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REVIEW

Review on safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongniartii*

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Abstract
The commercial use of entomopathogenic fungi and their products as mycoinsecticides necessitates their registration. Worldwide, several registration guidelines are available, however, most of them focus on similar or even the same safety issues. With respect to the two entomopathogenic fungi, *Beauveria bassiana* (Bals.-Criv.) Vuill. and *Beauveria brongniartii* (Sacc.) Petch, many commercial products have been developed, and numerous papers on different biological, environmental, toxicological and other safety aspects have been published during the past 30–40 years. The aim of the present review is to summarise these data. The following safety issues are presented: (1) identity of *Beauveria* spp.; (2) biological properties of *Beauveria* spp. (history, natural occurrence and geographical distribution, host range, mode of action, production of metabolites/toxins, effect of environmental factors); (3) analytical methods to determine and quantify residues; (4) fate and behaviour in the environment (mobility and persistence in air, water and soil); (5) effects on non-target organisms (non-target microorganisms, plants, soil organisms, aquatic organisms, predators, parasitoids, honey bees, earth worms and nontarget arthropods); (6) effects on vertebrates (fish, amphibia, reptiles and birds); and (7) effects on mammals and human health. Based on the present knowledge it is concluded that both *Beauveria* species are considered to be safe.

Keywords: Beauveria bassiana, Beauveria brongniartii, *occurrence, host range, toxins, environmental fate, safety, side-effects*

Introduction

The two entomopathogenic fungal species *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin and *Beauveria brongniartii* (Saccardo) Petch were described for the first time about 170 and 110 years ago, respectively. Since that time they have always been

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considered as fungi that can and should be used for control of pest insects. In the early days of biological control and especially microbial control, there was no concern for possible side-effects or safety considerations of these two species. Steinhaus (1957) was possibly the first who raised questions on the safety of microbial control products to man, other vertebrates and even to crops. He very carefully discussed different aspects of the scientific knowledge at that time. Although he concluded that microorganisms pathogenic to insects are in general harmless to man, animals and plants, he recommended that such products are subjected to appropriate State and Federal regulations. A few years later, Müller-Kögler (1965) published a book (unfortunately in German) on fungal diseases of insects, practical use for biological control and basics of insect mycology, in which some sections on side-effects of entomopathogenic fungi on humans and other warm-blooded animals as well as on beneficial insects were already included. In 1971, Heimpel summarised the knowledge on safety of insect pathogens, i.e. bacteria, viruses, protozoa, fungi and rickettsiae for man and vertebrates. He also emphasised the necessity for testing the safety of insect pathogens and said 'it seems incredible that so many good scientists have worked so long with insect pathogens without testing them for safety . . .'. He also mentioned registration guidelines of the USA and other countries at that time. A similar review was published two years later by Ignoffo (1973).

With the increasing interest in biological control of pest insects between 1980 and 1990, safety aspects were discussed in more detail (e.g. Austwick 1980; Burges 1981; Hall et al. 1982; Laird et al. 1990). Burges (1981) outlined the main principles and guidelines for testing the safety of insect pathogens and stated 'I believe that a pathogen should be registered as safe when there is reasonable evidence that it is so and in the absence of concrete evidence that it is not. A "no risk" situation does not exist, certainly not with chemical pesticides, and even with biological agents one cannot absolutely prove a negative'. First guidelines for the registration of entomopathogenic fungi under the auspices of the IOBC as an advisory document were published by Hall et al. (1982). The first book, dedicated alone to the safety of microbial insecticides was published by Laird et al. (1990), including sections on safety to domestic animals and wildlife (Saik et al. 1990) and to vertebrates and humans (Siegel & Shadduck 1990). In 1996, Cook and coauthors published an interesting framework for scientific safety evaluation of microorganisms intended for pest and plant disease control. The intention was to identify and discuss safety issues linked to microbial control agents which should stimulate and improve discussions on possible risks and risk management. Later reviews on safety of entomopathogenic fungi and especially also on *Beauveria* spp. were published by Goettel and Jaronski (1997), Goettel et al. (2001), Vestergaard et al. (2003) and Copping (2004). A good summary of various safety issues of *B. bassiana* strain GHA (128924) and strain ATCC 74040 (128818) can be found at www.epa.gov/pesticides/biopesticides/ingredients/factsheets/factsheet_128924.htm and 128818.htm.

Within the EU, safety regulations are documented in the Directive 91/414/EEC. A national registration of a microbial product is only possible after extensive testing (and inclusion of the microorganism in Annex I of the guideline). Annexes IIB and IIIB to Directive 91/414/EEC set out the requirements for the dossier to be submitted by an applicant, respectively, for the inclusion of an active substance consisting of micro-organisms or viruses in Annex I to that Directive and for the authorisation of a plant protection product based on preparations of micro-organisms or viruses. A guidance

for registration requirements of microbial pesticides is also published by the OECD (Anon. 2003).

The aim of the present review is to summarise and discuss our present knowledge on the safety, possible side-effects and the environmental behaviour of both *Beauveria* species, *B. bassiana* and *B. brongniartii*, as basis for further discussions within the registration process and the intended use of these fungi as mycoinsecticides.

Identity of *Beauveria* spp.

In 1954, MacLeod published the first, careful review of literature on the genera *Beauveria* and *Tritirachium*, including a taxonomic revision of both genera. The studies comprise cultural and morphological characteristics of numerous *Beauveria* isolates and species. Fourteen of formerly described species of *Beauveria* were described as synonyms of *B. bassiana* and *B. tenella. Beauveria stephanoderis*, *B. laxa*, *B. globulifera*, *B. effusa*, *B. vexans*, *B. doryphorae*, *B. delacroixii* and *B. acridiorum* were included as strains of *B. bassiana*, while *B. densa*, *B. melolonthae*, *B. brongniartii* and *B. shiotae* are strains of *B. tenella*. Later, De Hoog (1972) restricted the genus *Beauveria* Vuill. to three species: *B. bassiana*, *B. brongniartii* and *B. alba*. A comprehensive description of the species is presented, including a key to species, morphological characteristics, a huge list of former synonyms and some representative figures. The main synonymous names of *B. bassiana* and *B. brongniartii* are presented in Table I.

Further descriptions of *Beauveria* spp. are presented by Domsch et al. (1980) and Humber (1997). Recently, the taxonomy and phylogenetics of the genus *Beauveria* was discussed in detail (Rehner 2005; Rehner & Buckley 2005). *Beauveria bassiana* is characterised by white, later yellowish or occasionally reddish colonies. The reverse is uncoloured, or yellowish to pinkish. Conidiogenous cells consist of globose to

Table I. Main synonyms of *B. bassiana* and *B. brongniartii* (MacLeod 1954; De Hoog 1972; CABI Bioscience et al. 2006).

Fungus	Synonym
B. bassiana (Balsamo-Crivelli) Vuillemin (1912)	*Beauveria laxa* Petch (1931) *Beauveria globulifera* (Speg.) Picard (1914) *Botrytis bassiana* Balsamo (1835) *Botrytis necans* Massee (1914) *Botrytis bassiana* Sacc. subsp. *tenella* Delacroix (1937) *Botrytis bassiana* var. *lunzinensis* Szilvinyi (1941) *Botrytis brongniartii* subsp. *delacroixii* (Sacc. Delacroix (1893) *Botrytis effusa* Beauverie (1911) *Botrytis stephanoderis* Bally (1923) *Sporotrichum densum* Link (1809) *Sporotrichum globuliferum* Speggazzini (1880) *Sporotrichum minimum* Speggazzini (1881) *Isaria shiotae* Kuru (1931)
B. brongniartii (Saccardo) Petch (1926)	*Beauveria densa* Link (1914) *Beauveria tenella* (Sacc.) McLeod sensu McLeod (1954) *Botrytis tenella* Saccardo (1874) *Botrytis brongniartii* Saccardo (1892) *Botrytis melolonthae* (Sacc.) Ciferri (1929) *Isaria densa* Link (1892) *Sporotrichum epigaeum* Daszew (1912)

flask-shaped basal part and an up to 20-µm long rachis, mostly forming a zig-zag. Conidia are hyaline, globose to broadly ellipsoidal, generally 2–3 × 2–2.5 µm. The conidia are formed in clusters, like snow balls or cotton balls. *Beauveria brongniartii* is characterised by first white, later yellowish to pinkish or reddish colonies. The reverse is uncoloured, yellowish or pinkish. The conidiogenous cells also consist of a subglobose or flask-shaped basal part with a long rachis. In contrast to *B. bassiana*, the hyaline conidia are ellipsoidal, (2 −) 2.5–4.5 (−6) µm. Conidia are clustered, but also arranged in small groups or are solitary.

Recent investigations have demonstrated that there is a direct link between the genus *Beauveria* and the teleomorph genus *Cordyceps* (Ascomycota: Hypocreales, Clavicipitaceae) (Shimazu et al. 1988; Bo et al. 2002; Liu et al. 2002; Rehner 2005; Rehner & Buckley 2005). However, all *Beauveria* teleomorphs have been described so far from Asia.

Biological properties of *Beauveria* spp.

History

The most comprehensive study on the genera *Beauveria* and *Tritirachium*, the history of the genus *Beauveria* and of its species and the cultural and morphological characteristics are presented in detail by Steinhaus (1949), MacLeod (1954) and, later, by De Hoog (1972). The early history of *B. bassiana* started in 1835. It was Agostino Bassi di Lodi from Italy, who was the first to show that a fungus can cause a disease in insects, thus enunciating the germ theory of disease. He observed a disease in silkworms, *Bombyx mori*, which he called 'white muscardine' and started the first infection experiments. The fungus was then studied and described by the famous Italian naturalist Giuseppe Gabriel Balsamo-Crivelli in 1835, who gave it the name *Botrytis bassiana*, in honour of Bassi (Steinhaus 1949; Müller-Kögler 1965; Rehner 2005).

In 1911, Beauverie studied the fungus again and in 1912 Vuillemin created the new genus *Beauveria* in honor of Beauverie, of which the species *B. bassiana* became the type. *Beauveria brongniartii* has been described under different names by several investigators at the end of the 19th century (MacLeod 1954). An extensive study of this fungus with respect to its use against *Melolontha melolontha* was already published more than 100 years ago by Giard (1892).

Since the first descriptions of the genus *Beauveria*, both *B. bassiana* and *B. brongniartii* were used in biocontrol against pest insects. In a section on 'Fungous Infections', Steinhaus (1949) summarised the infection process, the development of the disease and the practical use of *B. bassiana* against some pest insects, mainly the European corn borer, *Ostrinia* (*Pyrausta*) *nubilalis*, and the codling moth, *Carpocapsa pomonella*. He also mentioned practical use of *Beauveria globulifera*, which later was included in *B. bassiana*, against the chinch bug, *Blissus leucopterus*.

In the book of Müller-Kögler (1965), 28 species and families of pest insects in agriculture, orchards, forestry, greenhouse and in the tropics were mentioned against which *B. bassiana* had been used for control purposes. The fungus *B. brongniartii* (=*B. tenella*) however, was applied mainly against *Melolontha* spp. and *Epilachna vigintioctomaculata*. A review on pest control of the fungi *Beauveria* and *Metarhizium* including basic as well as practical aspects was published later by Ferron (1981). At that time, the *B. bassiana* product 'Boverin' was extensively used in the USSR on

thousands of hectars mainly against the Colorado potato beetle, *Leptinotarsa decemlineata*, and the codling moth *Cydia pomonella*, while *B. brongniartii* was applied against *M. melolontha*. In China, *B. bassiana* was produced and widely used against *O. nubilalis* in corn, *Dendrolimus punctatus* on pines and *Nephotettix* leafhoppers on rice and tea (Hussey & Tinsley 1981).

The present state of *Beauveria* products registered or under commercial development is summarised in Table II (Butt et al. 2001; Wraight et al. 2001; Copping 2004; Zimmermann 2005; Kabaluk & Gazdik 2005).

Natural occurrence and geographical distribution

Beauveria bassiana is the most widely distributed species of the genus. It is generally found on infected insects both in temperate and tropical areas throughout the world. MacLeod (1954) mentioned that *B. bassiana* was isolated from 63 different insect species collected in various localities throughout Canada. He also reported that *Beauveria* strains were found within the lung tissues of 14 rodents. However, histological examination did not show that the fungus creates a pathological condition within the tissues.

