Chapter 9
Metabolite Toxicology of Fungal Biocontrol Agents

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Abstract Up to 300,000 potentially unique mycotoxins were reported by the Council of Agricultural Science Technology in 2003. Nevertheless, little information is available on fungal biological control agents (FBCAs) that have been developed or are being developed. The knowledge on fungal secondary metabolites and their toxicological significance depends on what is already known in the published scientific literature or in few cases becomes apparent by chance from high throughput screening programmes during product development. The purpose of this chapter is to describe what data basis is most frequently used for a routine evaluation of fungal metabolites and their residues in FBCAs. A decision scheme is discussed, which should be used to assess metabolite toxicity in the context of a worst-case scenario testing. This risk assessment procedure will help to identify the low risk, if any, of old and newly developed microbial pest control agents, and give support to applicants to market their FBCAs.

Contents

9.1 Introduction

Fungi secrete an array of natural metabolites (extrolites), mostly products of secondary metabolism, which serve different functions depending on the ecological habitat of the fungus (Butt 2002; Calvo et al. 2002; Vey et al. 2001). Secondary

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metabolism is commonly associated with fungal development (i.e. sporulation, cell differentiation). Calvo et al. (2002) classified the function of these products into three groups: (i) metabolites that activate sporulation; (ii) pigments required for spore structures; and (iii) toxic metabolites secreted by growing fungi (mycotoxins). The latter are of great interest to scientists and legislators, not only because of their powerful and varied biological effects, which can be used for medical or industrial purpose (Baker et al. 2007; Hoffmeister and Keller 2007; Nielson and Smedsgaard, 2003), but also to overcome obstacles in the registration and subsequent commercialization of fungal biological control agents (FBCAs).

The authorisation of BCAs is strictly regulated but there is still much debate in defining the criteria for registration of FBCAs, especially with respect to the potential of the micro-organism to produce metabolites, including toxins (points IIM 2.4 and IIM 3.5.2. OECD 2004; Strasser et al. 2000; Goettel et al. 2001; Strasser et al. 2008).

A global harmonization of registration procedures is still not realised, just because of the fact, that all "old" active BCAs in the EU will be put on the "green track (1097/2007/EC; EU 2007a)" and therefore will be listed in Annex I after examination by the rapporteur member state and the commission (Directive 91/414/EC, EU 1991; see 2008/113/EC; EU 2008a). The peer review will be organised by EFSA and will allow member states and EFSA to comment further on the scientific conclusions of the Draft assessment report. This "fast-track" authorisation has been given because unacceptable negative effects to humans and the environment were excluded (see also Table 9.1: Remarks and specific provisions). There is still no official "lesson learned document" available, which was expected to be published based on the experience of the judgement of 4th list substances (2229/2004/EC, EU 2004). Nevertheless, for the first time the Commission gave a clear indication that all already notified old active biocontrol agents do not have any harmful effects on human or animal health, or on groundwater or any unacceptable influence on the environment.

The focus of this chapter is to provide an overview about information available on fungal secondary metabolites (i.e. toxicants) and their effects, and to suggest how to deal with the registration of fungal BCAs for which no information about metabolites is available. A decision scheme for the assessment of potential relevant metabolites of fungal BCAs is presented, which will help to identify and to characterise low risk FBCAs.

9.2 Mycotoxins of Fungal Biocontrol Agents

In recent years, significant progress has been made in the development of fungal biocontrol agents (BCAs) for the suppression of pests (insects, nematodes), weeds and diseases of a wide range of forest, horticultural and agricultural crops (Butt et al. 2001). Nevertheless, relatively few of these products have reached the market: for example, at the time of writing this article only 24 FBCAs have been registered or

Table 9.1 Secondary metabolites from fungal biopesticide active products mentioned in the "Handbook of secondary fungal metabolites" (Cole and Schweikert 2003a, 2003b; Cole et al. 2003). All fungal biocontrol agents from the online forum of US EPA (2007a) and/or in Annex I (91/414/EC; EU 1991) or currently suggested for inclusion in Annex I (EU 2008a) were considered

