12 Toxic Metabolites of Fungal Biocontrol Agents

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Introduction

One of the major hurdles in the registration and subsequent commercialization of fungal biological control agents (BCAs) is risk assessment. This is a costly, controversial and contentious area, which may have deterred many entrepreneurs from investing in the development of fungal BCAs. Risk assessment and related issues are dealt with in more detail in Chapter 13. Of particular interest (and the focus of this chapter) are the biological properties of toxic metabolites secreted by fungal BCAs. Plant pathologists have generally defined toxins as low-molecular-weight products of microorganisms active in low concentrations (Graniti, 1972).

Fungi secrete a wide array of compounds with biological activity against other organisms, mostly products of secondary metabolism (Table 12.1). Secondary metabolites originate as derivatives from various intermediates in primary metabolism (Fig. 12.1). Most of these compounds arise from five main metabolic sources: (i) amino acids; (ii) the shikimic acid pathway for the biosynthesis of aromatic amino acids; (iii) the polyketide biosynthetic pathway from acetyl coenzyme A (CoA); (iv) the mevalonic acid pathway from acetyl CoA; and (v) polysaccharides and peptidopolysaccharides. Biosynthesis of fungal secondary metabolites is beyond the scope of this book, but has been briefly reviewed by Griffin (1994).

Fungal metabolites serve different functions, depending on the ecological niche of the fungus (Fig. 12.2). Some metabolites may be antibiotics to protect the BCA against antagonistic microorganisms, or may prevent growth of saprophytic microbes on the host after it is killed and thus improve the survival of the BCA. Mycoparasites, in particular, may exploit this strategy to displace plant pathogens or postharvest diseases. Some bioactive metabolites are also important pathogenicity determinants (Strasser *et al.*, 2000) and others have antifeedant/repellent properties that presumably deter mycophagous organisms. This chapter reviews the production and biological activity of selected metabolites of fungal BCAs. It is impossible to cover all metabolites,

 Fungi as Biocontrol Agents
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Table 12.1. Selected metabolites of some important fungal biological control agents (BCAs).

Fungal BCA	Main target	Metabolites produced *in vitro* and/or *in vivo*
Metarhizium anisopliae	Insects	Destruxins (> 27 types), swainsinone, cytochalasin C
Beauveria bassiana	Insects	Bassianin, beauvericin, bassianolide, beauverolides, tenellin
Beauveria brongniartii	Insects	Oosporein
Paecilomyces fumosoroseus	Insects	Beauvericin, beauverolides, pyridine-2,6-dicarboxylic acid
Verticillium lecanii	Insects	Dipcolonic acid, hydroxycarboxylic acid, cyclosporin
Tolypocladium spp.	Insects	Cyclosporin, efrapeptins (five types)
Hirsutella thompsonii	Insects and mites	Hirsutellin A, hirsutellin B, phomalactone
Trichoderma spp.	Fungi	Harzianic acid, alamethicins, tricholin, peptaibols, antibiotics, 6-pentyl-α-pyrone, massoilactone
Gliocladium spp.	Fungi	Viridin, gliovirin, glisoprenins, heptelidic acid
Fusarium spp.	Fungi, insects, and weeds	Trichothecenes, beauvericin, naphthazarins (e.g. fusarubin and anhydrofusarubin), fusaric acid,
Colletotrichum	Weeds	Colletotrichin

since the discovery of new compounds is an ongoing process, with many remaining to be discovered. A few selected examples will illustrate concepts or patterns that may apply to other fungal BCAs.

Toxins of Entomogenous Fungi

The most studied species of entomogenous fungi are *Metarhizium anisopliae* and *Beauveria bassiana*. Less studied, but equally important species of commercial importance are *Paecilomyces fumosoroseus*, *Tolypocladium* spp., *Verticillium lecanii* and *Hirsutella* spp. These fungi secrete an array of secondary metabolites, some of which are restricted to specific genera, while others are more ubiquitous. Here, attention is focused on a few representative examples from selected genera.

Destruxins

The first systematic study of toxin production by fungal entomopathogens *in vitro* was conducted on *M. anisopliae* and led to the discovery of destruxins A and B (Kodaira, 1961). Since then, many other compounds have been isolated from *M. anisopliae* (Table 12.2). Suzuki *et al.* (1971) first demonstrated the production of destruxins *in vivo*. Destruxins are quite disparate compounds, which may occur as isomers or congeners. Their basic structural backbone consists of five amino acids and an α-hydroxy acid. To date, 28 structurally different, related destruxins have been identified from three different sources; most congeners are produced by *M. anisopliae* (Strasser *et al.*, 2000). Other natural analogues of destruxins have been described, including roseotoxin (Engstrom *et al.*, 1975) and bursephalocids A and B (Kawazu *et al.*, 1993).

Recently, destruxins A4, A1 and A5 and homodestruxin B were isolated from the entomopathogenic fungus *Aschersonia* sp. (Krasnoff and Gibson, 1996). Destruxin B,

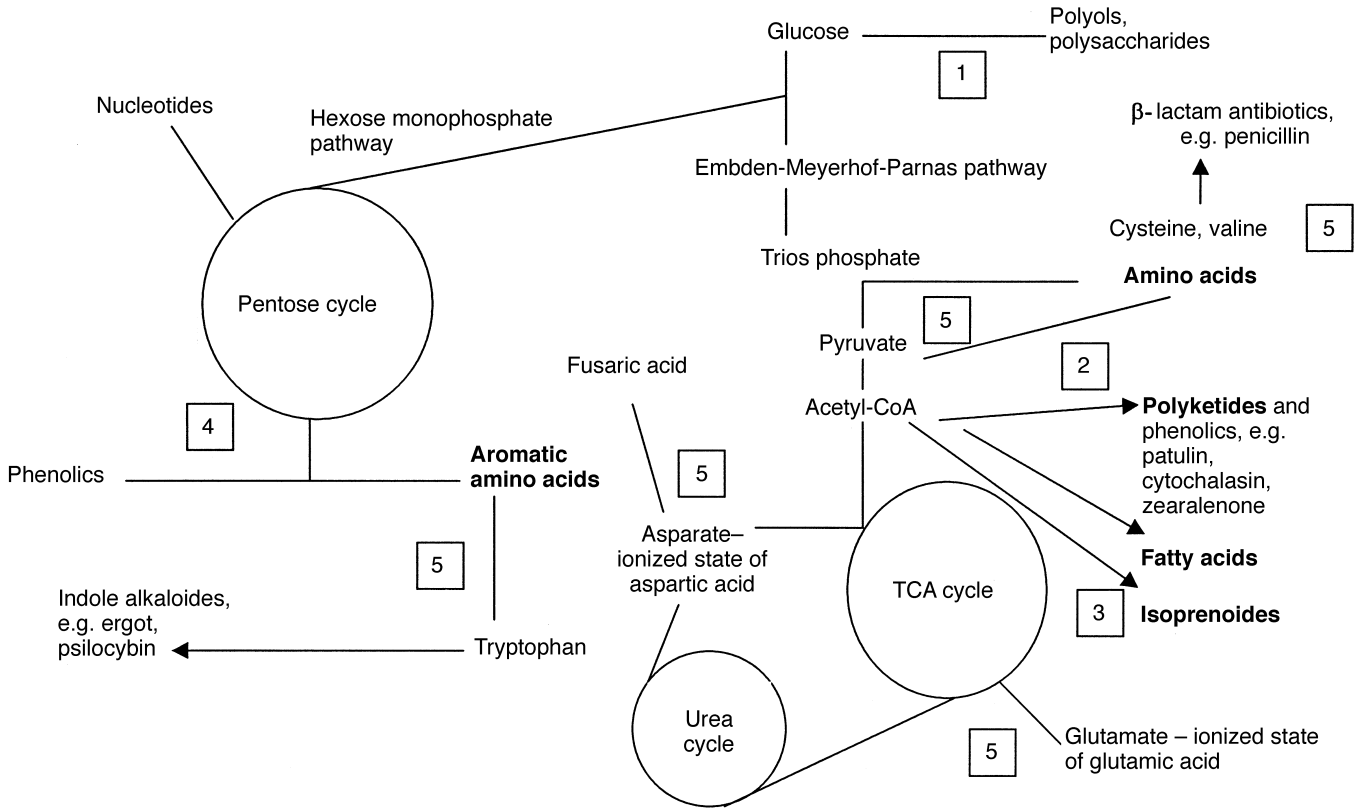

Fig. 12.1. Interrelationships of metabolic pathways in primary and secondary metabolism. The principal pathways of secondary metabolism are numbered as follows: (1) glucose-derived metabolites; (2) acetate–malonate pathway; (3) mevalonic acid pathway; (4) shikimic acid pathway; and (5) amino acid-derived pathways. TCA, tricarboxylic acid. (Adapted from Griffin 1994.)

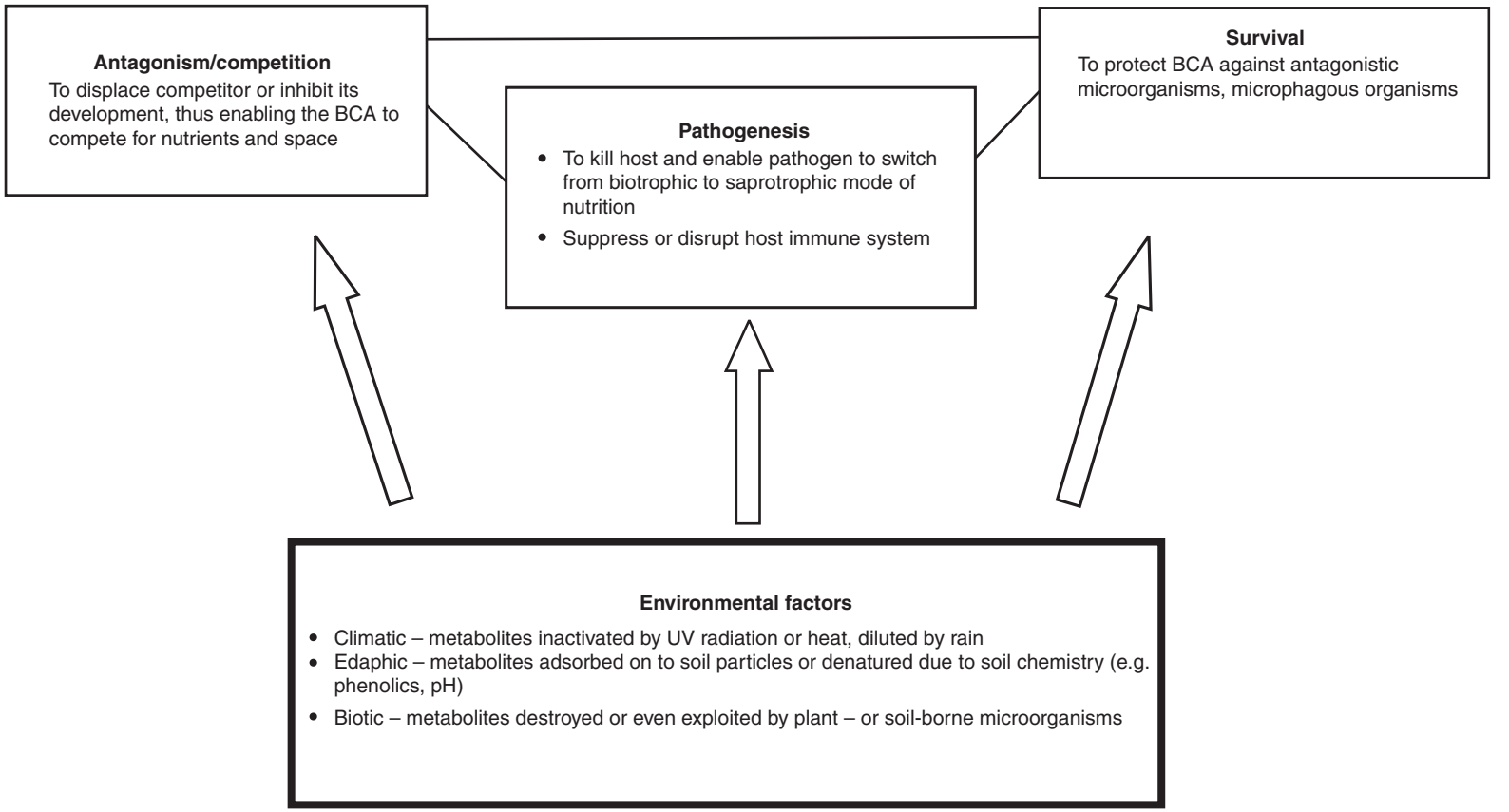

Fig. 12.2. Role of fungal metabolites and factors affecting their persistence. UV, ultraviolet.

Table 12.2. Destruxin analogues isolated from *Metarhizium anisopliae*.

Analogue	Reference
Desmethyl destruxins B, D and C	Suzuki *et al.*, 1970
Destruxins E, A1, A2, B1, B2, C2, D1, D2 and E1	Païs *et al.*, 1981
Destruxin E2 and chlorhydrin	Gupta *et al.*, 1989, 1991
Destruxins A3 and F and desmethyl destruxins A and C	Wahlman and Davidson, 1993

homodestruxin B and desmethyl destruxin B were also isolated from the plant-pathogenic fungi *Alternaria brassicae, Trichothecium roseum* and *Ophiosphaerella herpotricha* (Ayer and Pena-Rodriguez, 1987; Bains and Tewari, 1987; Gupta *et al.*, 1989; Buchwaldt and Jensen, 1991).

Insects vary in their susceptibility to destruxins, with some Lepidoptera being highly susceptible (Roberts, 1981; Charnley, 1984; Fargues and Robert, 1986; Samuels *et al.*, 1988a; Brousseau *et al.*, 1996; Thomsen *et al.*, 1996; Kershaw *et al.*, 1999). The median lethal dose (LD_{50}) of destruxin A and B injected into silkworm larvae was 0.015–0.030 mg g^{-1}, 24 h post-injection (Kodaira, 1961; Suzuki *et al.*, 1971; Tamura and Takahashi, 1971), but these compounds were ten- to 30-fold less active in waxmoth (*Galleria*) larvae (Roberts, 1966).

The effects of destruxins also vary between the species and specific developmental stages of the test organism and of the producing organism. For example, insects injected with low doses exhibit tetanus within 3 min and, at higher doses, tetanus paralysis is brief or absent and a flaccid paralysis occurs (Roberts, 1981). Toxins also affect insect growth. Larvae of the mustard beetle (*Phaedon cochleariae*) and potato lady beetle (*Epilachna sparsa*) grow slowly when exposed to leaves treated with destruxins compared with those fed on untreated leaves (Kodaira, 1961; Amiri *et al.*, 1999). The influence of destruxins on the larval growth, pupal weight and emergence rate of females was also observed in the eastern spruce bud-worm, *Choristoneura fumiferana* (Brousseau *et al.*, 1996).

Relationships between the chemical structure of destruxins and their biological activity have been investigated. Most workers note significant differences in the insecticidal activity of the compounds of this family, while others report no difference. Destruxins A and E appear to be the most toxic molecules and destruxin D the least toxic toward *Galleria* larvae (Fargues *et al.*, 1986; Vey and Quiot, 1989; Dumas *et al.*, 1994). Fargues and co-workers found that destruxins A and E were equally toxic, but more toxic than destruxin B when injected into or ingested by *Galleria* larvae. Housefly maggots appear to be more sensitive to destruxin E than to destruxin A or B (Robert and Fargues, 1986). Destruxin E was more toxic for *Galleria* larvae than for *Musca domestica*, which, in turn, was more susceptible to this toxin than the onion maggot fly, *Delia antiqua* (Roberts, 1981; Proprawski *et al.*, 1985; Fargues *et al.*, 1986; Robert and Fargues, 1986).

Some workers report that, depending on the type and host species involved, destruxin has no contact toxicity when applied to the integument (Fargues *et al.*, 1986), while others report contact toxicity (Poprawski *et al.*, 1994; Amiri *et al.*, 1999). Exactly how these compounds cross the insect cuticle remains unclear.

There is some evidence that destruxin E is systemic in plants, because the crucifer pest *Brevicoryne brassicae* (cabbage aphid) is repelled by cabbage leaves soaked in an 8.8 p.p.m. solution of destruxin E. *Myzus persicae* is also susceptible to destruxin

E (LD_{50} = 0.4 mg cm^{-2}), but not to the same degree as *B. brassicae*. In contrast, the cereal aphid *Rhopalosiphum padi* continued to feed on cereal leaves treated with destruxin E, even at relatively high doses, e.g. 6.6 mg cm^{-2} (Robert and Riba, 1989).