The occurrence and distribution of *B. bassiana* and *B. brongniartii* in various countries and areas are listed by Domsch et al. (1980). Reports of the occurrence of *B. bassiana* are from Turkey, the Ivory Coast, equatorial West Africa, central Africa, South Africa, the Bahamas, Nepal, east Siberia, New Zealand and Japan. Habitats for *B. bassiana* range from an alpine soil, to heathland, peat bogs, soils with savannah type vegetation, forest and cultivated soils, sand blows and dunes, desert soil and running

Table II. Mycopesticides of *Beauveria bassiana* and *B. brongniartii* registered or under commercial development (Butt et al. 2001; Wraight et al. 2001; Copping 2004; Kabaluk & Gazdik 2005; Zimmermann 2005).

Fungus	Product/Trade name	Company/Producer	Country/Origin
B. bassiana	Bio-Power	Stanes	India
	BotaniGard ES	Laverlam International (formerly	USA
	BotaniGard 22WP	Emerald BioAgriculture)	
	Boverol	Fytovita	Czech Republic
	Conidia	LST	Columbia
	Mycotrol ES	Laverlam International	USA
	Mycotrol-O	(formerly Emerald BioAgriculture)	
	Naturalis	Intrachem	Italy
	Naturalis-L	Andermatt Biocontrol	Switzerland
		Troy Biosciences Inc.	USA
	Ostrinil	Arysta (formerly NPP, Calliope)	France
	Proecol	Probioagro	Venezuela
	Racer BB	SOM Phytopharma	India
	Trichobass-L	AMC Chemical/Trichodex	Spain
	Trichobass-P		
B. brongniartii (=*B. tenella*)	Beauveria Schweizer	Lbu (formerly Eric Schweizer Seeds)	Switzerland
	Betel	Arysta (formerly NPP, Calliope)	France
	Biolisa-Kamikiri	Nitto Denko	Japan
	Engerlingspilz	Andermatt Biocontrol AG	Switzerland
	Melocont-Pilzgerste	Agrifutur-Kwizda	Italy–Austria

water. Also, isolations from the rhizoplane of peat bog plants, the rhizosphere of clover, dead bark, nests, feathers and droppings of free-living birds were mentioned.

Beauveria brongniartii is less common than *B. bassiana*, but also has a worldwide distribution in insects as well as in different habitats. It has been reported from open bogs, alpine habitats, forest soil in Hong Kong, terra rossa in Greece, a *Calluna* heath, an alpine grassland in Italy and sand dunes in the British Isles (see Domsch et al. 1980).

Beauveria bassiana has also been isolated from the surface and the interior of plants. Using selective media, *B. bassiana* was isolated from bark of elm trees and from soil at the base of elm trees (Doberski & Tribe 1980) and from the bark of *Carpinus caroliniana* (ironwood, hop hornbeam) (Bills & Polishook 1991). Recently, the species was also found naturally occurring on phylloplanes of various hedgerow plants (Meyling & Eilenberg 2006). Further information on the natural occurrence of *B. bassiana* in general and as an endophyte in various plant species are reported in *Host range* and *Effects on Plants*, respectively.

The description of the so-called 'Galleria-bait-bait' (Zimmermann 1986) and various selective media for isolation of *Beauveria* spp. from soil (see *Analytical methods to determine and quantify residues*) have lead to a huge increase in the findings of *B. bassiana* throughout the world (Table III).

Although *Beauveria* spp. are no common airborne fungi, *B. bassiana* has been isolated from the air. In a study on fungal biodiversity in the air of Turin, Italy, the species was found at a mean of 0.2 CFU m^{-3} during 10 months per year, while *B. brongniartii* was found at only <0.1 CFU m^{-3} during one month (Airaudi & Filipello-Marchisio 1996). The natural density of *B. bassiana* in the air of a forest in Japan ranged from 0 to 3.1×10^3 CFU m^{-2} day^{-1} (Shimazu et al. 2002). In the air of a hospital, the fungus was isolated together with 98 other fungal species (Rainer et al. 2000). Nolard (2004) mentioned *B. bassiana* in the air of humid dwellings of allergic patients. For natural occurrence in vertebrates including mammals and humans (see *Effects on vertebrates* (*fish, amphibia, reptiles and birds*) and *Effects on mammals and human health*). Recently, both *Beauveria* spp. were found in surface water-derived drinking water in Norway, but not in groundwater derived water (Hageskal et al. 2006).

Host range

Beauveria bassiana is a ubiquitous entomopathogenic fungus which has been found and isolated from a wide variety of insects from different orders (MacLeod 1954; Leatherdale 1970; Li 1988; Goettel et al. 1990). MacLeod (1954) mentioned about 60 insect species from which *Beauveria* strains have been isolated. The hosts of 106 species of entomopathogenic fungi known from Britain are catalogued by Leatherdale (1970). Listed hosts of *B. bassiana* are Heteroptera (*Picromerus bidens*, *Anthocoris nemorum*), Homoptera (*Eulecanium* spp.), Lepidoptera (*Hepialus* spp., *Hypocrita jacobaea*, *Cydia nigricans*), Coleoptera (*Lathrobium brunnipes*, *Calvia quattuordecimguttata*, *Phytodectra olivacea*, *Otiorhynchus sulcatus*, *Sitona lineatus*, *S. sulcifrons*, *S. macularius*, *S. hispidulus*, *Anthonomus pomorum*, *Hylaster ater*), Hymenoptera (Ichneumonidae, *Lasius fuliginosus*, *Vespula* spp., *Bombus pratorum*), Diptera (*Leria serrata*) and spiders.

Table III. Natural occurrence of *Beauveria bassiana* and *Beauveria brongniartii* in the soil in different countries and areas.

Location	Results	Reference
Canada	In 266 soil samples from 86 locations the most abundant species were *B. bassiana* (187 isolates) and *M. anisopliae* (357 isolates)	Bidochka et al. (1998)
Czech Republic: South Bohemia; arable soil	From 146 soil samples 25 strains of *B. bassiana* were isolated; no differences in soils from arable fields on conventional and organic farms	Landa et al. (2002)
Finland	From 590 soil samples, *B. bassiana* was isolated from 19.8%	Vänninen (1996)
Germany	In 100 soil samples from different locations and soil types, *B. bassiana* was found in 22%	Kleespies et al. (1989)
Italy: Southern part	In 188 soil samples, the most common entomopathogen was *B. bassiana*	Tarasco et al. (1997)
Japan	*B. bassiana* was often isolated from forest soils	Shimazu et al. (2002)
Macquarie Islands	In 163 subantarctic soils samples, 1 contained *B. bassiana*	Roddam and Rath (1997)
Nepal	*B. bassiana* was isolated from a few soil samples	Dhoj and Keller (2003)
New Zealand	*B. bassiana* was higher in pasture soils than in forest or cropland soils	Barker and Barker (1998)
Norway: Northern parts	Significantly higher occurrence of entomopathogenic fungi in soils from arable fields of organically managed farms compared to conventionally ones. Species found were *B. bassiana*, *M. anisopliae* and *Tolypocladium cylindrosporum*	Klingen et al. (2002)
Panama: Tropical forest	*B. bassiana* was detected in soil near colonies of leaf-cutting ants	Hughes et al. (2004)
Poland: Apple and plum orchards	*B. bassiana* was dominant in soil under sward in both kinds of orchards	Sapieha-Waszkiewicz et al. (2003)
Poland: Hop plantations and arable fields	*B. bassiana* in all soil types and areas available	Mietkiewski et al. (1996)
Poland: Mid-forest meadows (Sudety mountains)	*B. bassiana* and *B. brongniartii* were isolated from various areas	Mietkiewski et al. (1994)
Poland: Different soil types	*B. bassiana* was the dominant species in muck and loess	Tkaczuk and Mietkiewski (1996)
Spain: Alicante	*B. bassiana* was most frequently found in 21% of soils from 61 sites	Asensio et al. (2003)
Switzerland	Soil samples from 82 fields were analysed: *B. brongniartii* was limited to soil sites colonised by its host, *Melolontha melolontha*; *B. bassiana* was also isolated	Keller et al. (2003)
USA	Soil from 105 sites in 21 orchards: From 16 orchards mainly *B. bassiana* and *M. anisopliae* were isolated	Shapiro-Ilan et al. (2003)

Based on worldwide data, Li (1988) listed 707 species of insect hosts of *B. bassiana*. These comprise 521 genera and 149 families of 15 orders. In addition, 13 host species of Acarina distributed in seven genera and six families are listed. The insect orders in which *B. bassiana* has been found as a pathogen are as follows:

(1) Lepidoptera, (2) Coleoptera, (3) Hymenoptera, (4) Homoptera, (5) Diptera, (6) Hemiptera, (7) Orthoptera, (8) Siphonaptera, (9) Isoptera, (10) Thysanoptera, (11) Mantodea, (12) Neuroptera, (13) Dermaptera, (14) Blattariae, (15) Embioptera.

The host range and specificity of *B. bassiana* is listed by Goettel et al. (1990) as follows: Gastropoda, Acari, Orthoptera, Dermaptera, Isoptera, Blattaria, Thysanoptera, Homoptera, Heteroptera, Diptera, Coleoptera, Hymenoptera, Siphonaptera and Lepidoptera. Furthermore, several hundred *B. bassiana* isolates from numerous host insects are listed in the USDA ARS Entomopathogenic Fungus collection.

However, despite the prevalence of *B. bassiana* in a huge number of arthropods, it is known that most isolates of this fungus have a restricted host range (Goettel et al. 1990; Vestergaard et al. 2003), and there are several examples that *B. bassiana* isolates from a distinct host insect or from the soil are highly virulent against other target pests (Feng & Johnson 1990; Ekesi et al. 1998; Cottrell & Shapiro-Ilan 2003). Therefore, it is necessary to screen the virulence of different isolates against a target insect species in order to select the most virulent one.

In Europe, *B. brongniartii* mainly attacks the field and the forest cockchafer, *Melolontha melolontha* and *M. hippocastani*. However, the fungus may also occur on other insects. According to Leatherdale (1970), the listed hosts of *B. brongniartii* (=*B. tenella*) are: Heteroptera (Pentatomidae), Lepidoptera (*Hepialus lupulinus*), Coleoptera (Coccinellidae, Chrysomelidae, *Plateumaris braccata*, *Galerucella tenella*, Curculionidae, *Strophosomus sus*.), Hymenoptera (Formicidae, *Vespula* spp.) and spiders. Vestergaard et al. (2003) summarised the reports on the occurrence of *B. brongniartii* and mentioned the following hosts: Coleoptera (Cerambycidae, Curculionidae, Ipidae, Lucanidae, Nitidulidae), Lepidoptera (Pyralidae), Homoptera (Cicadidae), Hymenoptera (Vespidae), Phasmatodea and Orthoptera. However, isolates from non-coleopteran hosts have a large genetic distance to those from *Melolontha* spp. (Enkerli et al. 2001).

Mode of action

There is a huge number of publications dealing with the mode of action and the infection process. The first publications dealing with the infection of certain pest insects by *B. bassiana*, such as the silkworm, were compiled by Steinhaus (1949) and Müller-Kögler (1965). Therefore, in this section only the general aspects of the infection process are summarised (see e.g. Boucias & Pendland 1998 for more specific information). A comprehensive overview on the biochemical aspects of disease development, its physico-chemical aspects and the genetics and molecular biology of disease development is presented by Khachatourians (1998).

As in other entomopathogenic fungi, *Beauveria* species attack their host insects generally percutaneously. The infection pathway consists of the following steps: (1) attachment of the spore to the cuticle; (2) germination; (3) penetration through the cuticle; (4) overcoming the host response and immune defense reactions; (5) proliferation within the host by formation of hyphal bodies/blastospores, i.e. yeast like cells; (6) saprophytic outgrowth from the dead host and production of new conidia.

Attachment is due to the hydrophobicity of the conidia as well as the cuticular surfaces. In *B. bassiana*, conidia contain a hydrophobin-type protein on their exterior surfaces. Germination and successful infection depends on a number of factors, e.g.

susceptible host and host stage and certain environmental factors, such as optimal temperature and humidity. Germination is further influenced by certain cuticular lipids, such as short-chain fatty acids, aldehydes, wax esters, ketones and alcohols which may possess antimicrobial activity. However, the cuticle may also be coated with substances that are important for fungal recognition, like free amino acids or peptides, and may trigger attachment and germination. Generally, germination of *B. bassiana* conidia starts after about 10 h and is largely completed by 20 h at 20–25°C.

Generally the fungus penetrates thinner, non-sclerotised areas of the cuticle, like joints, between segments or the mouthparts. Before penetration, germ tubes may produce so-called appressoria and infection pegs. The penetration process is by mechnical means and by the production of several enzymes, including proteases, chitinases and lipases.

