CONFIDENTIAL

BIOLOGICAL METHODS

OF

MUSHROOM DISEASE

CONTROL

A review
on behalf of
The Horticultural Development Council
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Petersfield,
Hants.

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1. SUMMARY

Cultivated mushrooms, Agaricus bisporus, are susceptible to a number of pathogenic bacteria, fungi and viruses, which cause a range of disease symptoms.

Because of the high degree of environmental and nutritional control required for crop production, A.bisporus regimes are considered highly suited to the establishment of biological control organisms. The requirement for a beneficial microflora, leading to initiation of mushrooms, provides a unique opportunity for integration of beneficial organisms into the crop cycle.

The preferred strategy is the development of overall antibiosis of mushroom substrates to pathogens. This can be achieved by introduction and encouragement of beneficial 'cocktails' of organisms, rather than by concentrating on single pathogen - antagonist interactions.

The principle of biological control is environmentally attractive. It offers a viable alternative to chemical disease control. It is likely to become increasingly important where disease resistance to chemicals occurs or where chemicals are either ineffective or unavailable.

A range of bacteria and fungi have been successfully demonstrated for control of pathogens. Bacteria, particularly Pseudomonas spp. and Bacillus spp. have considerable potential for mushroom disease control, although, in the U.K., none are available commercially for any crop.

Current legislation requires further clarification and is a disincentive to the development of genetically manipulated organisms.

Commercial exploitation will depend on the development of reliable application techniques which meet requirements of repeatability, shelf-life and integration into normal husbandry practice.

Further advances in disease control in mushroom crops may be possible. This requires a better understanding of microbial dynamics in substrates, such as composts and soils, and the elucidation of fungal defence mechanisms.

Recommendations for future research and development in the areas of application technology, isolate screening, microbial dynamics and disease resistance mechanisms in fungi are as follows:

1) Legislation concerned with application of biological control organisms requires further clarification. In particular, the paradox of restricted use of biological control agents contrasting with the apparently uncontrolled use of some crop promoting and benign organisms requires explanation.

Action : Public sector

2) Formulation technology is the key to exploitation of biological disease control. Methods of applying organisms to crops and ways in which biological systems can be integrated into normal husbandry requires further study. This can be pursued via SAC project proposal SACA / CRU / 90.2. 'Mushrooms : Application of biological control organisms'.

Action : Industry

3) Further screening of existing isolates of potential biological control agents is required. In particular, assessment of long-term viability and efficacy against a range of diseases is required. Cross-protection and efficacy of 'cocktails' of organisms requires further study.

Action : Industry

4) The basic understanding of eco-physiological factors affecting microbial dynamics and establishment of individual organisms within substrates requires further study.

Action : Public sector

5) The biology of disease resistance, as opposed to antibiotic aggression, in fungi, is poorly understood. A study of interactions between defensive and aggressive fungi may provide a basis for future breeding developments.

Action : Public sector

This review was carried out on behalf of the Horticultural Development Council (HDC) by the author, under the auspices of the Scottish Office Agriculture and Fisheries Department (SOAFD).

2. INTRODUCTION

In the U.K., cultivation of edible mushrooms is almost exclusively concerned with one species, Agaricus bisporus (Lange) Sing. Production is dependent on complex processes of composting of growing substrates, colonisation of those substrates by the crop fungus and manipulation of the biological, chemical and physical environments to initiate cropping. These processes are fully described by Vedder, (1978) and Flegg, Spencer and Wood, (1985).

The crop is subject to antagonism by a number of fungal, bacterial and viral disease organisms which cause a range of symptoms. These generally result in economic loss of crop, the extent of which is directly related to the timing and severity of infection.

Mode of attack varies from pathogen to pathogen and in most cases is restricted to particular points in the life cycle of A. bisporus. In addition to specific disease causing organisms there are a range of fungi which may cause problems within the crop, primarily by competition. These have only a limited ability to colonise or antagonise the crop itself. Such fungi are widely referred to as 'weed moulds'.