Little is known about the functionally active groups in the different destruxin molecules. It has been suggested that an epoxy group in destruxin E increases potency, while a COOH group may decrease potency, such as in destruxin D (Dumas *et al.*, 1994). It appears that destruxins have a spectrum of other biological activities, such as disruption of the calcium balance in cells (Dumas *et al.*, 1996a) and inhibition of vacuolar adenosine triphosphatases (ATPases) (Muroi *et al.*, 1994; Bandani *et al.*, 2001); some of these activities are summarized in Table 12.3.

There is some evidence that insects can remove injected destruxins from circulation (Samuels *et al.*, 1988b). Insects differ in their ability to detoxify destruxins (Fargues *et al.*, 1985). The fat body seems to possess a greater affinity for this cyclic peptide than any other tissue or organ and is a more important detoxification system than the pericardial tissue (Lange *et al.*, 1991, 1992). When destruxin E is injected into *Locusta migratoria*, it is converted by the fat body into destruxin E-diol (which is inactive when injected in *Galleria mellonella*) and E-diol is secreted by the Malpighian tubules. Likewise destruxin A is transformed into the corresponding linear peptide (Lange *et al.*, 1991, 1992; Loutelier *et al.*, 1994, 1995).

The induction of paralysis by injection of haemolymph from insects infected by *M. anisopliae* suggested that destruxins are secreted during fungal infection (Kodaira, 1961; Roberts, 1966). The destruxin content amounted to 240 ng per silkworm larva 4 days after inoculation with spores. Sufficient amounts of destruxins are secreted into the haemolymph to induce a cytotoxic effect on host tissues (Vey *et al.*, 1986).

Al-Aïdroos and Roberts (1978) reported a link between the amount of destruxin produced by mutants of *M. anisopliae in vitro* and virulence against mosquito larvae. Recent studies by Amiri-Besheli *et al.* (2000) reveal inter- and intra-specific variation in destruxin production by the genus *Metarhizium*, both *in vivo* and *in vitro*. Specialized species, such as *Metarhizium album*, which is restricted to hemipteran insects, produce very little destruxin, while generalist species, such as *M. anisopliae* var. *anisopliae*, produce destruxins A, B and E, often in significant quantities. These observations suggest that destruxin may be significant in determining host specificity. The authors also argued that destruxins were possibly important pathogenicity determinants for some strains of *Metarhizium.*

Destruxins have antifeedant properties and are toxic to insects following absorption through the cuticle (Amiri *et al.*, 1999). They are also toxic to small mammals. The LD_{50} of destruxins A and B following intraperitoneal injection in mice was 1–1.35 mg kg^{-1} and 13.2–16.9 mg kg^{-1} within 1 h, respectively (Kodaira, 1961). In contrast, destruxins are less toxic to fish and amphibians. No lethal or teratogenic effect or postponement of emergence of the embryos was observed in the teleostean fish *Brachydanio rerio* H.B. (Debeaupuis and Lafont, 1985). The acute toxicity of destruxins on the amphibians *Xenopus laevis* Daudin and *Rana temporaria* L. is low, i.e. as a reference point, chemical pesticides, such as the fungicide thiram, show a stronger (lethal) effect (Fargues and Robert, 1986).

Recently, a neutral lipophilic extract (methylene chloride, pH 7.2) derived from *M. anisopliae* cultures was evaluated for toxicity and mutagenicity, using aquatic animal bioassays and the Ames test (Genthner *et al.*, 1998). The average LC_{50} of the extract obtained in static, acute 96 h tests conducted with 24-h-old *Mysidopsis bahia* was 2.41 mg l^{-1}. However, by partially purifying destruxins from the neutral extract,

Table 12.3. Some activities of destruxins (Dtx).

Effect/property	Reference
Dtx A, B and E cause *Gromphadorhina laevigata* (Dictyoptera) and *Bombyx mori* cell lines (Lepidoptera) to contract, become granulated and stop dividing. The cell line of *B. mori* was more susceptible to these destruxins than cells of *G. laevigata,* even at relatively low doses, i.e. 0.05 p.p.m. versus 1 p.p.m.	Quiot *et al.*, 1985
Dtx E effects on invertebrate cells include: aggregation of chromatin, deformation of nuclei, degradation of mitochondria and rough endoplasmic reticulum and impaired functioning of the ribosomes	
Interfere with haemocyte function and can prevent nodulation	Vey *et al.*, 1985; Huxham *et al.*, 1989
Trigger degranulation of isolated haemocytes of the crayfish (*Pacifastacus leniusculus*)	Cerenius *et al.*, 1990
Inhibit phagocytosis in plasmatocytes *in vitro* and in infected larvae	Vilcinskas *et al.*, 1997
Inhibit synthesis of DNA, RNA and proteins even at low doses, e.g. nucleotide synthesis of mouse P388 leukaemic cell lines.	Odier *et al.*, 1992
Dtx E acts on the midgut, Malpighian tubules and circulating haemocytes	Vey and Quiot, 1989; Dumas *et al.*, 1996 b
Block H^+-ATPase activity	Muroi *et al.*, 1994
Trigger phosphorylation of unidentified cellular proteins	Dumas *et al.*, 1996a
Alter enzyme activity, i.e. phenoloxidase, which is involved in melanin synthesis	Vey *et al.*, 1985; Huxham *et al.*, 1989; Cerenius *et al.*, 1990
Antiviral effects	Quiot *et al.*, 1980, 1985; Kopecky *et al.*, 1992; Sun *et al.*, 1994; Yeh *et al.*, 1996; Chen *et al.*, 1997
Cause rapid decrease in the transmembrane resting potential; directly or indirectly open endogenous Ca^{2+} channels within the muscle membrane of *Manduca sexta*	Bradfisch and Harmer, 1990
Prevent secretion of ecdysteroids by prothoracic glands of *Manduca*	Sloman and Reynolds, 1993
Inhibit fluid secretion by *Schistocerca gregaria* Malpighian tubules	James *et al.*, 1993

it was shown that destruxins alone were not responsible for the observed toxicity in mysids. After 3 months, no mortalities or adverse effects were observed in adult *Gambrusia affinis* fed a diet partially composed of a freeze-dried *M. anisopliae* culture. The same extract showed no mutagenicity in the Ames test using strains TA98 and TA100, with and without metabolic activation by rat liver S9. However, the extract was toxic to developing grass shrimp, *Palaemonetes pugio*, and frog, *X. laevis*, embryos; the LC_{50} values were 52 and 32 mg l^{-1}, respectively. The extract was toxic to juvenile mosquito fish, *G. affinis*, at an LC_{50} value of 141 mg l^{-1}. However, adult female

G. affinis surviving a 24 h exposure to 200 μg ml^{-1} of the neutral extract produced healthy broods.

In the case of the plant pathogen *A. brassicae*, destruxins play an important role in the infection of several *Brassica* species. These toxins induce chlorosis and have been isolated from infected leaf tissue (Ayer and Pena-Rodriguez, 1987; Bains and Tewari, 1987; Buchwaldt and Jensen, 1991; Buchwaldt and Green, 1992). Bains and Tewari (1987) suggested that the level of sensitivity of *Brassica* species to destruxin B was related to their degree of susceptibility to *A. brassicae*, with non-host plants being least affected. Likewise, Venkatasubbaiah *et al.* (1994) reported that destruxin B from *O. herpotricha* induced necrotic/chlorotic reactions in host but not in non-host plants.

Efrapeptins

The linear peptidic efrapeptins (types C to G) have been isolated only from *Tolypocladium* species (Weiser and Matha, 1988; Krasnoff and Gupta, 1991). Efrapeptins are inhibitors of intracellular protein transport and mitochondrial ATPases (Fricaud *et al.*, 1992; Krasnoff *et al.*, 1991). They arrest syncytium formation (SF) and have cytopathic effects (CPE) in Newcastle disease virus (NDV)- and vesicular stomatitis virus (VSV)-infected BHK cells, respectively, without profoundly affecting glycoprotein synthesis (Muroi *et al.*, 1996). Efrapeptins blocked cell-surface expression of NDV-HN and VSV-G glycoproteins, but did not suppress intoxication by ricin or diphtheria toxin even after prolonged pretreatment. Efrapeptins inhibit F-ATPase or ATP synthase, but their inhibitory effect on SF and CPE was independent of the intracellular ATP concentration (Muroi *et al.*, 1996).

Efrapeptins show insecticidal and miticidal effects against arthropod species such as spider mites, potato beetle, tobacco bud-worm and diamondback moth (Matha *et al.*, 1988; Krasnoff *et al.*, 1991). Dose-related antifeedant and insect growth inhibitory properties have also been reported by Bandani and Butt (1999). Furthermore, efrapeptins have limited antifungal and antibacterial activity. There is no information concerning the phytotoxicity and antiviral properties of the efrapeptins.

As with destruxin, there was inter- and intraspecific variation in efrapeptin production. In *Tolypocladium cylindrosporum, Tolypocladium niveum* and *Tolypocladium parasiticum*, efrapeptin production reached 116 mg l^{-1}, 80 mg l^{-1} and 2 mg l^{-1} in respective culture filtrates after 14 days' incubation. *Tolypocladium* species secreted low amounts of efrapeptins in the insect haemocoel during the infection process. Few fungal hyphae were detected in dead, infected *G. mellonella* and *Calliophora* sp., suggesting that death was to due to toxicosis. The LD_{50} values of the purified efrapeptin mixture for final-instar larvae of the Lepidoptera *G. mellonella* and *Manduca sexta* in injection assays were, on average, 30 and 47 ng, respectively (Bandani *et al.*, 2000).

Oosporein

The red-coloured dibenzoquinone oosporein is produced by a large number of soil fungi and the entomogenous fungi belonging to the genus *Beauveria* (Eyal *et al.*, 1994; Strasser *et al.*, 2000a, b). Oosporein is considered to react with proteins and amino acids through redox reactions by altering SH groups, resulting in enzyme malfunction (Wilson, 1971). Oosporein, like tenellin and bassianin, will inhibit erythrocyte mem-

brane ATPase activity in a dose-dependent manner by as much as 50% at 200 μg ml^{-1}. These pigments inhibited Ca^{2+}-ATPases to a greater extent than Na^{+}/K^{+}-ATPase activity. The ATPase-inhibitory activity of these pigments was not specific, but was probably a consequence of membrane disruption, since they all caused alterations in erythrocyte morphology and promoted varying degrees of cell lysis (Jeffs and Khachatourians, 1997). Oosporein is an antiviral compound, preferentially inhibiting herpes simplex virus-I DNA-polymerase (Terry *et al.*, 1992). The authors found that oosporein was a competitive inhibitor of dGTP or dCTP incorporation into DNA.

Oosporein is an effective antibiotic against Gram-positive bacteria, but has little effect on Gram-negative bacteria (Vining *et al.*, 1962; Brewer *et al.*, 1984; Taniguchi *et al.*, 1984; Wainwright *et al.*, 1986). It has no obvious antifungal properties but there are mixed reports on its phytotoxicity; some workers report plant growth-inhibiting and phytotoxic effects, while others report the contrary (Cole *et al.*, 1974; Brewer *et al.*, 1977; Strasser *et al.*, 2000). However, oosporein has been reported to cause avian gout in broiler chicks and turkeys (Cole *et al.*, 1974; Pegram and Wyatt, 1981; Pegram *et al.*, 1982; Manning and Wyatt, 1984; Brown *et al.*, 1987). Furthermore, oosporein has been found to be toxic to 1-day-old male chickens (LD_{50} = 6 mg kg^{-1}; Manning and Wyatt, 1984)). Toxicity studies of oosporein in mice and hamsters indicated an LD_{50} value of 0.5 mg kg^{-1} body weight, when injected intraperitoneally (Wainwright *et al.*, 1986). However, a daily oral administration of 7 mg kg^{-1} oosporein to mice over 47 days was non-lethal. Cytotoxicity tests on two different mammalian cell lines revealed that oosporein, at 600 ng ml^{-1}, had no adverse effect (Abendstein and Strasser, 2000). In addition, oosporein at 100 μg ml^{-1} had no effects on *in vitro* cell cultures of hamster tumour cells and baby hamster kidney cells (Wainwright *et al.*, 1986). Aleo *et al.* (1991) studied the nephrotoxic potential of oosporein using rat renal proximal tubules. The authors reported that tubule viability was altered, but there was no evidence to support a direct inhibitory effect on mitochondrial respiration at a maximum oosporein concentration of 306 μg ml^{-1}.

Strasser *et al.* (2000) found that oosporein was the only major secondary metabolite produced by three commercial strains of the entomopathogenic fungus *Beauveria brongniartii* in submerged cultures and on sterilized barley kernels. None of the other major toxins (bassianin, beauvericin and tenellin) normally produced by *Beauveria* species were detected by sensitive high-performance liquid chromatography (HPLC) and mass spectrometry (MS) techniques (Strasser *et al.*, 2000b). Both *in vitro* and *in vivo* studies on the distribution of oosporein revealed negligible amounts in the environment, suggesting that these particular strains of *B. brongniartii* pose no risk to humans and animals (Strasser *et al.*, 2000a). Laboratory experiments have shown that the maximum amount of oosporein produced in liquid batch reactors was 270 mg l^{-1}, after 4 days' incubation, while that produced on sterilized barley kernels ranged between 2.0 and 3.2 mg kg^{-1}, after 14 days' incubation (production time). The maximum amount of oosporein detected in cockchafer (*Melolontha melolontha*) larvae infected with *B. brongniartii* was 0.23 mg $larva^{-1}$. Melocont®-Pilzgerste, a commercial product based on *B. brongniartii*, was not phytotoxic to garden cress (*Lepidium sativum*), Hurd's grass (*Phleum pratense*) and potatoes (*Solanum tuberosum*), nor were fungal metabolites detected in these indicator plants and potato tubers (Strasser *et al.*, 2000; H. Strasser, unpublished data). No systemic effects (e.g. chlorosis, necrosis and stunting) of oosporein were observed in pasture turf treated with oosporein-enriched *B. brongniartii* culture broth, even several months after treatment (Strasser *et al.*, 2000).

Based on the results of laboratory and field experiments, a theoretical oosporein

concentration of 4.8–6.4 mg m^{-2} can be expected to be detected in the soil. This calculation is based on an average infestation density of 80 *M. melolontha* larvae m^{-2} and an average infection rate of 30–40%.

Beauvericin, bassianolide and beauveriolide

Beauvericin is a hexadepsipeptide, previously isolated from the entomopathogenic fungi *Beauveria* spp. and *Paecilomyces* spp. and the plant-pathogenic fungi *Fusarium* spp. and *Polyporus fumosoroseus* (Grove and Pople, 1980; Gupta *et al.*, 1991; Plattner and Nelson, 1994; Logrieco *et al.*, 1998; Munkvold *et al.*, 1998), and two analogues, A and B, were described by Gupta *et al.* (1995).

Beauvericin is structurally and functionally similar to the membrane-damaging antibiotics enniatins A, B and C and differs from these compounds with respect to the *N*-methylamino acids (Steinrauf, 1985). Beauvericin forms Na^+ and K^+ complexes, leading to increased permeability of natural and artificial membranes (Ovchinnikov *et al.*, 1971). Beauvericin shows antibiotic activity against several bacteria, such as *Bacillus subtilis*, *Escherichia coli*, *Mycobacterium phlei*, *Sarcinea lutea*, *Staphylococcus aureus* and *Streptococcus faecalis* (Ovchinnikov *et al.*, 1971). Furthermore, beauvericin has moderate insecticidal properties (Suzuki *et al.*, 1977; Kanaoka *et al.*, 1978; Champlin and Grula, 1979; Qadri *et al.*, 1989; Zizka and Weiser, 1993; Gupta *et al.*, 1995). However, some workers report that it has no toxicity for certain insects (Champlin and Grula, 1979).

Beauvericin is a specific cholesterol acyltransferase inhibitor of certain cell lines, induces programmed cell death similar to apoptosis and causes cytolysis, accompanied by internucleosomal DNA fragmentation into multiples of 200 base pairs (Ojcious *et al.*, 1991). It is toxic to brine shrimp (*Artemia salina* L.) with an LD_{50} = 2.8 μg ml^{-1} water (Moretti *et al.*, 1995), and to *M. bahia*, at an LD_{50} of 0.56 mg l^{-1}. The toxicity of beauvericin persists in sterile sea water for at least 3 but not 8 weeks (Genthner *et al.*, 1994). A non-polar extract of mycelia from *B. bassiana* containing beauvericin was toxic at an LC_{50} of 84.2 mg l^{-1}. Beauvericin toxicity was mostly investigated using insects, but recent reports show high *in vitro* toxicity towards murine (Ojcious *et al.*, 1991) and human cell lines (Di Paola *et al.*, 1994).

