

FUNGAL BIOLOGICAL CONTROL AGENTS

Tariq M. Butt from the University of Wales, Swansea, and Leonard G. Copping, a pesticide consultant from Saffron Walden, discuss whether these agents have a future in crop protection

Agriculture in developed countries undergoes continuous change often based on new introductions, some based on customer preferences and some based on ethical considerations. This is particularly true of crop protection. The global consensus to reduce inputs of chemical pesticide which are perceived as being hazardous by some consumers, has provided opportunities for the development of novel, benign, sustainable crop protection strategies. A great many chemical pesticides have been or are being phased out (*e.g.* organochlorine insecticides, methyl bromide) either because of potential human health risks, environmental pollution, effects on non-target organisms or the development of pest resistance. Today's new chemical pesticides are significantly more benign than yesterday's products, but will these products ever receive registrations on small area crops or in subsistence agriculture? Are there any alternatives to chemical crop protection? There is no doubt that there is a need to develop alternative control systems in the new future and these must be implemented to replace or complement conventional pesticide usage.

Delegates who attended recent Symposia at the University of Southampton (5–9th April 1998) and University of Wales Swansea (24–28th August 1999) felt that biological control agents (BCAs), particularly fungal agents, offered considerable potential (generally unexploited) for insect, disease and weed control. These international meetings provided a platform for industrial and academic researchers, extension workers, registration bodies and end users to discuss key issues and to share their problems and expectations. There are a number of biological agents commercially available for use in crop protection, most notably products based upon *Bacillus thuringiensis*, but it was clear from the presentations that considerable progress has been made in the development of fungal BCAs in the last decade. Several agents are currently being used in niche markets and many others are at various stages of commercialisation (Table 1a, b, c). It is these fungal agents that will be discussed in this article.

What are the perceived benefits and problems in developing fungal BCAs?

Natural methods of pest, weed and disease control are considered to be more labour intensive and less efficient than chemical pesticides. However, BCAs do offer several benefits.

- BCAs are a natural resource
- Use of BCAs in integrated crop protection (ICP) programmes offers a more sustainable method of crop production (ICP strategies demand the application of crop protection agents only when needed and only in the quantity that is needed to give cost effective control. The aim is to use the most benign agents to protect the crop and BCAs fit well into such strategies.)
- There is a high grower and retailer premium for pesticide free or “organic” produce
- Their reduced environmental impact and, therefore, increased biodiversity because BCAs are generally more target specific than chemicals.

Development and implementation of *de novo* crop protection programmes will take time and considerable investment. A key component is the education of growers and extension service workers on how best to use these new tools. But who should be responsible for the education programme? In the past, extension services ensured successful uptake of new, innovative crop protection strategies but these services have shrunk, are now often over-stretched and costly. This has not benefited small to medium size enterprises (SMEs) and research groups that often operate with limited resources.

There is little investment in the research and development of fungal BCAs compared with that spent on the discovery of chemical pesticides. Two reasons for this are that mycopesticides usually have a narrow host range and because they have given inconsistent or poor control in field trials. This has led to a greater emphasis on the search for broader spectrum biopesticides with improvements in the associated production, formulation and application technologies (Butt *et al.*, 1999; Copping, 1996; Copping, 1998; Copping and Menn, 2000). It must be remembered, however, that the advantage of extreme selectivity is lost if fungi are discovered that have a wider spectrum of biological effect and the environmental advantage may well be forfeited in the name of broad-spectrum control. Efforts are also being made to optimise the impact of these agents by integrating them with other novel crop protection strategies (Pickett *et al.*, 1995).

Technical issues

The key technical issues that need to be dealt with in the development of efficacious fungal BCAs include:

Table 1a. Fungi developed or being developed for the biological control of diseases (Burges, 1998; Butt et al., 1999, 2001).