Opportunities to protect the crop from disease are limited by a number of factors. The life cycle of the crop is short and, as such, time between application of any control measure and

harvesting of the crop is strictly limited. Problems are compounded by the fact that the crop is itself a fungus and the range of pesticides with activity against pathogens, but not against the crop, is inevitably small.

In recent years consumer awareness of potential pesticide residues in all crops has increased. This has emphasised the general benefit of maintaining a 'healthy image' which the mushroom crop currently enjoys. Furthermore, the hesitation of agro-chemical companies to develop new pesticides specifically for niche markets has encouraged the mushroom industry to examine alternative disease control strategies.

Over the past twenty years the use of biological methods of disease control has been developed for a number of crop / pathogen interactions. However, few have been fully commercialised despite a general awareness of the potential benefits. These encompass advantages of environmental safety, efficacy in trials, and nil residue.

The Horticultural Development Council commissioned this study to examine the rationale behind biological control of mushroom diseases. In particular, reasons for past successes and failure, possible opportunities and the future need for Research and Development are addressed.

3. PRINCIPLES OF BIOLOGICAL CONTROL OF DISEASES

Unlike conventional disease control strategies, some of which employ synthetic chemicals to prevent infection, biological control strategies employ natural means to protect crops. The most commonly referred-to process involves the challenging of any potential pathogen with an organism which can antagonise or kill it.

This predominant concept of biological control, whereby an undesirable organism may be eliminated, or the consequences of its presence reduced, by the introduction of one or more antagonists has been extensively reviewed by Baker (1987) and Baker and Cook (1974). The development of biological control of a wide range of pests and pathogens has been examined, although few appear to be commercially successful to date. Those showing most success are organisms for the control of insect pests.

Mushroom pest control is currently the subject of a separate HDC review, and outwith the scope of this paper.

In some cases however, fungi may be suitable as control agents of insect pests, and this is of direct relevance to studies of fungal/fungal interactions. Verticillium lecanii has been shown to give good control of whitefly and sciarid fly under glasshouse conditions (Hall and Burges, 1979, Hall 1980). Such fungal pest-control agents are now commercially available, as is Bacillus thuringiensis for control of caterpillars, (Koppert Ltd).

Biological control of fungal pathogens has not been developed to the same extent, although potential control has been shown in a wide range of situations, most commonly where plant pathogens, either in soil or on leaf surfaces, are target organisms.

Fungi which have been successfully used as biological control agents against fungal pathogens are typically soil colonists, (saprophytes), capable of antagonising target organisms by the productiion of toxins (Marois and Mitchell, 1981, Chand and Logan, 1984, Fokemma, 1973), or are aggressive fungi (necrotrophes). This group of fungi are generally able to grow saprophytically, but are able also to parasitise host mycelium following enzymic action or toxin production (Baker and Cook, 1974, Tu, 1980, Tu and Vaartaja, 1981, Spencer, 1980).

Verticillium lecanii has been shown to be capable of controlling fungal diseases, such as carnation rust (Uromyces dianthi) (Spencer, 1980), and bean rust (Uromyces appendiculatus)(Allen, 1983). Tu (1980) considered that Gliocladium virens has potential for control of Sclerotinia sclerotiorum but this has not been demonstrated on a large scale. G.virens showed considerable potential in small scale field trials by Tu and Vaartaja (1981), as a means of reducing the incidence of root rot of white beans caused by Rhizoctonia solani. G.virens was also shown to have potential in controlling carnation wilt (Fusarium oxysporum F.sp. dianthi (Ebben and Budge 1984). It remains unclear whether control can

be achieved on a commercial scale. Only in some studies has control been possible on a satisfactory scale. This has been reported for control of Fusarium oxysporum f.sp. radicis-lycopersici, the causal agent of crown rot of tomato, by the use of Trichodema harzianum or Penicillium finiculosum (Marois, Mitchell and Sonoda, 1981).