Another toxin secreted by *B. bassiana* is the cyclo-octadepsipeptide called bassianolide (Suzuki *et al.*, 1977). This chemical induces atonic symptoms in silkworm larvae fed on an artificial diet containing small amounts of this compound, but was lethal at higher doses (13 p.p.m.). Bassianolide, like beauvericin, is an ionophore antibiotic, but differs in its reaction to different cations (Suzuki *et al.*, 1977; Kanaoka *et al.*, 1978). Neither beauvericin nor bassianolide has been shown to exhibit mammalian or plant toxicity, but there may possibly be a synergistic effect with the structurally related mycotoxin moniliformin (Cole *et al.*, 1973). *Fusarium* species under certain conditions produce beauvericin, moniliformin, fumonisins (b1, b2) and fusaproliferin (Gupta *et al.*, 1991; Logrieco *et al.*, 1998; Munkvold *et al.*, 1998). Ingestion of *Fusarium*-contaminated grain by mammals and the subsequent uptake of these metabolites causes cancer of the oesophagus and heart problems (Marasas *et al.*, 1981). Whether beauvericin should be classified as a food toxin has not yet been determined.

Beauveria species also produce beauveriolides and beauverolides, which are peptides structurally related to beauvericin and bassianolide (Namatame *et al.*, 1999). Their toxicity to animals, plants and insects remains generally unknown. Where tested, they

give negative results, with the exception of beauveriolide I (Mochizuki *et al.*, 1993).

Bassianin and tenellin (two non-peptide toxins) have also been isolated from *Beauveria* species. These yellow-coloured secondary metabolites inhibit the erythrocyte membrane ATPases (Jeffs and Khachatourians, 1997). There is very little information on the effect of these toxins on target pests.

Hirsutellin

The hyphomycete *Hirsutella thompsonii* produces an extracellular insecticidal protein, hirsutellin A, which has been purified from culture filtrates during liquid fermentation (Mazet, 1992; Vey *et al.*, 1993). Liu *et al.* (1995) monitored the production of hirsutellin A by *H. thompsonii* during submerged fermentation. The peak level of exocellular production of hirsutellin A (13–14 μg ml^{-1}) occurred at the late exponential growth phase (39–45 h), as determined by densitometric analysis of the 16.3 kDa bands on SDS-PAGE gels and enzyme-linked immunosorbent assay (ELISA). Hirsutellin A production was directly correlated with mycelial growth. Twenty-one-hour culture filtrates were highly toxic to larvae of the greater wax-moth. Pure hirsutellin A at a concentration of 40 pmol was highly toxic to *G. mellonella* larvae.

The amino acid composition and the N-terminal sequence of hirsutellin A have been determined by Mazet and Vey (1995). The toxin appears to be distinct from other known proteins. It is not glycosylated and does not show proteolytic activity. The toxin is also antigenic, thermostable and not inactivated by treatments with proteolytic enzymes.

The hirsutellin A gene codes for a precursor of 164 amino acids, which includes a 34-amino-acid leader sequence, which, like those found in ribosomal-inhibiting proteins (RIPs), contains a signal and a pro sequence. The mature 130-amino-acid hirsutellin A, with a calculated M_r= 14,159 and pI = 9.21, is considered to be a stable hydrophilic protein. The sequence of hirsutellin A is unique and does not produce the secondary or tertiary structures characteristic of other fungal RIPs (Boucias *et al.*, 1998).

Hirsutellin A was tested using contact/residual leaf bioassay methodologies at concentrations of 0, 10, 32, 56 and 100 μg ml^{-1} against adult citrus rust mite, *Phyllocoptruta oleivora*, the natural host of the parasitic fungus, *H. thompsonii*. Mite mortality increased with an increase in hirsutellin A concentration, reaching virtually 100% at 100 μg ml^{-1}, using both leaf assay methods. The number of eggs found on leaf discs within a 3-day period decreased significantly with increasing concentrations of the toxin, suggesting that fecundity was affected prior to host death (Omoto and McCoy, 1998). Toxicity bioassays showed that wax-moth larvae injected with hirsutellin A at 1 mg toxin g^{-1} body weight caused a high mortality rate. Hirsutellin A was also toxic per os to neonatal mosquito (*Aedes aegypti*) larvae (Mazet and Vey, 1995). It is also capable of inhibiting protein translation, and possesses biological features similar to the well-characterized RIPs sarcin, mitogellin and restrictocin. Liu *et al.* (1996) reported that at 0.5 and 5.0 μM concentrations, hirsutellin A caused detectable cytopathic effects on *Spodoptera frugiperda* (Sf-9) cells within 2–4 h and completely inhibited Sf-9 cell growth 4 days after treatment. Electron-microscopic data showed that hirsutellin A-treated Sf-9 cells became hypotrophied, with disrupted internal organelles and cell membranes. At the same concentration, it effectively inhibited Brome mosaic virus protein synthesis of both rabbit reticulocyte and wheat germ in *in vitro* translation systems. The ribosomal RNA extracted from hirsutellin A-treated Sf-9 cells produced a

smaller RNA (about 528 bases) in addition to larger bands present in control and treated ribosomal preparations (Liu *et al.*, 1996).

Organic acids

Among organic acids, oxalic, kojic, cyclopyazonic, fusaric and 4′-hydroxymethylazoxybenzene-4-carboxylic acids have been isolated from fungi pathogenic to invertebrates, and are considered toxic to lepidopterans or dipterans (Roberts, 1981; Bidochka and Khachatourians, 1991; Khachatourians, 1996). Oxalic acid is an important pathogenicity determinant of some plant pathogens (Godoy *et al.*, 1990). It is also produced by *B. bassiana* (Kodaira, 1961) and is considered to be an important pathogenicity determinant because it can solubilize specific cuticular proteins. Bidochka and Khachatourians (1991) described this metabolite as a synergistic factor that enhances the hydrolytic activity of proteases and chitinase. The zygomycete *Entomophthora virulenta* produces azoxybenzene-4,4′-dicarboxylic acid and 4′-hydroxymethylazoxybenzene-4-carboxylic acid. The hydroxyacid is toxic for *Calliphora erythrocephala* when applied by injection, and is responsible for the insecticidal activity in culture filtrates (Claydon, 1978). This compound has structural similarities to the dichlorodiphenyltrichloroethane (DDT) group of insecticides (Roberts, 1981).

Toxins of Mycoparasites

Two of the most important commercial mycoparasites belong to the related genera *Trichoderma* and *Gliocladium* (Tomlin, 1997). These fungi produce a large variety of metabolites with diverse functions. However, the success of these BCAs as disease control agents can be partly attributed to the production of antifungal metabolites, aggressive growth habits and high competition for nutrients. This is also one of the reasons for the success of the naturally occurring mutant strain Fo47 of *Fusarium oxysporum*. This isolate protects plants from pathogenic strains of *F. oxysporum* and *Fusarium moniliforme* due to its rhizosphere competency (i.e. ability to colonize roots quickly) and competition for nutrients. It may displace plant-pathogenic strains through competitive exclusion, using antibiotics without harming the host plant.

Gliocladium virens strain GL-21 has been developed by Grace Biopesticides in collaboration with the US Department of Agriculture Agricultural Research Service (USDA-ARS) Biocontrol of Plant Diseases Laboratory in Beltsville, Maryland. The commercial product, SoilGard™, is an entirely biorational product, consisting of spores of this fungus, and is registered with the US Environmental Protection Agency (EPA) for control of damping-off and root-rot pathogens of ornamental and food-crop plants in greenhouses, nurseries and interior gardens. *G. virens* can parasitize some soil pathogens, such as *Rhizoctonia solani.* The *Gliocladium* will actually wrap itself around the pathogen and release enzymes that destroy the pathogen's wall, leaving the pathogen susceptible to attack. It also produces a broad-spectrum antibiotic, called gliotoxin, which kills many soil pathogens (see Chapter 2). Gliotoxin is sensitive to oxidation and probably poses no health risk because of rapid degradation. This is supported by the demonstrated lack of toxicity in oral and pulmonary studies conducted on rats (Anon, 1990).

Gliotoxin is not found in the SoilGard™ formulations, but, when the spores of

strain GL-21 begin to grow in the soil, they produce the antibiotic. SoilGard™ has a 'Caution' label, which is given to products which are least harmful and which may cause only slight irritation after normal exposures. It is exempt from tolerance for use on all food crops. SoilGard™ has the minimum re-entry interval allowed by the EPA, and has a 'zero day' preharvest interval.

Trichoderma harzianum is produced by several companies for the control of a wide range of plant-pathogenic fungi including *Botrytis cinerea* (Chapters 1 and 2). Several formulations based on selected strains of this fungus have been registered with the EPA. Toxicity testing on vertebrate species indicated no pathogenic or toxic effects, and the EPA has granted an exemption from tolerance for selected commercial strains.

In this review we focus on two important, controversial metabolites, peptaibols and gliotoxin. Other metabolites produced by these fungi will also be briefly reviewed, since they usually work in concert with peptaibols and gliotoxin.

Peptaibols

These are a family of short-chain polypeptides consisting of 15–20 residues or fewer. A high proportion of the amino acid residues are atypical, such as isovaline, hydroxyproline, ethylnorvaline and aminoisobutyric acid. There is a particularly high proportion of aminoisobutyric acid, which has a high tendency to form helices. This is borne out by the helical structures of the peptaibols. The chain has an alkyl N-terminus (usually acetyl) and a hydroxy-amino acid at the C-terminus.

Peptaibols generally exhibit antimicrobial activity and are referred to as antibiotic peptides. The main sources of the peptaibols known to date are fungi of the genera *Trichoderma* and *Emericellopsis*. The antimicrobial activity is thought to arise from their membrane activity and their ability to form pores in lipid membranes. The pores so formed cause leakage of ionic species across membranes, leading to loss of osmotic balance and cell death.

Peptaibols are usually secreted as microheterogeneous mixtures of peptides. Those secreted by *T. harzianum* include trichorzianins (El Hajji *et al.*, 1987; Rebuffat *et al.*, 1989), trichokindins (Iida *et al.*, 1994), trichorzins and harzianins (Rebuffat *et al.*, 1992; Goulard *et al.*, 1995; Duval *et al.*, 1998; Leclerc *et al.*, 1998). The trichokindins, which are 18-residue peptides containing one to three isovaline residues, induced Ca^{2+}-dependent catecholamine secretion from bovine adrenal medullary chromaffin cells (Iida *et al.*, 1994). *Trichoderma viridae* produces alamethicins (Kleinkauf and Rindfleisch, 1975; Brewer *et al.*, 1987) and trichotoxin (Irmscher *et al.*, 1978). Paracelsin, originally described as a metabolite of *Trichoderma resei*, is, in fact, produced by many *Trichoderma* species (Bruckner and Graf, 1983; Solfrizzo *et al.*, 1994). Paracelsin is highly toxic to *A. salina* larvae (calculated LD_{50} = 2.2 μM) (Solfrizzo *et al.*, 1994).

Lorito *et al.* (1996) reported synergism between peptaibols and cell-wall hydrolytic enzymes in the antagonism of phytopathogenic fungi by *T. harzianum*. β-Glucan synthase activity on isolated plasma membranes of *B. cinerea* was inhibited *in vitro* by the peptaibols trichorzianin TA and TB, but inhibition was reversed by the addition of phosphatidylcholine. β-Glucan synthesis *in vivo* (assayed by incorporation of [2-(3)*H*]glucose into cell-wall material) was inhibited by peptaibols, and this inhibition was synergized by exogenous *T. harzianum* β-1,3–glucanase. This synergism is, therefore, explained by an inhibition of the membrane-bound β-1,3-glucan synthase

of the host by the peptaibols, which inhibit the resynthesis of cell-wall β-glucans, sustain the disruptive action of β-glucanases and altogether enhance the fungicidal activity. Therefore cell-wall turnover is a major target of mycoparasitic antagonism.

Gliotoxin

The epidithiodiketopiperazine antibiotic gliotoxin was discovered in 1934 as an antifungal agent (Weindling, 1934) and then various biological activities were reported. It has subsequently been shown to have antimicrobial, antiviral and immunomodulating activities (Taylor, 1986). Furthermore, it is known to be an inhibitor of the platelet activating factor (PAF), which induces platelet aggregation (Okamoto *et al.*, 1986a, b; Yoshida *et al.*, 1988). The antifungal properties of gliotoxin are synergistically enhanced by the cell wall-degrading enzymes of *T. harzianum* and *G. virens* (Lorito *et al.*, 1994).

Gliotoxin is a toxic product of several moulds that cause a serious respiratory disease of poultry and humans. This toxin affects the immune system and inhibits many functional aspects of this system, and renders the host prone to disease-causing agents.

The relatively short period of bioactivity limits the use of this agent in certain applications. Wilhite and Straney (1966) examined the apparent transient accumulation of gliotoxin, a potential limitation in biocontrol activity. ^{35}S-pulse labelling of gliotoxin indicated that *G. virens* strain G20-4VIB synthesizes this compound only within a short 16 h period, during replicative growth. An apparent lack of gliotoxin production in later growth phases was due to the cessation of synthesis, rather than to increased gliotoxin catabolism. Media-transfer experiments indicated that cessation of gliotoxin synthesis could not be explained by gliotoxin feedback inhibition, a diffusible inhibitor or a change in the nutritional status of the medium over a 2 h period. These results demonstrate that the regulation of gliotoxin biosynthesis is a major determinant in the kinetics of gliotoxin appearance and points out the need for further study on the regulation of gene expression (Wilhite and Straney, 1966).

Other important metabolites of Trichoderma and Gliocladium *species*

Trichoderma and *Gliocladium* secrete diverse secondary metabolites with antibiotic properties, including polyketides, terpenoids, polypeptides and metabolites derived from α-amino acids (Taylor, 1986). Thus *T. harzianum* produces harzianic acid (Sawa *et al.*, 1994), the terpenoid cyclonerodiol and the corresponding octaketide keto-diol (Ghisalberti and Rowland, 1993), 6-pentyl-α-pyrone antibiotics (Graeme-Cook and Faull, 1991), and a new sesquiterpene antibiotic, heptelidic acid, also produced by *G. virens* (Itoh *et al.*, 1980). Heptelidic acid has antibiotic activity against Gram-positive and Gram-negative bacteria and inhibits the growth of some anaerobic bacteria (Itoh *et al.*, 1980).

T. harzianum produces unidentified volatile metabolites with fungistatic effects on *Agaricus bisporus* (Mumpuni *et al.*, 1998). T-2 toxin and related trichothecenes are secreted by *Trichoderma* species (Ueno, 1984). The production by *T. harzianum* of a novel trichothecene, harzianum A, which exhibits modest antifungal activity, has been reported (Corley *et al.*, 1994). Furthermore, a novel antifungal protein from *T. viride*, tricholin, has been described (Lin *et al.*, 1994). This ribosome-inactivating protein causes cessa-

tion of growth and uptake of amino acids, and is active against *R. solani*. Cyclonerodiol and koninginins have been purified and characterized from strains of *Trichoderma koningii* (e.g. Ghisalberti and Rowland, 1993). Some of these compounds have been purified from culture filtrates of a *T. harzianum* strain isolated from wheat roots and were active against the take-all fungus, *Gaeumannomyces graminis* var. *tritici*.

Antifungal antibiotics produced by *T. harzianum* are not sufficient to explain its mycoparasitic activity. Ultraviolet light-induced mutants with altered antibiotic production revealed that those strains with elevated antibiotic production did increase inhibition of hyphal growth of phytopathogenic fungi, but there was no correlation between this factor and colonization ability (Graeme-Cook and Faull, 1991).

In addition to secondary metabolites, *Trichoderma* and *Gliocladium* spp. also produce different classes of fungal cell-wall-hydrolytic enzymes such as chitinases, β1,3-glucanases and proteases, which play an important role in mycoparasitism (Schirmbock *et al.*, 1994). Cell-wall-degrading enzymes produced by *T. harzianum* and *G. virens* inhibit spore germination of *B. cinerea in vitro* (Lorito *et al.*, 1994). The role of *T. harzianum* endochitinase has been studied, using constructed strains carrying multiple copies of the gene encoding for this enzyme and by using gene disrupters. The level of chitinase activity increased strongly in multi-copy strains, while gene disrupters had practically no activity. However, comparative observations regarding the efficacy of the strains generated as BCAs revealed no major differences (Carsolio *et al.*, 1999).

Hydrolytic enzymes and peptaibol antibiotics are produced in parallel by *T. harzianum* in the same cultural conditions when cell walls of *B. cinerea* are introduced in fresh medium. When enzymes and peptaibols were tested together, an antifungal synergistic action on spore germination and hyphal elongation, measured by the reduction of 50% of the effective dose value, was noted. These data revealed that parallel formation and synergism of hydrolytic enzymes and antibiotics may have an important role in the antagonistic action of *T. harzianum* (Schirmbock *et al.*, 1994). Investigations on the molecular bases for this synergism showed that it is peptaibol inhibition of the membrane-bound β-1,3-glucan synthase of *B. cinerea* that strengthens the disruptive activity of β-glucanases (Lorito *et al.*, 1996).

