1 Introduction – Fungal Biological Control Agents: Progress, Problems and Potential

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There is increasing interest in the exploitation of fungi for the control of invertebrate pests, weeds and diseases, as evidenced by the number of commercial products available and under development (Tables 1.1–1.3). Fungal biological control is an exciting and rapidly developing research area with implications for plant productivity, animal and human health and food production. This area includes a number of important disciplines, such as pathology, ecology, genetics, physiology, mass production, formulation and application strategies. The research, development and final commercialization of fungal biological control agents (BCAs) continue to confront a number of obstacles, ranging from elucidating important basic biological knowledge to socio-economic factors. Considerable advances have been made in separate areas but it is important to integrate and communicate these new findings.

In this chapter we present a brief overview of some of the social and economic reasons for developing fungal BCAs, highlighting the commercial perspective. We also outline the main steps in developing fungal BCAs and draw attention to the chapters that correspond with each step in the commercialization process.

There is considerable interest in the exploitation of naturally occurring organisms, such as bacteria, viruses and fungi, for the control of crop pests, weeds and diseases. Although this chapter focuses on fungal BCAs, many of the concepts apply to other beneficial organisms which offer environmentally friendly alternatives to chemical pesticides. Fungal BCAs could be used where chemical pesticides are banned (e.g. organochlorines) or being phased out (e.g. methyl bromide) or where pests have developed resistance to conventional pesticides (see Chapter 2). It is generally recognized that some chemical pesticides contaminate groundwater and enter food-chains that have an impact on a wide range of organisms. Furthermore, pesticides can pose hazards to animal health and to the user spraying the chemical. Consumer perceptions worldwide are that chemical usage in agricultural production needs to be significantly

Table 1.1. Fungi developed or being developed for the biological control of diseases (data from Burges, 1998; Butt and Copping, 2000).

Product	Fungus	Target	Producer
Mycoparasites			
Rotstop	*Phlebiopsis* (=*Peniophora*) *gigantea*	*Heterobasidium annosus*	Kemira Agro Oy, Finland
Primastop	*Gliocladium catenulatum*	Several plant diseases	Kemira, Agro Oy, Finland
SoilGard (= GlioGard)	*Gliocladium virens*	Several plant diseases Damping off and root pathogens	ThermoTrilogy, USA
Cotans WG	*Coniothyrium minitans*	*Sclerotinia* spp.	Prophyta, Germany; KONI, Germany
AQ10 Biofungicide	*Ampelomyces quisqualis*	Powdery mildews	Ecogen Inc., USA
YieldPlus	*Cryptococcus albidus*	*Botrytis* spp., *Penicillium* spp.	Anchor Yeast, South Africa
Aspire	*Candida oleophila*	*Botrytis* spp., *Penicillium* spp.	Ecogen Inc., USA
Endothia parasitica	*Endothia parasitica* (non-pathogenic strain)	*Endothia parasitica* (chestnut blight)	CNICM
Fusaclean	*Fusarium oxysporium*	*Fusarium oxysporium*	Natural Plant Protection, France
Biofox C	*F. oxysporium*	*F. oxysporium, Fusarium moniliforme*	SIAPA, Italy
Polygandron polyversum	*Pythium oligandrum*	*Pythium ultimum*	Plant Protection Institute, Slovak Republic
Trichoderma 2000	*Trichoderma harzianum*	*Rhizoctonia solani, Sclerotium rolfsii, Pythium*	Mycontrol (EfA1) Ltd, Israel
Trichopel	*T. harzianum*	Wide range of fungal diseases	Agrimm Technologies Ltd, New Zealand
T-22 and T-22HB Bio-Trek, RootShield	*T. harzianum*	*Pythium, Rhizoctonia, Fusarium, Sclerotinia*	BioWorks (= TGT Inc.), Geneva, USA
Supresivit	*T. harzianum*	Various fungi	Borregaard and Reitzel, Denmark; Fytovita, Czech Republic
Trichodowels, Trichoject, Trichoseal and others	*T. harzianum* and *Trichoderma viride*	*Chondrostereum purpureum* and other soil and foliar pathogens	Agrimms Biologicals, New Zealand, and others
Binab T	*T. harzianum, Trichoderma polysporum*	Fungi causing wilt, wood decay and take-all	Bio-Innovation, Sweden
Trichodex	*T. harzianum*	Fungal diseases, e.g. *Botrytis cinerea*	Makhteshim-Agan, Israel, several European companies, e.g. DeCeuster, Belgium

Table 1.2. Fungi developed or being developed for the biological control of pests (data from Burges, 1998; Butt and Copping, 2000).