The use of bacteria as a means of controlling fungal pathogens has not received the same attention as the use of fungal antagonists. Bacteria were considered by Newhook (1951), who demonstrated inhibition of Botrytis cinerea by a number of bacterial antagonists. In some instances, bacteria have shown potential as control agents against other targets (Chand and Logan, 1984, Howell and Stipanovich, 1980). Pseudomonas syringae, for instance, has been shown to exhibit marked antagonism towards Ceratocystis ulmi, (syn.Ophiostoma ulmi) the causal agent of Dutch Elm disease, both in laboratory tests and practice (Myers and Strobel, 1983).

Similarly, a range of rhizosphere bacteria have been shown to be potential control agents against Fusarium oxysporum f.sp dianthi on carnation (Sneh, 1981).

Pathogenesis of Agaricus bisporus by bacteria and fungi within casing and compost is similar, in some respects, to that of soil plant-pathogen relationships. A fundamental difference between natural soils and mushroom substrates occurs in that while soil microbial populations vary from site to site, mushroom substrates typically have a more uniform microbial population.

This is principally due to standardisation of environments, in terms of temperature, humidity, and carbon dioxide (Hayes, 1980).

In field situations the success of soil borne plant pathogens appears to be influenced by an ability to interact successfully with chemical, physical and biological factors in the immediate environment (Marois, Mitchell and Sonoda, 1981). The influence of the microflora is apparently related to the total soil population of fungi and bacteria, rather than to the presence or absence of specific species within the substrate. Marois and Mitchell (1981) demonstrated that there is a negative relationship between the ability of Fusarium oxysporum to infect host plants and total soil populations of fungi and bacteria. No relationship between infection of host plants and the isolation of specific species was observed by these authors. The influence of total soil microfloral populations, which vary between sites, on the ability of soil borne pathogens to infect hosts may account for the lack of field scale success of biological control of soil borne plant diseases in some instances.

The biological control of fungal diseases of A.bisporus has received little attention. This may be due to the current availability of fungicides effective against many of the major pathogens, (Van Zaayen, 1983, Fletcher, Hims and Hall, 1983, (Fletcher, White and Gaze, 1989). Resistance to fungicides, such as Chlorothalonil, has been recorded in some fungal pathogens as it has done with previously effective fungicides

(Gaze, personal communication). Such a build-up of resistance to fungicides was particularly rapid in the case of benzimidazole derivatiives (Samuels and Johnston, 1980, Fletcher, Connolly, Mountfield and Jacobs, 1980, Fletcher and Yarham, 1976).

Resistance to fungicides, or a possibility of this occurring, is a major incentive to the development of alternative strategies, such as biological control.

In some cases the use of organisms to counter others within a crop may be considered crude. A preferred course of action may be to identify active processes of biological control and specifically mimic them. In short, extract or synthesise an enzyme or antibiotic and use such a pure product within the crop.

In reality, this is seldom possible because of the wide range of chemico-physical interactions, such as pH, ion chelation or enzymic and antibiotic action which make up the active processes in many biological systems. In addition, the problems associated with legislation covering application of antibiotics to crops may be overwhelming. This is despite some research successes, (Liao, Tu and Jeng, 1979, Stoller, 1978). This is more fully considered in Section 6.

While the term biological control has been coined to encompass use of antagonists capable of countering pathogens, described above, a further approach is less evident.

The value of disease resistance by crops has long been acknowledged for plants, and this has been the subject of extensive research. With regard to fungi, such as A.bisporus, there is very limited information on natural disease resistance mechanisms. In the case of plants, this may be interactive by, for instance, the production of antimicrobial compounds when exposed to potential pathogens. Such interaction has been the subject of various reviews, (Dixon, 1986, Darvill and Albersheim, 1984, Lamb, Lawton, Dron and Dixon, 1989).

In other situations, plants may be passive, but resist infection by the normal presence of antimicrobial compounds. Typical of this is the presence of glycoalkaloids in potato as a passive defence mechanism, (Sinden, Goth and O'Brian, 1973, Percival, personal communication).