In conclusion, the biocontrol efficacy of *Trichoderma* spp. seems to be a combination of antibiosis, lysis, competition, mycoparasitism and promotion of plant growth (Ghisalberti and Sivasithamparam, 1991).

Besides the metabolites already mentioned, *Gliocladium* spp. are able to produce other antibiotics such as the fungistatic compound viridin (Jones and Hancock, 1987) and gliovirin (Stipanovic and Howell, 1982). *Gliocladium* species also produce a chitinase inhibitor in liquid culture called argifin. The IC_{50} value of argifin against *Lucilia cuprina* chitinase was 3.7 μM. It arrested the moult of cockroach larvae upon injection into the ventral abdominal part (Omura *et al.*, 2000). Various other metabolites have also been discovered in culture broths, including verticillin, glisoprenin (Joshi *et al.*, 1999) and polyketide antibiotics (Kohno *et al.*, 1999). Glisoprenins inhibit the formation of appressoria by the phytopathogenic fungus *Magnaporthe grisea* on inductive surfaces (Thines *et al.*, 1998).

Toxins of Mycoherbicides

Some microbes (phytopathogenic and non-phytopathogenic bacteria and fungi) and secondary microbial products (phytotoxins) exhibit potential as biological weed control agents (see Chapter 6; Lax *et al.*, 1988). Collectively these organisms and natural products are called bioherbicides. Fungi with potential bioherbicidal activity are termed mycoherbicides. These organisms are generally applied to weeds in a similar manner to synthetic herbicides, i.e. spray applications to weed surfaces. This use of pathogens for weed control differs from that of 'classical biological control', where organisms are released and allowed to spread to host plants via natural dispersion in the environment. Mycoherbicidal organisms are usually applied directly to host weeds or narcotic plants (Chapters 1 and 6) at relatively high fungal spore and/or propagule concentrations. Since many of the fungi evaluated as mycoherbicides have little or no potential to propagate to epidemic levels during the next season following the first application, re-application of the same organism is required for each growing season.

The initiative for using phytopathogens and phytotoxins and other microorganisms as biological weed control agents (bioherbicides) began about three decades ago. Since then, numerous fungi have been screened for phytotoxic potential and several dozen fungi have been more closely examined as mycoherbicides. These concepts and organisms and their phytotoxins have been reviewed in books (Hoagland, 1990; TeBeest, 1991). It is apparent, from the vast amount of research currently being conducted in this area, that many more fungal and bacterial weed pathogens and phytotoxins from pathogenic and non-pathogenic microorganisms will be discovered that possess useful bioherbicidal activity. Although most bioherbicidal products have been targeted at agronomic weeds, they may also be useful for weed control in non-agronomic areas (recreational areas, forests, rights of way, lawns, gardens, etc.), where synthetic herbicides are either not registered or their use is cost-prohibitive.

Interest in these organisms (either directly or as sources of naturally occurring phytotoxins) has also increased recently, due to the search for less persistent, more selective and more environmentally benign herbicides. Pathogens also have potential for use in integrated weed management programmes, if the organisms can tolerate various agricultural chemicals. Genetic engineering and microbial strain selection can be used to increase pathogen virulence, alter host range and enhance interactions with other chemical regulators or synergists. Although many fungi have been discovered that have potential as mycoherbicides for the control of many weed species (see Table 12.4), there has been little research aimed at risk assessment of these microbes or their chemical products with regard to living targets other than weeds and soil and water quality. Futhermore, to date, only a very few organisms possessing mycoherbicidal activity have been patented for use as weed control agents. Some plant pathogens that have been or are being developed for commercial use include: *Phytophthora palmivora* (DeVine) for the control of strangler vine in citrus groves, *Colletotrichum gloeosporioides* f. sp. *aeschynomene* (Collego) for the control of northern joint-vetch in rice and soybean fields (Templeton and Heiny, 1989), *C. gloeosporioides* f. sp. *malvae* (BioMal), a foliar pathogen for round-leaved mallow (*Malva pusilla*) control and *Alternaria cassiae* for control of sicklepod (*Cassia obtusifolia*) (Hoagland, 2000).

Overall, a few major points are evident. Most of these organisms with mycoherbicidal potential have not been exhaustively examined for their phytotoxic mode of action, i.e. digestive enzymes, identification of specific phytotoxins, elucidation of interactions of multiple phytotoxins, etc. Furthermore, the toxicity of specific phytotoxins

Table 12.4. Selected examples of mycoherbicides for economically important terrestrial and aquatic weeds. (Adapted from Charudattan, 1990.)

Weed	Pathogen	Reference
Velvetleaf (*Abutilon theophrasti*)	*Colletotrichum coccodes*	Hodgson *et al.*, 1988
	Fusarium lateritium	Walker, 1981
Giant ragweed (*Ambrosia trifida*)	*F. lateritium*	Anon., 1989
Wild oat (*Avena fatua*)	*Septoria tritici* Desm. f. sp. *avenae*	Madariaga and Scharen, 1985
Common lambsquarters	*Ascochyta caulina* Sacc.	Scheepens and van Zon, 1982
(*Chenopodium album*)	*Cercospora chenopodii* Fres.	Scheepens and van Zon, 1982
	Cercospora dubia (Riess) Wint.	Scheepens and van Zon, 1982
Field bindweed	*Phomopsis convolvulus*	Anon., 1989,
(*Convolvulus arvensis)*		Vogelgsang *et al.*, 1998
Yellow nutsedge	*Cercospora caricis* Oud.	Anon., 1989
(*Cyperus esculentus)*		
Purple nutsedge (*C. rotundus*)	*Phyllachora cyperi* Rehm.	Anon., 1989
Large crabgrass	*Pyricularia grisea* (Cke.) Sacc.	Anon., 1989
(*Digitaria sanguinalis*)		
Barnyard-grass	*Cochliobolus lunatus* Nelson &	Scheepens, 1987
(*Echinochloa crus-galli*)	Haasis	
Water hyacinth	*Alternaria eichhorniae* Nag Raj	Shabana, 1987
(*Eichhornia crassipes*)	& Ponnappa	
Goosegrass (*Eleusine indica*)	*Bipolaris setariae* (Saw.)	Anon., 1989
	Shoemaker	
Common purslane	*Dichotomophthora indica* Rao	Baudoin, 1986
(*Portulaca oleracea*)	*Dichotomophthora portulacae*	Klisiewicz, 1985
	Mehrlich & Fitzpatrick ex	
	M.B. Ellis	
Itchgrass (*Rottboellia*	*Curvularia* sp.	Evans and Ellison, 1988
cochinchinensis)	*Phaeoseptoria* sp.	Evans and Ellison, 1988
Johnsongrass	*Sphacelotheca holci* Jack.	Massion and Lindow, 1986
(*Sorghum halepense*)	*Bipolaris halepense* Chiang,	Chiang *et al.*, 1989
	Leonard & Van Dyke	Winder and van Dyke, 1989
	Bipolaris sorghicola (Lefebvre	Anon., 1989
	& Sherwin) Alcorn	Anon., 1989
	Colletotrichum graminicola	
	(Ces.) G.W. Wils.	
	Gloeocercospora sorghi D. Hain	
	& Edg.	
Sicklepod (*Cassia obtusifolia*)	*Pseudocercospora nigricans*	Hofmeister and Charudattan, 1987

to non-target organisms has only rarely been investigated and most of these organisms have not been examined for other potentially harmful effects or the production of harmful non-phytotoxic chemicals. Mass production of mycoherbicides and their distribution and use on a wide agricultural scale could pose a significant exposure problem to humans, animals and the environment. Whether this also results in elevated amounts of secondary metabolites has still to be determined. Risk assessment will increase as more mycoherbicides are discovered, evaluated, patented for use and used to control various weeds.

Phytopathogenic fungi

Plant-pathogenic fungi produce a range of phytotoxins that interfere with plant metabolism and produce results ranging from whole-plant death to subtle effects on gene expression. Fungal phytotoxins must interact with a plant component (e.g. enzyme or membrane receptor), but, if that component is missing or altered, the compound will have no effect. Thus, fungal phytotoxins and/or their targets are important determinants of pathogen host range. Indeed, some phytotoxins are non-specific while others are host-specific. Non-specific phytotoxins produced by pathogenic fungi have not been critically evaluated for possible roles in plant disease; however, several of the host-specific phytotoxins have undergone extensive analysis, such as the maculosins (Bobylev *et al.*, 1996; Table 12.5). Some of these compounds will be discussed below, but, ironically, very little information is available on the production and properties of phytotoxins from commercialized mycoherbicides.

Host-specific phytotoxins

Chemically, the host-specific toxins are a diverse group of low-molecular-weight secondary metabolites. For example, HC- and AM-toxins are cyclic tetrapeptides of M_r 436 and 445, respectively. HS-toxin is a sesquiterpene galactofuranoside (M_r 884), T-toxin is a linear polyketol (M_r 768), AK-toxin is an ester of epoxy-decatrienoic acid (M_r 413), and AAL-toxin is a dimethylheptadecapentol ester of propane tricarboxylic acid (M_r 508). Several of these phytotoxins are found in culture filtrates as families of isomers or congeners.

Table 12.5. Examples of host-specific toxins produced by phytopathogenic fungi. (Adapted from Yoder and Turgeon, 1985; Hoagland, 1990.)

Fungus[a]	Toxin	Host and some properties of the toxin
[b]*Cochliobolus victoriae*	HV	Specific for oats. Pathogenicity determinant. Induces tissue leakage
Cochliobolus carbonum	HC	Specific for maize. Targets include plasma membrane
Cochliobolus nicotianae	Colletotrichin	Toxic to tobacco
Cochliobolus heterostrophus	T	Toxic to maize. Mitochondria of susceptible maize cultivars become leaky
Alternaria alternata f. sp. *lycopersici*	AAL	Disrupts pyrimidine synthesis in tomato by inhibiting aspartate carbamoyl transferase. Structural similarity to fumonisin B_1 suggests possible inhibition of ceramide synthase
A. alternata f. sp. *fragariae*	AF	Toxic to strawberry
A. alternata f. sp. *mali*	AM	Induces necrotic spots on leaves and apple fruit
A. alternata f. sp. *kikuchiana*	AK	Causes necrosis of Japanese pear
A. alternata f. sp. *terreus*	Acetylaranotin	Inhibits plant growth
A. alternata f. sp. *citri*	ACTG	Toxic to mandarine oranges

[a]Each phytotoxin affects only susceptible genotypes of the plant and is an important pathogenicity determinant.
[b]*Cochliobolus* designates the teleomorph of *Helminthosporium, Biolaris* and *Drechslera*.

Host-specific phytotoxins cause the visible and physiological changes that are characterisitic of infected plants. Gross physiological effects include changes in respiration, cell permeability, protein synthesis and CO_2 fixation. Most of the changes appear to be secondary, relative to the primary or initial biochemical lesions, as indicated by the single-gene control of sensitivity and by experiments with isolated organelles (Scheffer and Livingston, 1984). Host-specific phytotoxins are, for the most part, those that significantly affect only one plant species, the species to which the producing microorganism is a pathogen. Some host-specific phytotoxins are only toxic to certain cultivars of the host-plant species. For example, tomato plants homozygous for the *Asc* locus are resistant (*Asc1*^{1}/*Asc1*^{1} and *Asc1*^{2}/*Asc1*^{2}) or sensitive (*asc*/*asc*) to the fungal pathogen *Alternaria alternata* f. sp. *lycopersici* and its host-specific AAL-toxins (Abbas *et al.*, 1995b). Heterozygous plants are resistant to the fungus, but resistant (*Asc1*^{2}/*asc*) or sensitive (*Asc1*^{1}/*asc*) to high toxin concentrations. In susceptible plant tissues, AAL toxins and the structurally related fumonisins of *F. moniliforme* lead to the accumulation of free sphingoid bases by inhibition of ceramide synthase, a key enzyme in *de novo* sphingolipid biosynthesis, and cause apoptosis-like symptoms. Recently, free sphingoid bases and their metabolites have been shown to function as second messengers that regulate cell death (Merrill *et al.*, 1997).

All known host-specific phytotoxins are derived from fungal pathogens. Fewer than 20 host-specific phytotoxins that affect crops have been reported (Scheffer and Livingston, 1984). These phytotoxins have been reported to possess the same host range as the pathogens producing them. Only one of the host-specific phytotoxins affecting crops that has been tested on weed species is AAL-toxin. Other studies have demonstrated that it is not a host-specific phytotoxin and that it is highly phytotoxic to a wide range of weed species (Abbas *et al.*, 1992b, 1993b; Tanaka *et al.*, 1993). Destruxin B (discussed above) was reported to be host-specific (Bains and Tewari, 1987); however, it was later demonstrated to be non-host-specific (Buchwalt and Green, 1992). Colletotrichin, a product of several *Colletotrichum* species, is toxic to cucumber, tobacco and solanaceous weed species, including nightshades and horsenettle (*Solanum* spp.) (Gobhara *et al.*, 1978; Duke *et al.*, 1992). The first ultrastructural symptom of phytotoxicity is the loss of structural integrity of the plasma membrane. Lipid peroxidation is associated with the membrane damage; however, radical-quenching agents do not protect the plant cells from the toxin. Colletotrichin reduces or prevents the phytotoxicity of the synthetic herbicide acifluorfen (Gobhara *et al.*, 1978). Acifluorfen requires the activity of a plasma membrane-associated redox enzyme for its activity (Jacobs *et al.*, 1991). Thus, colletotrichin may interfere with the function of this plasma membrane and other membranes.

Non-specific phytotoxins

These compounds are produced by both specialist and generalist plant pathogens. For example, *A. alternata* f. sp *citri*, causal agent of brown spot disease of tangerines and mandarins, simultaneously produces host-specific toxins and non-specific phytotoxins (e.g. tentoxin and tenuazonic acid) in culture broth (Kono *et al.*, 1986). In this section most attention will focus on toxins of *Fusarium* because of the different niches this fungus occupies, principally as a saprophyte, plant pathogen, insect pathogen and fungal BCA (Teetor-Barsch and Roberts, 1983; Desjardins, 1992). There has been interest in exploiting different *Fusarium* species for use as mycoparasites, mycoinsecticides

and mycoherbicides. One benign BCA strain, Fusaclean™, has been commercialized by NPP (France) to promote plant growth by displacing plant-pathogenic strains of *Fusarium* (see Chapter 1). Other strains are being developed for the control of gorse (Morin *et al.*, 2000) and narcotics (see http://mycoherbicide.net).

The phytopathogenic species of *Fusarium* produce a wide variety of chemical contaminants of plant tissues including trichothecenes (T2-toxin and others), fumonisins, naphthazarins, fusaric acid and related pyridine derivatives (Desjardins, 1992). The phytotoxicity of *Fusarium* spp. and some of their natural products has been reviewed (Hoagland and Abbas, 1995). The exact role of some of these compounds remains unclear, but some are insecticidal (Grove and Pople, 1980), suggesting that they may offer some protection to the plants against phytophagous insects (Miller *et al.*, 1985; Strongman *et al.*, 1988).

Trichothecenes

These are produced by several common moulds, including species in the genera *Acremonium* (*Cephalosporium*), *Cylindrocarpon, Dendrodochium, Myrothecium, Trichoderma, Trichothecium* and, most numerously, *Fusarium*. Trichothecenes are composed of a tetracyclic sesquiterpene skeleton containing a six-membered oxane ring, a stable 12–13-epoxide group and a 9,10-olefinic bond. They have been classified into four groups. *Fusarium* spp. contain several well-known trichothecenes, including two members of group A with high mammalian toxicity, diacetoxyscirpenol (DAS) and T2-toxin, and toxins in group B, including deoxynivalenol (DON) and nivalenol. DON is the most common and possibly least toxic of these. Trichothecenes cause diarrhoea, severe haemorhages and immunotoxic effects and are also strong inhibitors of protein synthesis in mammalian cells. However, DON received its common name, vomitoxin, from the vomiting that generally accompanies trichothecene poisoning (D'Mello, 1997). DON is not mutagenic, but has clastogenic neurotoxic and immunotoxic effects (van Egmond and Speijers, 1990; Eriksen and Alexander, 1998). One typical feature of the toxicity of T2 is that it causes adverse cardiovascular effects in some experimental animal species, including pigs and monkeys. However, insufficient data exist on whether it is a carcinogen. Immunotoxic effects have also been reported in humans (Eriksen and Alexander, 1998).