Product	Fungus	Target	Producer
Entomogenous fungi			
Mycotal	*Verticillium lecanii*	Whitefly and thrips	Koppert, the Netherlands
Vertalec	*V. lecanii*	Aphids	Koppert, the Netherlands
Biogreen	*Metarhizium anisopliae*	Scarab larvae on pasture	Bio-care Technology, Australia
Metaquino	*M. anisopliae*	Spittlebugs	Brazil
Bio-Path	*M. anisopliae*	Cockroaches	EcoScience, USA
Bio-Blast	*M. anisopliae*	Termites	Ecoscience, USA
Cobican	*M. anisopliae*	Sugar-cane spittlebug	Probioagro, Venezuela
Conidia	*Beauveria bassiana*	Coffee-berry borer	Live Systems Technology, Colombia
Ostrinil	*B. bassiana*	Corn-borer	Natural Plant Protection (NPP), France
CornGuard	*B. bassiana*	European corn-borer	Mycotech, USA
Mycotrol GH	*B. bassiana*	Grasshoppers, locusts	Mycotech, USA
Mycotrol WP & BotaniGard	*B. bassiana*	Whitefly, aphids, thrips	Mycotech, USA
Naturalis-L	*B. bassiana*	Cotton pests including boll-worms	Troy Biosciences, USA
Proecol	*B. bassiana*	Army worm	Probioagro, Venezuela
Boverin	*B. bassiana*	Colorado beetle	Former USSR
Boverol	*B. bassiana*	Colorado beetle	Czechoslovakia
Boverosil	*B. bassiana*	Colorado beetle	Czechoslovakia
Engerlingspilz	*Beauveria brongniartii*	Cockchafers	Andermatt, Switzerland
Schweizer	*B. brongniartii* *Beauveria* spp.	Cockchafers	Eric Schweizer, Switzerland
Melocont	*B. brongniartii*	Cockchafers	Kwizda, Austria
Green Muscle	*Metarhizium flavoviride*	Locusts, grasshoppers	CABI BioScience, UK
PFR-97	*Paecilomyces fumosoroseus*	Whitefly	ECO-tek, USA
Pae-Sin	*P. fumosoroseus*	Whitefly	Agrobionsa, Mexico
Laginex	*Lagenidium giganteum*	Mosquito larvae	AgraQuest, USA
Nematophagous fungi			
DiTera	*Myrothecium verrucaria*	Plant-parasitic nematodes	Valent (Sumitomo), USA, Japan
Product under development	*Duddingtonia flagrans*	Animal-parasitic nematodes	Christian Hansen, Denmark
Product under development	*Verticillium chlamydosporium*	Plant-parasitic nematodes	DeCeuster, Belgium

reduced. In order to satisfy this demand, biological control strategies, especially for the growing organic market, are urgently required. Several members of the European Union (EU) (Sweden, Denmark, the Netherlands) decided in the mid–late 1980s to decrease the chemical input in agriculture by 50% within a 10-year period, but these countries may be unable to meet their goals unless a greater impetus is given to the development of new, environmentally friendly pest and disease management strategies

Table 1.3. Fungal agents being developed or commercially available for the biological control of weeds (data from Templeton and Heiny, 1989; Butt *et al.*, 1999; Butt and Copping, 2000).

Product	Commercial name	Supplier or country where registered	Target weed
Acremonium diospyri		USA	Persimmon (*Diospyros virginiana*) in Oklahoma rangeland
Alternaria zinniae		Italy	Noogoora burr (*Xanthium occidentale*)
Alternaria eichhornia		India	Water hyacinth (*Eichhornia crassipes*)
Alternaria cassiae	Casst	USA	Sicklepod (*Cassia obtusifolia*) and coffee senna (*Cassia occidentalis*) in soybeans and groundnuts
Cercospora rodmanii	ABG 5003	Abbott Labs, USA	Water hyacinth (*Eichhornia crassipes*)
Colletotrichum coccodes	Velgo	USA, Canada	Velvet-leaf (*Abutilon theophrasti*) in maize and soybeans
Colletotrichum gloeosporioides f. sp. *cuscutae*	Luboa 2	PR China	*Cuscuta chinensis, Cuscuta australis* in soybeans
C. gloeosporioides f. sp. *malvae*	Biomal	Canada	Mallow (*Malva pusilla*) in wheat and lentils
C. gloeosporioides f. sp. *aeschynomene*	Collego	Encore Technologies, USA	Northern joint vetch (*Aeschynomene virginica*) in rice
Colletotrichum orbiculare		Australia	Spiny burr (*Xanthium spinosum*)
Chondrosterium purpureum	BioChon	Koppert, the Netherlands	Black cherry (*Prunus serotina*) in forestry in the Netherlands
Phytophthora palmivora	Devine	Sumitomo, Valent, USA	Milkweed vine (*Morrenia odorata*) in Florida citrus

(Matteson, 1995). Plant protection is at present partly trapped between the increasing number of prohibited chemical compounds and the lack of safe, efficient alternatives.

