Development and commercialization of biocontrol agents

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Summary

The development of biological control agents into commercial products requires business decisions as well as technical developments. Most of the business decisions relating to the market place are similar to those for conventional products. The need to protect intellectual property which is in the public domain, through patenting is considered. Development from pilot scale to commercial scale and downstream processing are discussed. Improved formulation which may overcome environmental constraints, and reduce end use concentration requirements of biocontrol organisms, appears to be the area of greatest need for development.

Introduction

Classical biocontrol agents become freely available, once introduced. Successful classical biocontrol agents spread and locate their target. There is little scope for involvement of private firms in their production and distribution.

Inundative biocontrol agents are, by definition, applied at high concentration, and although they may occur naturally in the environment, it is unlikely that they could be produced in large quantities or in a useable form without laboratory facilities or by untrained people. Moreover, it is possible to protect the novel use of a species by patents. While such agents could be produced and supplied by some government agencies, they generally do not have the facilities for large scale production or the distribution systems for such products.

Private firms have shown an increasing interest in the development of inundative biocontrol agents in the last ten years, in spite of the fact that biologicals make up only about 0.5% of the pesticide market (5). This is due to a number of factors: public concern about non-target damage and environmental contamination; development costs of conventional chemical pesticides; resistance and cross-resistance to conventional products; and the growing body of research results in government and university laboratories on the use of naturally occurring organisms.

In this paper I will discuss some commercial factors and technological considerations involved in the development of biopesticide products, generally using mycoherbicides as examples.

Commercial factors in mycoherbicide development

Market Place

The decision to develop a mycoherbicide involves many of the same considerations associated with the development of a conventional herbicide. Principally, what is the size and stability of the market? This requires knowledge of the weed's distribution, particularly in relation to crops, its seasonal occurrence, and alternative forms available for control.

Mycoherbicide development has lower relative costs than conventional herbicides. For instance, $US2m for Collego®one of the first mycoherbicides (Templeton, personal communication, 1983) compared with about $US30m for a conventional herbicide in 1981. Thus, there is considerable scope for development of mycoherbicides for small niche markets, as well as for large scale problem weeds. Furthermore, once a company has a fermentation production facility (see below) producing microorganisms, and downstream processing in place, it is feasible to produce a range of products with the same equipment.

Patents, secrecy, confidentiality

While major chemical companies have the financial resources to undertake the fundamen-

tal research into biopesticides, most of the basic research has been done in university and government laboratories where the technical skills in basic plant pathology, insect pathology, weed ecology and physiology are well established. Much of the research done "in-house" in private firms remains secret or protected by confidentiality agreements. Researchers at universities and government institutions have traditionally been expected to publish the results of their work, hence there is a need to protect this intellectual property if it is to be used by only one company. Patents can provide this protection and provide the tangible substance of negotiations with a private firm. Clearly, a private company will be less interested in developing a product if the relevant information about the microorganism and its use is in the public domain and unprotected by patents.

Technical

Basic research on the microorganism being used should have established identity, host range, biology and efficacy in laboratory, glasshouse, and ideally in some field tests. The major input of the private firm is to scale-up production, including separating the product (e.g., spores) from fermentation liquor and other products (e.g., mycelium) and downstream development of a stable product with shelf-life.

Mass production

Submerged liquid culture

Mass production of fungi and bacteria is most readily achieved by industrial scale fermentation, at least in the developed world. Fermentation technology has developed rapidly over the last thirty years and existing equipment can be utilized for production of biocontrol agents. The commercial mycoherbicides College®and Devine®are produced by this method (see 6,13), as well as *Verticillium lecanii* (as blastospores) for aphid and whitefly control and some strains of *Beauveria bassiana* for insect control (14).

Some fungi which do not sporulate in submerged culture may produce mycelium in submerged culture that can be dried (8) and applied as fragments or pellets in the field; among them is *Metarhizium anisopliae* produced by Bayer as BIO 1020®. Such fungi may sporulate following dew (10,12). Walker and Connick (16) describe the production of sodium alginate pellets of dried mycelium for a mycoherbicide (see two phase systems below).