Lambs will consume a wheat diet containing DON at 15.6 mg kg^{-1} of body weight for 28 days. DON does not appear to alter their feed consumption, weight gain or feed efficiency. Oral administration of DON showed that it was rapidly and primarily excreted in urine, essentially unchanged (95%). Incubation of DON with ruminal microorganisms *in vitro* for 48 h resulted in partial conversion to de-epoxy DON. These results indicate that the impact of DON on ruminants is lower than initially suspected. DON caused no organ damage to animals. Extremely low amounts of DON (< 4 ng ml^{-1}) were transmitted to milk after a single oral dose of 920 mg to a dairy cow (Diekman and Green, 1992). For the USA, the Food and Drug Admininstration (FDA) have suggested guidelines as to tolerable levels of DON in wheat for milling and human/animal consumption (Wood, 1992).

Macrocyclic trichothecenes also possess a wide range of phytotoxic specificity. Verrucarin A and J and trichoverrin B show phytotoxicity in wheat coleoptile bioassays at concentrations of 10^{-7} M (Cutler and Jarvis, 1985). Roridin A exhibited phytotoxicity on tobacco, maize and bean seedlings (Cutler and Jarvis, 1985). Simple

trichothecenes, such as neosoliniol monoacetate produced by *Fusarium tricinctum* showed phytotoxicity at 10^{-6} M, and diacetoxy-scripenol severely injured pea seedlings at 2.7×10^{-5} M, but was not toxic to wheat (Brain *et al.*, 1961).

Zearalenone (ZEN)

Zearalenone and zearalenol are both oestrogenic resorcylic acid lactones produced by *Fusarium* spp. (Diekman and Green, 1992). Despite their structural dissimilarity to the steroidal oestrogens, ZEN and several of its derivatives possess oestrogenic activity. ZEN undergoes a folding such that hydroxyl or potential hydroxyl groups become appropriately orientated to facilitate binding to tissue receptors that normally bind oestrogens. Similar binding affinities for ZEN have been determined for the oestrogen receptor in uteruses of sheep and calves (Diekman and Green, 1992).

Poultry show little reaction to ZEN ingestion. However, pigs are strongly affected, with symptoms in prepubertal gilts including enlarged mammae, swelling of the uterus and vulva and atrophy of the ovaries. In severe cases, prolapse of the vulva and rectum may occur. Boars exhibit enlarged mammae and atrophied testes (Flannigan, 1991). *In vivo* studies have revealed that ZEN was rapidly metabolized in animals and humans and eliminated mainly as water-soluble glucuronides. Free and conjugated forms of ZEN have been found in the milk of lactating cows under experimental conditions. That high oral doses of the toxin are required to elicit such a response indicates that consumption of contaminated feed by dairy cows would not result in a health hazard to humans (Wood, 1992).

Zearalenone has been shown to be genotoxic and carcinogenic in mice but not rats, affects reproduction in low doses and produces hormonal effects in mice and rats. Hormonal effects were produced in monkeys by α-zearalanol a compound closely related to α-zearalenol, which is a major metabolite of zearalenone (van Egmond and Speijers, 1990; Eriksen and Alexander, 1998).

Enniatins

Enniatins are cyclohexadepsipeptides produced by various strains of *Fusarium*. They consist of three residues of D-2–hydroxy-isovaleric acid (D-HIV) and an *N*-methyl-L-branched-chain amino acid, which are arranged in an alternated fashion. They are synthesized by enniatin synthetase, a 350 kDa multifunctional enzyme. Enniatins exhibit antibiotic properties towards a number of bacteria and fungi due to their ionophoretic properties. Furthermore, they are reported to act as inhibitors of cholesterol acyl transferase in mammalians and act as phytotoxins during infection of plants by *Fusaria*. Enniatins A/A1 extracted from *Fusarium avenaceum* also appear to have insecticidal properties (Strongman *et al.*, 1988).

Fumonisins

Fumonisin B_1 is both a toxin and a phytotoxin produced by both saprophytic *Fusarium* species (Abbas *et al.*, 1991, 1992b) and an *A. alternata* strain known to cause stem canker on certain tomato varieties (Chen *et al.*, 1992). Fumonisin B_1 (FB_1), first isolated from

F. moniliforme MRC 826 (Bezuidenhout *et al.*, 1988), is a hydroxylated, long-chain alkylamine with two tricarboxylic acid moieties attached. It is highly phytotoxic to most weed and crop species tested (Abbas *et al.*, 1992a, b; van Asch *et al.*, 1992; Tanaka *et al.*, 1993). Ultrastructural and physiological studies of jimson-weed leaves with FB_1 revealed that it caused light-dependent plasma membrane and tonoplast disruption through an unknown mechanism (Abbas *et al.*, 1992a; Tanaka *et al.*, 1993). Although the fumonisins and AAL-toxin have similar activity, AAL-toxin is generally more potent than FB_1 (Tanaka *et al.*, 1993). Aminoalcohols, hydrolysis products of the fumonisins, have very low phytotoxicity. FB_1 is also a potent mammalian toxin (Shier *et al.*, 1991; Abbas *et al.*, 1993a), and in animal systems the mechanism of action is apparently altered sphingolipid synthesis (Wang *et al.*, 1991). The mode of action of FB_1 in plants and mammals is inhibition of ceramide synthase (Hoagland and Abbas, 1995). Sphingolipids themselves, at relatively high concentrations, can cause phytotoxicity symptoms similar to those of fumonisins and AAL-toxin (Vesonder *et al.*, 1992a, b; Tanaka *et al.*, 1993). For example, sphingosine and phytosphingosine required micromolar concentrations to cause effects similar to those of nanomolar levels of FB_1 and AAL-toxin.

Other phytotoxins from Fusarium *spp.*

Fusaric acid is produced by both the virulent plant pathogen *F. oxysporum*, the cause of wilt in several species, and by non-pathogenic *Fusarium* species that grow saprophytically on maize kernels (Abbas *et al.*, 1989). Recently, fusaric acid has been demonstrated to be herbicidal against several weed species, including jimson-weed (Abbas *et al.*, 1991, 1995a) and duckweed (Vesonder *et al.*, 1992a).

Moniliformin can be obtained from isolates of saprophytic *Fusarium* species, as well as pathogenic isolates of *F. oxysporum* (Abbas *et al.*, 1989). Moniliformin causes growth inhibition, necrosis and chlorosis in many weed species. (Cole *et al.*, 1974; Abbas *et al.*, 1991, 1995a; Vesonder *et al.*, 1992a). However, it lacks selectivity and is highly toxic to mammals. This compound was used as a chemical structure template in the design and synthesis of many compounds for herbicide discovery (Fischer and Bellus, 1983).

Other fungal phytotoxins

AAL-toxin is a hydroxylated long-chain alkylamine containing one tricarboxylic acid moiety. It is a close analogue of the fumonisins and is produced by *A. alternata* f. sp. *lycopersici*. In susceptible varieties of tomatoes, it causes rapid wilting and necrosis (Abbas *et al.*, 1995b). Its physiological effects appear to be identical to those of the fumonisins, and it is thus suspect for mammalian toxicity.

Cercosporin is a red compound produced by several species of *Cercospora* (Nasini *et al.*, 1977). Isocercosporin, a closely related compound, is produced by *Scolecotrichum graminis*, the agent of leaf streak in orchardgrass (*Dactylis glomerata* L.) (Tabuchi *et al.*, 1991). These are photoactivated, active-oxygen-producing toxins (Hartman *et al.*, 1988). Similar compounds, such as hypericin (a plant product) (Knox and Dodge, 1985), have been suggested as herbicides; however, such compounds may have high risk factors since they are toxic to all life forms that exist in sunlight and an oxygen atmosphere.

As mentioned earlier, destruxins are produced by *A. brassicae* and a few other plant pathogens (Buchwaldt and Jenson, 1991; Buchwaldt and Green, 1992). These compounds cause necrotic and chlorotic symptoms in susceptible species (Buchwaldt and Green, 1992).

Aflatoxin

A last example of a natural product with multiple biological activities (phytotoxic and carcinogenic) to be presented is aflatoxin. Aflatoxins are a group of mycotoxins produced by certain strains of *Aspergillus flavus, Aspergillus parasiticus* and *Aspergillus nominius*. Although these fungi are ubiquitous, not all strains produce aflatoxin. Toxin titre is also dependent on environmental conditions related to fungal growth, such as moisture, temperature and nutrient availability. These mycotoxins occur naturally on commodities, including groundnuts and groundnut meal, cotton-seed meal, maize, dried chilli peppers, etc. There are numerous members of this mycotoxin group, with B_1 being one of the most potent mutagens and carcinogens yet discovered. Other analogues or derivatives are less toxic (Merck Index, 1996). Aflatoxin is currently recognized as an extremely toxic contaminant in various food and feed products and as a risk factor for liver cancer in humans (Wogan, 1992).

Biological testing of aflatoxins on plants has shown that these compounds also possess phytotoxic activity. In 1965, AB_1 was shown to have phytotoxic effects on plant tissues (Lilley, 1965; Schoental and White, 1965). Since then, many other reports have demonstrated the phytotoxicity of these compounds in a variety of plant and plant-tissue bioassays (McLean, 1994). These compounds are absorbed by plants, translocated to various plant parts and distributed within specific cellular compartments (McLean, 1993; McLean *et al.*, 1994). Studies on the metabolism of these compounds in plants has been controversial, i.e. some researchers report a lack of metabolism by plants (Mertz *et al.*, 1980; Reiss, 1984), while others have detected metabolic products in aflatoxin-treated plants (Howes *et al.*, 1991; McLean, 1993). As is the case with many other chemicals, it is highly probable that the uptake, translocation and metabolism of aflatoxins are dependent upon the test-plant species.

Toxins and Risk Assessment – Conclusions

Most fungal BCAs are widespread soil inhabitants. We believe that epizootics/epidemics induced naturally or artificially through inundative introductions of BCAs do not pose a risk to human or animal health. This is partly because the BCAs are subject to a wide range of regulatory (climatic and biotic) controls, so inoculum levels generally subside over time (see Chapters 3 and 4). As far as we are aware, there are no documented accounts of toxin levels rising as a result of artificially induced or natural epizootics/epidemics, nor are there any reports of BCA metabolites entering the food-chain. These observations should not lead to complacency, but to rationalization of risk assessment (see Chapters 13 and 14). For example, it should not be assumed that all strains produce similar metabolites, because recent studies clearly show inter- and intraspecific variation in quantity and types of toxin produced (Amiri-Besheli *et al.*, 2000; Bandani *et al.*, 2000). Furthermore, what is detected in liquid culture may have no link to what is produced in the target host or released into the environment.

In our opinion, both the efficacy of the fungal BCA and data on the fate of both inoculum and any major metabolites secreted are essential. The fact that some strains produce few, if any, metabolites may encourage some companies to select these organisms, except when these compounds play an important role in pathogenesis/antagonism. More information also needs to be generated on the fate of bioactive metabolites in the environment. We know that gliotoxins are inactivated by oxidation, but is this true for other metabolites?

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References

Abbas, H.K., Mirocha, C.J., Kommedahl, T., Vesonder, R.F. and Golinski, P. (1989) Production of trichothecene and non-trichothecene mycotoxins by *Fusarium* species isolated from maize in Minnesota. *Mycopathologia* 108, 55–58.

Abbas, H.K., Boyette, C.D., Hoagland, R.E. and Vesonder, R.F. (1991) Bioherbicidal activity of *Fusarium moniliforme* (Sheldon) and its phytotoxin fumonisin. *Weed Science* 39, 673–677.

Abbas, H.K., Paul, R.N., Boyette, C.D., Duke, S.O. and Vesonder, R.F. (1992a) Physiological and ultrastructural effects of fumonisin on jimsonweed leaves. *Canadian Journal of Botany* 70, 1824–1833.

Abbas, H.K., Vesonder, R.F., Boyette, C.D., Hoagland, R.E. and Krick, T. (1992b) Production of fumonisins by *Fusarium moniliforme* cultures isolated from jimsonweed in Mississippi. *Journal of Phytopathology* 136, 199–203.

Abbas, H.K., Gelderbloom, W.C.A., Cawood, M.F. and Shier, W.T. (1993a) Biological activities of fumonisins, mycotoxins from *Fusarium moniliforme*, in jimsonweed (*Datura stramonium* L.) and mammalian cell cultures. *Toxicon* 31, 345–353.

Abbas, H.K., Vesonder, R.F., Boyette, C.D. and Petterson, S.W. (1993b) Phytotoxicity of AAL-toxin and other compounds produced by *Alternaria alternata* to jimsonweed (*Datura stramonium*). *Canadian Journal of Botany* 71, 155–160.

Abbas, H.K., Boyette, C.D. and Hoagland, R.E. (1995a) Phytotoxicity of *Fusarium* isolates and their phytotoxins fumonisin, fusaric acid and moniliformin on jimsonweed. *Phytoprotection* 76, 17–25.

Abbas, H.K., Tanaka, T. and Duke, S.O. (1995b) Pathogenicity and/or phytotoxicity of *Alternaria alternata* and its AAL-toxin, *Fusarium moniliforme* and its fumonisin B1 on tomato varieties. *Journal of Phytopathology* 143, 329–334.

Abendstein, D. and Strasser, H. (2000) Considerations on toxic metabolites produced by *Beauveria brongniartii*. In: Keller, S. (ed.) *Integrated Control of Soil Pests*. IOBC Bulletin, Avignon, France, pp. 789–796.

Al-Aïdroos, K. and Roberts, D.W. (1978) Mutants of *Metarhizium anisopliae* with increased virulence toward mosquito larvae. *Canadian Journal of Genetics and Cytology* 20, 211–219.

Aleo, M.D., Wyatt, R.D. and Schnellmann, R.G. (1991) Mitochondrial dysfunction is an early event in ochratoxin A but not oosporein toxicity to rat renal proximal tubules. *Toxicology and Applied Pharmacology* 107, 73–80.

Amiri, B., Ibrahim, L. and Butt, T.M. (1999) Antifeedant properties of destruxins and their use with the entomogenous fungus *Metarhizium anisopliae* for improved control of crucifer pests. *Biocontrol Science and Technology* 9, 487–498.

Amiri-Besheli, B., Khambay, B., Cameron, S., Deadman, M. and Butt, T.M. (2000) Inter- and intra-specific variation in destruxin production by the insect pathogenic *Metarhizium*, and its significance to pathogenesis. *Mycological Research* 104, 447–452.

Anon. (1989) Discovery and development of plant pathogens for biological control of weeds. In: Reg. Res. Proj. SRCS 8801 (S-136), Plant Pathology Department, University of Florida, Gainesville, p. 44.

Anon. (1990) Pesticide Fact Sheet No. 217: '*Gliocladium virens*' *GL-21*. Environmental Protection Agency, Washington, DC. Office of Pesticide Programs Report No. EPA/540/FS-91/118.

van Asch, M.A.J., Rijkenberg, F.H.J. and Coutinho, T.A. (1992) Phytotoxicity of fumonisin B1, moniliformin, and T-2 toxin to corn callus cultures. *Phytopathology* 82, 1330–1332.

Ayer, W.A. and Pena-Rodriguez, M. (1987) Metabolites products by *Alternaria brassicae*, the black spot pathogen of canola. Part 1, the phythotoxic components. *Journal of Natural Products* 50, 400–407.

Bains, P.S. and Tewari, J.P. (1987) Purification, chemical characterisation and host-specificity of the toxin produced by *Alternaria brassicae*. *Physiological Molecular Plant Pathology* 30, 259–271.

Bandani, A.R. and Butt, T.M. (1999) Insecticidal, antifeedant, growth inhibitory activities of efrapeptins, metabolites of the entomogenous fungus *Tolypocladium*. *Biocontrol Science and Technology* 9, 499–506.

Bandani, A.R., Khambay, B.P.S., Faull, J., Newton, R., Deadman, M. and Butt, T.M. (2000) Production of efrapeptins by *Tolypocladium* species and evaluation of their insecticidal and antimicrobial properties. *Mycological Research* 104, 537–544.

Bandani, A.R., Amiri, B., Butt, T.M. and Gordon-Weekes, R. (2001) Effects of efrapeptin and destruxin, metabolites of entomogenous fungi, on the hydrolytic activity of a vacuolar type ATPase from the brush border membrane vesicles of *Galleria mellonella* midgut and on plant membrane bound hydrolytic enzymes. *Biochemica et Biophysica Acta* 1510, 367–377.

Baudoin, A.B.A.M. (1986) First report on *Dichotomophthora indica* on common purslane in Virginia. *Plant Disease* 70, 352.

Bezuidenhout, S.C., Gelderblom, W.C.A., Gorst-Allman, C.P., Horak, R.M., Marasas, W.E.O., Spiteller, G. and Vleggaar, R. (1988) Structure elucidation of the fumonisins, mycotoxins from *Fusarium moniliforme*. *Journal of the Chemical Society. Chemical Communications* 11, 743–745.

Bidochka, M.J. and Khachatourians, G.G. (1991) The implication of metabolic acids produced by *Beauveria bassiana* in pathogenesis of the migratory grasshopper, *Melanoplus sanguinipes*. *Journal of Insect Pathology* 58, 106–117.