The penetration of the cuticle layers and the beginning of invasion is accompanied by several host response activities, e.g. by production of phenoloxidase and certain hemocytes and melanisation. The interactions between the penetrating fungus and the insect immune system are a complex process and comprise many molecular and cellular reactions (Vilcinskas & Götz 1999). During the infection process, *Beauveria* spp. produce proteolytic enzymes and toxins, while the host insects respond with cellular and humoral defence reactions. These reactions consist of the production of antifungal compounds, inducible protease inhibitors and proteins, which detoxify fungal toxins in the insect.

After successful penetration, the fungus produces hyphal bodies, i.e. yeast like cells, which are distributed passively in the hemolymph, enabling the fungus to invade other tissues of the host insect by extensive vegetative growth and the production of toxins. For example, during this stage, the metabolite oosporein is produced by *B. brongniartii*, which is visible by turning its host cadaver red. During its invasion of the insect body, the fungus depletes nutrients in the hemolymph and the fat body. This process is followed by the death of the insect and the end of the pathogenesis (Boucias & Pendland 1998).

The incubation period depends on the host, the host stage, temperature and virulence of the fungus strain. In aphids, it may take 3–4 days, while in scarab larvae, 2–4 weeks. After the host death and under humid conditions, the fungus starts its saprophytic phase by emerging out of the host body and producing conidia on the exterior surface of the cadaver. Under very dry conditions, the fungus may also persist in the hyphal stage inside the cadaver or, e.g. in locusts in Africa, produce its conidia inside the body.

During the incubation period, the fungus may affect its host insect throughout behavioural and feeding changes, the reduction of body weight or fecundity, malformations or behavioural fever (Müller-Kögler 1965; Ekesi 2001; Ouedraogo et al. 2003).

Production of metabolites/toxins

Microorganisms, and especially fungi, produce a wide variety of compounds or metabolites, mostly within their secondary metabolism, which generally have diverse activities and functions. Therefore, it is not surprising that entomopathogenic fungi are also able to produce different metabolites. With respect to registration and risk assessment, these metabolites and their special activities are of particular toxicological

interest. In the following chapter, the most important metabolites of *B. bassiana* and *B. brongniartii* are presented and their occurrence and general activities are outlined.

One of the first comprehensive overviews on the toxins of entomopathogenic fungi is presented by Roberts (1981). The author summarises the knowledge of toxic metabolites produced by well-known entomopathogenic Deuteromycetes, such as *Beauveria*, *Metarhizium*, *Nomuraea, Aspergillus*, *Paecilomyces* and *Verticillium*, the ascomycete *Cordyceps* and the genus *Entomophthora* sensu lato. He presented four principle objectives of studies on fungal metabolites toxic to insects, which are still important: (1) to elucidate the mode of action; (2) to search for new chemicals for use in pest control; (3) to evaluate the safety of specific fungi proposed for use in pest control; and (4) to conduct basic chemistry studies on natural products. Today, we should add the search for new drugs and pharmaceuticals in human medicine. These objectives demonstrate that fungal metabolites generally cannot only be considered as safety issues. A recent review on toxic metabolites of entomopathogenic fungi including those used for other biocontrol purposes was given by Vey et al. (2001).

Both *Beauveria* spp. produce several toxic compounds *in vitro* and *in vivo* (e.g. Mazet et al. 1994; Strasser et al. 2000; Vey et al. 2001). These are presented in Table IV. A majority of these insecticidal molecules are low molecular weight secondary metabolites, mainly cyclic peptides such as beauvericin and bassianolide, and the pigments bassianin and tenellin. There is also evidence that melanising macromolecular toxins are secreted during mycosis in the haemolymph (Fuguet & Vey 2004). One toxic macromolecule was identified as a hydrophilic, chitosanase-like protein (Fuguet et al. 2004). Additionally, the secondary metabolite cyclosporin A is produced by *B. bassiana*, which was originally found in *Tolypocladium inflatum* (Boucias & Pendland 1998). The main metabolite produced by *B. brongniartii* is oosporein.

Beauvericin is the most important compound which was reported first from *B. bassiana.* Beauvericin is a toxic cyclic hexadepsipeptide and comprises a cyclic repeating sequence of three molecules of *N*-methyl phenylalanine alternating with three molecules of 2-hydroxyisovaleric acid. Not all isolates of *B. bassiana* produce beauvericin *in vitro* (for corresponding literature see Roberts, 1981). A review on the activity of beauvericin and two other metabolites, bassianolide and beauveriolide, is given by Strasser et al. (2000) and Vey et al. (2001). Beauvericin has also been isolated from other fungi, such as *Paecilomyces fumosoroseus* (see Roberts 1981), *Paecilomyces tenuipes* (Nilanonta et al. 2000) and, especially from members of the genus *Fusarium*

Table IV. Major metabolites produced by *Beauveria* spp.

Fungus	Metabolite	Reference
B. bassiana	Beauvericin, bassianin, bassianolide, beauverolides, beauveriolides, tenellin, oosporein	Strasser et al. (2000); Vey et al. (2001)
	Oxalic acid	Roberts (1981)
	Bassiacridin	Quesada-Moraga and Vey (2004)
B. brongniartii	Oosporein	Strasser et al. (2000); Vey et al. (2001)
	Oxalic acid	Müller-Kögler (1965); Roberts (1981)
B. felina	Destruxin B	Kim et al. (2002)

(e.g. Gupta et al. 1991; Bottalico et al. 1995; Logrieco et al. 1998). Gupta et al. (1991) detected beauvericin in cultures of *Fusarium moniliforme* var. *subglutinans* and *F. semitectum*, and later, a co-occurrence of beauvericin with fumonisin B_1 in *F. moniliforme* was reported (Bottalico et al. 1995). From 94 *Fusarium* isolates tested belonging to 25 taxa, the following species produced beauvericin: *F. acuminatum* var. *acuminatum*, *F. acuminatum* var. *armeniacum*, *F. anthophilum*, *F. avenaceum*, *F. beomiforme*, *F. dlamini*, *F. equiseti*, *F. longipes*, *F. nygamai*, *F. oxysporum*, *F. poae*, *F. sambucinum*, and *F. subglutinans* (Logrieco et al. 1998). The metabolite has also been found as a natural contaminant of maize in Italy, Austria, Poland, South Africa and the USA and was detected in all maize hybrids (Pascale et al. 2002). These results and other published data (Munkvold et al. 1998; Fotso et al. 2002; Logrieco et al. 2002; Moretti et al. 2002) confirm that beauvericin is a common metabolite of many phytopathogenic *Fusarium* species and occurs in diverse foods and feeds contaminated with *Fusarium* species.

Investigations of beauvericin have demonstrated that this metabolite has insectidal, antibiotic, cytotoxic, and ionophoric properties. According to Roberts (1981), some toxic effects have been noticed against bacteria, mosquito larvae, brine shrimp and adult houseflies, but not against silkworm larvae at 1000 ppm in artificial diet. Recently, Ganassi et al. (2002) reported some effects of beauvericin on the aphid *Schizaphis graminum*. Antimycobacterial (*Mycobacterium tuberculosis*) and antiplasmodial (*Plasmodium falciparum*) activity of beauvericin and beauvericin A isolated from *P. tenuipes* BCC 1614 was reported by Nilanonta et al. (2000).

Beauvericin is a specific cholesterol acyltransferase inhibitor and is toxic towards *Artemia salina* larvae and against insect, murine and human cell lines. It can induce programmed cell death similar to apoptosis and causes cytolysis (Logrieco et al. 1998; Vey et al. 2001; Pascale et al. 2002). Investigations on the effect of beauvericin to the insect cell line SF-9 from the lepidopteran *Spodoptera frugiperda* revealed a clear cytotoxicity (Calo et al. 2003; Fornelli et al. 2004). One micromolar concentration of beauvericin caused about 10% decrease in the number of viable cells, and the effect increased at higher concentrations. However, in time-course experiments, no effect of beauvericin at 30 μM was noticed on cell viability (Calo et al. 2003). Cytotoxicity of beauvericin to two human cell lines of myeloid origin was reported by Calo et al. (2004). After an exposure time of 24 h, a decline in viability of cells was observed at a concentration of 10 μM or higher. In turkeys, fumonisin B-1 and beauvericin may affect the immune functions by suppressing proliferation and inducing apoptosis of peripheral blood lymphocytes (Dombrink-Kurtzman 2003).

Beauvericin did not cause any symptoms on roots of melon, tomato, wheat and barley, however, it showed high toxicity towards protoplasts of these plants (see Moretti et al. 2002).

With respect to the natural occurrence of beauvericin in *Fusarium*-contaminated food, feeding trials were conducted with broilers to study the effect of diets containing the mycotoxins moniliformin and beauvericin from natural contamination in the field (Zollitsch et al. 2003). The results indicate that dosages of up to 2.7 mg moniliformin and 12 mg beauvericin per kg diet showed no effect on any of the traits observed.

Whether beauvericin should be seen as a food mycotoxin is not yet clear, because it mostly co-occurrs with other *Fusarium* metabolites, such as fumonisins. In any case, it is likely that beauvericin found and isolated from foods and feeds in nature derives from *Fusarium* species rather than from *B. bassiana*.

Bassianin and tenellin are two yellow-coloured non-peptide secondary metabolites which inhibit the erythrocyte membrane ATPases (Jeffs & Khatchatourians 1997). There is very little published information on these two metabolites (see Strasser et al. 2000).

Bassianolide is another cyclo-octadepsipeptide produced by *B. bassiana* with ionophoric and antibiotic activity similar to beauvericin (see Strasser et al. 2000; Vey et al. 2001). The biosynthesis of a structurally related substance, called PF1022, was reported by Weckwerth et al. (2000). PF1022 has strong anthelmintic properties and was found in a fungus producing only sterile mycelium.

Bassiacridin is a toxic protein, that was purified from a strain of *B. bassiana* infecting locusts (Queseda-Moraga & Vey 2004). Injection of fourth instar nymphs of *Locusta migratoria* with the pure protein at relatively low dosage (3.3 μg toxin/g body weight) caused nearly 50% mortality. This insecticidal protein showed specific activity against locusts and has a limited similarity to a chitin binding protein from yeasts.

Beauveriolides and beauverolides are peptides with a similar structure to beauvericin and bassianolide (Namatame et al. 1999, 2004). Beauveriolides seem to have potential as drugs in human medicine. Beauveriolides I and III isolated from culture broth of *Beauveria* sp. (FO-6979) showed potent inhibitory activity of lipid droplet accumulation in primary mouse peritoneal macrophages. They are the first microbial cyclodepsipeptides with demonstrated *in vivo* antiartherosclerotic effects and show promise as potential lead compounds as antiartherosclerotic agents (Namatame et al. 2004). Recently, a patent for production of beauveriolide I or III by *Beauveria* sp. (FO-6979) on selective media was granted (Omura & Tomoda 2005).

Oosporein is the major secondary metabolite produced by *B. brongniartii* and is also produced by many isolates of *B. bassiana*. Comprehensive overviews on oosporein are presented by Strasser et al. (2000), Vey et al. (2001) and Seger et al. (2005a,b). This red-coloured pigment is a dihydroxybenzoquinone, which is also produced by many soil fungi. It is an antiviral compound and has antibiotic activity against gram-positive bacteria, but little effect on gram-negative bacteria. Obviously, oosporein has no antifungal and phytotoxic effects. It has been reported to cause avian gout in broiler chicks and turkeys and was found to be toxic to 1-day-old chicks. Furthermore, studies on its toxicity in mice and hamsters indicated an LD_{50} value of 0.5 mg kg^{-1} body weight after intraperitoneal injection (Manning & Wyatt 1984; Vey et al. 2001). *In vivo* and *in vitro* studies on the distribution of oosporein in the environment revealed negligible amounts present. The maximum amount of oosporein produced in a culture medium was 300 mg L^{-1}, 3.2 mg kg^{-1} in the commercial product 'Melocont®-Pilzgerste' (Agrifutur-Kwizda), 200 μg in a mycosed larva, 0.02 mg m^{-2} in soil enriched with the commercial product and 6.4 mg m^{-2} in soil enriched by mycosed larvae (Strasser et al. 2000). These results demonstrate that the quantity of oosporein produced by these fungi *in vivo* is usually much less than that secreted in nutrient rich liquid media. No fungal metabolites, such as oosporein, were detected in potato plants and tubers after application of 'Melocont®-Pilzgerste' into the soil of a potato field (Abendstein et al. 2000; Strasser et al. 2000; Seger et al. 2005c).