A demonstration that a potential mycoherbicide fungus will sporulate in submerged liquid culture may increase commercial interest in its development. It may be possible to achieve this in shake flasks. Oxygen mass transfer is typically a limiting factor for aerobic processes, as its solubility in water is only about 6 ppm. Oxygen transfer can be increased by increasing boundary layer resistance by agitation. Orbital shakers, with low liquid to air ratios in the flasks, are superior to reciprocating shakers. However, a pilot bioreactor (fermentor) allows greater oxygen input and better control of temperature, pH, agitation and foaming. The minimum size of pilot bioreactors is one litre and although they do not exactly mimic larger reactors, they are useful research tools.

From a commercial point of view, the basic medium used to grow the fungus should be inexpensive, readily available and have the appropriate nutrient balance. Rate of growth of the fungus is also important. The longer the process, the greater the chance of contamination and the greater the cost. Stowell (13) has estimated fermentation costs of a 75000 litre unit at $US4000-$US9000 per day.

Production of product per unit volume is an important consideration, as this directly relates to total fermentation time required. The average yield of spores from filamentous fungi reported by Vezina *et al.* (15) was about 3×10^{11} spores per litre (i.e., 3×10^{8} spores per ml). Thus, if a mycoherbicide from a putative "average" fungus was being applied at 1×10^{6} spores ml^{-1} at 100 l ha^{-1} for weed control, 1×10^{11} spores are required and 0.3 l of fermentation capacity (for a given number of days) is required for each hectare treated.

Overriding this is a physical limitation to the number of spores that will fit in to a given volume, so that production per unit volume is, ultimately, limited. The greatest scope for economies in production may come from the end use concentration being reduced through formulation developments.

Harvesting spores may require separation from mycelium by filtration and centrifugation. Following their recovery, it is usually necessary to dry spores for long-term storage.

Shelf-life is an important commercial consideration. The longer the shelf-life of products, the greater the flexibility of use of available bioreactors. Baker and Hennis (4) have suggested a minimum shelf-life of one year at room temperature or warmer. Drying usually provides shelf-life and may be achieved by pan (with or without heating) spray/freeze drying. Inert materials, as well as protectants such as oligosaccharides, may be added at this point to absorb moisture and hasten drying; slow drying may lead to contamination problems.

Solid state culture

Solid substrate fermentation has been widely used to produce fermented foods in Japan. Media used to contain some liquid; a 'solid' substrate itself may be relatively inert (e.g., paper, wood, vermiculite) allowing for use of defined nutrient levels. On the other hand, some nutritive solid substrates may be available locally at low costs (e.g., coffee pulp, sorghum grain, straw, groundnut shells). Particle size, moisture content and temperature may all need to be controlled for successful production. Equipment used may be bags, trays or rotating drums.

Industrial submerged culture fermentation production requires considerable capital investment. Production on solid media may be relatively costly in terms of labour and materials in the western industrial environment, but not necessarily so where labour is less costly and suitable raw material is freely available.

There are a number of examples of bioinsecticide production on solid media from USSR, The People's Republic of China and Czechoslovakia (3).

Two phase systems

A two phase system has been used for *B. bassiana* and *M. anisopliae* production in the USSR, where mycelium produced in deep tank fermentation is allowed to sporulate in shallow open trays (7,11). Walker and Riley (17) described a similar preparation method for *Alternaria cassiae* for control of the weed *Cassia obtusifolia*. In Brazil, *M. anisopliae* is produced on autoclaved rice or wheat bran in autoclavable plastic bags following inoculation with blastopores produced in liquid shake culture (1,2).

Formulation

Because many plant pathogenic fungi have a requirement for free water (or dew period) for infection, recent efforts by many mycoherbicide researchers have been directed towards overcoming this dew requirement via formulation. In particular, formulating fungal spores within the aqueous phase of an invert emulsion in oil (or oil mixtures) (9). Although the technique has been shown to overcome the need for dew in some fungi, there are disadvantages with the method: the amount of oil required adds considerably to the cost of the product; non target contamination by oil; and the application of the viscous material requires special equipment. "Crop oils" of vegetable and petroleum origin have been used in association with conventional pesticides at much lower concentrations (1-2%). They may have a place in biopesticide development.

Any solute in water will decrease its rate of evaporation, but propylene glycol and polyethylene glycol have been used specifically for this purpose. It should be noted that a supplement may act in more than one way, say as a humectant, as well as another, e.g., a dispersing agent. The sensitivity of spores to any ingredient will override other considerations and viability and pathogenicity tests must be made continually as a formulation is developed.