Some Benefits and Problems in Developing Fungal BCAs

Natural methods of pest, weed and disease control may be more labour-intensive and less efficient than chemical pesticides but they can lead to:

- job and wealth creation because of the numerous niche markets they would have to satisfy if chemicals were phased out altogether;
- more sustainable methods of crop production;
- more income for the grower because of the premium on pesticide-free and organic produce.

De novo development and implementation of alternative crop protection programmes will take time and investment as well as re-education, particularly of growers and extension service workers (see Chapter 14). Maintaining biodiversity is of paramount importance from an amenity (i.e. public interest in natural systems) and from a medical (i.e. potential source of useful medicines) point of view. Development of natural agents could have many spin-offs, such as the development of pharmaceu-

Table 1.4. Bioactive compounds of pharmaceutical importance

Compound	Source	Function
Swainsinone	*Metarhizium anisopliae*	Inhibitor of α-mannosidase II, inhibits metastasis and tumour growth
Cytochalasin C	*M. anisopliae*	Inhibits cytokines
SN-C (protein-bound polysaccharide)	*Cordyceps* (teleomorph of many entomogenous fungi)	Antitumour activity
Bassiatin	*Beauveria bassiana*	Platelet aggregation inhibitor
Viridiofungins	*Trichoderma viride*	Inhibitor of squalene synthetase
Zearalenone	*Fusarium* spp.	Oestrogenic
Cyclosporin	*Tolypocladium* spp.	Immunosuppressant

tical drugs, research tools and safer agrochemicals. Table 1.4 lists some compounds of medical interest isolated from fungal BCAs.

Unfortunately, there is relatively little investment in the research and development of microorganisms compared with that spent on the discovery of chemical pesticides (Whipps and Lumsden, 1989). Two reasons for this are that BCAs usually have a narrow host range and often give inconsistent and poor control in field trials. Consequently, more attention is being given to the selection of broad-spectrum BCAs and improvements in the production, formulation and application technologies (Butt *et al.*, 1999). Efforts are also being made to optimize the impact of these agents by integrating them with other novel crop protection strategies (Pickett *et al.*, 1995; see also Chapter 3).

The Commercial Perspective

One major factor to consider is the market potential of BCAs. Currently, only specialized, niche markets exist. Their full potential has not been realized because of the following:

1. Absence of strong incentives to develop these agents and/or discourage chemical pesticides.
2. Availability of new, biodegradable chemical pesticides.
3. Absence or breakdown of the infrastructure, which facilitates transfer of new technologies and research knowledge to the end-user (i.e. grower).
4. Absence of a universally acceptable registration procedure.
5. Restrictions in the use of exotic BCAs.
6. Lack of robust and reliable field effects.
7. Very few growers or extension workers know how to use BCAs.

Progress is also slow because the main producers of BCAs are often small–medium-size enterprises (SMEs), which have limited resources for the effective development and marketing of products. According to Lisansky (1999), some of the characteristics of successful companies are:

- Low production costs. This remains the key to cost-effective products and yet it attracts neither research money nor speculative investment. Cost-competitive products will succeed, sometimes even where control is imperfect.

- Good market research. This is essential because markets for BCAs are smaller and generally require more input than markets for chemicals. Companies must take a very precise look at their markets and know who will buy and use their products. Experience in agrochemicals is not sufficient nor is simple awareness of socio-economic trends, e.g. expansion of the organic farming sector and public sensitivity to health risks and environmental pollution.
- Corporate commitment. Good companies commit funds to ensure that a good, cost-effective product will reach the market, i.e. they do not enter the market half-heartedly. The commitment is not limited to sale of products but includes the follow-through to ensure that end-users will be successful when using BCAs.
- Good management. This is important to ensure that the company remains focused and does not diffuse its resources (i.e. spread the risk).

According to Lisansky (1999), companies can make three fundamental mistakes in their approach to BCAs:

- They believe what is said about BCAs in print, namely that they are easy, quick and cheap to make and are in great, yet untapped, demand.
- They overestimate their own capabilities, believing that they, unlike nearly 200 of their predecessors, will avoid the pitfalls and pick the winners.
- They under-budget in time and resources and try to succeed on the cheap.