Fungal biocontrol agent (intended use)	Permit	Secondary metabolites (biological activity)	Remarks/specific provisions	Product name(s)
Alternaria destruens 059 (Herbicide)	US	Dehydroaltenusin (Anti-tumor effects)	*A. destruens* is minimally toxic and nonpathogenic to mammals. Consequently, no cumulative effects from the residues of this product with other related microbial pesticides are anticipated (US EPA 2005a)	Smolder G, Smolder WP
Ampelomyces quisqualis AQ 10 (Fungicide)	EU/US	No relevant metabolites	Standing Committee concluded . . . [no toxins are involved (EU 2005a)]	AQ 10

Table 9.1 (continued)

Fungal biocontrol agent (intended use)	Permit	Secondary metabolites (biological activity)	Remarks/specific provisions	Product name(s)
Aspergillus flavus AF36 (Cottonseed protector)	US	Ditryptophenaline Aflavinine; (antiinsectan activity) Seven indol derivates, e.g. 20-Hydroxyaflavinine, Aflazvazole; (antifeeding activity) Paspalinine and two more related compounds, e.g. Alfatrem; (tremorgenic in rodents) Aflatoxin B1, B2, B_{2a}, G1, $G_{2a,}$ M1, M2 (hepatocarciogen) Parasiticol (cause biliary hyperplasia in organs) O-Methylsterigmatocystin Dihydro- O-methylsterigmatocystin (negligible effects in cell culture) Aspertoxin (in developing chicken embryo – 100% killed after injection of 2.0 μg/egg) Ergosta-4,6,8(14),22-tetraen-3-one 24-Ethylcholesta-4,6,8(14),22-tetraen-3-one Aspervalvin Cyclopiazonic acid (potent inhibitor of Ca^{2+} activated ATPase)	This strain of *A. flavus* does not produce aflatoxin (atoxigenic strain; US EPA 2003a)	*Aspergillus flavus* AF36
A. flavus NRRL 21882 (Peanut protector)	US	See above	This strain of *A. flavus* does not produce aflatoxin (US EPA2004)	Aflaguard

Table 9.1 (continued)

Fungal biocontrol agent (intended use)	Permit	Secondary metabolites (biological activity)	Remarks/specific provisions	Product name(s)
Beauveria bassiana ATCC 74040 (Insecticide)	EU*/US	Isoleucylisoleucyl anhydride cyclo-(L-Isoleucyl-L-valine) cyclo-(L-Alanyl-L-proline)	No risks expected. Residues of the fungus are not expected to remain on treated food or feed (US EPA 1999a). Based on the Commission review report – the BCA will be listed in Annex I (SANCO/1546/2008; EU 2008a)	Troy Boverin, Fermone Naturalis L-225
B. bassiana GHA (Insecticide)	EU*/US	See above	No risks expected (US EPA 2000a). Based on the Commission review report – the BCA will be listed in Annex I (SANCO/1547/2008; EU 2008a)	Mycotrol ES, Botanigard 22WP, Organigard ES
B. bassiana 447 (Insecticide)	US	See above	No risks expected. EPA will require additional studies if the bait stations are proposed for outdoor or other additional uses (US EPA 2002a)	"Baits motel stay a while-rest forever"
B. bassiana HF23 (Insecticide)	US	See above	Residues do not remain on treated food (US EPA 2006a) Confirmatory analyses of five batches were requested to demonstrate that beauvericin and other unintentional ingredients, are within levels required for quality assurance and quality control (US EPA 2006b)	*Beauveria bassiana* HF23

Table 9.1 (continued)