Conclusion

The development of biopesticides will be driven by consumer demand. Many microorganisms pathogenic to insect pests and weeds are known. Mass production by existing fermentation technology and equipment is usually possible. The main technical limitation to the development of many biocontrol agents appears to be formulation. Improved formulation has the potential to overcome dew requirements for mycoherbicides, as well as to reduce end-use concentration required and therefore, reduce production costs.

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References

1. Aquino, de L.M.N., Cavalcanti, V.A., Sena, R.C. and Queiroz, G.F. (1975). A

new technique for the production of the fungus *Metarhizium anisopliae*. *Boletim Tecnio CODECAP* (Brazil) 4, 1-31.
2. Aquino, de L.M.N., Vital, A.F., Calvalcanti, V.A. and Nascimento, M.G. (1977). Culture of *Metarhizium anisopliae* (Metchn) Sorokin in polypropylene bags. *Boletim Tecnico CODECAP* (Brazil) 5, 7-11.
3. Auld, B.A. (1992). Mass production, formulation and application of fungi as biocontrol agents. Proceedings international workshop on locust and grasshopper control. Contonou, Benin. I.I.T.A. and I.I.B.C., (In press).
4. Baker, C.A. and Hennis, J.M.S. (1990). Commercial production and formulation of microbial biocontrol agents. *In* 'New directions in biological control: alternatives for suppressing agricultural pests and diseases', ed R.R. Baker and P.E. Dunn, pp. 333-34, Alan R Liss, New York.
5. Carlton, B.C. (1990). Economic considerations in marketing and application of biocontrol agents. *In* 'New directions in biological control: alternatives for suppressing agricultural pests and diseases', ed R.R. Baker and P.E. Dunn, pp. 419-34, Alan R Liss, New York.
6. Churchill, B.W. (1982). Mass production of microorganisms for biological control. *In* 'Biological control of weeds with plant pathogens', ed R. Charudattan and H.L. Walker. pp. 134-56, Wiley, New York.
7. Goral, V.M. and Lappa, N.V. (1973). In-depth and surface culture of the green muscardine fungus. *Zashchita Rastyeni* 1, 19.
8. Peireira, R.M. and Roberts, D.W. (1990). Dry mycelium preparations of entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana*. *Journal of Invertebrate Pathology* 56, 39-46.
9. Quimby, P.C., Fulgham, F.E., Boyette, C.D. and Connick, W.J. (1989). An invert emulsion replaces dew in biocontrol of sicklepod – A preliminary study. *Pesticide Formulations and Application Systems* 8, 264-70.
10. Roberts, D.W. and Wraight, S.P. (1986). Current status on the use of insect pathogens as biocontrol agents in agriculture: fungi. *In* 'Fundamental and applied aspects of invertebrate pathology', ed T.A. Sampson, J.M. Vlak and D. Peters. 4th International Colloquium of Invertebrate Pathology, Wageninen, pp. 510-3.
11. Roberts, D.W. and Yendol, W. (1981). Use of fungi for microbial control of insects. *In* 'Microbial control of insects and mites', ed H.D. Burgess and N.W. Hussey, pp. 125-49, Academic Press, New York.
12. Roberts, D.W., Dunn, H.M., Ramsay, G., Sweeney, A.W., Dunn, N.W., McCabe, B. and Soper, R.S. (1987). A procedure for preservation of the mosquito pathogen *Culicinomyces clavisporus*. *Applied Microbiology and Biotechnology* 26, 186-8.
13. Stowell, L.J. (1991). Submerged fermentation of biological herbicides. *In*, 'Microbial control of weeds', ed D.O. Te Beest. pp. 225-261, Routledge, Chapman & Hall, New York.
14. Thomas, K.C., Khachatourians, G.G. and Ingleden, W.M. (1987). Production and properties of *Beauveria bassiania* conidia cultivated in submerged culture. *Canadian Journal of Microbiology* 33, 12-20.
15. Vezina, C., Singh, K. and Sehgal, S.M. (1965). Sporulation of filamentous fungi in submerged culture. *Mycologia* 57, 722-36.
16. Walker, H.L. and Connick, W.J. (1983). Sodium alginate for production and formulation of mycoherbicides. *Weed Science* 31, 333-8.
17. Walker, H.L. and Riley, J.A. (1982). Evaluation of *Alternaria cassiae* for the biocontrol of siklepod (*Cassia obtusifolia*). *Weed Science* 30, 651-4.