Oxalic acid is secreted by *B. bassiana* and *B. brongniartii* (Müller-Kögler 1965; Roberts 1981). It is considered an important pathogenicity determinant because it can solubilise specific cuticular proteins (see Vey et al. 2001). In the grasshopper *Melanoplus sanguinipes*, a synergistic activity between oxalic acid and *B. bassiana* conidia was observed (Bidochka & Khachatourians 1991), however the acid was not related to virulence in grasshoppers (Bidochka & Khachatourians 1993). Cell-free culture supernatants of *B. bassiana* containing oxalic acid caused mortality in several tick species after dipping (Kirkland et al. 2005), which support the hypothesis that oxalic acid secretion by *B. bassiana*, coupled to a reduction in the pH of the medium, acts as a potent acaricidal factor during pathogenesis.

Effect of environmental factors (temperature, humidity, solar radiation)

The propagation and survival of any microorganism in the environment is strongly affected by several abiotic and biotic factors. The most important abiotic environmental constraints for fungi are temperature, humidity or moisture and solar radiation. These factors are also responsible for effective, commercial use of entomopathogenic fungi. The importance of these ecological parameters was recognised early (Clerk & Madelin 1965; Müller-Kögler 1965; Roberts & Campbell 1977; Keller & Zimmermann 1989; Fuxa 1995). This section summarises some general observations of the effect of temperature, humidity and solar radiation on the activity and longevity of *B. bassiana* and *B. brongniartii* in the laboratory as well as in the field.

Temperature. Temperature can affect an entomopathogen in different ways by influencing the germination, growth and viability of the fungus on and in the host insect and in the environment. High temperatures may inactivate an entomopathogen before contact with the pest insect or may reduce or accelerate the growth within an insect depending on the temperature requirements of the entomopathogen and the host insect. In contrast, low temperatures may reduce or stop the germination and growth, thus impair or prolong a successful infection, e.g. against soil dwelling pest insects.

In *B. bassiana,* the optimum temperature is 23–28°C, the minimum 5–10°C and the maximum about 30–38°C, depending on the isolates tested (Müller-Kögler 1965; Roberts & Campbell 1977). These values have been substantiated later by other scientists (e.g. Hywel-Jones & Gillespie 1990; Fargues et al. 1997; Hallsworth & Magan 1999). Among African isolates of *B. bassiana*, germination, radial growth and sporulation of all isolates were retarded at 15 and 35°C, while the optimum temperature of different isolates was between 20 and 30°C (Tefera & Pringle 2003) or 25–30°C (Ekesi et al. 1999). In contrast, *B. bassiana* isolates from subantarctic soils of Macquarie Island germinated at 5°C (Roddam & Rath 1997). The thermal death point of *B. bassiana* spores is at 50°C for 10 min (Walstad et al. 1970). In *B. brongniartii* the temperature range for growth and sporulation is between 2 and 33°C with an optimum of 22–23°C (Müller-Kögler 1965; Roberts & Campbell 1977). Growth of *B. brongniartii* has been noticed during storage at 2°C (Aregger 1992).

Humidity. Humidity is a very important environmental factor affecting the efficacy and survival of entomopathogens. Spore germination on the insect cuticle and sporulation

after outgrowth of the dead host insect require high moisture. On the other hand, high or low humidity in conjunction with high temperature may affect the viability and persistence of fungal spores. We have to distinguish between the macroclimate or macrohumidity and the microclimate or microhumidity on leaf or insect surfaces when considering moisture effects in the field. Generally, the range of relative humidity (RH) for germination of *B. bassiana* conidia is 100–92% (Walstad et al. 1970; Hallsworth & Magan 1999). A slight reduction in germination occurs at 99% RH, while germination and growth is retarded at 94% and 92%. However, fungal infections of insects have been noticed at relatively low macrohumidities of 60–70%. Presumably the microhumidity at the surface of the host integument or foliage was higher in these cases. Successful infection at low relative humidities has also been observed with oil formulations (Prior et al. 1988; Bateman et al. 1993; Vidal et al. 2003). Obviously, fungal spores are able to germinate and infect the insect when covered by a thin oil layer on the insect cuticle independent of the surrounding relative humidity. The longevity of conidia at different temperatures is also strongly affected by the relative humidity and moisture content of the conidia. For example, the viability of dry conidia of *B. bassiana* was 635 days at 8°C and 0% RH in contrast to 28–56 days at 25°C and 75.8% RH. A lower RH increases the longevity of spores even at higher temperature (Clerk & Madelin 1965).

Solar radiation. Sunlight, especially UV-B (290–330 nm) and UV-A (330–400 nm), is the most detrimental environmental factor affecting the field persistence of fungal insecticides. The results presented in the literature reveal that entomopathogens are inactivated within hours or days when exposed to sunlight (Gardner et al. 1977). In laboratory experiments under simulated sunlight, 99% of all *B. bassiana* conidia were inactivated at UV-C after nearly 16 min, and at UV-A and UV-B after about 31 min (Krieg et al. 1981). After irradiation with simulated sunlight, Ignoffo and Garcia (1992) found a half life of *B. bassiana* conidia of about 2 h. The influence of simulated sunlight (295–1100 nm) on the survival of conidia of 65 isolates of *B. bassiana* demonstrated that the survival decreased with increasing exposure, i.e. an exposure for 2 h or more was detrimental to all isolates tested (Fargues et al. 1996). In the laboratory, the survival of conidia applied in water onto glass coverslips and on crested wheatgrass (*Agropyron cristatum*) was reduced by greater than 95% after 15 min exposure to UV-B radiation (Inglis et al. 1995). Conidial survival in oil was more pronounced on glass (74% mortality after 60 min) than on leaves (97% mortality after 60 min). Significant differences in susceptibility to simulated sunlight among isolates of *B. bassiana* were demonstrated by Morley-Davies et al. (1995). Also, diffuse sunlight has inactivating abilities as demonstrated in the entomopathogenic fungus *Paecilomyces fumosoroseus* (Smits et al. 1996).

The detrimental effects of sunlight implicating the relatively short persistence of microbial control agents after application have led to incorporation of various UV-protectants to conidial formulations (e.g. Inglis et al. 1995; Edgington et al. 2000; Cohen et al. 2001; Leland & Behle 2005).

Analytical methods to determine and quantify residues

There are several methods and techniques for selective isolation of entomopathogenic fungi, including *B. bassiana* and *B. brongniartii* (Goettel & Inglis 1997). As larvae of

Galleria mellonella are notoriously sensitive to entomopathogenic fungi, the 'Galleria bait method' (Zimmermann 1986) is generally used for qualitative analysis and to indirectly isolate fungi from soil. The use of selective media in combination with the soil serial dilution plating method or a leaf washing technique gives quantitative results. Different selective media for reisolation of *B. bassiana* and *B. brongniartii* from soil or plants have been used (Veen & Ferron 1966; Müller-Kögler & Stein 1970; Joussier & Catroux 1976; Doberski & Tribe 1980; Beilharz et al. 1982; Chase et al. 1986; Strasser et al. 1996). As selective agents, the first media contained oxgall, rose Bengal, crystal violet, cycloheximide and antibiotics, such as streptomycin, chloramphenicol and/or tetracycline. In 1982, Beilharz et al. found, that media based on oatmeal agar containing the fungicide Dodine® (*N*-dodecylguanidine monoacetate) considerably improved the selectivity for *B. bassiana* and *M. anisopliae*. Later, the addition of benomyl was suggested by Chase et al. (1986). For *B. brongniartii*, Strasser et al. (1996) demonstrated that a nutrient medium containing cycloheximide (0.05%), Dodine® (0.1%) and antibiotics was best suited for selective isolation of this fungus from soil. In all cases, the minimum number for recovery of both *Beauveria* species is about 10^2 conidia or propagules per 1 g of soil.

During a study on the microbial flora in heavy metal polluted soils, Bååth (1991) found that entomogenous fungi were highly tolerant to copper. Therefore, a copper-based medium could also be useful for selective isolation of these fungi.

A variety of different biochemical and molecular methods has been developed to identify and distinguish among strains of entomopathogenic fungi (see Bidochka 2001; Rehner & Buckley 2005). Neuveglise et al. (1997) found that 28s rDNA group-I introns are a powerful tool for identifying strains of *B. brongniartii.* Also, strain-specific microsatellite markers in *B. brongniartii* (Enkerli et al. 2001) and in *B. bassiana* (Rehner & Buckley 2003) have been identified. These microsatellite markers can also be applied to study fungus populations and to monitor the fate of specific strains in the environment (Enkerli et al. 2004).

Fate and behaviour in the environment

Studies on the fate and behaviour of a microbial control agent are important with respect to its ecological safety, e.g. potential unintended effects on non-target organisms, including their displacement, unintended distribution in the environment or contamination of groundwater.

Mobility and persistence in air

Conidia of *B. bassiana* and *B. brongniartii* are dry, of small size and are produced in powdery clusters. Therefore, it is obvious that these types of conidia are easily transported by air. However, there are only few observations of *Beauveria* as an airborne fungus, but these results document that *Beauveria* spp., especially *B. bassiana*, do occur naturally in the air (Airaudi & Filipello-Marchisio 1996; Rainer et al. 2000; Shimazu et al. 2002; Lackner et al. 2004; Nolard 2004; see *Natural occurrence and geographical distribution* and *Effects on mammals and human health*).

The transportation of fungal spores through the air by insects has been documented in many cases. Insects may function as a mechanical carrier or vector for *B. bassiana*, e.g. in order to transmit the fungus from a contamination device into the pest

population (for references see Kreutz et al. 2004). For example, sap beetles were contaminated with *B. bassiana* in an autoinoculative device and transferred the fungus to overwintering sites (Dowd & Vega 2003). The species was also isolated from free flying sap beetles caught in traps. Similar transmission of *B. bassiana* in laboratory and semi-field experiments has been demonstrated with the bark beetle, *Ips typographus* (Kreutz et al. 2004). *Carpophilus freemani* is a fungivore that is frequently found in European corn borer, *Ostrinia nubilalis*, tunnels in corn. Beetles fed *B. bassiana* excreted viable conidia in 14% of their fecal droppings and, thus, may transfer the fungus both via their fecal droppings and mechanically (Bruck & Lewis 2002a).

The viability of *B. bassiana* spores in the air is mainly affected by temperature, humidity and sunlight (see *Effect of environmental factors* (*temperature, humidity, solar radiation*)). The longevity of dry conidia on glass surfaces decreases as the storage temperature increases from 8 to 25°C and by exposure to light. High storage humidities reduce the germination sooner than low humidities (Clerk & Madelin 1965). Undoubtedly, natural sunlight, i.e. the UV-B (290–330 nm) and UV-A (330–400 nm) component, is one of the most important factors affecting the survival of fungal spores in the air and on plants. A rapid inactivation of *B. bassiana* conidia by ultraviolet radiation within hours has been demonstrated in the laboratory (e.g. Krieg et al. 1981; Fargues et al. 1996). Studies on the persistence of *B. bassiana* conidia on plant surfaces, such as soybean foliage, have demonstrated that one-half of the original activity was lost between 5 and 10 days post application (Gardner et al. 1977).

Viability on the plant surface may also be influenced by the plant type. Field evaluations of *B. bassiana* revealed a conidia persistence and infectivity up to 26 days on foliage of lettuce and celery. However, the number of colony forming units (CFUs) recovered on lettuce was significantly higher than on celery leaves (Kouassi et al. 2003). The viability of conidia of *B. bassiana* on phylloplanes of alfalfa (*Medicago sativa*) and crested wheatgrass (*Agropyron cristatum*) was reduced after four days by more than 75%, at 16 days more than 99% of the conidia on wheatgrass leaves and 28–85% on alfalfa leaves were destroyed (Inglis et al. 1993).

Mobility and persistence in water

There are different aspects on the mobility and persistence of fungal spores in water: (1) water can be used for longterm storage of fungi under laboratory conditions, (2) water is responsible for migration/percolation of spores into the soil, and (3) water as raindrops is responsible for dispersal.

Storage of fungal cultures in water is an easy and cheap method, which was used more than 60 years ago by Castellani (1939). Boesewinkel (1976) was able to store 650 plant pathogenic and saprophytic fungi successfully in sterile, distilled water at room temperature for periods up to seven years. Several entomopathogenic fungi were viable after storage in sterile aqueous solutions of 0.675% NaCl at 4°C for 2–3 years (Müller-Kögler & Zimmermann 1980).