Several fungal BCAs have been or are being developed as commercial BCAs, often with global markets in mind (see Tables 1.1–1.3). In order to survive, many SMEs market products of other companies or produce BCAs under licence. Presumably, this mutualism will decline as the use of BCAs increases (i.e. the market expands) and it becomes more lucrative for individual companies to develop their own agents.

The Search for and Development of Commercially Viable Fungal BCAs

This usually entails several steps:

1. Isolation of BCAs from the environment. Some methods for the isolation of fungi are given in Butt and Goettel (2000).
2. Studies to generate knowledge of the ecology, physiology and taxonomy of potential fungal BCAs. It is important to identify organisms as some resemble less desirable organisms, e.g. the entomogenous fungus *Metarhizium anisopliae* can be mistaken for *Aspergillus* species (cause of aspergillosis). This information is also essential to meet the registration requirements. Knowledge of BCA ecology can contribute to a better understanding of the effect of environmental factors on the survival and distribution of BCAs. This in turn can enable scientists to predict when to apply inoculum and/or promote habitats that encourage amplification of natural inoculum and the induction of epidemics/epizootics (see Chapters 2–6). Biochemical and molecular markers help to monitor pathogens in the field (Chapter 7). Such markers can also help to distinguish between exotic and native isolates, as well helping to elucidate how epidemics/epizootics develop in the field.
3. For effective pest, weed and disease control, laboratory and field bioassays will help identify the most antagonistic/virulent, ecologically fit strains. Dose–mortality

studies determine the minimum amount of inoculum required to cause disease in pests/weeds or to suppress plant pathogens. Such studies also indicate the time it will take for BCAs to have an impact on target organisms. Overall, ecological fitness is a fundamental requirement because of the relatively narrow window of environmental parameters, particularly relative humidity, over which many fungal BCAs are able to grow effectively in the natural environment. This should not be confused with biological fitness, which refers to the ability of the organism to reproduce successfully. It is possible that during culture in artificial media fungi lose properties that facilitate survival and infection in the field. Genetic manipulation, specific production systems and formulations may help overcome some of these problems (see Chapters 8–10).

4. Economical, mass production of stable inoculum is vital for the successful development of fungal BCAs. With entomogenous fungi, attenuation of virulence/ antagonism is a poorly understood phenomenon and research is under way at a number of universities to elucidate the underlying mechanisms. SMEs are investigating methods for reducing their production costs and increasing both the inoculum yield and the shelf-life (Chapters 9 and 10).

5. Formulation can improve the field efficacy of the pathogen by protecting against desiccation and harmful ultraviolet (UV) radiation. Some formulations can enhance fungal virulence by improving spore attachment to the host surface, diluting the fungistatic compounds in the epicuticular waxes and stimulating germination. Progress in this area is reviewed in Chapter 10.

6. Application strategies can have a profound impact on the efficacy of fungal BCAs but this has often been a neglected area. This is because pest, weed and disease control is dose-related. Not all the inoculum will infect the host; some is removed due to natural causes (e.g. during preening by the insect, shed during ecdysis), destroyed by UV irradiation or washed off by rain. Some of the strategies and tools for delivering the inoculum are dealt with in Chapter 11.

7. One of the big economic hurdles in the commercialization of fungal BCAs is in risk assessment. Risk assessment trials are essential for registration purposes. Fungal BCAs must be shown to be safe both to humans and to other non-target organisms. Besides the high cost of conducting the trials, additional technical protocols are often required by the registration authorities. All this takes a lot of time and is expensive, sometimes prohibitively so for SMEs. The cost of delay in getting a product to the market is often higher than one might imagine – in fact, it is the current discounted cash value of every single year's sales coming 1 year later than expected. Some companies have gone bankrupt waiting for sales to start. The toxins and safety of fungal BCAs are reviewed in Chapters 12 and 13, respectively.

8. Last, but not least, are the training processes whereby fungal BCAs are integrated into a unified crop protection programme that is easy to manage by the grower or other end-user. Integrated, sustainable crop and animal protection management programmes often require imagination and daring – for example, the use of bees that not only pollinate flowers but concomitantly spread beneficial fungi for pest and disease control (Vanneste, 1996; Butt *et al.*, 1998). Many challenges still remain to be dealt with at the technical, agronomic, socio-economic and political levels; these are discussed in the final chapter of this book with recommendations on how we can proceed to accelerate commercialization and effectively deploy fungal BCAs.

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