Fungal biocontrol agent (intended use)	Permit	Secondary metabolites (biological activity)	Remarks/specific provisions	Product name(s)
Candida oleophila isolate I-182 (Fungicide)	US	Ergosterol 5,6-Dihydroergosterol 4-alpha-Methylzymosterol Sitosterol	No risks expected. Fact Sheet (US EPA 2000b)	Aspire
Candida oleophila strain O (Fungicide)	EU*	See above	2007/380/EC: no open questions published (EU 2007b)	Bionext
Chondrostereum purpureum HQ1 (Herbicide)	US	No relevant metabolites	No information available (US EPA 2005c)	Myco-tech paste
Chondrostereum purpureum PFC2139 (Herbicide)	US	No relevant metabolites	No known reports that fungus produce any toxins harmful to animals, and which has caused adverse effects in people (CAN 2007)	Chontrol paste
Colletotrichum gloeosporioides f. sp. *Aeschynomene* (Herbicide)	US	No relevant metabolites	No harmful endpoints when tested in laboratory rodents for toxicity and pathogenicity by the oral, dermal, and pulmonary routes (US EPA 2006c)	Collego
Coniothyrium minitans CON/M/91-08 (Fungicide)	US	Palmarumycin C_1 to C_6 Palmarumycin C_8 to C_{16} (biological activity against bacteria, fungi and garden cress)	The review did not reveal any open questions or concerns (2003/79/EC; EU 2003) *C. minitans* has not been reported to produce toxins or antibiotics (US EPA 2001c)	Contans WG
Gliocladium virens GL- 21 (Fungicide)	US	Viridin (strong antifungal activity) Viridol	No risks expected (US EPA 2000c)	WRC-AP-1

Table 9.1 (continued)

Fungal biocontrol agent (intended use)	Permit	Secondary metabolites (biological activity)	Remarks/specific provisions	Product name(s)
G. catenulatum J1446 (Fungicide)	EU/US	No relevant metabolites	The review did not reveal any open questions or concerns (EU 2005a). According to a literature search on April 2000 there is no indication of toxin production by *G. catenulatum*. No cell toxicity was detected in an FL cell tests or in the *Escherichia coli* WP2/CM871 DNA-repair test. No gliotoxin was detected by HPLC analysis (EU 2005b). There are no reports indicating that *G. catenulatum* strain J1446 produces any toxins or antibiotics (US EPA 2002b)	Primastop, Prestop
Lecanicillium muscarium (formerly *Verticillium lecanii)* (Insecticide)	EU*	23,24,25,26,27-Pentanorlanost-8-ene-3beta,22-diol	Based on the Commission review report – the BCA will be listed in Annex I (SANCO/1861/2008; EU 2008a)	Mycotal
Metarhizium anisopliae ESF1 (Insecticide)	US	Cytochalasin C (strong inhibitor of mammalian cellular functions) Zygosporin A (Teratogenicity effects in chicken, see also cytochalasin C)	No information available (US EPA 2001a)	Bioblast

Table 9.1 (continued)

Fungal biocontrol agent (intended use)	Permit	Secondary metabolites (biological activity)	Remarks/specific provisions	Product name(s)
M. anisopliae F52 (Insecticide, Acaricide)	EU*/US	See above	Although destruxin A, B and E secretion, often in significant quantities, are reported, ... [no harm is expected to humans or the environment when BCAs containing *M. anisopliae* strain F52 are used according to label instructions (US EPA 2003b)]. BCA will be listed in Annex I (SANCO/1862/2008; EU 2008a)	Taenure, Tick-Ex (G, EC);
M. anisopliae BIPESCO5 (Insecticide, Acaricide)	EU*/US	See above	Based on the Commission review report the BCA will be listed in Annex I (SANCO/1862/2008; EU 2008a)	GRANMET-P (GR), WP
Muscodor albus QST20799 (Bactericide, Fungicide, Nematicide)	US	A number of volatiles, mainly alcohols, acids, and esters (inhibit and kill plant pathogenic and other organisms)	Re-evaluation in March 2008 (US EPA 2005d)	Arabesque, Andante, Glissade
Paecilomyces fumosoroseus Apopka strain 97 or PFR 97 or CG 170, ATCC20874 (Insecticide)	EU/US	Paecilospirone	Each fermentation broth should be checked by HPLC to ensure that no secondary metabolites are present (2001/47/EC; EU 2001b). There are no expected health risks from use of this fungus as a pesticide (US EPA 1999b)	PFR-97 (Preferal)

Table 9.1 (continued)