The occurrence of toxins in both saprophytic and parasitic fungi has been extensively reported. In particular, members of the Eurotiaceae, (Penicillium spp. and Aspergillus spp.) have been shown to produce a number of antimicrobial toxins. (Berdy, 1974, Ciegler, Kadis and Ajl, 1971).

These have generally been viewed as a method of attack rather than defence. A.bisporus is known to produce antibiotics, (Szmidt, 1985), but mode of action and spectrum of activity remains unclear.

4. DISEASES AFFECTING AGARICUS BISPORUS

A.bisporus is susceptible to attack by a range of pathogenic organisms. Complete descriptions are provided by a number of authors including Fletcher et.al. (1989), Fletcher (1990), Vedder (1978), Flegg, Spencer and Wood, (1985).

The crop is principally affected by either viral, bacterial or fungal pathogens. In the case of virus infection the organism is specific (obligate) to the host mycelium and has no ability to grow elsewhere. By contrast, the most common bacterial and fungal pathogens of mushrooms are generally capable of sustained growth in the absence of the host (facultative necrotrophes). In addition, a range of 'weed mould' fungi may cause yield loss by competition for nutrients or become mildly pathogenic under certain conditions. Such weed moulds and the principal pathogens are summarised in Tables 4.1 and 4.2.

A.bisporus degrades its substrate in order to obtain nutrients for growth, (Wood and Fermor, 1985). This is typical of the way in which Basidiomycete fungi fit into the ecology of litter decomposition in natural environments. As such, fungi, particularly members of the Agaricaceae, including A.bisporus, supercede others in a continuous chain of degrading-organisms. In nature, as nutrients are exhausted, Agaric fungi are themselves superceded by others which can utilise remaining substrate or which can colonise any senescing fungal tissue.

This chain of competition is harnessed in commercial mushroom production by accelerating early degradation of compost constituents and then halting the competitive processes for as long as possible in order to maximise the 'window of opportunity' for growth of A.bisporus.

It was this philosophy of mushroom production which was considered by Baker and Cook, (1974). These authors likened the entire mushroom cropping cycle to a complete biological control process. As such, pathogenesis of A.bisporus can be considered as part of the normal chain of colonisation or degradation, but which occurs at an undesirable time. This implies that pathogenesis results from a breakdown of the biological defences of the entire cropping system. In turn, biological control of pathogens, by introduction of beneficial organisms, is a process intended to re-establish the 'window of opportunity' for the crop.

Principal pathogens and competitors of Agaricus bisporus

Type	Species (Common Name)	Pathogen location	Method of dispersal/source
Table 4.1			
Fungal [pathogenic]			
	Verticillium fungicola (Dry bubble)	Sporophore tissue	Aerial, passive e.g. pickers, flies, mites/crop waste contaminated casing and soil.
	Mycogone perniciosa (Wet bubble)	Sporophore tissue and casing layer	Water splash, aerial in dust and passive/ crop was contaminate casing and soil.
	Cladobotryum dendroides syn: Dactylium dendroides (Cobweb)	Casing layer	Water splash, aerial/ passive/contaminated casing and soil
	Diehliomyces microsporus (False truffle)	Compost, casing	Water splash, aerial/ crop waste and soil.
	Trichoderma spp. including T. harzianum T. viride T. koningii Penicillium spp.	- Compost and casing	Aerial, passive and via water/soil and timber.

Table 4.1 continued

Principal pathogens and competitors of *Agaricus bisporus*

Type	Species (Common Name)	Pathogen location	Method of dispersal/source
Fungal [Competitive]			
	Coprinus spp. (Ink cap)	Compost	Plant debris, soils / compost
	Botrytis cristallina (Brown mould) syn. *Peziza ostracoderma*	Casing surface	Aerial / soils and dust
	Chaetomium olivaceum (Olive green mould)	Compost	Aerial / soils
	Pythium oligandrum	Compost	Water splash/straw
	Papulospora byssina (Brown plaster mould)	Casing and compost	Aerial/soil and timber