Product	Fungus	Target	Producer
Mycoparasites			
Rotstop	<i>Phlebiopsis (=Peniophora) gigantea</i>	<i>Heterobasidium annosus</i>	Kemira Agro Oy, Finland
Primastop	<i>Gliocladium catenulatum</i>	Several plant diseases	Kemira, Agro Oy, Finland
SoilGard (= GlioGard)	<i>Gliocladium virens</i>	Several plant diseases Damping off & root pathogens	ThermoTrilogy, USA
Cotans WG	<i>Coniothyrium minitans</i>	<i>Sclerotinia</i> species	Prophyta, Germany. KONI, Germany
AQ10 Biofungicide	<i>Ampelomyces quisqualis</i>	Powdery mildews	Ecogen Inc.USA
YIELDPLUS	<i>Cryptococcus albidus</i>	<i>Botrytis</i> spp., <i>Penicillium</i> spp	Anchor Yeast, S. Africa
Aspire	<i>Candida oleophila</i>	<i>Botrytis</i> spp., <i>Penicillium</i> spp.	Ecogen Inc.USA
Fusaclean	<i>Fusarium oxysporium</i>	<i>Fusarium oxysporium</i>	Natural Plant Protection, France
Biofox C	<i>Fusarium oxysporium</i>	<i>Fusarium oxysporium</i> , <i>F. moniliforme</i>	SIAPA, Italy
Polygandron Polyversum	<i>Pythium oligandrum</i>	<i>Pythium ultimum</i>	Plant Protection Institute, Slovak Republic
Trichoderma 2000	<i>Trichoderma harzianum</i>	<i>Rhizoctonia solani</i> , <i>Sclerotium rolfsii</i> , <i>Pythium</i>	Mycontrol (Efa1)Ltd, Israel
Trichopel	<i>Trichoderma harzianum</i>	Wide range of fungal diseases	Agrimm Technologies Ltd, New Zealand
T-22 and T-22HB Bio-Trek, RootShield	<i>Trichoderma harzianum</i>	<i>Pythium</i> , <i>Rhizoctonia</i> , <i>Fusarium</i> , <i>Sclerotinia</i>	BioWorks (=TGT Inc) Geneva, USA
Trichodowels, Trichobject, Trichoseal & others	" & <i>T. viride</i>	<i>Chondrostereum purpureum</i> & other soil & foliar pathogens	Agrimms Biologicals, New Zealand & others
Binab T	<i>Trichoderma harzianum</i> , <i>T. polysporum</i>	Fungi causing Wilt, wood decay and take all	Bio-Innovation, Sweden
Trichodex	<i>Trichoderma harzianum</i>	Fungal diseases; e.g. <i>Botrytis cinerea</i>	Makhteshim-Agan, Several European companies e.g. DeCeuster, Belgium.

Speed of action

One major criticism of fungal BCAs is that they act slowly and, therefore, give limited protection to crops. Clearly, more aggressive strains of fungal BCAs can be sought i.e. those which work more quickly and require a lower inoculum. Factors that determine pathogen virulence (virulence determinants) should be identified and used in strain selection and quality control. Fortunately, some progress has been made in this area with enzymes and metabolites having been identified which are important virulence or antagonistic determinants.

Greater ecological fitness

All fungi perform well in the laboratory but most are less effective in the field. Fungal BCAs must be "primed" for field conditions, i.e. strains must tolerate a wide range of climatic (fluctuating temperatures, humidities, UV light), edaphic (soil types) and biotic (antagonists) factors. The ecological fitness of strains can be improved through physiological manipulation of endogenous reserves and use of

appropriate formulations. However, if a fungal product works quickly then the manipulation of the growing environment to provide the necessary humidity and temperature for the fungus to work may be possible particularly in protected crops. It is important, however, to ensure that this period is short enough not to compromise any disease control strategy (i.e. not encourage diseases like *Botrytis cinerea*)

Production

- production costs must be reduced so the end product competes with conventional pesticides on cost grounds (it is true that in countries such as the USA registration of biologicals is a shorter, less expensive process allowing the economic development of an effective product for use in a small niche market)
- virulence and ecological fitness must be retained or improved
- products must be easy to handle and package
- products must have a shelf life that is acceptable to the user.