Fungal biocontrol agent (intended use)	Permit	Secondary metabolites (biological activity)	Remarks/specific provisions	Product name(s)
P. fumosoroseus Fe 9901 (Insecticide)	EU	No relevant metabolites	Completeness check of dossier passed (2008/565/EC; EU 2008b)	FUTURECO – NOFLY
P. lilacinus strain 251 (Nematicide)	EU/US	No relevant metabolites	Although many species of *Paecilomyces* release potent toxins, no such toxins (mycotoxins or paecilotoxins) have been detected in this strain (. . ..as shown by chromatographic analyses and lack of toxicity to mammals and other organisms; US EPA 2005e). No relevant toxic metabolite/residue mentioned (2008/44/EC; EU 2008c)	Melocon WG
Phlebiopsis gigantea (Fungicide)	EU*	No relevant metabolites	Based on the Commission review report – the BCA will be listed in Annex I (SANCO/1863/2008; EU 2008a)	ROTSTOP, ROTEX

Table 9.1 (continued)

Fungal biocontrol agent (intended use)	Permit	Secondary metabolites (biological activity)	Remarks/specific provisions	Product name(s)
Pseudozyma flocculosa PF-A22 (Fungicide)	EU*/US	Three fungitoxic unsaturated C-17 fatty acids: (9-heptadecenoic acid, 6-methyl-9-heptadecenoic acid and 4-methyl-7,11-heptadecadienoic acid) fungitoxins acyclic norterpene (2, 6, 10, 14, 18-pentamethyl-2, 6, 8, 10, 12, 14, 17-nonadecaheptene-1,19-diol)	No harm is expected if humans eat food containing residues of *Pseudozyma flocculosa* strain PF-A22 UL (US EPA 2002c)	Sporodex L
Puccinia thlaspeos strain woad (dyer's woad rust) (Herbicide)	US	No relevant metabolites	There is a reasonable certainty that no harm will result from human exposure to *P. thlaspeos* "strain woad". All toxicity study requirements concerning human risk assessment were waived (US EPA 2002d)	Woad Warrior
Pythium oligandrum DV74 (Fungicide)	EU*/US	No relevant metabolites	No harmful health effects to humans are expected: no evidence that the fungus is toxic to humans or other mammals, ... and no adverse environmental effects are expected (US EPA 2007b) Based on the Commission review report – the BCA will be listed in Annex I (SANCO/1864/2008; EU 2008a)	DV 74

Table 9.1 (continued)

Fungal biocontrol agent (intended use)	Permit	Secondary metabolites (biological activity)	Remarks/specific provisions	Product name(s)
*Trichoderma asperellum (*formerly *T. harzianum* ICC012 (Fungicide)	EU*	No relevant metabolites	Based on the Commission review report – the BCA will be listed in Annex I (SANCO/1842/2008; EU 2008a)	Remedier
*T. asperellum (*formerly *T. viride)* T11, TV1, T25 (Fungicide)	EU*	No relevant metabolites	Based on the Commission review report – the BCA will be listed in Annex I (SANCO/1868/2008; EU 2008a)	TV1 and T11
T. atroviride (formerly *T. harzianum*) T-11 (Fungicide)	EU*	No relevant metabolites	Based on the Commission review report – the BCA will be listed in Annex I (SANCO/1841/2008; EU 2008a)	Binab T pellets component, (see also *T. polysporum*)
T. atroviride I-1237 (Fungicide)	EU*	No relevant metabolites	Completeness check of dossier passed (2008/565/EC; EU 2008b)	I-1237 (Agrauxine)
T. atroviride (formerly *T. harzianum*) IMI 206040; (= *T. viride* ATCC 20476) (Fungicide)	EU*/US	No relevant metabolites	Based on the Commission review report – the BCA will be listed in Annex I (SANCO/1866/2008; EU 2008a); see also US EPA (2000e)	Binab component, (see also *T. polysporum*)

Table 9.1 (continued)