Table 4.1 continued

Type	Species (Common Name)	Pathogen location	Method of dispersal/source
Viral			
	MV1 Isometric 25 nm MV1 Isometric 29 nm MV1 Bacilliform 19 nm x 50 nm MV1 Isometric 35 nm MV1 Isometric 40 nm - 50 nm - Club shaped	- Within A.bisporus cells	- transfer of infected spores or mycelium
Bacterial			
	Pseudomonas tolaasii (Blotch) Ps. gingeri (Ginger blotch)	- Cap surface	- Water splash, passive transfer/ natural peat or soil populations
	Pseudomonas spp. (Mummy)	Within A.bisporus cells	- Mycelial transfer
	Ps. cichorii syn.Ps.agarici (Drippy gill)		Water splash / peat or soil
	Pseudomonas spp. (Bacterial pit)	Cap surface	- Water splash, probably associated with mites

Table 4.2

Crop phases in relation to disease susceptibility *

Crop stage	Most significant disease risk
Pre-wet	none
Phase I	none
Phase II	Medium : susceptible to competitive fungi if hygiene and environment control are poor.
Spawning	High : virus
Spawn running	High : virus and compost colonists, e.g. D.microsporus
Casing	High : M.perniciosa, C. dendroides.
Case-running	Medium : Dependent on hygiene against competitive fungi.
CACing / ruffling	High : virus.
Initiation	High : V.fungicola, M.perniciosa,
Cropping	Medium (High at watering) : particular risk of Blotch under high humidity conditions. V.fungicola capable of rapid build-up.
Clear-out	High : risk of disease spread to other crops unless disinfection is good

* Unless otherwise stated this assessment assumes operational filtration and hygiene measures.

5. OPPORTUNITIES TO CONTROL DISEASES BY BIOLOGICAL METHODS

Mode of action of potential biological control agents varies from species to species. This includes antimicrobial (antibiotic) activity, competition for nutrients, enzymic action, localised pH effects or formation of iron-pigment complexes, (siderophores). The principal effects on any target pathogen of these factors are likely to be, respectively : -

i) Metabolic disruption

ii) Reduced growth

iii) Cellular cleaving

iv) Sub-optimal growth / competitive ability

v) Induced toxicity, particularly of metal elements

(Baker, 1987, Nevell and Wainwright, 1986).

It is crucial to the success of biological control that such activity of a potential organism is effective against the target, but not against the host crop.

That the development of composting procedures for mushroom production is, in effect, a biological control process protecting the 'window of opportunity' for growth of A.bisporus has been previously discussed in section 4 (Baker and Cook, 1974). Because A.bisporus relies on the presence of beneficial organisms, particularly in the casing layer, (Hayes, Randle and Last, 1969, Rainey, Cole, Fermor and Wood, 1990), it is of paramount importance that such populations are not disrupted by the introduction of any other organism.

Therefore, it is a logical objective that any biological control agent should be able to integrate into the beneficial microflora associated with A.bisporus, at the same time as contributing to antibiosis of any potential pathogens (Cresswell and Hayes, 1978, Szmidt, 1985, Szmidt and Dix, 1990). This situation is not unique and a similar approach has been adopted for establishment and protection of mycorrhizal fungi (Linderman, 1988).

The various pathogens noted in Section 4 tend to occur under different environments within the cropping cycle. These can be broadly summarised as :- compost, casing, surface and host mycelium. The opportunities to challenge pathogens using other organisms is different for each of these environments and these are discussed separately, below.

5.1 Compost

The multi-phase composting process normally adopted for commercial mushroom production is, microbiologically, extremely complex (Fermor, Randle & Smith, 1985). The process involves a wide range of organisms in sequential colonisation of the material. Such organisms are now widely acknowledged as contributing to the nutrition of the crop by provision of bacterial polysaccharide and microbial protein which is then utilised by A.bisporus. In addition, they are responsible for final chemical composition, and physical structure of the medium.