Virulence

Fungi often become attenuated (lose virulence or antagonistic characteristics) when maintained on artificial media. Cultural conditions must be identified which retain virulence without increasing production costs. At present little progress has been made in this area partly because the underlying mechanisms for attenuation have not been elucidated.

Formulation

For any crop protection agent, an efficient formulation is essential to translate laboratory activity into adequate field performance. Little progress has been made in this area despite the fact that formulations will improve the field efficacy of fungal BCAs and expand their market opportunities beyond high value, niche markets. New, more effective formulation components must be found (e.g. UV protectants, humectants, carriers, virulence-enhancing factors and “stickers”). In all cases, it is essential that these formulants be compatible with other BCAs (viruses, bacteria and entomophilic nematodes) which may be sprayed at the same time as the fungal BCA. Green Muscle (*Metarhizium anisopliae* var *acridium*) formulated in oil and applied at very low volumes is a good example of a fungal BCA that uses innovative formulation characteristics to enhance its effectiveness against locusts (Bateman and Luke, 2000).

Application

BCA products should be capable of application through standard hydraulic nozzles or application equipment with few special application requirements. Growers will not readily invest in new spray equipment to apply a BCA, nor will they accept a very different spray regime or more frequent applications than is normal practice

Improved targeting

Cost-effective control of pests, weeds and diseases demands efficient targeting of the BCA. Recently it has been shown that honey bee-mediated delivery of the insect pathogen, *Metarhizium anisopliae*, increased pollen beetle control (*Meligethes* spp) in oilseed rape (Butt *et al.*, 1998). The bees were more efficient than conventional sprayers in delivering the inoculum to the pest infested flowers. There is also evidence that the use of systems that attract insects to a trap that contains a fungal entomopathogen where they are contaminated allowing them to take the BCA to other members of the species is also showing promise particularly for communal insects. “Push-pull” pest control strategies entail insect pests being driven out of the cash crop with the application of feeding deterrents and being drawn into a trap crop where they are controlled by inundation with pathogens or other benign control agents. To encourage pests into the trap crop, lures such as favoured plant

Table 1b. Fungi developed or being developed for the biological control of pests (Burges, 1998; Butt *et al.*, 1999, 2001).

Product	Fungus	Target	Producer
Entomogenous Fungi			
Mycotal	<i>Verticillium lecanii</i>	Whitefly and thrips	Koppert, The Netherlands
Vertalec	<i>Verticillium lecanii</i>	Aphids	Koppert, The Netherlands
BIO 1020 (withdrawn ?)	<i>Metarhizium anisopliae</i>	Vine weevil	Licensed to Taensa, USA
Biogreen	<i>Metarhizium anisopliae</i>	scarab larvae on pasture	Bio-care Technology, Australia
Metaquino	<i>Metarhizium anisopliae</i>	spittle bugs	Brazil
Bio-Path	<i>Metarhizium anisopliae</i>	cockroaches	EcoScience, USA
Bio-Blast	<i>Metarhizium anisopliae</i>	termites	Ecoscience, USA
Cobican	<i>Metarhizium anisopliae</i>	Sugarcane spittle bug	Probioagro, Venezuela
Conidia	<i>Beauveria bassiana</i>	Coffee berry borer	Live Systems Technology, Colombia
Ostrinil	<i>Beauveria bassiana</i>	Corn borer	Natural Plant Protection (NPP), France
CornGuard	<i>Beauveria bassiana</i>	European corn borer	Mycotech, USA
Mycotrol GH	<i>Beauveria bassiana</i>	grasshoppers, locusts	Mycotech, USA
Mycotrol WP & BotaniGard	<i>Beauveria bassiana</i>	whitefly, aphids, thrips	Mycotech, USA
Naturalis-L	<i>Beauveria bassiana</i>	cotton pests including bollworms	Troy Biosciences, USA
Proecol	<i>Beauveria bassiana</i>	Army worm	Probioagro, Venezuela
Boverin	<i>Beauveria bassiana</i>	Colorado beetle	former USSR
Boverol	<i>Beauveria bassiana</i>	Colorado beetle	Czechoslovakia
Boverosil	<i>Beauveria bassiana</i>	Colorado beetle	Czechoslovakia
Engerlingspilz	<i>Beauveria brongniartii</i>	Cockchafer	Andermatt, Switzerland
Schweizer Beauveria	<i>Beauveria brongniartii</i>	Cockchafers	Eric Schweizer, Switzerland
Melocont	<i>Beauveria brongniartii</i>	Cockchafers	Kwizda, Austria
Green Muscle	<i>Metarhizium flavoviride</i>	Locusts, grasshoppers	CABI – BioScience, UK
PFR-97	<i>Paecilomyces fumosoroseus</i>	whitefly	ECO-tek, USA
Pae-Sin	<i>Paecilomyces fumosoroseus</i>	Whitefly	Agrobionsa, Mexico
Laginex	<i>Lagenidium giganteum</i>	Mosquito larvae	AgraQuest, USA