Water is responsible for percolation of spores into soil and will be discussed in *Mobility and persistence in soil*. Rainfall plays an active role in the dispersal of *B. bassiana* from the soil to the surface of whorl-stage corn. Increased levels of crop residues reduce the amount of fungal transfer to the surface of young maize (Bruck & Lewis 2002b). Recently, both *Beauveria* spp. were found in surface water-derived drinking water in Norway (Hageskal et al. 2006).

Mobility and persistence in soil

The mobility of fungal spores in the soil is mainly due to water/rain and soil arthropods. In contrast, persistence depends on several abiotic and biotic factors. These are specific soil properties, temperature, moisture and water, and agrochemicals as abiotic factors and soil microorganisms as well as soil arthropods as biotic factors (Keller & Zimmermann 1989).

Mobility. The mobility of fungal spores in and into the soil is of relevance for two different reasons: (1) for effective biocontrol of soil dwelling pests, which mostly feed on roots of their host plants. In this case, the fungus has to be introduced into the root area in order to come into contact with the host insect; (2) for environmental safety considerations, i.e. it has to be demonstrated that the fungus only percolates to the root area and does not reach or contaminate the ground water. In this respect, Marshall and Bitton (1980) pointed out that microbial adhesion is of fundamental significance in the function and interaction between microorganisms, i.e. attachment of microorganisms to surfaces ensures that they are not eliminated from the particular ecosystem. This means that attachment may be a prerequisite for the relationship between micro- and macroorganisms.

Concerning *B. bassiana* and *B. brongniartii,* it has been proven that these fungi occur naturally in the soil throughout the world (see *Natural occurrrence and geographical distribution*). It has further been demonstrated that *B. bassiana* could be found from 0–5 to 20–25 cm depth in different arable soils (Mietkiewski et al. 1995). The question however is, how deep can these fungi percolate into the soil. First experiments with other fungal species show that the spore and soil type may affect the migration. Spores of *Zygorrhynchus* and *Gliomastix* wash readily through a sand column up to 30–40 cm, whereas *Penicillium* spores show very little movement (5–10 cm) (Burges 1950). Spores that have a mucilaginous coat wash down readily, while spores which have a waxy non-wetting coat remain on the surface (Burges 1950). According to Hepple (1960), water is responsible for vertical movement of spores of *Mucor ramannianus*, but only over short distances. About 75% of conidia of the entomopathogenic fungus *Nomuraea rileyi* layered on a 10.5-cm column of sand were recovered in the filtrate after exposure to the equivalent of 16.25 cm of rain (Ignoffo et al. 1977). However, no conidia were recovered in filtrate from a silt-loam soil and over 90% of the recovered conidia were in the upper 2 cm of the column. The spores had probably been adsorbed on clay or organic particles. Studies on the vertical movement of wet and dry spores of *M. anisopliae* through a 30-cm sand column revealed that less than one spore per 1 mL effluent was found (Zimmermann 1992). The effect of percolating water on spore movement through soil was also studied using a plant-pathogenic isolate of *Fusarium oxysporum* f. sp. *niveum*. Formulations were placed on soil columns and artificial rain was applied. In general, 10-fold fewer CFU were recorded at an 8–10 cm depth compared with a 0–2 cm depth (Gracia-Garza & Fravel 1998).

Vertical movement of commercially formulated conidia of *B. bassiana* was measured in four, sifted soil types in 30.6 cm columns (Storey & Gardner 1987). When applied as an aqueous suspension to the soil, >90% of the viable CFU's were recovered in the upper 15.2 cm in two soil types. Approximately 12.5% of the CFU's moved through the 30.6 cm column of the two other types and were collected in the effluent. When the vertical movement of formulated *B. bassiana* conidia was investigated in

undisturbed soil types, the migration was considerably less than that observed in columns of sifted soil. The majority, i.e. >94% of the conidia remained in the upper 5 cm of all four soil profiles (Storey & Gardner 1988; Storey et al. 1989). These observations indicate that the soil type, the soil pore structure (sifted versus undisturbed soil) and probably also the shape and size of spores affect their movement through the soil. Finally, movement in horizontal and vertical direction is also possible by Collembola (Dromph 2003) and earthworms (Hozzank et al. 2003).

Persistence. As already mentioned, persistence of fungal spores in the soil is affected by several biotic and abiotic factors. In this respect, one main aspect is soil fungistasis. The term describes the phenomenon, whereby (a) viable fungal propagules, not under the influence of endogenous or constitutive dormancy, do not germinate in non-sterile soil at temperature and moisture favourable for germination, or (b) growth of fungal hyphae is retarded or terminated by conditions of the soil environment other than temperature and moisture (Watson & Ford 1972). A widespread fungistasis in soils was found and postulated about 50 years ago (Dobbs & Hinson 1953). Soil fungistasis has been shown to be general in natural soils and to be a dynamic phenomenon.

First experiments concerning the survival of *B. bassiana* in the soil were carried out by Huber (1958) and later by Wartenberg and Freund (1961). The authors found that antibiotic microorganisms suppress the germination of conidia of *B. bassiana* in soil. They concluded that *B. bassiana* is a weak saprophyte and speak of a 'conservation effect' induced by antibiotic microorganisms, such as Actinomycetes. According to Clerk (1969), several authors have reported that conidia of *B. bassiana* are subject to fungistatic effects in natural soils. However, the nature of the inhibitor(s) responsible for soil fungistasis is still unknown, although several authors consider that inhibitory substances released by soil microorganisms play a major role in fungistasis. Clerk (1969) found that conidia of *B. bassiana* are able to germinate in sterilised soil or in soil stimulated by an external source of nutrients, i.e. the presence of insects in the soil might influence the behaviour of conidia. Conidial germination and hyphal growth of *B. bassiana* was inhibited in unsterilised aqueous extracts of soil. Extracts of the deepest soil layer were less inhibiting than extracts of overlaying layers of humus-rich soil. The inhibition was reduced by autoclaving and by filtering the extracts, indicating that the soil microbiota has an impact on the activity of *Beauveria* spp. When *B. bassiana* and *B. brongniartii* were stored at 4°C in sterile soil for preservation purposes, Müller-Kögler and Zimmermann (1980) found that the *B. bassiana* isolate was still viable after six years, the *B. brongniartii* isolate after four years with decreased viability after six years.

According to Lingg and Donaldson (1981), the viability of *B. bassiana* conidia in soil was primarily dependent on temperature and soil water content. Conidia half-lives ranged from 14 days at 25°C and 75% water saturation to 276 days at 10°C and 25% water saturation. Conidia held at −15°C exhibited little or no loss in viability. Conidia were not recoverable after 10 days from soils at 55°C. Conidia survival in nonsterile soil amended with carbon (wheat and pea straw, gluciose, chitin) and/or nitrogen (KNO_3, NH_4Cl, urea, ammonium tartrate) sources was greatly decreased with often complete loss in less than 22 days, whereas conidia in sterile soil treated in the same manner showed dramatic increase. The fungistatic effect in amended nonsterile soil was possibly related to *Penicillium urticae*, which was often isolated and which produced a water-soluble inhibitor of *B. bassiana*. Groden and Lockwood

(1991) noticed a fungistatic effect on *B. bassiana* in two soils from different areas. Fungistatic levels varied between years and increased with increasing pH in soil. A loss of fungistatic mechanisms by sterilisation was postulated by McDowell et al. (1990). Sterilising soil before bioassays resulted in a 10- and 1000-fold reduction in LC_{50} values required to kill first and third instars of *Elasmopalpus lignosellus* (Lepidoptera: Pyralidae), respectively. These findings are also supported by Rosin et al. (1996) who found that soil containing fresh manure was detrimental to *B. bassiana*, whereas high rates of composted manure were beneficial. Obviously, certain factors in the fresh manure reduce the survival of *B. bassiana*.

The persistence of *B. bassiana* conidia in artificially contaminated soil was investigated under laboratory and field conditions (Müller-Kögler & Zimmermann 1986). When starting the experiment in October, the number of viable conidia decreased from about 10^6 g^{-1} dry soil to about 10^4 or 10^3 after one year according to the soil depth. When the experiment was started in May, the corresponding values were 10^7 at the beginning to about 10^5 conidia g^{-1} dry soil after 1 year. Storey et al. (1989) estimated the persistence of applied conidia to be about 200 days, while the granular formulation of conidia persisted for a longer period. The persistence of *B. bassiana* blastospores in soil and their protection by clay-coating was investigated by Fargues et al. (1983). Naked blastospores of *B. bassiana* were inactivated after 3 weeks incubation in soil, while clay-coated blastospores were still active after two months at 20°C. Clay coating is a protection against biodegradation of fungal propagules by soil bacteria and protozoa. The authors stated that antagonists implicated in lysis of blastospores must be considered an important part of the environmental response to a massive introduction of a fungus for biocontrol. Studdert et al. (1990) studied the relationship between soil water potential and temperature on survival of *B. bassiana* clay coated and noncoated conidia in two nonsterile soils. The longest mean half-life value was 44.4 weeks for conidia in sandy loam at −10 bars (0.0 bars = saturation) and 10°C. Clay-coating increase the survival of conidia. Survival was longer in the low organic soil compared to the high organic peat. The results suggest that conidia survival is affected by several physical factors and the soil microbiota. With an experimental biodegradation method, Fargues and Robert (1985) found that inocula of *B. bassiana* were substantially degraded and subject to 70–80% dry weight loss after six months at 19°C. In another experiment, conidia were spread on test areas as water suspensions at a rate of 10^{10} spores m^{-2}. After one year, the mean counts were about only 0.2% for *B. bassiana* of the originally spread spore amount. In loamy soil, most of the spores were found at 0–5 cm, while in humus, they were found in deeper soil layers at 5–15 and 15–20 cm (Tyni-Juslin & Vänninen 1990). Inglis et al. (1997) investigated the influence of three formulations (water, oil, and 15% oil emulsion) and two crops (alfalfa and crested wheatgrass) on the deposition and subsequent persistence of *B. bassiana* conidia in soil. Reductions during winter after 225–272 days were less than one order of magnitude. Neither crop nor formulation influenced conidial persistence in a clay–loam soil.

Investigations on the persistence of *B. brongniartii* were done mainly in Switzerland within the frame of experiments for cockchafer control. Applications of *B. brongiartii* fungus kernels from May to August generally resulted in an increase of $1–5 \times 10^3$ CFU g^{-1} dry soil compared to untreated control plots (Kessler et al. 2003). Soils treated in October and November yielded no increase. Soil temperatures between 20 and 25°C and a high clay content had a positive effect on the occurrence and density

of *B. brongniartii*, whereas temperatures above 27°C had a negative influence. The survival of *B. brongniartii* in soil was further examined for over 16 months after application of fungus kernels in different soil types in Switzerland (Kessler et al. 2004). In the absence of the host insect, *M. melolontha*, the reduction in the CFU in soil was nearly 90%. In soils with high organic content, the decline was more pronounced. When grubs of *M. melolontha* were present, the survival was significantly longer. The rapid decrease of the fungus in soil without the host reveals the high specificity of the fungus and that a saprophytic multiplication is unlikely (Längle et al. 2005).

Monitoring of introduced microorganisms in the environment is essential not only for the development of new biocontrol agents, but also for understanding of their interactions with the living environment, their ecological impact and safety assessments. Several years after *B. brongniartii* was applied against *M. melolontha* in various field tests in Switzerland, isolates were recovered from soil and subjected to genetic analyses using specific microsatellite markers (Enkerli et al. 2004). The applied *B. brongniartii* strains were detected at all sites up to 14 years after their application. In addition to the applied strains, indigenous and mutated or intermediate strains were also isolated. The results suggested that applied and indigenous strains of *B. brongniartii* could coexist in the same habitat. These observations are supported by Castrillo et al. (2004), who observed a large number of vegetative compatibility groups (VCG) among strains of *B. bassiana* and a very low level of recombination which may be a barrier preventing genetic exchange between dissimilar strains in the field.

Effects on non-target organisms

Effects on other microorganisms

Investigations on the natural prevalence of *B. bassiana* have shown that this fungus widely occurs in the soil as well as on insects in the aerial environment. This means that there is a long lasting evolutionary coexistence with other microorganisms that includes different forms of interactions.