Because of this rapid flux in the compost microflora it is unlikely that any organism introduced early in the composting

cycle will remain active towards the end, when A.bisporus is itself introduced. Thermophilic actinomycetes and thermophilic bacteria, present at the completion of Phase II composting, were considered by Stanek (1972) to contribute to a reduced susceptibility to invasion by competitive fungi. These types of organisms appeared to be mutually stimulatory and enzymically complementary, with the result that they positively contribute to availability of nutrients to A.bisporus. That such organisms may be suitable as supplements to compost, as biological control agents, is supported by results of Tautorus and Townsley (1982), Raffle (1990) and Raffle and Szmidt (in press).

5.2 Casing

The role of the casing layer in development of A.bisporus sporophores involves a combination of physical, chemical and microbiological factors.

Peat, modified to a pH of 6 - 8.5 is the most widespread material used for casing of commercial crops. In contrast to the nutritionally rich composts prepared for vegetative growth of A.bisporus, casing materials generally have a low nutrient content and a different microflora.

Eger (1961) demonstrated that the microflora of the casing layer contributes to the process of sporophore initiation in A.bisporus. The vital role of such populations was demonstrated by Hayes et. al. (1969).

These workers identified the bacterium Pseudomonas putida as playing a major role in initiation. This view was supported by Hume and Hayes (1972). The way in which casing layer colonists aid A.bisporus sporophore production remains unclear. However, it is considered to be by removal or reduction in concentration of self-inhibitors produced by A.bisporus (Flegg and Wood, 1985). These authors summarised work which suggests that one or more organic substances may be involved. Further evidence suggests that that chelation, particularly of iron, may also be important (Hayes, 1972).

Although Pseudomonas putida appears to be the most important species involved in initiation of A.bisporus other members of the same family are generally also present. Pseudomonas spp. make up the predominant group of casing colonists (Preece and Wong, 1982, Hayes et. al. 1969, Long and Jacobs, 1974).

Pseudomonas spp., particularly species group 3 - 5, may produce antibiotics and have a chelation ability (Howell and Stipanovic, 1980, Gandy, 1968, Nevell and Wainwright, 1990). Bacteria belonging to this group have been evaluated as biological control agents for plant diseases, (Sakthivel and Gnanamanickam, 1987, Weller and Cook, 1983, (Gurusiddaiah, Weller, Sarkar and Cook, 1986). Henry, Lynch and Farmor, (1991) have also demonstrated their potential as antagonists of mushroom pathogens.

Therefore, such bacteria are prime candidates as biological control agents within mushroom crops. The potential benefits of

such bacteria to the crop suggests that introduced strains may readily become established as part of the normal casing layer microbial population (Szmidt, 1985).

The use of aggressive (necrotrophic) fungi as control agents within casing has also been examined. Gandy (1981) failed to achieve satisfactory control of Verticillium fungicola using Trichoderma viride and T.polysporum because of antagonism of A.bisporus. This was despite previous studies showing significant antagonism of V.fungicola in laboratory experiments (De Troghoff and Ricard, 1976). Similarly, Acremonium strictum, antagonistic towards Mycogone perniciosa in plate cultures, is an unsatisfactory control agent because of its ability to antagonise A.bisporus (Gandy, 1979).

Such aggressive fungi represent a range of potentially harmful organisms, including all the major fungal pathogens of A.bisporus and have no valid role within mushroom environments. Similarly, highly competitive fungi, such as Penicillium spp., Geotrichum spp. and Chaetomium spp. are unsuitable as control agents because of competition for nutrients with A.bisporus.

These observations are in contrast to most results of phyto-pathogenic interactions. In most plant diseases, the ability of aggressive soil-colonising fungi to infect the host does not come into question, (Moody and Gindrat, 1977, Baker, 1987, Whipps and Budge, 1990)

Verticillium fungicola has been isolated from un-used peat (Matthews, personal communication) and M.perniciosa may colonise

casing material prior to use if spores, either carried in dust or in contaminated water, are inadvertantly introduced (Fletcher and Ganney, 1968).