Table 1c. Fungi being developed or commercially available for the biological control of weeds (Butt et al., 1999, 2001; Templeton & Heiny, 1994).

Product	Commercial Name	Supplier or Country Where Registered	Target Weed
<i>Alternaria cassiae</i>	Casst	USA	sicklepod (<i>Cassia obtusifolia</i>) and coffee senna (<i>C. occidentalis</i>) in soybeans and peanuts
<i>Cercospora rodmanii</i>	'ABG 5003'	Abbott Labs, USA	Water hyacinth (<i>Eichhornia crassipes</i>)
<i>Colletotrichum coccodes</i>	Velgo	USA, Canada	Velvetleaf (<i>Abutilon theophrasti</i>) in corn and soybeans
<i>Colletotrichum gloeosporioides</i> f. sp. <i>cuscutae</i>	Luboa 2	PR China	<i>Cuscuta chinensis</i> , <i>C. australis</i> in soybeans
<i>Colletotrichum gloeosporioides</i> f. sp. <i>Malvae</i>	Biomal	Canada	Mallow (<i>Malva pusilla</i>) in wheat and lentils
<i>Colletotrichum gloeosporioides</i> f. sp. <i>aeschynomene</i>	Collego	Encore Technologies, USA	Northern jointvetch (<i>Aeschynomene virginica</i>) in rice
<i>Chondrostereum purpureum</i>	BioChon	Koppert, The Netherlands	Black cherry (<i>Prunus serotina</i>) in forestry in The Netherlands
<i>Phytophthora palmivora</i>	Devine	Sumitomo, Valent, USA	Milkweed vine (<i>Morrenia odorata</i>) in Florida citrus

varieties (*i.e.* those more attractive than the crop) and chemical attractants (sex pheromones and gustatory stimulants) would be used. Feeding attractants incorporated into formulations may be useful to encourage insects to feed on BCAs. As yet, very few inexpensive but effective lures and deterrents have been developed for commercial use.

Packaging and storage

Both farmer and distributor will be deterred from using a novel BCA if the shelf life, storage requirements and packaging are different from those of conventional chemicals. Some BCAs have a specific need for refrigeration, but very few distributors in Europe have such facilities and even fewer would be prepared to invest in them although some specialist distributors for certain macro-organism bio-control agents (predators and parasites) already have such refrigerated storage and distribution facilities world-wide.

Bioactive compounds

Many fungi produce biologically active secondary metabolites, some of which are very toxic and this is a major concern with all fungal BCAs as their presence would represent a health risk. Very little is known about many of these compounds and the following areas need urgent investigation:

- **Screening for bioactive compounds from fungal BCAs.** Techniques must be developed for the identification of toxin producers and the selection of strains that are good crop protection agents but not toxin producers. The methodologies and tools developed would help detect toxins in foodstuffs and the environment (target and non-target hosts, plant, soil, water).
- **Determining the role of bioactive compounds.** Are they pathogenicity determinants? Do they help in the survival of the BCA? Are they waste products?
- **The mode of action of bioactive compounds.** Are they i) a risk to living systems and ii) do they have any commercial

value as pharmaceuticals, agrochemicals or research tools?

