14 Fungal Biological Control Agents – Appraisal and Recommendations

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It is clear from an analysis and synthesis of the preceding chapters that considerable progress has been made in the development of fungal biological control agents (BCAs). This has been achieved in part through multidisciplinary approaches, often involving multinational research groups. Although niche markets exist for fungal BCAs, considerable progress still needs to be made in the following areas:

- Technical – production, formulation and application systems.
- Agronomic – integration of BCAs into cropping systems.
- Socio-economic – public perception, economic feasibility.
- Political – improved registration procedures, improved extension services, support for small–medium-size enterprises (SMEs) and organic growers.

In this chapter we summarize our analysis of the preceding chapters, briefly discuss the technical and agronomic issues, including the perception of SMEs, and, where appropriate, make recommendations. The socio-economic and political issues are outside the scope of this book. Some of these issues have recently been reviewed by Butt and Copping (2000). It should be stressed that all these issues have to be put in perspective: if there is no market, the issues become irrelevant. Numerous niche markets do exist for fungal BCAs. The estimated world market for pesticides in 1995 was *c.* $29 billion, with the biopesticide share being *c.* $380 million (Menn and Hall, 1999). The growth rate for biopesticides is expected to increase over the next 10 years, with fungal BCAs probably having a substantial share of this market (Moore and Prior, 1993).

Technical Issues

The following need to be dealt with for the development of more efficacious fungal BCAs.

Improved speed of action of the fungal BCA

One major criticism of fungal BCAs is that they act slowly and give limited protection to crops from pests and diseases. For effective pest control, more aggressive strains of fungal BCAs should be sought, i.e which work more quickly and require less inoculum. Virulence determinants should be identified and used in strain selection and quality control. Some progress has been made in this area; enzymes and metabolites have been identified which are important determinants of virulence or antagonism (e.g. Butt *et al.*, 1998; Amiri-Besheli *et al.*, 2000; Bandani *et al.*, 2000, see also Chapter 8). For disease control, priming of inocula, particularly of spores, needs to receive more attention, especially with regard to effective control in the rhizosphere and phyllosphere.

Greater ecological fitness

Many fungi that perform well in the laboratory are less effective in the field. The quality of fungal BCAs prior to use has received very little attention. For effective field performance, the formulations must consistently be able to tolerate a wide range of climatic (fluctuating temperatures, humidities, ultraviolet (UV) light), edaphic (soil types) and biotic (antagonists) factors. The ecological fitness of strains can be improved through physiological manipulation of growth conditions to channel useful endogenous reserves into the inoculum (Magan, Chapter 9). The physiologically modified inoculum will germinate at humidities slightly lower than unmodified inoculum. This, combined with improved formulations, could greatly improve the field efficacy of fungal BCAs.

Cost-effective production of fungal BCAs

Production costs need to be reduced to allow competitive pricing with conventional pesticides. The products must remain viable for up to 2 years under commercial storage conditions and maintain biocontrol efficacy. The end-product should be easy to handle and package. Cultural conditions must be identified, without increasing production costs, to overcome problems of loss of biocontrol. For pest control, particular attention needs to be paid to problems of attenuation of virulence. At present, little progress has been made in this area, partly because the underlying mechanisms of attenuation have not been elucidated.

Improved formulation of fungal BCAs

Good results are often obtained in trials carried out with fresh inoculum but when the inocula have been formulated control is often less effective. For any crop protection agent, efficient formulation is necessary to translate laboratory activity into adequate field performance (Burges, 1998; see also Chapter 10). In many cases the formulation of BCAs is very different from that of chemicals. This has not often been appreciated and has resulted in less progress being achieved in this area. For BCAs, different methods of preparation, from wet pastes to fluidized-bed drying, spray drying and freeze-drying, have not been investigated exhaustively. During this process, additives can be incorporated to enable viability to be conserved. Some progress has been made, but it is evident that investment is needed to improve field efficacy of fungal BCAs and expand their niche (and ultimately market). While experience exists with pesticide formulations, less knowledge is available in relation to biological material. Furthermore, formulation with appropriate targeting and spraying systems could further improve the consistency and efficacy of BCAs (see Chapters 10 and 11). New, more effective formulation additives and appropriate stickers need to be investigated that are compatible with other BCAs (viruses, bacteria and entomophilic nematodes). The net result will be that the farmer can integrate BCA products in spray regimes in a single tank mix or at different times without one formulation/BCA affecting the other, and concomitantly reducing pesticide use.