Bobylev, M.M., Bobyleva, L.I. and Strobel, G.A. (1996) Synthesis and bioactivity of analogs of maculosin, a host-specific phytotoxin produced by *Alternaria alternata* on spotted knapweed (*Centaurea maculosa*). *Journal of Agricultural Food Chemistry* 44, 3960–3964.

Boucias, D.G., Farmerie, W.G. and Pendland, J.C. (1998) Cloning and sequencing of cDNA of the insecticidal toxin Hirsutellin A. *Journal Invertebrate Pathology* 72, 258–261.

Bradfisch, G.A. and Harmer, S.L. (1990) Omega conotoxin GVIIA and nifedipine inhibit the depolarizing action of the fungal metabolite destruxin B on muscle from the tobacco budworm *Heliothis virescens*. *Toxicon* 28, 1249–1254.

Brain, B., Dawkins, A.W., Grove, J.F., Henning, H.G., Lowe, D. and Norris, G.L.F. (1961) Phytotoxic compounds produced by *Fusarium equeseti*. *Journal of Experimental Botany* 12, 1–7.

Brewer, D., Maass, W.S.G. and Taylor, A. (1977) The effect on fungal growth of some 2,5-dihydroxy-1,4–benzoquinones. *Canadian Journal of Microbiology* 23, 845–851.

Brewer, D., Jen, W.C., Jones, G.A. and Taylor, A. (1984) The antibacterial activity of some naturally occurring 2,5-dihydroxy-1,4-benzoquinones. *Canadian Journal of Microbiology* 30, 1068–1072.

Brewer, D., Mason, F.G. and Taylor, A. (1987) The production of alamethicins by *Trichoderma* spp. *Canadian Journal of Microbiology* 33, 619–625.

Brousseau, C., Charpentier, G. and Belloncik, (1996) Susceptibility of spruce budworm, *Choristoneura fumiferana* Clemens, to destruxins, cyclodepsipeptidic mycotoxins of *Metarhizium anisopliae*. *Journal of Invertebrate Pathology* 68, 180–182.

Brown, T.P., Fletcher, O.J., Osuna, O. and Wyatt, R.D. (1987) Microscopic and ultrastructural renal pathology of oosporein-induced toxicosis in broiler chicks. *Avian Diseases* 31, 868–877.

Bruckner, H. and Graf, H. (1983) Paracelsin, a peptide antibiotic containing alpha-aminoisobutyric acid, isolated from *Trichoderma reesei* Simmons. Part A. *Experientia* 39, 528–530.

Buchwaldt, L. and Green, H. (1992) Phytotoxicity of destruxin B and its possible role in the pathogenesis of *Alternaria brassicae. Plant Pathololgy* 4, 55–63.

Buchwaldt, L. and Jensen, J.S. (1991) HPLC purification of destruxin produced by *Alternaria brassicae* in culture and leaves of *Brassica napus. Phytochemistry* 30, 2311–2316.

Carsolio, C., Benhamou, N., Haran, S., Cortès, C., Gutièrrez, A., Chet, I. and Herrera-Estrella, A. (1999) Role of *Trichoderma harzanium* endochitinase gene, ech 42, in mycoparasitism. *Applied Environmental Microbiology* 65, 929–935.

Cerenius, L., Thorn Quist, P.O., Vey, A., Johanson, M.W. and Soderhall, K. (1990) The effect of the fungal toxin destruxin E on isolated crayfish haemocytes. *Journal of Insect Physiology* 36, 785–789.

Champlin, F.R. and Grula, E.A. (1979) Noninvolvement of beauvericin in the entomopathogenicity of *Beauveria bassiana. Applied and Environmental Microbiology* 37, 1122–1125.

Charnley, A.K. (1984) Physiological aspects of destructive pathogenesis in insects by fungi: a speculative view. In: Anderson, J.M., Rayner, A.D.M. and Walton, D.W.H. (eds) *Invertebrate Microbial Interactions*. Cambridge University Press, Cambridge, pp. 229–270.

Charudattan, R. (1990) Pathogens with potential for weed control. In: Hoagland, R.E. (ed.) *Microbes and Microbial Products as Herbicides.* American Chemical Society Symposium Series No. 439, ACS Books, Washington, DC, pp. 132–154.

Chen, H.C., Chou, C.K., Sun, C.M. and Yeh, S.F. (1997) Suppressive effect of destruxin B on hepatitis B virus surface antigen gene expression in human hepatoma cells. *Antiviral Research* 34, 137–144.

Chen, J., Miroscha, C.J., Xie, W., Hogge, L. and Olson, D. (1992) Production of the mycotoxin fumonisin B1 by *Alternaria alternata* f. sp. *lycopersici*. *Environmental Microbiology* 58, 3928–3931.

Chiang, M.Y., Leonard, K.J. and van Dyke, C.G. (1989) *Bipolaris halepense*: a new species from *Sorghum halepense* (Johnsongrass). *Mycologia* 81, 532–538.

Claydon, N. (1978) Insecticidal secondary metabolites from the entomogenous fungi: *Entomophthora virulenta. Journal of Invertebrate Pathology* 32, 319–324.

Cole, R.J., Kirksey, J.W., Cutler, H.G., Doupnik, B.L. and Peckham, J.C. (1973) A toxin from *Fusarium moniliforme*: effects on plants and animals. *Science* 179, 3124–1326.

Cole, R.J., Kirksey, J.W., Cutler, H.G. and Davis, E.E. (1974) Toxic effects of oosporein from *Chaetomium trilaterale. Journal of Agricultural and Food Chemistry* 22, 517–520.

Corley, D.G., Miller-Wideman, M. and Durley, R.C. (1994) Isolation and structure of harzianum A: a new trichothecene from *Trichoderma harzianum*. *Journal of Natural Products* 57, 422–425.

Cutler, H.G. and Jarvis, B.B. (1985) Preliminary observations on the effects of macrocylic trichothecens on plant growth. *Environmental and Experimental Botany* 25, 115–128.

Debeaupuis, J.P and Lafont, P. (1985) Effet de quelques mycotoxines et substances génotoxiques de synthèse sur le développement de l'embryon et de la larve de *Brachydanio rerio. Comptes Rendus de l'Académie des Sciences, Série 3 (Paris)* 300, 167–170.

Desjardins, A.E. (1992) Genetic approaches to the chemical ecology of phytopathogenic *Fusarium* species. In: Bhatnagar, D., Lillehoj, E.B. and Arora, D. (eds) *Handbook of Applied Mycology*, Vol.5, *Mycotoxins in Ecological Systems.* Marcel Dekker, New York, pp. 333–357.

Diekman, M.A. and Green, M.L. (1992) Mycotoxins and reproduction in domestic livestock. *Journal of Animal Science* 70, 1615–1627.

Di Paola, R., Nenna, S., Fornelli, F., Moretti, A., Logrieco, A., Caiaffa, M.F., Bottalico, A., Tursi, A. and Macchia, L. (1994) Cytotoxicity of beauvericin on human B-lymphocyte cell lines. *Allergy and Clinical Immunology News* 2, p. 256.

D'Mello, F.J.P. (ed.) (1997) *Handbook of Plant and Fungal Toxicants.* CRC Press, Boca Raton, Florida.

Duke, S.O., Gobhara, M., Paul, R.N. and Duke, M.V. (1992) Colletotrichin causes rapid membrane damage to plants. *Journal of Phytopathology* 134, 289–305.

Dumas, C., Robert, P., Pais, M., Vey, A. and Quiot, J.-M. (1994) Insecticidal and cytotoxic effects of natural and hemisynthetic destruxins. *Comparative Biochemistry and Physiology* 108C, 195–203.

Dumas, C., Matha, V., Quiot, J.-M. and Vey, A. (1996a) Effect of destruxins, cyclic depsipeptide mycotoxins, on calcium balance and phosphorylation of intracellular proteins in lepidopteran cell lines. *Comparative Biochemistry and Physiology* 114C, 213–219.

Dumas, C., Ravallec, M., Matha, V. and Vey, A. (1996b) Comparative study of the cytological aspects of the mode of action of destruxins and other peptidic fungal metabolites on target epithelial cells. *Journal of Invertebrate Pathology* 67, 137–146.

Duval, D., Riddell, F.G., Rebuffat, S., Platzer, N. and Bodo, B. (1998) Ionophoric activity of the antibiotic peptaibol trichorzin PA VI: a 23Na- and 35Cl-NMR study. *Biochimica et Biophysica Acta* 1372, 370–378.

van Egmond, H.P. and Speijers, G.J.A. (1990) Food contaminants, naturally occurring toxicants in foodstuffs. 1. Mycotoxins. *Food Laboratory News*, 20, 38–45.

El Hajji, M., Rebuffat, S., Lecommandeur, D. and Dodo, B. (1987) Isolation and sequence determination of trichorzianines A antifungal peptides from *Trichoderma harzianum*. *International Journal of Peptide and Protein Research*. 29, 207–215.

Engstrom, G., Delance, J., Richard, A.L. and Baetz, J. (1975) Purification and characterization of roseotoxin B, a toxic cyclodepsipeptide from *Trichothecium roseum. Journal of Agriculture and Food Chemistry* 23, 244–253.

Eriksen, G.S. and Alexander, J. (1998) Fusarium *Toxins in Cereals – A Risk Assessment.* Nordic Council of Ministers, TemaNord Food 502, Copenhagen, Denmark.

Evans, H. and Ellison, C. (1988) Preliminary work on the development of a mycoherbicide to control *Rottboellia cochinchinensis*. In: DelFosse, E.S. (ed.) *Proceedings VII International Symposium on Biological Control of Weeds.* 1st Sper. Patologia Vegetale (MAF), Rome, p. 76.

Eyal, J., Mabud, A., Fischbein, K.L., Walter, J.F., Osborne, L.S. and Landa, Z. (1994) Assessment of *Beauveria bassiana* Nov. EO-1 strain, which produces a red pigment for microbial control. *Applied Biochemistry and Biotechnology* 44, 65–80.

Fargues, J. and Robert, P.-H. (1986) Potentialités insecticides des destruxines, mycotoxines produites par l'hyphomycete entomopathogéne *Metarhizium anisopliae* (Metsch.) Sorok. In: *Proceedings 4ème Congrès Protection cultures et santé en milieu tropical, Marseille, France,* pp. 357–362.

Fargues, J., Robert, P.-H. and Vey, A. (1985) Effet des destruxines A, B et E dans la pathogénèse de *Metarhizium anisopliae* chez les larves de Coléoptères Scarabeidae. *Entomophaga* 30, 353–364.

Fargues, J., Robert, P.-H., Vey, A. and Païs, M. (1986) Toxicité relative de la destruxine E pour le Lépidoptère *Galleria mellonella. Comptes Rendus de l'Académie des Sciences, Paris Série III* 303, 83–86.

Fischer, H.-P. and Bellus, D. (1983) Phytotoxicants from microorganisms and related compounds. *Pesticide Science* 14, 334–346.

Flannigan, B. (1991) Mycotoxins. In: *Toxic Substances in Crop Plants.* Royal Society of Chemists, pp. 226–257.

Fricaud, A.C., Walters, A.J., Whitehouse, D.G. and Moore, A. (1992) The role(s) of adenylate kinase and the adenylate carrier in the regulation of plant mitochondrial respiratory activity. *Biochimica et Biophysica Acta* 1099, 253–261.

Genthner, F.J., Cripe, G.M. and Crosby, D.J. (1994) Effect of *Beauveria bassiana* and its toxins on *Mysidopsis bahia* (Mysidacea). *Archives of Environmental Contamination and Toxicology* 26, 90–94.

Genthner, F.J., Chancy, C.A., Couch, J.A., Foss, S.S., Middaugh, D.P., George, S.E., Warren, M.A. and Bantle, J.A. (1998) Toxicity and pathogenicity testing of the insect pest control fungus, *Metarhizium anisopliae*. *Archives of Environmental Contamination and Toxicology* 35, 317–324.

Ghisalberti, E.L. and Rowland, C.Y. (1993) Antifungal metabolites from *Trichoderma harzianum*. *Journal of Natural Products* 56, 1799–1804.

Ghisalberti, E.L. and Sivasithamparam, K. (1991) Antifungal antibiotics produced by *Trichoderma* spp. *Soil Biology and Biochemistry* 23, 1011–1020.

Gobhara, M., Kosuge, Y., Yamaaki, S., Kimura, Y., Suzuki, A. and Tamura, S. (1978) Isolation, structures and biological activities of colletotrichins, phytotoxic substances from *Colletotrichum nicotianae. Agricultural and Biological Chemistry* 42, 1037–1043.

Godoy, G., Steadman, J.R., Dickman, M.B. and Dam, R. (1990) Use of mutants to demonstrate the role of oxalic acid in pathogenicity of *Sclerotinia sclerotiorum* on *Phaseolus vulgaris. Physiological and Molecular Plant Pathology* 37, 179–191.

Goulard, C., Hlimi, S., Rebuffat, S. and Bodo, B. (1995) Trichorzins HA and MA, antibiotic peptides from *Trichoderma harzianum*. I. Fermentation, isolation and biological properties. *Journal of Antibiotics* (*Tokyo*) 48, 1248–1253.

Graeme-Cook, K.A. and Faull, J.L. (1991) Effect of ultraviolet-induced mutants of *Trichoderma harzianum* with altered antibiotic production on selected pathogens *in vitro*. *Canadian Journal of Microbiology* 37, 659–664.

Graniti, A. (1972) The evolution of the toxin concept in plant pathology. In: Woods, R.K.S., Ballio, A. and Graniti, A. (eds) *Phytotoxins in Plant Diseases.* Academic Press, New York, pp. 1–18.

Griffin, D.H. (1994) *Fungal Physiology.* Wiley-Liss, New York.

Grove, J.F. and Pople, M. (1980) The insecticidal activity of beauvericin and the enniatin complex. *Mycopathologia* 70, 103–105.

Gupta, S., Roberts, D.W. and Renwick, J.A.A. (1989) Preparative isolation of destruxins from *Metarhizium anisopliae* by high performance liquid chromatography. *Journal of Liquid Chromatography* 12, 383–395.

Gupta, S., Krasnoff, S.B., Underwood, N.L., Renwick, J.A. and Roberts, D.W. (1991) Isolation of beauvericin as an insect toxin from *Fusarium semitectum* and *Fusarium moniliforme* var. *subglutinans*. *Mycopathologia* 115, 185–189.

Gupta, S., Montillor, C. and Hwang, Y.-S. (1995) Isolation of novel beauvericin analogues from the fungus *Beauveria bassiana. Journal of Natural Products* 58, 733–738.

Hartman, P.E., Dixon, W.J., Dahl, T.A. and Daub, M.E. (1988) Multiple modes of photodynamic action by cercosporin. *Phytochemistry and Photobiology* 44, 699–703.

Hoagland, R.E. (ed.) (1990) *Microbes and Microbial Products as Herbicides.* ACS Books, American Chemical Society, Washington, DC.

Hoagland, R.E. (2000) Plant pathogens and microbial products as agents for biological weed control. In: Tewari, J.P., Lakhanpal, T.N., Singh, J., Gupta, R. and Chamola, B.P. (eds) *Advances in Microbial Biotechnology.* APH, New Delhi, India.

Hoagland, R.E. and Abbas, H.K. (1995) Phytotoxicity of *Fusarium* spp. and their natural products. In: Greene, D.W. (ed.) *Proceedings of the Plant Growth Regulator Society of America.* PGRSA Press, La Grange, Georgia, pp. 109–114.

Hodgson, R.H., Wymore, L.A., Watson, A.K., Snyder, R.H. and Collette, A. (1988) Efficacy

of *Colletotrichum coccodes* and thidiazuron for velvetleaf (*Abutilon theophrasti*) control in soybean (*Glycine max*). *Weed Technology* 2, 473–480.

Hofmeister, F.M. and Charudattan, R. (1987) *Pseudocercospora nigricans*, a pathogen of sicklepod (*Cassia obtusifolia*) with biocontrol potential. *Plant Disease* 71, 44–46.

Howes, A.W., Dutton, M.F. and Chuturgoon, A.A. (1991) Metabolism of aflatoxin B1 by *Petroselinum crispum* (parsley). *Mycopathologia* 113, 25–29.

Huxham, I.M., Lackie, A.M. and McCorkindale, N.J. (1989) Inhibitory effect of cyclodepsipeptides, destruxins, from the fungus *Metarhizium anisopliae*, on cellular immunity in insects. *Journal of Insect Physiology* 35, 97–105.