From the viewpoint of safety, the main concern is that microorganisms applied for biological control could potentially pre-empt or displace other nontarget microorganisms. After application on plants or in the soil, biocontrol agents should be able to survive and maintain themselves for biocontrol activity, but they should not interfere with the resident microbiota. For example, studies on inundative release of an atoxigenic strain of *Aspergillus flavus* into the soil of cotton fields showed that the native, toxigenic isolates were almost completely displaced (Cotty 1994). Yet, in a risk analysis case study using *Fusarium* species, the effect of antagonistic *Fusarium oxysporum* to control *Fusarium* wilts on the resident soil microbiota revealed that the introduction of wild-type and genetically manipulated antagonistic strains of *Fusarium oxysporum,* released alone or in mixture, did not interfere with the microbial equilibrium of a natural soil (Gullino et al. 1995). Similar results were also obtained by Wang et al. (2004) in *B. bassiana* and Enkerli et al. (2004) in *B. brongniartii*. Wang et al. (2004) monitored the fate of inundatively applied strains of *B. bassiana* against *Dendrolimus punctatus* in southwest China. During one year, the indigenous and exotic strains were reisolated, but the indigenous strains predominated in the local environment, indicating that they were not displaced by the exotic ones. Enkerli et al. (2004) studied the behaviour of introduced *B. brongniartii* strains for control of *M. melolontha* grubs in

Switzerland. The results suggested that applied and indigenous strains of *B. brongniartii* could coexist in the same habitat. Furthermore, in *B. bassiana*, a genetic exchange between indigenous and introduced strains in the field is unlikely due to the large number of vegetative compatibility groups (Castrillo et al. 2004).

There are several reports on interactions of *Beauveria* spp. with hyperparasitic, antagonistic and especially, phytopathogenic fungi. A hyperparasitic fungus attacking *B. bassiana* and *B. brongniartii* is the ascomycete *Syspastospora parasitica*, formerly known as *Melanospora parasitica* (Müller-Kögler 1961; Markova 1991; Posada et al. 2004). Lingg and Donaldson (1981) reported that the survival of conidia of *B. bassiana* in nonsterile soil amended with carbon sources, nitrogen sources or combinations of both was possibly related to *Penicillium urticae*, which produced a water-soluble inhibitor of *B. bassiana*. There are also various interactions with *Clonostachys* spp. and *Trichoderma* spp. which may suppress or overgrow *B. bassiana in vitro* (Krauss et al. 2004; Zimmermann unpublished). Meanwhile, there is an increased interest to test and use *B. bassiana* also against plant pathogens (Ownley et al. 2004). According to the experiments, *B. bassiana* isolate 11–98 could reduce *Rhizoctonia solani* damping-off of tomato in greenhouse tests, and also protect cotton against a seedling disease complex in some sites. Laine and Nuorteva (1970) had previously observed that *B. bassiana* and *B. brongniartii* (*B. tenella*) had a strong antagonistic effect on *Fomes* (*Heterobasidium*) *annosus*. *Beauveria bassiana* had also an antagonistic activity against *Ophiostoma ulmi* (*Ceratocystis ulmi*) (Gemma et al. 1984) and significantly reduced the disease incidence of *Phoma betae*, the blackleg of beet (Roberti et al. 1993). Under greenhouse conditions, *B. bassiana* and *B. brongniartii* were antagonistic to *Pythium ultimum*, *P. debaryanum* and *Septoria* (*Leptosphaeria*) *nodorum*, while *Pythium irregulare*, *Phoma betae*, *Phoma exigua* var. *foveata* and *Rhizoctonia solani* showed resistance to both *Beauveria* species (Vesely & Koubova 1994). The mycelial growth of three phytopathogens of the genera *Fusarium*, *Armillaria* und *Rosellinia* was significantly reduced by filtrates of *B. bassiana* (Reisenzein & Tiefenbrunner 1997), and metabolites from *B. bassiana* produced in liquid culture inhibited the growth of several *Fusarium* spp. (Langbauer et al. 1996). Mycelial growth and conidial germination of *Botrytis cinerea* and *Fusarium oxysporum* were inhibited by a culture filtrate of *B. bassiana* (Bark et al. 1996).

Effects on plants

Both *Beauveria* species are typical soil dwelling fungi and are known to be entomopathogens. Nevertheless, the question of possible phytopathogenic side-effects or any other interactions with plants should be raised. In the past 100 years, *B. bassiana* and *B. brongniartii* have been used for biocontrol of so many leaf- and root-feeding pest insects, that there was ample opportunity of observation of detrimental effects of these fungi in plants. In summarising the past literature, Müller-Kögler (1965) concluded that side-effects or any phytopathogenic activity on plants are not known. We can presently come to the same conclusion. The natural occurrence on plants is reviewed in *Natural occurrence and geographical distribution*.

Recent research has demonstrated that there are various tri-trophic interactions between the plant, the pest insect feeding on the plant and entomopathogenic fungi attacking these herbivores. Elliot et al. (2000) hypothesise whether plants use

entomopathogens as bodyguards against herbivores. The most interesting interactions are as follows:

1. Plants may affect the infection by the entomopathogen;
2. Plants may affect the persistence of the entomopathogen;
3. *B. bassiana* can persist as an endophyte within plants.

There are several reports indicating that the plant species may affect the infectivity and persistence of *B. bassiana*. Ramoska and Todd (1985) found that chinch bugs, *Blissus leucopterus*, fed on sorghum and corn were more resistant to *B. bassiana* than those on barley, and *Bemisia argentifolii* reared on cotton was significantly less susceptible to the fungus than white flies reared on melon (Poprawski & Walker 2000). On foliage of lettuce and celery, the mortality of *Lygus lineolaris* adults seven days post treatment was 91 and 78%, respectively (Kouassi et al. 2003). Nymphs of *Trialeurodes vaporariorum* were highly susceptible to *B. bassiana* on cucumber plants, while insects reared on tomato plants were significantly less susceptible (Poprawski et al. 2000). It is assumed that sequestered tomatine by *T. vaporariorum* nymphs may explain the inhibition of *B. bassiana* after the penetration process, as tomatine was demonstrated *in vitro* to have a detrimental effect on *B. bassiana.*

Beauveria bassiana has been reported to be an endophyte of certain plants, especially corn (Bing & Lewis 1992). Studies have demonstrated that the fungus, applied to whorl-stage corn by foliar application or injection, colonised, translocated and persisted in corn plants. Some conidia of *B. bassiana* are able to germinate on the leaf surface of corn and penetrate it. Virulence bioassays demonstrated that *B. bassiana* does not lose virulence against *Ostrinia nubilalis* once it has colonised corn (Wagner & Lewis 2000). In the greenhouse, *B. bassiana* was applied as a liquid seed treatment to Bt transgenic corn hybrids and their near isolines (2×10^{10} conidia mL^{-1}), and no significant differences in seed germination or presence of root pathogens were observed (Lewis et al. 2001). Recent experiments revealed that *B. bassiana* may also function as an endophyte in cocoa seedlings (Posada & Vega 2005), in banana tissue culture plants (Dubois et al. 2005) and in opium poppy, *Papaver somniferum* (Quesada-Moraga et al. 2006).

Another aspect of the tri-trophic interactions between plants, entomopathogenic fungi and pest insects is the fact that toxic metabolites of *Beauveria* spp. may enter the plants. As already mentioned in *Production of metabolites/toxins*, beauvericin derived from *Fusarium* species and not from *B. bassiana* is obviously widespread in maize and food. Beauvericin did not cause any symptoms on roots of melon, tomato, wheat and barley, however, it showed high toxicity towards the protoplasts of these plants (see Moretti et al. 2002). The phytotoxic potential of *B. brongniartii* and its main metabolite oosporein were evaluated against seed potatoes (*Solanum tuberosum*) *in vitro* and *in situ*. The weight of haulm and tubers was unaffected by *B. brongniartii*, and no oosporein was detected in the potatoes. Therefore, the species pose no risks to potato plants or tubers (Abendstein et al. 2000; Seger et al. 2005a; See *Production of metabolites/toxins*).

Effects on soil organisms

As mentioned, *B. bassiana* and *B. brongniartii* are commonly found in soil. They generally have a broad host range and are often used for biocontrol of soil dwelling

pests. Therefore, possible interactions or effects on other non-target soil inhabiting invertebrates should be noticed.

In 1964, Samšiňák found that the mites *Tyrophagus putrescentiae* and *Acarus siro* are not susceptible to *B. bassiana*. *Tyrophagus putrescentiae* feeds on insects and also on dead, *Beauveria*-infected larvae. This mite species is also able to transmit spores of *B. bassiana* from fungus-infected larvae of *Galleria mellonella* to healthy ones. *Beauveria bassiana* was also found in high frequency on a great number of the collembolan *Onychiurus subtenuis* (Visser et al. 1987). The authors concluded that there was no indication that *B. bassiana* killed the collembolan. The collembolan *Folsomia candida* was not susceptible to *B. bassiana*. It consumed and inactivated the insect pathogen without causing mortality or any other harmful effects (Broza et al. 2001). Pathogenicity tests of *B. bassiana* and *B. brongniartii* were conducted against adults of *Folsomia fimetaria*, *Hypogastrura assimilis* and *Proisotoma minuta* (Dromph & Vestergaard 2002). By dipping the collembolans in 1×10^7 conidia mL^{-1} suspension and in one case also in 1×10^8 conidia mL^{-1}, none of the fungal isolates increased mortality over the controls. After continuous exposure of *F. fimetaria* and *P. minuta* to conidia of *B. brongniartii* for 14 days at 20°C in sphagnum containing 1×10^8 conidia g^{-1} wet weight, one of the *B. brongniartii* isolates increased the mortality significantly. In a test of the attractiveness of these fungi for the three collembolan species, *B. brongniartii* was found to be more attractive than baker's yeast. Mites were also observed feeding on *B. brongniartii* killed white grubs of *Melolontha* spp. without any sign of infection (Zimmermann, unpubl.).

Dispersal of entomopathogenic fungi by Collembolans has been demonstrated by several authors (Samšiňáková & Samšiňák 1970; Zimmermann & Bode 1983; Dromph 2001, 2003). For example, *B. bassiana* is distributed both in a horizontal and vertical direction by the mite *Sancassania phyllognathi* which is resistant to fungal infection (Samšiňáková & Samšiňák 1970). The transmission of spores of *B. bassiana* and *B. brongniartii* to a susceptible host, *Tenebrio molitor*, by the collembolans *F. fimetaria*, *H. assimilis* and *P. minuta* was also demonstrated (Dromph 2003).

These findings show that there are no or very low detrimental effects on the tested soil-dwelling collembolans and mites. In contrast, collembolans can act as vectors of *Beauveria* spp. and thus may play an important role for the dispersal and transmission of these fungi in soil.

Effects on aquatic organisms

No toxicity or pathogenicity was observed in *Daphnia magna* when exposed to 1×10^9 conidia of *B. bassiana* strain GHA per litre for 21 days (Goettel & Jaronski 1997). Strain GHA was also not infectious against the grass shrimp, *Palaemonetes pugio*, after percutaneous and oral contamination (Genthner et al. 1994b). In the mysid shrimp *Americamysis bahia* (formerly *Mysidopsis bahia*) *B. bassiana* conidia caused high mortalities, but these were attributed to a high particulate density since heat-killed controls also proved lethal (Genthner et al. 1994a).

Beauvericin has been found to be highly toxic towards *Artemia salina* larvae and murine cell lines and can induce apoptosis (Pascale et al. 2002). In the mysid *A. bahia*, beauvericin was toxic at an LC_{50} of 0.56 mg L^{-1} (Genthner et al. 1994a). To my knowledge there are no published studies regarding effects of other *Beauveria* metabolites as well as of *B. brongniartii* on aquatic organisms.

Effects on predators, parasitoids, honey bees, earthworms and other non-target arthropods

It is well known that *B. bassiana* has a wide host range, occurring on several hundred arthropod species; however, host specificity is really a strain-specific trait. For example, *B. bassiana* isolates from the lady beetle, *Olla v-nigrum*, were pathogenic to adult *O. v-nigrum* but not to adults of the Asian lady beetle, *Harmonia axyridis* (Cottrell & Shapiro-Ilan 2003). The GHA strain of *B. bassiana* was not significantly pathogenic to either *O. v-nigrum* or *H. axyridis*. In contrast, *B. brongniartii* has a much narrower host range being mostly restricted to members of the coleopteran family Scarabaeidae.

The practical use of these fungi in different crop protection systems raises the question of possible side-effects on non-target organisms. This is especially important when commercial products of these fungi are used on wide areas, e.g. for control of grasshoppers on meadows or of the European cockchafer species, or when *B. bassiana* is used together with beneficial insects in glasshouses.