Effective targeting of fungal BCAs

This is needed for the efficacious control of pests, weeds and diseases. Because control is dose-related, strategies need to be devised to ensure that sufficient amounts of the pathogen contact the target. Autodissemination/autoinoculation devices are being tested (Pell *et al.*, 1993; Vega *et al.*, 2000); insects are attracted to a device by specific olfactory and visual cues, they become contaminated with a BCA and, on leaving the device, they then act as vectors of the BCA, which could be for the control of pests, weeds or diseases. Recently, honey-bee-mediated dissemination of BCAs has been patented (Gross *et al.*, 1994); the bees can be more efficient than conventional sprayers in delivering the inoculum to the pest-infested flowers – trials with the insect pathogen *Metarhizium anisopliae* increased pollen beetle (*Meligethes* spp.) control in oil-seed rape (Butt *et al.*, 1998). To optimize the impact of the BCA, particularly for pest control, it could be used in the 'push–pull' pest control strategy. Briefly, this entails insect pests being driven ('pushed') out of the main crop with feeding deterrents and drawn ('pulled') into a trap crop, where they could be controlled by inundation with pathogens. To encourage pests into the trap crop, lures, such as favoured plant varieties that are more attractive than the crop and chemical attractants such as sex pheromones and gustatory stimulants, could be used. Feeding attractants incorporated into the formulation may be useful for encouraging insects to feed on the BCA. As yet, very few inexpensive, effective lures and deterrents have been developed for commercial use. Recently, Amiri *et al.* (1999) demonstrated that antifeedants can drive pests to the underside of leaves treated with *M. anisopliae*. The abaxial surface of leaves was a conducive environment for fungal infection of mustard-beetle larvae. Ideally, BCA products should be capable of application through the standard hydraulic sprayer or application equipment that is common in a particular market and should have as few unique requirements as possible. Growers are unlikely to invest in new spray equipment solely to apply a BCA, nor are they going to accept a very different spray regime or more frequent applications than is normal practice. Therefore, knowledge is required of how well formulations of BCAs survive such applications and how, using standard application techniques, targeting to prevent wastage can be included as part of the strategy for delivery of BCAs for effective control.

Improved packaging, shelf-life and sales

The formulation type and packaging materials must be similar to those with which the grower is already familiar. The grower will also want to purchase his/her BCAs through the same distribution chain as his/her agrochemicals. Distributors will want to handle BCA formulations in the same way as their normal chemical stock, and expect the product to be packaged in standard sizes and types of containers as used throughout the agrochemical industry. Storage stability must be such that product purchased at the start of one season is good for the whole of that season and the next, without any special storage requirements. If the shelf-life of a BCA formulation is very limited, a distributor may be prepared to buy only small quantities, thus limiting availability, or will only stock products on a consignment basis, which is a major inconvenience to the BCA supplier. Furthermore, some BCAs have a specific need for refrigeration for conserving viability; few distributors in Europe have such facilities and even fewer would be prepared to invest in them.

Understanding tritrophic interactions

There is growing evidence that the host plant can affect the efficacy of BCAs through dilution of the inoculum during growth, physical interference of the inoculum (trapping spores in epicuticular waxes) and via exudates and allelochemicals (Inyang *et al.*, 1998, 1999a, b). Some plants when ingested by insects can increase the susceptibility of insects to fungal infection (T.M. Butt, unpublished observations; see also Chapter 3). The exact mechanisms for this are unknown. This is an important area for future research.

Population dynamics

Although information has been generated on selected pests, weeds and diseases, comparatively little work has been done on the environmental fate of fungal BCAs and their impact on target organisms and plants. There is an urgent need to study the relationships between entomogenous fungi and their host(s) in the field and to identify vulnerable stages of the target. This could optimize the impact of BCAs. Studies on population dynamics could also provide invaluable information on fungal persistence in the environment, the fate of the inoculum and any genetic shift due to parasexual or sexual recombination (Leal-Bertioli *et al.*, 2000). Indeed, registration documentation requires information on the environmental fate of and potential side-effects in non-target fauna and flora. Besides models, there is a need to develop molecular markers to characterize fungal strains (Leal *et al.*, 1994; Driver *et al.*, 2000). The models could be useful in the study of epizootics/epidemics and lead to more effective deployment of fungal BCAs. When combined with safety studies (i.e. impact on non-target organisms), they could lead to timely applications of the pathogen to minimize any negative impact on beneficial non-target organisms, thus optimizing the impact of each component. Furthermore, these studies would reveal if pests could develop resistance to fungal BCAs. Evidence is growing that insects possess potent antifungal peptides, which could also be specificity determinants (Ekengren and Hultmark, 1999; T.M. Butt, unpublished observations).

Bioactive compounds

Bioactive compounds of BCAs, sometimes referred to as toxins, are a major concern, because some people believe they are a health risk (Strasser *et al.*, 2000). Very little is known about these compounds. The following topics need urgent study:

1. Development of the methods and tools to screen for bioactive compounds from fungal BCAs. This would facilitate rapid screening of toxins from BCAs and identification of strains that are efficacious BCAs but low toxin producers. The methodologies and tools developed could also help detect toxins in foodstuffs and the environment (target and non-target hosts, plant, soil, water).
2. Studies to determine the role of bioactive compounds. Are they pathogenicity determinants? Do they help in the survival of the BCA? Are they waste products?
3. Studies of the mode of action of bioactive compounds to see: (i) if they pose a risk to living systems; and (ii) if they have any commercial value as pharmaceutical drugs, agrochemicals or research tools.