Iida, A., Sanekata, M., Fujita, T., Tanaka, H., Enoki, A., Fuse, G., Kanai, M., Rudewicz, P.J. and Tachikawa, E. (1994) Fungal metabolites. XVI. Structure of new peptaibols, trichokindins I–VII, from the fungus *Trichoderma harzianum*. *Chemical and Pharmaceutical Bulletin* (*Tokyo*) 42, 1070–1075.

Irmscher, G., Bovermann, G., Boheim, G. and Jung, G. (1978) Trichotoxin A 40, a new membrane-exciting peptide. Part A. Isolation, characterization and conformation. *Biochimica et Biophysica Acta* 507, 470–484.

Itoh,Y., Kodama, K., Furuya, K., Takahashi, S., Haneishi, T., Takiguchi, Y. and Arai, M. (1980) A new sesquiterpene antibiotic, heptelidic acid: producing organisms, fermentation, isolation and characterization. *Journal of Antibiotics* (*Tokyo*) 33, 468–473.

Jacobs, J.M., Jacobs, N.J., Sherman, T.D. and Duke, S.O. (1991) Effect of diphenyl ether herbicides on oxidation of protoporphyrinogen to protoporphyrin in organellar and plasma membrane-enriched fractions of barley. *Plant Physiology* 97, 197–203.

James, P.J., Kershaw, M.J., Reynolds, S.E. and Charnley, A.K. (1993) Inhibition of desert locust (*Shistocerca gregaria*) Malpighian tubule fluid secretion by destruxins, cyclic peptide toxins from the insect pathogenic fungus *Metarhizium anisopliae*. *Journal of Insect Physiology* 39, 797–804.

Jeffs, L.B. and Khachatourians, G.G. (1997) Toxic properties of *Beauveria* pigments on erythrocyte membranes. *Toxicon* 35, 1351–1356.

Jones, R.W. and Hancock, J.G. (1987) Conversion of viridin to viridiol by viridin producing fungi. *Canadian Journal of Microbiology* 33, 963–966.

Joshi B.K., Gloer J.B. and Wicklow D.T. (1999). New verticillin and glisoprenin analogues from *Gliocladium catenulatum*, a mycoparasite of *Aspergillus flavus* sclerotia. *Journal of Natural Products* 62, 730–733.

Kanaoka, M., Isogai, A., Murakoshi, S., Ichione, M., Suzuki, A. and Tamura, S. (1978) Bassianolide, a new insecticidal cylcodepsipeptide from *Beauveria bassiana* and *Verticillium lecanii*. *Agricultural and Biological Chemistry* 42, 629–635.

Kawazu, K., Murakami, T., Ono, Y., Kanzaki, H., Kobayashi, A., Mikawa, T. and Yoshikawa, N. (1993) Isolation and characterization of two novel nematicidal depsipeptides from an imperfect fungus, strain D1084. *Bioscience, Biotechnology and Biochemistry* 57, 98–101.

Kershaw, M.J., Moorhouse, E.R., Bateman, R., Reynolds, S.E. and Charnley, A.K. (1999) The role of destruxins in the pathogenicity of *Metarhizium anisopliae* for three species of insects. *Journal of Invertebrate Pathology* 74, 213–223.

Khachatourians, G.G. (1996) Biochemistry and molecular biology of entomopathogenic fungi. In: Howard, D.H. and Miller, J.D. (eds) *The Mycota VI. Human and Animal Relationships.* Springer Verlag, Berlin, pp. 331–363.

Kleinkauf, H. and Rindfleisch, H. (1975) Non-ribosomal biosynthesis of the cyclic octadecapeptide alamethicin. *Acta Microbiol. Acad. Sci. Hung.* 22, 411–418.

Klisiewicz, J.M. (1985) Growth and reproduction of *Dichotomophthora portulacae* and its biological activity on purslane. *Plant Disease* 69, 761–762.

Knox, J.P. and Dodge, A.D. (1985) Isolation and activity of the photodynamic pigment hypericin. *Plant Cell Environment* 8, 19–25.

Kodaira, Y. (1961) Biochemical studies on the muscardine fungi in the silkworms, *Bombyx mori.*

Journal of the Faculty of Textile Science and Technology, Sinshu University, Sericult. 5, 1–68.

Kohno, J., Asai, Y., Nishio, M., Sakurai, M., Kawano, K., Hiramatsu, H., Kameda, N., Kishi, N., Okuda, T. and Komatsubara, S. (1999) TMC-171A, B, C and TMC-154, novel polyketide antibiotics produced by *Gliocladium* sp. TC 1304 and TC 1282. *Journal of Antibiotics* (*Tokyo*) 52 (12), 1114–1123.

Kono, Y., Gardner, J.M. and Takeuchi, S. (1986) Nonselective phytotoxins simultaneously produced with host-selective ACTG-toxins by a pathotype of *Alternaria citri* causing brown spot disease of mandarins. *Agricultural and Biological Chemistry* 50, 2401–2403.

Kopecky, J., Matha, V. and Jegorov, A. (1992) The inhibitory effect of destruxin A on the arboviruses in *Aedes albopictus* C6/36 cell line. *Comparative Biochemistry and Physiology* 103C, 23–25.

Krasnoff, S. and Gibson, D.M. (1996) New destruxins from the entomopathogenic fungus *Aschersonia* sp. *Journal of Natural Products* 59, 485–489.

Krasnoff, B., Gupta, S.B., St Leger, R.J., Renwick, J.A. and Roberts, D.W. (1991) Antifungal and insecticidal properties of efrapeptins: metabolites of the fungus *Tolypocladium niveum. Journal of Invertebrate Pathology* 58, 180–188.

Krasnoff, S.B. and Gupta, S. (1991) Identification and biosynthesis of efrapeptins in the fungus *Tolypocladium geodes* Gams (Deuteromycotina: Hyphomycetes). *Journal of Chemical Ecology* 17, 1953–1960.

Lange, C., Mulheim, C., Cherton, J.C., Cassier, P., Vey, A. and Pais, M. (1991) Desorption of ions from locust tissue – behaviour of E-destruxin using positive-ion fast atom bombardment mass spectrometry. *Rapid Communications in Mass Spectrometry* 5, 169–174.

Lange, C., Loutelier, C., Cherton, J.-C., Cassier, P., Vey, A. and Pais, M. (1992) Desorption of ions from locust tissues. II. Metabolites of E-destruxin using negative-ion fast-atom bombardment mass spectrometry. *Rapid Communications in Mass Spectrometry* 6, 28–33.

Lax, A.R., Sheperd, H.S. and Edwards, J.V. (1988) Tentoxin, a chlorosis-inducing toxin from *Alternaria* as a potential herbicide. *Weed Technology* 2, 540–544.

Leclerc, G., Rebuffat, S. and Bodo, B. (1998) Directed biosynthesis of peptaibol antibiotics in two *Trichoderma* strains. II. Structure elucidation. *Journal of Antibiotics* (*Tokyo*) 51, 178–183.

Lilley, L.J. (1965) Induction of chromosome aberrations by aflatoxins. *Nature* 207, 433–434.

Lin, A., Lee, T.M. and Rern, J.C. (1994) Tricholin, a new antifungal agent from *Trichoderma viride* and its action in biological control of *Rhizoctonia solani. Journal of Antibiotics* (*Tokyo*) 47, 799–805.

Liu, J.-C., Boucias, D.G., Pendland, J.C., Liu, W.-Z. and Maruniak, J. (1996) The mode of action of Hirsutellin A on eukaryotic cells. *Journal of Invertebrate Pathology* 67, 224–228.

Liu, W.-Z., Boucias, D.G. and McCoy, C.W. (1995) Extraction and characterization of the insecticidal toxin hirsutellin A produced by *Hirsutella thompsonii* var. *thompsonii. Experimental Mycology* 19, 254–262.

Logrieco, A., Moretti, A., Castella, G., Kostecki, M., Golinski, P., Ritieni, A. and Chelkowski, J. (1998) Beauvericin production by *Fusarium* species. *Applied Environmental Microbiology* 64, 3084–3088.

Lorito, M., Peterbauer, C., Hayes, C.K. and Harman, G.E. (1994) Synergistic action between fungal cell wall degrading enzymes and different antifungal compounds enhances inhibition of spore germination. *Microbiology* 140, 623–629.

Lorito, M., Farkas, V., Rebuffat, S., Bodo, B. and Kubicek, C.P. (1996) Cell wall synthesis is a major target of mycoparasitic antagonism by *Trichoderma harzianum*. *Journal of Bacteriology* 178, 6382–6385.

Loutelier, C., Lange, C., Cassier, P., Vey, A. and Cherton, J.C. (1994) Non-extractive study of E- and A-destruxines in the locust, *Locusta migratoria* L. III: Direct high performance liquid chromatography analysis and parallel FAB-MS monitoring. *Journal of Chromatography B, Biomedical Applications* 656, 281–292.

Loutelier, C., Marcual, A., Cassier, P., Cherton, J.-C. and Lange, C. (1995) Desorption of ions from locust tissues. III. Study of a metabolite of A-destruxin using fast-atom bombardment linked-scan mass spectrometry. *Rapid Communications Mass Spectrometry* 9, 408–413.

Madariaga, R.B. and Scharen, A.L. (1985) *Septoria tritici* blotch in Chilean wild oat. *Plant Disease* 69, 126–127.

Manning, R.O. and Wyatt, R.D. (1984) Comparative toxicity of *Chaetomium* contaminated corn and various chemical forms of oosporein in broiler chickens. *Poultry Science* 63, 251–259.

Marasas, W.F.O., Wehner, F.C., van Rensburg, S.J. and van Schalkwyk, D.J. (1981) Mycoflora of corn produced in human esophageal cancer areas in Transkei, Southern Africa. *Phytopathology* 71, 792–796.

Massion, C.L. and Lindow, S.E. (1986) Effects of *Sphacelotheca holci* infection on morphology and competitiveness of johnsongrass (*Sorghum halepense*). *Weed Science* 34, 883–888.

Matha, V., Weiser, J. and Olejnicek, J. (1988) The effect of tolypin in *Tolypocladium niveum* crude extract against mosquito and blackfly larvae in the laboratory. *Folia Parasitologia* 35, 381–383.

Mazet, I. (1992) Recherches sur les hirsutellines, toxines protéiques produites par *Hirsutella thompsonii* Fisher, champignon parasite d'acariens phytophages. Doctoral dissertation, Université de Montpellier 2, France (cited in Liu *et al.*, 1996).

Mazet, I. and Vey, A. (1995) Hirsutellin A, a toxic protein produced *in vitro* by *Hirsutella thompsonii*. *Microbiology* 141, 1343–1348.

McLean, M. (1993) Towards an understanding of the effects of aflatoxin B1 on plant tissue. PhD, thesis, University of Natal, Durban, South Africa.

McLean, M. (1994) The phytotoxic effects of aflatoxin B1: a review (1984–1994) *South African Journal of Science* 90, 385–390.

McLean, M., Watt, M.P., Berjak, P., Dutton, M.F. and Snyman, C. (1994) Effects of aflatoxin B1 on *in vitro* cultures of *Nicotiana tabacum* var. Samsun. II: Root and shoot development in tobacco plantlets. *Mycopathologia* 125, 107–117.

Merck Index (1996) *Aflatoxins B*, ed. S. Budavari. Merck and Co., Inc. Whitehouse Station, New Jersey, p. 33.

Merrill, A.H. Jr, Schmelz, E.-M., Dillehay, D.L., Spiegel, A., Shayman, J.A., Schroeder, J.J., Riley, R.T. and Wang, E. (1997) Sphingolipids. The enigmatic lipid class: biochemistry, physiology and pathophysiology. *Toxicology and Applied Pharmacology* 142, 208–225.

Mertz, D., Lee, D., Zuber, M. and Lillehoj, E.B. (1980) Uptake and metabolism of aflatoxin by *Zea mays*. *Journal of Agricultural and Food Chemistry* 28, 963–966.

Miller, J.D., Strongman, D. and Whitney, N.J. (1985) Observations on fungi associated with spruce budworm infested balsam fir needles. *Canadian Journal of Forestry Research* 15, 896–901.

Mochizuki, K., Ohmori, K., Tamura, H., Shizuri, Y., Nishiyama, S., Miyoshi, E. and Yamamura, S. (1993) The structures of bioactive cyclodepsipeptides, Beauveriollides I and II, metabolites of entomopathogenic fungi *Beauveria* sp. *Bulletin of the Chemical Society of Japan* 66, 3041–3046.

Moretti, A., Logrieco, A., Bottalico, A., Ritienti, A., Randazzo, G. and Corda, P. (1995) Beauvericin production by *Fusarium subglutinans* from different geographical areas. *Mycological Research* 99, 282–286.

Morin, L., Gianotti, A.F. and Lauren, D.R. (2000) Trichothecene production and pathogenicity of *Fusarium tumidum*, a candidate bioherbicide for gorse and broom in New Zealand. *Mycological Research* 104, 993–999.

Mumpuni, A., Sharma, H.S.S. and Brown, A. (1998) Effect of metabolites produced by *Trichoderma harzianum* biotypes and *Agaricus bisporus* on their respective growth radii in culture. *Applied Environmental Microbiology* 64, 5053–5056.

Munkvold, G., Stahr, H.M., Logrieco, A., Moretti, A. and Ritieni, A. (1998) Occurrence of

fusaproliferin and beauvericin in *Fusarium*-contaminated livestock feed in Iowa. *Applied Environmental Microbiology* 64, 3923–3926.

Muroi, M., Shiragami, N. and Takatsuki, A. (1994) Destruxin B, a specific and readily reversible inhibitor of vacuolar type H+ translocating ATPase. *Biochemical and Biophysical Research Communications* 205, 1358–1365.

Muroi, M., Kaneko, N., Suzuki, K., Nishio, T., Oku, T., Sato, T. and Takatsuki, A. (1996) Efrapeptins block exocytic but not endocytic trafficking of proteins. *Biochemical and Biophysical Research Communications* 227, 800–809.

Namatame, I., Tomoda, H., Tabata, N., Si, S.Y. and Omura, S. (1999) Structure elucidation of fungal Beauveriolide-III, a novel inhibitor of lipid droplet formation in mouse macrophages. *Journal of Antibiotics* 52, 7–12.

Nasini, G., Locci, L., Camarda, L., Merlini, R. and Nasini, G. (1977) Screening of the genus *Cercospora* for secondary metabolites. *Phytochemistry* 16, 243–247.

Odier, F., Vey, A. and Bureau, J.P. (1992) *In vitro* effects of fungal cyclodepsipeptides on leukemic cells: study of destruxins A, B and E. *Biology of the Cell* 74, 267–271.

Ojcious, D.M., Zychlinsky, L., Zheng, M. and Yong, D.-E. (1991) Ionophore-induced apoptosis: role of DNA fragmentation and calcium fluxes. *Experimental Cell Research* 197, 43–49.

Okamoto, M., Yoshida, K., Uchida, I., Nishikawa, M., Kohsaka, M. and Aoki, H. (1986a) Studies of platelet activating factor (PAF) antagonists from microbial products. I. Bisdethio bis(methylthio)gliotoxin and its derivatives. *Chemical and Pharmaceutical Bulletin* (*Tokyo*) 34, 340–344.

Okamoto, M., Yoshida, K., Uchida, I., Kohsaka, M. and Aoki, H. (1986b) Studies of platelet factor (PAF) antagonists from microbial products. II. Pharmacological studies of FR-49175 in animal models. *Chemical and Pharmaceutical Bulletin* (*Tokyo*) 34, 345–348.

Omoto, C. and McCoy, C.W. (1998) Toxicity of purified fungal toxin hirsutellin A to the citrus rust mite *Phyllocoptruta oleivora. Journal of Invertebrate Pathology* 72, 319–322.

Omura S., Arai, N., Yamaguchi, Y., Masuma, R., Iwai, Y., Namikoshi, M., Turberg, A., Kolbl, H. and Shiomi, K. (2000) Argifin, a new chitinase inhibitor, produced by *Gliocladium* sp. FTD-0668. I. Taxonomy, fermentation, and biological activities. *Journal of Antibiotics* (*Tokyo*) 53 (6), 603–608.

Ovchinnikov, Y.A., Ivanov, V.T. and Mikhaleva, I.I. (1971) The synthesis and some properties of beauvericin. *Tetrahedron Letters* 2, 159–162.

Païs, M., Das, B.C. and Ferron, P. (1981) Depsipeptides from *Metarhizium anisopliae*. *Phytochemistry* 20, 715–723.

Pegram, R.A. and Wyatt, R.D. (1981) Avian gout caused by oosporein, a mycotoxin produced by *Chaetomium trilaterale. Poultry Science* 60, 2429–2440.

Pegram, R.A., Wyatt, R.D. and Smith, T.L. (1982) Oosporein toxicosis in turkey poult. *Avian Diseases* 26, 47–49.