Fungal biocontrol agent (intended use)	Permit	Secondary metabolites (biological activity)	Remarks/specific provisions	Product name(s)
T. polysporum ATCC 20475 + (*T. atroviride* ATCC 20476) (Fungicide)	EU*/US	Viridin, (strong antifungal activity) Viridol	Based on the Commission review report – the BCA will be listed in Annex I (SANCO/1867/2008; EU 2008a); see also US EPA (2000e)	Binab T WP
T. harzianum RIFAI ITEM 908 (Fungicide)	EU*	Koninginin A, (inhibits growth of etiolated wheat coleoptiles at 10^{-3} M) Koninginin C, (inhibits growth of etiolated wheat coleoptiles at 10^{-3} M)	Based on the Commission review report – the BCA will be listed in Annex I (SANCO/1840/2008; EU 2008a)	TRICOVER
T. harzianum RIFAI T-39 (Fungicide)	US	See above	Based on its low toxicity potential, an additional FQPA safety factor is not required for residues of *T. harzianum* strain T-39 (US EPA 2000d)	TRICHODEX
T. harzianum RIFAI T-22 (KRL-AG2) (Fungicide)	EU*/US	See above	No risks ecpected (US EPA 2001b) Based on the Commission review report – the BCA will be listed in Annex I (SANCO/1839/2008; EU 2008a)	TRIANUM P, T-22 G, T-22 WP, Fafard Growing mix
T. gamsii (formerly T. viride) ICC080 (Fungicide)	EU*	Trichodermin, (strong inhibitor of protein synthesis in mammalian cells, antifungal antibiotic) Viridin (strong antifungal activity) Viridol	Based on the Commission review report – the BCA will be listed in Annex I (SANCO/1868/2008; EU 2008a)	Remedier

Table 9.1 (continued)

Fungal biocontrol agent (intended use)	Permit	Secondary metabolites (biological activity)	Remarks/specific provisions	Product name(s)
Verticillium albo-atrum (formerly V. dalia) WCS850 (Fungicide)	EU*/US	No relevant metabolites	No mechanism of pathogenicity or toxicity in mammals has been identified for this organism, no cumulative effects from the residues of this product with other related microbial pesticides are anticipated (US EPA 2005b)	Dutch Trig

EU Active ingredients and products listed in Annex I Directive 91/414 (Europe)
EU* Active ingredients and products currently suggested for inclusion in Annex I (2008/113/EC 2008a)
US Active ingredients and products containing them US EPA (2007a)

will be registered in the near future in the European Union under the harmonized registration procedure of Council Directive 91/414/EEC (EU 1991). Likewise, only 33 FBCAs have been approved under the Pest Control Products Act in Canada and U.S. Pesticide Data Requirements, respectively (Kabaluk and Gazdik 2005). Today, only 39 FBCAs (i.e. insecticides, fungicides and herbicides), comprising 21 fungal genera are registered in the EU, USA and Canada (Table 9.1).

The existence of fungal secondary metabolites and their toxicological significance, apart from accessible information in the literature, often becomes known only by chance, when acute toxicity studies with products based on FBCAs had led to negative effects (Rochon and Belliveau 2006) and applicants in the registration process were committed to isolate and characterise the toxicants.

Based on two examples, the difficulties related to the registration of potential mycotoxin-producing FBCAs are presented. The type of data that need to be produced and will later be available to the target audience will be described. The circumstances under which authorities will ask for data on relevant metabolites (mycotoxins) will be detailed.

In 2003 Cole and co-authors published the comprehensive standard work "Handbook of fungal secondary metabolites" (Cole and Schweikert 2003a, 2003b; Cole et al. 2003). The authors' aim was to offer data on all major groups of secondary fungal metabolites assigned to fungal species. Although economically important groups (e.g. the aflatoxins, trichothecenes, fumonisins) have been included in the series, most of the relevant secondary metabolites of commercialised FBCAs (e.g. beauvericine, beauveriolide, destruxins, gliovirin, glioprennins, heptelidic acids, oosporein and viridian) are missing (Table 9.1). This comprehensive handbook is an example for all other relevant publications and demonstrates how difficult it is to get a precise overview on secondary metabolites produced by specific, potential fungal biocontrol agents.

A literature search in the database ISI Web of Knowledge (Thomson Reuters 2008) for all reported secondary fungal metabolites and for all FBACs listed in the online forum of US EPA (2007a) and/or in Annex I (91/414/EC, EU 1991) or currently suggested for inclusion in Annex I (2008/113/EC, EU 2008a) resulted in one new identified secondary metabolite per FBCA and per year on average (Table 9.2). The timeframe for this research was defined for the last 5 years, from January 2003 until August 2008.