Safety

Safety is a major concern for all crop protection products and more studies are needed to evaluate the risks involved in the use of fungal BCAs. The focus should be on:

- allergic properties
- risks of toxic metabolites
- genetic recombination and displacement of natural strains
- effect on biodiversity (*i.e.* impact on non-target organisms)

These data would be useful for registration purposes and could reduce development costs considerably.

The industrial perspective

The market potential of biopesticides is key to their success and currently they are only used in specialised, niche markets. There are many reasons for this restricted adoption of BCAs in crop protection:

- There are no strong incentives to develop these agents and/or discourage chemical pesticides. The supermarket code of practice and the possible introduction of pesticide taxes may be key drivers for BCA use.
- Newly introduced chemical pesticides have good environmental profiles with few side effects and low persistence.
- There is no universally acceptable registration procedure
- The use of exotic BCAs is restricted
- Field performance is unreliable and unpredictable. In part this can be attributed to insufficient information for growers and poor storage and distribution conditions.
- The infrastructure which facilitates transfer of new technologies and research knowledge to the "end user" (*i.e.* grower) is either absent or has broken down

Product introduction is also slow because the main producers are often small to medium size enterprises (SMEs) with limited resources for effective development and marketing. Some of the characteristics of successful companies according to Lisansky (1999) are:

Low production costs

This remains the key to cost-effective products 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 biologicals 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 cognisance of socio-economic trends (e.g. expansion of organic farming sector and public sensitivity to health risks and environmental pollution).

Corporate commitment

Good companies generate funds to ensure that a good, cost-effective product will reach the market; i.e. they do not enter the scene half-heartedly. The commitment is not limited to sale of products but the follow through to ensure that end-users feel comfortable in using BCAs.

Good management

This is also important to ensure that the company remains focused and does not diffuse its resources (i.e. spread the risk).

Interested parties must co-operate even more

The successful deployment of fungal BCAs depends on close co-operation between all interested parties. This includes researchers developing BCAs, manufacturers who will produce the agent, growers who wish to use the BCAs and government agencies who often fund the research. The latter undoubtedly are central to the success of fungal BCAs. The other players believe governments could:

- Strengthen extension services to accelerate “technology transfer” from research institutes/industry to the grower.
- Streamline or refine policies and/or procedures to reduce product development time and/or costs e.g. reform registration procedures, and
- Support research which bridges theory and practice

More and more SMEs are taking the lead in reaching out to “end users”. Besides providing technical support to growers they are using all channels of communication e.g. providing information on product use *via* websites, publishing literature on BCAs and specialists (Butt, Goettel and Papierok, 1999). Several SMEs are working closely to reduce the time and cost to develop new BCAs, to increase market size and to reduce distribution costs. A good example of co-operation is illustrated by the EU-funded

BIPESCO project (FAIR6-CT98-4105). Four companies, Prophyta (Germany), Agrifutur (Italy), Kwizda (Austria) and Eric Schweizer (Switzerland) are working closely to develop fungal BCAs for control of subterranean pests such as cockchafer and vine weevil grubs. Although each company has fungal products on the market, they are using their complementary expertise to reduce production costs and improve formulation, shelf life and application strategies. For more details on this project see <http://bipesco.uibk.ac.at>.