Generally, there is a difference between the physiological host range and the ecological host range (Hajek & Butler 2000). The physiological host range demonstrates the range of insect species that can be infected in the laboratory, while the ecological host range demonstrates which insects can be infected in nature or under field conditions. Non-target insects which are infected under laboratory conditions, may not necessarily be infected in nature. This topic was also discussed in detail by Hajek and Goettel (2000) and Jaronski et al. (2003).

There are numerous papers on the effect of *B. bassiana* and *B. brongniartii* on beneficial and other non-target organisms. Examples are presented in Table V. Most of the studies were done in the laboratory and only a few in the field. One of the first comprehensive reports was given by Goettel et al (1990), who listed the effects of *B. bassiana* on nontarget invertebrates, such as bees and other pollinators, silkworms, predators and parasitoids. Further, general information is mentioned by Goettel et al. (1997, 2001) and Vestergaard et al. (2003). The last authors conclude that despite the wide host range of *B. bassiana*, evidence to date suggests that this fungus can be used with minimal impact on nontarget organisms, especially when isolate selection and spacio-temporal factors are taken into consideration. *Beauveria brongniartii* has a narrower host range, mainly including Scarabaeidae, and occurs worldwide in soil habitat. Laboratory bioassays demonstrated that it was possible to infect collembolans, cicindellid and carabid beetles unter stress conditions, while honey bees and earthworms were not affected (Table V). Data from field investigations did not reveal any indication of possible adverse effects on vertebrates, honeybees, beneficial insects, earthworms and plants (Vestergaard et al. 2003).

Effects on vertebrates (fish, amphibia, reptiles and birds)

Possible side-effects of entomopathogenic fungi on vertebrates and men were summarised by Müller-Kögler (1967) nearly 40 years ago. Today, detailed vertebrate safety tests are included in the registration process of commercial *Beauveria* products and have been conducted with several isolates of *B. bassiana*. Generally, both

Table V. Examples of effects of *B. bassiana* and *B. brongniartii* (strains and formulations) on beneficial and nontarget organisms.

Beneficial organism	Fungus (Strain/ Formulation)	Lab./ Field Trials (L/F)	Results/Observations	Reference
Amblyseius cucumeris	*B. bassiana* (Naturalis-L, BotaniGard WP)	L/F	No detrimental effect when sprayed onto excised cucumber leaves	Jacobson et al. (2001)
Aphidius colemani *Orius insidiosus* *Phytoseiulus persimilis* *Encarsia formosa*	*B. bassiana* (commercial formulation, strain JW-1)	L	Highly susceptible under laboratory conditions, lower infection rates in greenhouse	Ludwig and Oetting (2001)
Apis mellifera	*B. bassiana*	F	Conidia were applied in bee hives: low mortality and no noticeable effect on behaviour, larvae and colony characteristics	Alves et al. (1996)
Apis mellifera	*B. bassiana* (unformulated spore preparation)	L	*B. bassiana* reduced bee longevity at the two highest concentrations tested and caused mycosis at 10^6–10^8 spores per bee	Vandenberg (1990)
Apis mellifera	*B. bassiana* (Naturalis-L, Bio-Power)	L	30-day dietary and contact studies had no significant effect; LC_{50} (23 days, ingestion) 9.285 μg/bee	Copping (2004)
Apis mellifera	*B. brongniartii*	F	No negative effects noticed	Wallner (1988)
Arthropod and nematode populations	*B. bassiana* (Naturalis-L)	F	Chlorpyrifos had a stronger negative impact than the microbial treatment	Wang et al. (2001)
Bembidion lampros *Agonum dorsale*	*B. bassiana*	F/L	A negligible number was infected; low susceptibility of both species	Riedel and Steenberg (1998)
Bombus terrestris	*B. bassiana*	L/F	Able to infect bumblebees; it appears that there are no risks if the fungus is incorporated into soil or sprayed onto plants that are not attractive to bumblebees	Hokkanen et al. (2003)
Carabidae: *Calanthus micropterus* *C. piceus* *Carabus violaceus* *Cychrus caraboides* *Leistus ruefescens* *Nebria brevicollis,* *Pterostichus oblongopunctatus,* *P. niger*	*B. bassiana*	L	No adverse effects noticed	Hicks et al. (2001)
Carabidae, Staphylinidae	*B. bassiana*	F	Infection levels in adult ground beetles and rove beetles were low (Carabidae max. 7.6% and Staphylinidae max. 7.0%); an epizootic in the staphylinid *Anotylus rugosus* (67%) and *Gyrohypnus angustatus* (37%) was observed	Steenberg et al. (1995)

Table V (*Continued*)

Beneficial organism	Fungus (Strain/ Formulation)	Lab./ Field Trials (L/F)	Results/Observations	Reference
Cephalonomia tarsalis	*B. bassiana*	–	3 h exposure to 100 and 500 mg kg^{-1} wheat resulted in 52.5 and 68.6% mortality	Lord (2001)
Chrysoperla carnea	*B. bassiana*	L	Temperature, starvation and nutrition stresses significantly affected the susceptibility; nutrition stress caused the most increase in adult and larval mortality	Donegan and Lighthart (1989)
Coleomegilla maculate	*B. bassiana* (isolate ARSEF 3113)	L/F	No mortality was observed	Pingel and Lewis (1996)
Coleomegilla maculate and *Eriopis connexa*	*B. bassiana* (isolate ARSEF 731)	L	Mortality after direct application of spores; exposure via sprayed leaf surfaces resulted in no infection	Magalhaes et al. (1988)
Coleomegilla maculate lengi	*B. bassiana* (10 isolates)	L	6 isolates were highly virulent, 3 isolates caused low mortality	Todorova et al. (2000)
Diadegma semiclausum	*B. bassiana*	L	Detrimental effects on cocoon production and emergence depending on concentration	Furlong (2004)
Formica polyctena	*B. brongniartii*	F	No negative effects noticed	Dombrow (1988)
Earthworms: *Lumbricus terrestris* and others	*B. brongniartii* (commercial product of barley grains)	L/F	No effect in lab and in field noticed	Hozzank et al. (2003)
Earthworms: *Lumbricus terrestris*	*B. brongniartii*	L	No effect on earth worms noticed	Arregger-Zavadil (1992)
Earthworms: *Aporrectodea caliginosa*	*B. bassiana* (Bb64)	L	No effect on hatching rate of cocoons	Nuutinen et al. (1991)
Lysiphlebus testaceipe Aphidius colmani	*B. bassiana*	F	No significant impacts on both parasitoids	Murphy et al. (1999)
Megachile rotundata	*B. bassiana* (strain for grasshopper control)	L	Spray-application of flowering alfalfa in pots: female and male mortality averaged 9%; no difference in treatment and control; however *B. bassiana* grew out from dead bees	Goettel and Johnson (1992)
Nontarget arthropods (forests)	*B. brongniartii*	F	Only 1.1% of 10.165 collected insects and spiders were infected	Baltensweiler and Cerutti (1986)
Nontarget arthropods (forests)	*B. brongniartii*	F	1671 nontarget specimens were collected: 3.4% of them were infected, mainly species from Araneae, Thysanoptera, Homoptera, Coleoptera and Lepidoptera	Back et al. (1988)
Nontarget arthropods (major predators, parasitoids and pollinators on rangeland)	*B. bassiana* (strain GHA)	F	No statistical differences in the abundance of aerial insects	Brinkman and Fuller (1999)

Table V (*Continued*)

Beneficial organism	Fungus (Strain/ Formulation)	Lab./ Field Trials (L/F)	Results/Observations	Reference
Nontarget arthropods (forests)	*B. bassiana* (emulsifiable concentrate)	F	From 3615 invertebrates collected, only 2.8% became infected; *B. bassiana* could be applied to forest soil without a significant negative impact on forest-dwelling invertebrate population	Parker et al. (1997)
Non-target beetle communities	*B. bassiana* (strain SP 16)	F	No detectable effects	Ivie et al. (2002)
Perillus bioculatus	*B. bassiana* (six isolates)	L	5 isolates were highly pathogenic, isolate IPP46 showed low pathogenicity	Todorova et al. (2002)
Pimelia senegalensis *Trachyderma hispida* *Bracon hebetor* *Apoanagyrus lopezi*	*B. bassiana*	L	No infection in *P. senegalensis* and *T. hispida*; 100% mortality in the parasitoids *B. hebetor* and *A. lopezi*	Danfa et al. (1999)
Poecilus versicolor	*B. brongniartii* (Melocont-Pilzgerste, Melocont-WP, and Melocont-WG)	L	No significant negative effects on *P. versicolor* could be observed	Traugott et al. (2005)
Predatory mites: *O. insidiosus*	*B. bassiana* (Botanigard ES)	F	Can be used	Shipp et al. (2003)
A. colemani *Dacnusa sibiria* ***Parasites:*** *Encarsia formosa* *Eretmocerus eremicus* *Aphidoletes aphidimyza*			Not recommended during application of *B.bassiana* Used with caution during application of *B. bassiana*	
Prorops nasuta	*B. bassiana* (3 isolates)	L	Strain 25 caused the lowest infection level	De La Rosa et al. (2000)
Serangium parcesetosum	*B. bassiana*	L	The predator had significantly lower survivorship when sprayed with *B. bassiana* than with *P. fumosoroseus*; feeding on *B. bassiana* contaminated prey caused 86% mortality	Poprawski et al. (1998)

Beauveria species have been proven to be non-toxic and non-infectious to vertebrates, however, in a few cases, infections of *B. bassiana* have also been noticed.

Fish

Safety tests against fish for *B. bassiana* were reported for the isolate GHA by Goettel and Jaronski (1997) and for the product Naturalis-L® as well as for *B. brongniartii* by Copping (2004). No adverse effects of strain GHA were observed in embryos and larvae of the fish *Pimephales promelas*, when exposed for 31 days to 1×10^9 CFU L^{-1}. Naturalis-L® did not affect fish embryos, larvae or adults; the LC_{50} (31 days) for rainbow trout was 7300 mg L^{-1}. In contrast, when developing embryos of the inland

silverside fish, *Menidia beryllina*, were exposed to conidia of *B. bassiana*, various adverse effects were observed in embryos and larvae (Genthner & Middaugh 1992; Middaugh & Genthner 1994). In a strain of *B. brongniartii* (IMBST 95.031 and 95.041, Austria) the LC_{50} (at 30 days) for rainbow trout was 7200 mg L^{-1}, while the no observed effect level (NOEL) at 30 days was 3000 mg L^{-1} (Copping 2004).

Amphibia

A fungal suspension of *B. bassiana* was fed to the leopard frog, *Rana pipiens* via gastric incubation. The dosage was 9.8×10^8 conidia corresponding to 2.22×10^{12} conidia for a 70-kg human. No mortality or fungus recovery was recorded in any of the tissues. The viscera were free of mycelial growth. Viability of spores was established in fecal washings of pellets (Donovan-Peluso et al. 1980).

Reptiles

A fungus attributed to be *B. bassiana* was observed to cause infections in a captive American alligator (Fromtling et al. 1979), and *B. bassiana* was implicated in causing a pulmonary disease in captive tortoises (Georg et al. 1962; Gonzales-Cabo 1995). The reptiles were in captivity and under temperature stress which may explain their susceptibility to the fungus. When a tortoise was kept at 22°C and injected with 0.5 mL of 10^6 spores of *B. bassiana* into the lung, no mortality was observed, while a second contaminated tortoise died when kept only at 16°C (see Müller-Kögler 1967).

Birds

Birds may become exposed to entomopathogenic fungi directly by consuming spores deposited on their food, or indirectly by consuming fungus-infected insects. The concern about possible side-effects in birds is more than 100 years old. Müller-Kögler (1967) mentioned that according to E. Devaux (in Giard 1892), chickens, fed white grubs of *Melolontha* sp. infected with *B. brongniartii* (*B. tenella*), did not demonstrate any side-effects. However, precise examinations were not carried out at that time. Later, young *Falco sparvensis* were fed with 5×10^6 spores of *B. bassiana* per kg body weight (Althouse et al. 1997). No differences were found among any treatments and the control in growth, body mass or survival. Male and female ring-necked pheasants (*Phasianus colchicus*) were challenged per os with conidia of *B. bassiana* (Johnson et al. 2002). In both sexes, the weight gain at 17 and 25 days was not significantly different between challenged and control groups. Histopathological changes were generally undetectable. In 1987, a large field trial was carried out in Germany with *B. brongniartii* blastospores against the forest cockchafer *Melolontha hippocastani* ($1.5–2.8 \times 10^{14}$ blastospores ha^{-1}). During this experiment, no side-effects on birds, especially young ones, were noticed (Havelka & Ruge 1988). According to Copping (2004), the non-target bird toxicity for *B. bassiana* strain ATCC 74040 is: Oral LD_{50} (5 days) for quail >2000 mg kg^{-1} daily (by gavage); for *B. brongniartii* strain IMBST 95.031 and 95.041 (Austria): Dietary LD_{50} (5 days) for quail and mallard ducks >4000 mg kg^{-1}.