Since fungi are the subject of numerous high-throughput screening programmes (Nielsen and Smedsgaard 2003), and metabolic profiling techniques are used to build up and complete comprehensive metabolite data bases (i.e. using NMR spectroscopy and chemometric tools; Seger and Sturm 2007; Holmes et al. 2006), new fungal metabolites are found permanently. One example is the recently re-opened discussion for the safety of the genus *Metarhizium.* Krasnoff et al. (2006, 2008) presented data on two compounds (i.e. fusarin C analogues) which exhibited mutagenic activity in the Ames assay. The authors concluded that "the impact of these findings on the use of *M. anisopliae* as biocontrol agents is currently unknown and need further investigation". The subjects of this study were knockout mutant strains of *M. anisopliae,* which were characterised as metabolite overproducing isolates. From

Table 9.2 New reported secondary metabolites from fungal biopesticide active products published in peer reviewed Journals. All fungal biocontrol agents from the online forum of US EPA 12/2007 and/or in Annex I (91/414/EC; EU 1991) or currently suggested for inclusion in Annex I (EU 2008a) were considered. The following search terms were always checked in the database "ISI web of knowledge" (Thomson Reuters 2008) for the last 5 years: Species name combined with the general search terms (toxin* OR mycotoxin* OR metabolite* OR bioactive; the asterisk stands for the plural, e.g. toxins)

Fungal biocontrol agent	ISI hits/hits with new metabolites	Novel secondary metabolites	References
Alternaria destruens	No records found		
Ampelomyces quisqualis	6/2	Two new sulfur-containing phenolic compounds: (7-hydroxy-5-hydroxymethyl-2H-benzo[1,4]thiazin-3-one and 2,5-dihydroxy-3-methanesulfinylbenzyl alcohol) Ampelopyrone, Desmethyldiaportinol, Desmethyldichlorodiaportim, Macrosporin-7-O-sulfate, 3-O-Methylalaternin-7-O-sulfate, Ampelanol	Zhang et al. (2008) Aly et al. (2008)
Aspergillus flavus	336/0		
Beauveria bassiana	65/1	Novel beauvericin derivate (beauvericins G1–3, beauvericins H1–3) cytotoxicity (haptotaxis).	Xu et al. (2007)
Candida oleophila	2/0		
Chondrostereum purpureum	1/0		
Colletotrichum gloeosporioides f. sp. *Aeschynomene*	23/2	Tetraol (-)-1 Cis-4-hydroxy-6-deoxyscytalone and (4R)-4,8-dihydroxy-a-tetralone	Femenia-Rios et al. (2006), Inacio et al. (2006)

Table 9.2 (continued)

Fungal biocontrol agent	ISI hits/hits with new metabolites	Novel secondary metabolites	References
Coniothyrium minitans	5/1	Macrosphelide A Palmarumycin C_1 (MW 350.03; Palmarumycins)	McQuilken et al. (2003)
Gliocladium virens	6/1	Gliotoxin	Anitha and Murugesan (2005)
G. catenulatum	No records found		
Lecanicillium muscarium (formerly Verticillium lecanii)	10/0		
Metarhizium anisopliae	64/4	Helvolic acid and 1,2 dihdrohelvolic acid (antibacterial compound) Two analogues of fusarin C (i.e. 7-desmethyl and (8Z)-fusarin C) Serinocyclins A and B (cyclic heptapeptides)	Lee et al. (2008) Krasnoff et al. (2006, 2008, Moon et al. (2008) Krasnoff et al. (2007)
Muscodor albus	8/1	Bioactive volatile organic compounds (VOCs)	Strobel (2006)
Paecilomyces fumosoroseus	8/0		
P. lilacinus strain	4/0		
Phlebiopsis gigantea	1/0		
Pseudozyma flocculosa	1/1	Flocculosin (novel glycolipid)	Cheng et al. (2003)
Puccinia thlaspeos	No records found		
Pythium oligandrum	3/0		
Trichoderma asperellum	3/0		
T. atroviride	See above		

Table 9.2 (continued)