One of the major potential markets for fungal BCAs is the organic farming sector although it must be remembered that true organic farming argues against any input at all and the energy inputs associated with fermentation is considerable. Nevertheless, the Soil Association does allow the application of *Bacillus thuringiensis* as a ‘natural’ insecticide. According to Nic Lampkin (University of Wales Aberystwyth), organic farming accounts for nearly 125,000 holdings and 3 million hectares in Western Europe. He further argues that, although this represents only 2% of the agricultural land area, continued growth at 25% per annum could lead to 10% of EU agriculture being organic by 2005. Given the restrictions on the use of pesticides, genetically modified crops and animal health products in organic farming and the emphasis on the use of renewable resources, BCAs have a significant role to play in organic farming. However, it soon became clear at the International Symposium held at the University of Wales Swansea (August 1999) that there were several camps in the organic farming community. Whereas some growers readily accepted BCAs others had reservations or felt that they should be used as a last resort. Representatives from various organic farming organisations felt that there were risks associated with strategies that apply BCAs simply as a substitute for conventional inputs. They felt that BCAs in organic farming need to be seen in the context of multi-strategy approaches to controlling weeds, pests and diseases, which build on the use of preventive, ecosystem management practices and the encouragement of naturally occurring predators and other beneficial organisms.

Overseas growers or grower organisations are also becoming interested in novel pest control strategies. Recently, the senior author was invited by the Kenya Flower Council to address growers about the use of fungi for the control of insect pests. Although some growers had reservations, the majority of growers were very interested in fungal BCAs and recognised the need to reduce the use of pesticide chemical inputs. Certain growers, such as HomeGrown (Kenya) Ltd, had already taken steps to reduce pesticide use. They also appointed an Integrated Crop Management manager, Louise Labuschagne, to oversee this.

Some work has begun to investigate the use of BCAs in conjunction with low dose chemical applications. There is compelling evidence that such strategies do, in fact, reduce chemical inputs but also achieve effective pest control. This is particularly true of low doses of herbicides in combination with myco-herbicides. Regrettably this work is not often supported by the chemical manufacturer and development by academics is slow.

It must be remembered that it may well be that

application systems other than conventional spraying may be a more effective method of delivering BCAs. The use of baits to attract insect pests has been touched upon as one alternative. In addition, it is possible to manipulate the environment around the crop plant such that the fungal pesticide thrives and protects the crop plant throughout its life. Such practice demands detailed knowledge of the crop, the BCA and the interactions between the two but clearly offers advantages in delivery and long-term pest control. Much of the discussion has concentrated on conventional application techniques but the future may well be different and it is possible that the success of BCAs will be dependent upon such innovative approaches.

In conclusion, there are numerous niche markets for fungal BCAs. However, they have to be integrated with complementary control strategies (e.g. use of baits to attract pests to the control agent). Scientists will have to be a lot more imaginative in developing strategies which optimise the impact of fungal BCAs.

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Dr. Tariq Butt has been interested in the development of fungi as biological control agents for over 20 years. He has worked for the USDA and IACR-Rothamsted and recently accepted a lectureship at the University of Wales Swansea where he continues his research on improving the production, formulation and application of fungi for pest control. He has also been investigating ways of integrating fungi with other pest control strategies to reduce pesticide inputs. He has developed species specific molecular markers to monitor fungi in the field, and unravelled some of the mysteries surrounding attenuation of virulence in fungi. Tariq has also been studying the bioactive metabolites produced by fungal biocontrol agents and assessing the risks they pose to human and animal health. He has published over 50 refereed articles in international journals and several book chapters.

Leonard G Copping worked in Discovery Research in the agrochemical industry for twenty years before establishing himself as a technical editor, consultant and author. He has become increasingly interested in alternative methods of pest, disease and weed control embracing the use of natural products, molecular biology and micro- and macro-organisms. He is currently editor of *Focus on BioPesticidesPLUS* for the RSC and the *BioPesticide Manual* for BCPC and sits on the editorial boards of *Pest Management Science* and *Pesticide Outlook*.

BIOACTIVE FUNGAL METABOLITES: IMPACT AND EXPLOITATION

University of Wales Swansea, 22-27 April 2001

This 5-day International Symposium sponsored by the British Mycological Society will cover the whole range of activities of bioactive fungal metabolites:

Sessions include:

- Overview
- Fungi as a source of novel bioactive compounds
- Tools and methods to study fungal metabolites
- Optimising the production of bioactive metabolites
- Mycotoxins
- Risk assessment for Fungal Biological Control Agents
- Network discussions

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