Safety

Safety is a major concern of all parties developing fungal BCAs. More studies are needed to evaluate the risks involved in the use of fungal BCAs, and particular attention should focus on: (i) allergenic properties; (ii) risks of toxic metabolites; (iii) genetic recombination and displacement of natural strains; and (iv) effect on biodiversity (i.e. impact on non-target organisms). These data would be useful for registration purposes and could reduce development costs considerably.

Agronomic Issues

There are still many agronomic issues that need to be resolved, a few of which are discussed below.

Compatibility of fungal BCAs with other BCAs and agrochemicals

This needs investigation so that growers know which agents can be used in the same tank mix. For example, entomogenous fungi may be harmful to entomophilic nematodes, or fungicides used for disease control may kill entomogenous fungi. Industry should work closely with researchers and extension service workers in resolving these matters before the products reach the market.

Development of crop protection strategies

The problem faced by developers of fungal BCAs for the control of disease is complex and difficult. Crops are grown under a variety of climatic and environmental conditions, and temperature, rainfall, soil type, crop variety and pathogen can change from farm to farm or some even within one field. The producer of a crop protection product has to be able to give some assurance to the farmer that the product will be robust in order for the product to be used. The availability of effective chemical controls for foliar pathogens has made it unlikely that a biological agent will compete effectively. It is therefore not surprising that the majority of effort in research has been concentrated on soil-borne or postharvest diseases. Even in these situations, the lack of robustness has limited the penetration of such products.

Natural plant resistance and transgenic plants

There is still considerable scope for exploiting plant natural resistance to pests and diseases and integrating this into an overall sustainable crop protection strategy. However, the widening opportunities offered by genetic modification have influenced research and development quite dramatically during the past decade. Large companies are more likely to support this area because of the intellectual property rights (IPR) afforded by producing unique products. Widespread use of transgenic plants is also seen as an opportunity and a potentially major constraint, through engineering for both herbicide tolerance and for inclusion of insecticidal activity. Both have potential, either indirectly, by removal of non-crop plants through widespread herbicide usage, or directly, by continuous expression of insecticidal genes, to induce resistance in target organisms. There may also be potential effects from reducing the effectiveness of more conventionally applied microbial agents. It is important that risk assessment of these new agents is improved, supported by ecological research to assess both the beneficial and potentially non-beneficial effects of the new technology.

Organic farming

Organic farming currently forms a minor part of European agriculture but is rapidly expanding. In some countries, it has gained acceptance on up to 8% of farms. Recent emphasis in policy towards more environmentally friendly farming practices and the importance of surplus reduction has led to more attention to organic farming and the establishment of specific policy provisions, e.g. European Community (EC) regulation 2078/92 (agricultural production methods compatible with the requirements of the protection of the environment and the maintenance of the countryside) and EC regulation 2092/91 (certification of organic food). The premium prices for organically grown produce offer some incentive to farmers to convert to organic farming. But there is an urgent need for more investment in this area, particularly in topics that would benefit both organic and conventional growers, e.g. funding of projects that integrate agronomic practices used by organic growers with natural agents for increased productivity. Another area for collaboration is in the development of tools and strategies to monitor pest influxes (e.g. traps or trap crops) and optimize the impact of artificially introduced BCAs and natural predators and parasitoids (e.g. application of BCAs at night, when conditions favour these organisms but minimize risk to parasitoids).

Perception of SMEs

Large commercial companies have relatively little interest in wild-type microbial agents, because these have more limited markets than chemical pesticides and, even where markets have been established, they may not be able to protect their IPR sufficiently to make the initial investment. These conclusions point to opportunities for exploitation in markets where there are problems of resistance to conventional pesticides or in niche markets where there are no viable conventional alternatives.

There is a lack of international harmonization in the registration process for microbials, which drives up costs. Although the toxicological criteria for safety are similar in most countries (usually a tier testing system), there is little international harmony in the methodology, interpretation and presentation of the dossiers. This variation makes costs higher for SMEs, while having less effect on larger companies with an established presence in many countries (Lisansky, 1999). Clearly, this must be redressed before significant progress can be made.

A common misconception is the widespread tendency to classify fungal BCAs as one-to-one substitutes for chemical pesticides. This may be true for some, but more attention should be paid to the biological and ecological characteristics of BCAs. In other words, the chemical paradigm must be replaced by a biological paradigm, constructed on the innate characteristics of the microbial agents and not trying to circumvent them by making them chemical analogues. In essence, more account should be taken of the strengths of microbial agents, rather than forcing them into a standard of assessment that, within the chemical paradigm, emphasizes some particular weaknesses relative to chemical insecticides. Furthermore, it must be remembered that there are common hurdles that need to be overcome in the successful development of BCAs for fungal disease, pest and weed control.

Finally, if there were to be any key messages to convey to government bodies regarding fungal BCAs, these would be for governments to:

- strengthen extension services to accelerate technology transfer from research institutes and industry to the grower;
- streamline or refine policies and/or procedures to reduce product development time and/or costs, e.g. harmonize registration procedures;
- support interphase research that bridges theory and practice.

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