Plattner, R.D. and Nelson, P.E. (1994) Production of beauvericin by a strain of *Fusarium proliferatum* isolated from corn fodder for swine. *Applied Environmental Microbiology* 60, 3894–3896.

Poprawski, T.J., Robert, P.H. and Maniana, N.K. (1985) Susceptibility of the onion maggot *Delia antiqua* (Diptera Antomyiidae) to the mycotoxin destruxin E. *Canadian Entomologist* 117, 801–802.

Poprawski, T.J., Robert, P.H. and Maniania, N.K. (1994) Contact toxicity of the mycotoxin destruxin E to *Empoasca vitis. Journal of Applied Entomology* 117, 135–143.

Qadri, S., Mohiuddin, S., Anwar, N., Rizki, Y.M., Qureshi, S.A. and Anwarullah, M. (1989) Larvicidal activity of β-exotoxin and beauvericin against two dipterous species. *Pakistan Journal of Scientific and Industrial Research* 32, 467–470.

Quiot, J.-M., Vey, A., Vago, C. and Pais, M. (1980) Action antivirale d'une mycotoxine. Etude d'une toxine de l'hyphomycète *Metarhizium anisopliae* (Metsch.) Sorok. en culture cellulaire. *Comptes Rendus de l'Academie des Sciences, Série D* (*Paris*) 291, 763–766.

Quiot, J.-M., Vey, A. and Vago, C. (1985) Effects of mycotoxins on invertebrate cells *in vitro*. *Advances in Cell Culture* 4, 199–212.

Rebuffat, S., El Hajji, M., Hennig, P., Davoust, D. and Bodo, B. (1989) Isolation, sequence, and conformation of seven trichorzianines B from *Trichoderma harzianum*. *International Journal of Peptide and Protein Research* 34, 200–210.

Rebuffat, S., Duclohier, H., Auvin-Guette, C., Molle, G., Spach, G. and Bodo, B. (1992) Membrane-modifying properties of the pore-forming peptaibols saturnisporin SA IV and harzianin HA V. *FEMS Microbiology Immunology* 5, 151–160.

Reiss, J. (1984) Failure of plant tissue to metabolize aflatoxin B1? *Mycopathologia* 85, 43–44.

Robert, P.-H. and Fargues, J. (1986) Toxicité par ingestion des destruxines A, B et E pour les larves de *Musca domestica* L. *Comptes Rendus de l'Académie des Sciences, Paris, Serie III* 303, 641–643.

Robert, P.H. and Riba, G. (1989) Toxic and repulsive effect of spray, *per os* and systemic application of destruxin E to aphids. *Mycopathologia* 108, 170–183.

Roberts, D.W. (1966) Toxins from the entomogenous fungus *Metarhizium anisopliae*. II. Symptoms and detection in moribund hosts. *Journal of Invertebrate Pathology* 8, 222–227.

Roberts, D.W. (1981) Toxins of entomopathogenic fungi. In: Burges H.D. (ed.) *Microbial Control of Pests and Plant Diseases 1970–1980*. Academic Press, New York, pp. 441–464.

Samuels, R.I., Reynolds, S.E. and Charnley, A.K. (1988a) Calcium channel activation of insect muscle by destruxins, insecticidal compounds produced by the entomopathogenic fungus, *Metarhizium anisopliae*. *Comparative Biochemistry and Physiology* 90C, 403–412.

Samuels, R.I., Charnley, K. and Reynolds, S.E. (1988b) Application of reversed-phase HPLC in separation and detection of the cyclodepsipeptide toxins produced by the entomopathogenic fungus *Metarhizium anisopliae*. *Journal of Chromatographic Science* 26, 15–19.

Sawa, R., Mori, Y., Inuma, H., Naganawa, H., Kamada, M., Yoshida, S., Furutani, H., Kajimura, Y., Fuwa, T. and Takeuci, T. (1994) Harzianic acid, a new microbial antibiotic from a fungus. *Journal of Antibiotics* (*Tokyo*) 47, 731–732.

Scheepens, P.C. (1987) Joint action of *Cochliobolus lunatus* and atrazine on *Echinochloa crus-galli* (L.) Beauv. *Weed Research* 27, 43–47.

Scheepens, P.C. and van Zon, H.C.J. (1982) Microbial herbicides. In: Kurstak, E. (ed.) *Microbial and Viral Pesticides.* Marcel Dekker, New York, pp. 623–641.

Scheffer, R.P. and Livingston, R.S. (1984) Host-selective toxins and their role in plant diseases. *Science* 223, 17–21.

Schirmbock, M., Lorito, M., Wang, Y.L., Hayes, C.K., Arisan-Atac, I., Scala, F., Harman, G.E. and Kubicek, C.P. (1994) Parallel formation and synergism of hydrolytic enzymes and peptaibol antibiotics, molecular mechanisms involved in the antagonistic action of *Trichoderma harzianum* against phytopathogenic fungi. *Applied Environmental Microbiology* 60, 4364–4370.

Schoental, R. and White, A.F. (1965) Aflatoxins and 'albinism' in plants. *Nature* 205, 57–58.

Shabana, Y.M.N.E. (1987) Biological control of water weeds by using plant pathogens. Dissertation, Mansoura University, El-Mansoura, Egypt.

Shier, W.T., Abbas, H.K. and Mirocha, C.J. (1991) Toxicity of the mycotoxins fumonisins B1 and B2 and *Alternaria alternata* f. sp. *lycopersici* toxin (AAL) in cultured mammalian cells. *Mycopathologia* 116, 97–104.

Sloman, I.S. and Reynolds, S.E. (1993) Inhibition of ecdysteroid secretion from *Manduca sexta* prothoracic glands *in vitro* by destruxins – cyclic depsipeptide toxins from the insect pathogenic fungi *Metarhizium anisopliae*. *Insect Biochemistry and Molecular Biology* 23, 43–46.

Solfrizzo, M., Altomare, C., Visconti, A., Bottalico, A. and Perrone, G. (1994) Detection of peptaibols and their hydrolysis products in cultures of *Trichoderma* species. *Natural Toxins* 2, 360–365.

Steinrauf, L.K. (1985) Beauvericin and the other enniatins. In: Sigel, H. and Sigel, A. (eds)

Metal Ions in Biological Systems – Antibiotics and their Complexes. Marcel Dekker, New York, pp. 139–171.

Stipanovic, R.D. and Howell, C.R. (1982) The structure of gliovirin a new antibiotic from *Gliocladium virens. Journal of Antibiotics* (*Tokyo*) 35, 1326–1330.

Strasser, H., Vey, A. and Butt, T.M. (2000) Are there any risks in using entomopathogenic fungi for pest control, with particular reference to the bioactive metabolites of *Metarhizium, Tolypocladium* and *Beauveria* species? *Biocontrol Science and Technology* 10, 717–735.

Strongman, D.B., Strunz, G.M., Giguere, P., Yu, C.-M. and Calhoun, L. (1988) Enniatins from *Fusarium avenaceum* isolated from balsam fir foliage and their toxicity to spruce budworm larvae, *Choristoneura fumiferana* Clem. *Journal of Chemical Ecology* 14, 753–764.

Sun, C., Chen, H. and Yeh, S.F. (1994) Suppressive effects of metabolites from *Alternaria brassicae* on the hepatitis B surface antigen. *Planta Medica* 60, 87–88.

Suzuki, A., Taguchi, H. and Tamura, S. (1970) Isolation and structure elucidation of three new insecticidal cyclodepsipeptides, destruxins C and D and desmethyldestruxin B produced by *Metarhizium anisopliae*. *Agricultural and Biological Chemistry* 34, 813–816.

Suzuki, A., Kawakami, K. and Tamura, S. (1971) Detection of destruxins in silkworm larvae infected with *Metarhizium anisopliae*. *Agricultural and Biological Chemistry* 35, 1641–1643.

Suzuki, A., Kanaoka, M., Isogai, A., Murakoshi, S., Ichinoe, M. and Tamura, S. (1977) Bassianolide, a new insecticidal cyclodepsipeptide from *Beauveria bassiana* and *Verticillium lecanii*. *Tetrahedron Letters* 25, 2167–2170.

Tabuchi, H., Tajimi, A. and Ichihara, A. (1991) (+)-Isocerosporin, a phytotoxic compound isolated from *Scolecotrichum graminis* Fuckel. *Agricultural and Biological Chemistry* 55, 2675–2676.

Tamura, S. and Takahashi, N. (1971) Destruxins and piercidens. In: Jacobson, M. and Grosby, D.G. (eds) *Naturally Occurring Insecticides.* Marcel Dekker, New York, pp. 499–539.

Tanaka, T.H., Abbas, H.K. and Duke, S.O. (1993) Phytotoxin structure–activity relationships of fumonisins, aminopentals, sphingolipids and AAL-toxin in a duckweed (*Lemna pausiocostata* L.) bioassay. *Phytochemistry* 33, 779–785.

Taniguchi, M., Kawaguchi, T., Tanaka, T. and Oi, S. (1984) Antimicrobial and respiration inhibitory activities of oosporein. *Agricultural and Biological Chemistry* 48, 1065–1067.

Taylor, A. (1986) Some aspects of the chemistry and biology of the genus *Hypocrea* and its anamorphs, *Trichoderma* and *Gliocladium*. *Proceedings of the Nova Scotia Institute of Science* 36, 27–58.

TeBeest, D.O. (ed.) (1991) *Microbial Control of Weeds.* Chapman & Hall, New York.

Teetor-Barsch, G. and Roberts, D.W. (1983) Entomogenous *Fusarium* species. *Mycopathologia* 84, 3–16.

Templeton, G.E. and Heiny, D.K. (1989) Improvement of fungi to enhance mycoherbicide potential. In: Whipps, J.M. and Lumsden, R.D. (eds) *Biotechnology of Fungi for Improving Plant Growth.* Cambridge University Press, Cambridge, pp. 127–151.

Terry, B.J., Liu, W.-C., Cianci, C.W., Proszynski, E., Fernandes, P. and Meyers, E. (1992) Inhibition of herpes simplex virus type I DNA polymerase by the natural product oosporein. *Journal of Antibiotics* 2, 286–288.

Thines, E., Eilbert, F., Anke, H. and Sterner, O. (1998) Glisoprenins C, D and E, new inhibitors of appressorium formation in *Magnaporthe grisea*, from cultures of *Gliocladium roseum*. 1. Production and biological activities. *Journal of Antibiotics* (*Tokyo*) 51, 117–122.

Thomsen, L., Eilenberg, J. and Esberg, P. (1996) Effects of destruxins on *Pieris brassicae* and *Agrotis segetum*. *IOBC Bulletin* 19, 190–195.

Tomlin, C.D. (1997) *The Pesticide Manual.* BCPC Publications, Bracknell, UK, 1606 pp.

Ueno,Y. (1984) Toxicological features of T-2 toxin and related trichothecenes. *Fundamental. Applied Toxicology* 4, 124–132.

Venkatsubbaiah, P., Tisserat, N.A. and Chilton, W.S. (1994) Metabolites of *Ophiosphaerella herpotricha. Mycopathologia* 128, 155–159.

Vesonder, R.F., Labeda, D.P. and Peterson, R.E. (1992a) Phytotoxic activity of selected water-

soluble metabolites of *Fusarium* against *Lemna minor* L. (duckweed). *Mycopathologia* 118, 185–189.

Vesonder, R.F., Peterson, R.E., Lebeda, D. and Abbas, H.K. (1992b) Comparative phytotoxicity of fumonisins, AAL-toxin and yeast sphingolipids in *Lemna minor* L. (duckweed). *Archives of Environmental Contamination and Toxicology* 23, 464–467.

Vey, A. and Quiot, J.-M. (1989) Etude *in vitro* et chez l'insecte hôte des destruxines, toxines cyclodepsipeptidiques produites par le champignon entomopathogène *Metarhizium anisopliae*. *Canadian Journal of Microbiology* 35, 1000–1008.

Vey, A., Quiot, J.-M., Vago, C. and Fargues, J. (1985) Effet immunodépresseur de toxines fongiques: inhibition de la réaction d'encapsulement multicellulaire par les destruxines. *Comptes Rendus de l'Académie des Sciences, Série 3* (*Paris*) 300, 647–651.

Vey, A., Quiot, J.-M. and Païs, M. (1986) Toxémie d'origine fongique chez les invertébrés et ses conséquences cytotoxiques: étude sur l'infection à *Metarhizium anisopliae* (Hyphomycète, Moniliales) chez les lépidoptères et les coléoptères. *Comptes Rendus de la Société de Biologie* 180, 105–112.

Vey, A., Quiot, J.-M., Mazet, I. and McCoy, C.W. (1993) Toxicity and pathology of crude broth filtrate produced by *Hirsutella thompsonii* var. *thompsonii* in shake culture. *Journal of Invertebrate Pathology* 61, 131–137.

Vilcinskas, A., Matha, V. and Gotz, P. (1997) Inhibition of phagocytic activity of plasmatocytes isolated from *Galleria mellonella* by entomogenous fungi and their secondary metabolites. *Journal of Insect Physiology* 43, 475–483.

Vining, L.C., Kelleher, W.J. and Schwarting, A.E. (1962) Oosporein production by a strain of *Beauveria bassiana* originally identified as *Amanita muscaria*. *Canadian Journal of Microbiology* 8, 931–933.

Vogelgsang, S., Watson, A.K., Ditommaso, A. and Hurle, K. (1998) Effect of the pre-emergence bioherbicide *Phomopsis convolvulus* on seedling and established plant growth of *Convolvulus arvensis. Weed Research* 38, 175–182.

Wahlman, M. and Davidson, B.S. (1993) New destruxins from the entomopathgenic fungus *Metarhizium anisopliae*. *Journal of Natural Products* 56, 643–647.

Wainwright, M., Betts, R.P. and Teale, D.M. (1986). Antibiotic activity of oosporein from *Verticillium psalliotae*. *Transactions of the British Mycological Society* 86, 168–170.

Walker, H.L. (1981) *Fusarium lateritium*: a pathogen of spurred anoda (*Anoda cristata*), prickly sida (*Sida spinosa*), and velvetleaf (*Abutilon theophrasti*). *Weed Science* 29, 629–631.

Wang, E., Norred, W.P., Bacon, C.W., Riley, R.T. and Merrill, A.H. (1991) Inhibition of sphingolipid biosynthesis by fumonisins. *Journal of Biological Chemistry* 266, 14486–14490.

Weindling, R. (1934) Studies in a toxic principle effect in the parasitic association of *Trichoderma lignorum* on *Rhizoctonia solani* and other soil fungi. *Phytopathology* 24, 1153–1179.

Weiser, J. and Matha, V. (1988) Tolypin, a new insecticidal metabolite of fungi of the genus *Tolypocladium*. *Journal of Invertebrate Pathology* 51, 94–96.

Wilhite, S.E. and Straney, D.C. (1966) Timing of gliotoxin biosynthesis in the fungal biological control agent *Gliocladium virens* (*Trichoderma virens*). *Applied Microbiology and Biotechnology* 45, 513–518.

Wilson, B.J. (1971) Miscellaneous *Aspergillus* toxins. In: Ciegler, A., Kadis, S. and Aje, S.J. (eds) *Microbial Toxins: a Comprehensive Treatise,* Vol. 6. Academic Press, New York, pp. 288–289.

Winder, R.S. and van Dyke, G.C. (1989) The pathogenicity, virulence, and biocontrol potential of two *Bipolaris* species on johnsongrass (*Sorghum halepense*). *Weed Science* 38, 84–89.

Wogan, H. (1992) Aflatoxins as risk factors for hepatocellular carcinoma in humans. *Canadian Research* 52, 2114–2118.

Wood, G.E. (1992) Mycotoxins in foods and feeds in the United States. *Journal of Animal Science* 70, 3941–3949.

Yeh, S.F., Pan, W., Ong, G.T., Chiou, A.J., Chuang, C.C., Chiou, S.H. and Wu, S.H. (1996) Study of the structure–activity correlation in destruxins, a class of cyclodepsipeptides pos-

sessing suppressive effect on the generation of hepatitis virus surface antigen in human hepatoma cells. *Biochemical and Biophysical Research Communications* 229, 65–72.

Yoder, O.C. and Turgeon, B.G. (1985) Molecular bases of fungal pathogenicity to plants. In: Bennet, J.W. and Lasure, L. (eds) *Gene Manipulations in Fungi.* Academic Press, New York, USA, pp. 417–441.

Yoshida, K., Okamoto, M., Shimazaki, N. and Hemmi, K. (1988) PAF inhibitors of microbial origin. Studies on diketopiperazine derivatives. *Progresses in Biochemistry and Pharmacology* 22, 66–80.

Zizka, J. and Weiser, J. (1993) Effect of beauvericin, a toxic metabolite of *Beauveria bassiana*, on the ultrastructure of *Culex pipiens autogenicus* larvae. *Cytobios* 75, 13–19.