5.3 Surface

In many instances pathogens enter mushroom environments after cropping has begun. In such cases aerially dispersed propagules may land on, or be transferred directly to, mushroom tissue.

Both V. fungicola and M. perniciosa can be spread in this way (Gandy, 1985, Fletcher, White and Gaze, 1989). Each of these fungi produce thin-walled phialoconidia, on verticillate conidiophores, which are readily spread within and between cropping environments. In each case germination can readily take place. M.perniciosa also produces melanised thick walled verrucose conidia which are highly resistant to attack and dessication. These appear to be the normal resting spore of this pathogen, although other morphological forms are also reported (Fletcher and Ganney, 1968, Vincent-Davies, 1972, Holland and Cooke, 1991).

Factors affecting germination of the resting spore of M.perniciosa are not well understood (Holland and Cooke, 1990, Vincent-Davies 1972). However, once germinated, developing mycelium is susceptible to antagonism by bacteria, (Szmidt, unpublished). Similarly, thin-walled phialoconidia of both

V.fungicola and M.perniciosa are likely to be susceptible to antagonism.

Incoming pathogen propagules should ideally be challenged by biological control agents immediately on coming into contact with the crop or its substrate. Consequently, the presence of an extensive microflora on A.bisporus cap surfaces offers a potential location for control organisms. The surface microflora is made up of predominantly Pseudomonas spp. bacteria (Preece and Wong, 1982).

As for casing material, Pseudomonas spp. appears to be an attractive group of organisms for investigation as potential surface biological control agents. However, this group also contains bacteria capable of antagonising A.bisporus. In particular Ps. tolaasii and Ps. gingeri may cause severe blotch on mushroom caps (Paine, 1919, Gandy, 1968, Wong and Preece, 1980, Wong and Preece, 1979, Wong, Fletcher, Unsworth and Preece, 1982). These potentially pathogenic organisms may be part of the natural microflora, but only produce disease symptoms when populations climb to excessive levels, possibly because of changes in environmental conditions (Fahy, Nair and Bradley, 1981, Wong and Preece, 1980).

The existence of naturally established populations of bacterial pathogens, particularly Ps. tolaasii, within casing materials, such as peat implies that they may be difficult to challenge by retrospective introduction of biological control agents. An alternative approach may be to use closely related

or avirulent strains of the pathogen to swamp the ecological niche usually occupied by the pathogen (Baker, 1987).

5.4 Host mycelium

The most important disease syndrome in which the pathogen directly invades and multiplies within A.bisporus mycelium is virus. Despite the various types of virus particle which can infect the crop (Atkey, 1985) the principles and opportunities for control are similar for each.

Virus particles are unable to grow outside of host cells and require anastomosis between cultures to transfer the pathogen. Alternatively they may spread by dispersal of infected A.bisporus spores.

Theoretically, the only available technique, other than isolation of susceptible crops, for protection against virus is the use of cross-protection. This technique involves infecting the host with an attenuated strain of the virus which is avirulent. Presence of such non-pathogenic virus particles then prevents establishment of pathogenic strains.

This technique is widely available for cross protection of tomato varieties susceptible to tomato mosaic virus (TMV) by inoculating young plants with attenuated strain MII-16, (Gibbs and Harrison, 1976). Similarly, virus infection of citrus trees can be prevented by use of attenuated strains, (Baker, 1987).

A further development of the attenuation techniques, now available to breeders, lies in genetic engineering. Plants modified to produce virus-coat-protein may be self-protected against virus invasion, (Sunderland, 1990).

However, there is no apparent use of these techniques to prevent infection of fungal mycelium by virus particles. While isolation of crops from potential sources of virus is desirable, use of attenuation techniques may have a future role in 'virus-break' rotations.