Fungal biocontrol agent	ISI hits/hits with new metabolites	Novel secondary metabolites	References
T. polysporum	25/4	Oxiranyldecene; viridenepoxydiol Viridepyronone Trichovirin I Trichodermamides A and B	Chovanec et al. (2005), Evidente et al. (2003), Brückner and Koza (2003), Reino et al. (2008)
T. harzianum	75/4	T39 butenolide, T22 azaphilone Anthraquinones and xanthone derivatives Nonanoic acid Antibiotics	Vinale et al. (2006), Reino et al. (2008) Aneja et al. (2005), El-Hasan et al. (2008)
T. gamsii	No records found		
Verticillium albo-atrum (formerly V. dalia)	6/0		

the scientific point of view the authors raised the right questions, and based on this case study it is understandable why strain-specific data on relevant metabolites are required for a risk assessment of FBCAs. Simply because *Metarhizium anisopliae* var. *anisopliae* relevant data could be presented to regulation authority in spring 2008 (i.e. PMRA Canada), the ongoing safety evaluation for this potential BCA in Canada was not suspended. Otherwise, new labour- and cost-intensive studies would have to be presented by the applicant to demonstrate, that their specific production strain is safe and does not produce these fusarin-like mycotoxins.

It is understandable that regulatory authorities want to have a stringent procedure for the assessment of potentially toxic metabolic byproducts by candidate fungi (Rochon and Belliveau 2006). In order not to hinder the marketing of potentially low risk products (see Laengle and Strasser 2010), REBECA experts proposed a tiered scheme to be able to assess the risks of potential metabolites of FBCAs.

9.3 Standard Procedure for Toxic Metabolite Assessment

REBECA proposed a decision scheme (see Chapter 13), which has been tested initially in case studies on fungal toxic metabolites [i.e. alamethicin, antiamoebins, destruxins (type A, B, E), elsionchrome A, gliotoxin, paracelsin, oosporein (Boss et al. 2007; Favilla et al. 2006; Ganassi et al. 2007; Skrobek and Butt 2005; Skrobek et al. 2006) and selected crude extracts from *Beauveria brongniartii, Metarhizium anisopliae*, *Paecilomyces lilacinus*, *Stagonospora convolvuli*, *Trichoderma harzianum* and *Verticillum lecanii* (Boss et al. 2007; Butt et al. 2004; Skrobek and Butt 2005; Skrobek et al. 2006)].

Based on RAFBCA results (EU funded project QLK1-CT2001-01391; Strasser et al. 2007; 2008) and the outcome of REBECA workshops and conferences held in Innsbruck (April 2006), Salzau (September 2006), Alès (June 2007) and Brussels (September 2007), REBECA experts emphasized as a final recommendation that potential effects of relevant metabolites produced by micro-organisms should be handled according to the pre-submission data request (see Chapter 15) and the tiered scheme presented in Chapter 15.

9.4 Conclusion

While microbials are often reported to pose low risks to the environment (OECD 2007), it is critical for the credibility of microbial pest control products to underline such generic statements with solid data. The “decision tree”, presented in Chapter 15, permits the unbiased generation of a risk assessment of metabolites produced by FBCAs, which have a high toxicological relevance on the basis of scientific data. Unfortunately, only few standardised bioassays are available (such as effect-based ones) to evaluate metabolites and their specific toxicity. To provide a sound basis for a verification of the proposed decision scheme for future risk assessment of FBCAs, crude extracts from a number of well-known mycoparasitic,

entomopathogenic or phytopathogenic fungi as well as from new isolates (bacteria included) should be tested (Strasser et al. 2008). Crude extracts, produced from fractions of polar and/or non-polar solvents from different production batches, and selected metabolites displaying different structural nature and mode of actions, should be made available from isolates where analyses showed activity of suspected toxic metabolites. Cultivation methods and extraction protocols have to be developed to meet the conditions for high-level production of toxins. Furthermore, bioassays have to be established and/or standardised to be able to define tolerance levels of metabolite toxicity (i.e. genotoxicity, cytotoxicity and ecotoxicity), because crude extracts are not expected to show zero toxicity.

The benefits and pitfalls in the use of crude extract analysis were extensively discussed by RAFBCA and REBECA experts (Strasser et al. 2008, Chapter 15). Rationales to increase the budget for more publicly funded projects (possibly with matching funds from the industry) for metabolite analysis and their risk assessment have been proposed for many years because consumers, animals and the environment require more protection. These new long-term projects will result in a generic safety registration of each particular agent and will also help to define the basis for new alternative regulation concepts.

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