Effects on mammals and human health

Safety of entomopathogenic fungi, especially *B. bassiana* and *B. brongniartii*, to mammals and humans is of primary concern and has to be considered as one of the main potential hazards of using fungi as biocontrol agents. Therefore, it is not unusual that allergic, pathogenic or toxic risks for humans and mammals have been stressed in many papers (Steinhaus 1957; Müller-Kögler 1967; Ignoffo 1973; Austwick 1980; Burges 1981; Saik et al. 1990; Siegel & Shadduck 1990; Goettel et al. 1997, 2001; Vestergaard et al. 2003). Recently, some papers from South Korea on the addition of *B. bassiana* to human food documents a totally new aspect of this fungus. Yoon et al. (2003b) reported that extracts of *B. bassiana* synnemata had anticoagulant and immune system modulating activity, which could provide beneficial physiological activities for humans. In another paper, Yoon et al. (2003a) found that *B. bassiana* synnemata could be used as an additive to wheat flour for the preparation of noodle and bread.

Allergy

Allergies are caused by certain protein and polysaccharide antigens, so all types of microorganisms are potentially allergenic to man. Generally, a wide range of allergic reactions to various fungi can occur. According to Nolard (2004), 5–15% of the population suffering from respiratory allergy have been sensitised to one or several moulds. Exposure to fungus associated antigens may cause sensitisation, and later exposures can elicit reactions like respiratory distress, lachrymation or erythema. The main route of sensitisation is respiratory. Allergies relevant to the safety of biological pesticides are placed in Type I and III. Type I allergy is an immediate response to relatively small amounts of an inhaled allergen resulting in rhinitis, heavy asthma or lacrymation. Type III allergy is a delayed response 4–8 h after exposure to a relatively heavy inhaled dose of the allergen, causing fever, headache and weakness (Austwick 1980; Burges 1981). Basics of fungal allergy are summarised by Gumowski et al. (1991).

Nolard (2004) differenciates between 'outdoor air fungal allergy', 'indoor air fungal allergy' and 'fungal allergies in work environments'. Concerning *B. bassiana* and *B. brongniartii*, allergies or allergic reactions could occur in workers in production facilities who are exposed repeatedly to high concentrations of spores and when the fungi are released into the air in an inhalable form after application for biocontrol purposes. Because the conidia of both fungi are 'dry', relatively small (2–3 μm, globose, in *B. bassiana* and 2–6 μm, oval, in *B. brongniartii*) and produced in dusty clusters, they are easily spread by air and may reach the lower respiratory tract after inhalation (Austwick 1980).

The natural occurrence of *B. bassiana* in the air has already been mentioned in *Natural occurrence and geographical distribution* and *Mobility and persistence in air*. MacLeod (1954) reported, that *B. bassiana* was found within the lung tissues of 14 rodents. But, histological examination has not shown that the fungus could readily create a pathological condition within the tissues. In a survey of fungi from sputum of patients hospitalised from chronic pulmonary disease, more than 15% of more than 3000 sputum samples were found to contain one or more colonies of *B. bassiana* (Pore et al. 1970) and from 103 sputum specimens from male telephone workers, five contained *Beauveria* sp. (Comstock et al. 1974). Gumowski et al. (1991) listed about

100 fungal genera associated with allergy; *B. bassiana* was not mentioned at that time. However, there are some documents that indicate *B. bassiana* may cause allergies and/or allergic reactions. Müller-Kögler (1967) mentions several cases of allergic reactions in humans caused by this species, especially during the production process. Generally, headache, weakness and fever were noticed. High fever and a reaction similar to an anaphylactic shock were noticed by Dr Samšiňáková, Praha, and her assistant in 1965 when producing a spore powder of *B. bassiana*. However, when she was working with dry and dusty blastospores of *B. bassiana*, such reactions were never observed. These observations confirm that fungal conidia contain more allergenicity causing factors compared to hyphae and to submerged culture produced blastospores (Müller-Kögler 1967). Allergic reactions in workers were also reported by Mel'nikova and Murza (1980). In contrast to these findings, no incidents of human hypersensitivity reaction were noticed by workers of the company Mycotech during many years of mass production of the fungus (Goettel & Jaronski 1997). Inhalation experiments with *B. brongniartii* (*B. tenella*) and albino-mice ('Rüdiger') were reported by Müller-Kögler (1967). In preliminary experiments, mice were treated two times per week by dusting 50 mg conidia in a 10-L flask. They died within a short time. But the reactions were less harmful after only one treatment per week. There is no doubt, that the spore dose was rather high corresponding to 5 g m^{-3}. After histological examination, the fungus was not found in mouse lungs. In further inhalation experiments with *B. bassiana* and various animals, the fungus did not migrate from the lungs into other organs, and the lungs were completely rid of the fungus 4–5 days later (Mel'nikova & Murza 1980). Some inhalation and irritant data on *B. bassiana* and *B. brongniartii* are also presented by Copping (2004). *Beauveria* bassiana strain ATCC 74040: inhalation: LC_{50}: rats $>1.2 \times 10^8$ CFU/animal. Possible irritant to eyes, skin and respiratory system. *Beauveria brongniartii* strain IMBST 95.031 and 95.041 (Austria): Acute dermal LD_{50}: rats >2000 mg kg^{-1}. Mildly irritant to skin of rabbits.

These investigations and findings reveal that conidia of *Beauveria* species have allergenic potential. In molecular studies, *B. bassiana* crude extracts possess numerous IgE reactive proteins, some of which are cross-reactive among allergens from other fungi (Westwood et al. 2005). A strongly reactive potential *B. bassiana* specific allergen (35 kDa) was identified and confirmed by intradermal skin testing.

Pathogenicity/Toxicity

Besides allergy, one of the main concerns in the use of entomopathogenic fungi is the risk of infection to humans or mammals. On the other hand, *B. bassiana* itself or fungus diseased larvae of *Bombyx mori* have been used as medicants for hundreds of years in Chinese medicine (Müller-Kögler 1965).

Natural occurrence. Beauveria species have been rarely identified as agents of human infections, and in an overview on the emergence of less common, but medically important fungal pathogens on recipients, the genus *Beauveria* is not mentioned (Walsh et al. 2004; Strasser & Kirchmair 2006). Nevertheless, there are some cases where *B. bassiana* has been reported as the cause of mycotic keratitis in humans (Sachs et al. 1985; Low et al. 1997; Kisla et al. 2000; Sigler 2003) and in a rabbit cornea (Ishibashi et al. 1987). For example, Sachs et al. (1985) described the first case of *B. bassiana* keratitis in a patient following the removal of a corneal foreign body.

However, the patient was treated with topical corticosteroids and antibiotics prior to the identification of mycotic elements. This therapy may have depressed the normal protective mechanisms. A fungal keratitis due to *B. bassiana* was also described in an 82-year-old woman (Kisla et al. 2000). The patient was treated successfully with topical natamycin and oral fluconazole.

The first documented human deep tissue infection with a *Beauveria* sp. in a patient receiving immunosuppressive therapy was reported by Henke et al. (2002). Antifungal therapy with itraconazole (200 mg orally twice daily) was successful. Recently, a second case of disseminated *Beauveria* infection in an immunosuppressed patient with acute lymphoblastic leukemia was reported from New Zealand (Tucker et al. 2004). The infection was successfully treated with amphotericin B and itraconazole. In both cases, the *Beauveria* isolates were unable to grow at 37°C, and the New Zealand isolate even did not grow at 35°C. In both cases, the patients were not exposed directly to the fungus, e.g. by use of a *B. bassiana* product. Thus, we can only speculate how these patients came into contact with the fungus. Tucker et al. (2004) hypothesised that their patient was exposed to *B. bassiana* while living in an agricultural area. Recently, the first case of empyema caused by *B. bassiana* in a 51-year-old man was reported in Turkey (Gürcan et al. 2006). The authors think that prolonged air leakage after lung operation was the primary cause of infection. The patient recovered without any antifungal treatment after the air leakage was secured. The isolated strain of *B. bassiana* did not grow at 37°C.

Experimental data. During the development and registration of *B. bassiana* products for biocontrol, the fungus has been extensively tested for safety against several mammals. The first experiments were conducted by Schaerffenberg (1968). They included injection, inhalation and feeding tests with adult white rats. No toxic or pathogenic but allergic reactions were noticed. A commercially used strain of *B. bassiana* was administered to albino rats intragastrically and intraperitoneally and to rabbits intravenously (Mel'nikova & Murza 1980). The LD_{50} number of spores per animal was more than 1.1×10^{10}, 2.2×10^{10} and 4.0×10^{10}, respectively. Single administration of Boverin® dust intraperitoneally and intragastrically to albino rats resulted in an LD_{50} of 0.6 ± 0.1 g kg^{-1} and more than 10 g kg^{-1}, respectively. The potential pathogenicity of B. bassiana to mice was studied by intramuscular injection of 2×10^8 (high) and 2×10^5 (low) conidia (Semalulu et al. 1992). It was concluded that *B. bassiana* does not cause infection, multiply, nor survive for more than 3 days when injected into healthy mice.

Summaries and EPA evaluations of toxicity/pathogenicity data are available for *B. bassiana* strains GHA and ATCC 74040 on the EPA website: http://www.epa.gov/oppbppd1/biopesticides/ingredients/tech_docs/tech_128924.htm and /tech_128818.htm. The experiences of Mycotech Corporation (now Laverlam International, Butte MT) with the safety testing of their *B. bassiana* strain GHA are also presented by Goettel and Jaronski (1997). No infectivity or toxicity was demonstrated in any of the tests with rats and other vertebrates, The fungus was cleared from rats within 7 days after intratracheal application and within 3 days after intraperitoneal or peroral application. In an ocular irritation test with conidial powder, a moderate reaction, such as redness or swelling with no signs of infection was observed. Ocular introduction of two formulations, an oil flowable and an emulsifiable suspension,

resulted in minimal irritation and dermal application of conidia to rabbits showed only transitory erythema.

Some precise data on mammalian pathogenicity/toxicity of both *Beauveria* species are also presented by Copping (2004): *B. bassiana* strain ATCC 74040: Mammalian toxicity: No infectivity or pathogenicity was observed in rats after 21 days exposure to 1.8×10^9 colony forming units kg^{-1}. Acute oral LD_{50}: rats $>18 \times 10^8$ CFU kg^{-1}. Acute dermal LD_{50}: rats >2000 mg kg^{-1}. Inhalation: LC_{50}: rats $>1.2 \times 10^8$ CFU/animal. Skin and eye: Possible irritant to eyes, skin and respiratory system. Dermal, oral and inhalation studies with Naturalis-L® on rats indicated that the fungus is non-toxic and non-pathogenic. *Beauveria brongniartii* strain IMBST 95.031 and 95.041 (Austria): Acute oral LD_{50}: rats >5000 mg kg^{-1}. In rats, there was no toxicity, infectivity or pathogenicity from a single dose of 1.1×10^9 CFU kg^{-1}.

Conclusions

Beauveria bassiana and *B. brongniartii* (=*B. tenella*) are two well-known entomopathogenic fungi which have a worldwide distribution, and are used for biological control of pest insects for more than 100 years. This means that tons of fungus material of both species have been produced and used during that time. The products have passed the registration requirements in several countries, and are still widely used for biocontrol of pest insects. The present review documents, that there is a broad knowledge on *B. bassiana* and *B. brongniartii*, i.e. on their biology, their fate and behaviour in the environment, their effects on non-target organisms and on vertebrates, including mammals and humans. So far, no serious detrimental effects have been observed after application of these fungi. On the basis of our actual and presented knowledge, both *Beauveria* species, *B. bassiana* and *B. brongniartii*, should be considered as safe. Nevertheless, to avoid possible risks, certain vertebrate pathogenicity/toxicity tests and relevant studies on non-target organisms should be made within future registrations of new strains. Additionally, known protection measures during the production process and application are necessary to avoid allergic reactions. However, all risks can never be excluded. It is hoped, that this review provides a fundamental basis of knowledge for scientists, as well as for producers, potential registrants and regulatory authorities for their future work and decisions on the development and registration of these two fungi.

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