Other pathogens infecting directly A.bisporus mycelium include bacterial pathogens. The most significant disease is mummy, thought to be caused by a mycelial invasion of Pseudomonas spp. (Fletcher, White & Gaze, 1989). Gandy (1985) considered that etiology of the disease is unclear and control strategies are therefore difficult to consider. Because of the uncertain relationship between Pseudomonas spp. and this disease there may be an undefined risk in using some types of bacteria as potential biological control agents.

Induced resistance to fungal diseases has been demonstrated in plants into which complete L-form bacterial antagonists have been introduced. Such biotechnological techniques do not involve genetic engineering per se and may hold future potential for protection of A.bisporus. (Amijee, personal communication).

A.bisporus does not have any well documented method of disease resistance. Various workers have demonstrated an ability of the host fungus to antagonise other species in agar

plate cultures (Gandy, 1985). Toxin production has been reported for Psalliota spp. (syn. Agaricus) (Wilkins, 1946, Brian, 1951). The nature of antimicrobial substances produced by A.bisporus remains unclear, but there is evidence that it is lipidic in nature, (Szmidt, 1985).

The presence of antimicrobial activity in A.bisporus mycelium offers a potential means of breeding for disease resistance. Before this is possible, the exact nature of any active biochemical, mode of action and spectrum of activity need to be elucidated.

6. SOURCES OF POTENTIAL ANTAGONISTS

Various protocols for selection of potential control organisms have been adopted. These range from selection and screening of naturally occurring populations to genetic manipulation of organisms, (Baker, 1987).

A further approach is to consider organisms cited in literature, for suitability in any chosen environment. This approach has the benefit of identifying organisms for which significant work on physiology, function and biochemistry has already been completed, (Seddon, personal communication).

Isolation of organisms capable of controlling mushroom pathogens either in the laboratory or under commercial conditions has been considered by those listed in Table 6.1.

No organism is currently approved for commercial application to U.K. crops, because of problems with efficacy and legislation governing approval. Isolates cannot simply be taken 'off-the-shelf' and be made available to growers. Further development work is required before biological control strategies can be used with confidence.

In order to develop isolates in the strategy preferred by Baker (1987) a 'cocktail' approach to using more than one organisms may be beneficial. This concept of modifying the natural positive effects of substrates, such as casing

material, on the host crop, rather than direct challenge of one organism against another may make implementation of pesticide regulations with respect to biological disease control less of an issue. This approach is dincreasingly adodpted for plant protection (Lindeman, 19865, Lindeman, 1988).

Table 6.1 Selected biological control interactions of possible value against mushroom pathogens

Disease	Control organism	Laboratory
Blotch	Pseudomonas spp.	IHR Littlehampton AGC Ltd. (Fermor and Lynch, 1988)
"	Pseudomonas spp.	B and CRI, Australia (Fahy, Nair and Bradley, 1981)
"	Ps. putida Ps. spp.	Punjab University (Munjal, Khanna and Garcha 1989)
Wet bubble	Pseudomonas spp.	University of Stirling Scottish Agricultural College (Szmidt, 1985, Szmidt and Dix, 1990)
False truffle	Bacillus licheniformis	Scottish Agricultural College University of Strathclyde (Raffle, 1990)
Various	B. subtilis	AGC Ltd (Rodgers, personal communication)
"	B. brevis	University of Aberdeen. Department of Agriculture (Seddon, personal communication).
"	Various	CAB International Institute of Biological Control (CIBC)
"	"	National Collection of Plant Pathogenic Bacteria
"	B.substilis Streptomyces graminofaciens	Humboldt - Univ. Berlin (Bochow, 1989)
"	Various	Botany Dept. Cambridge University.

7. PROBLEMS ASSOCIATED WITH APPLICATION OF BIOLOGICAL CONTROL ORGANISMS

The major problems with exploitation of organisms shown to have potential for controlling pathogens under laboratory conditions are : -

i) Efficacy under commercial conditions

ii) Application technology

iii) Legislation.

These are summarised as follows;

7.1 Efficacy

Laboratory screening and development protocols may be readily devised to identify organisms with an ability to antagonise pathogens but which have no deleterious effects on the host, A.bisporus.

However, activity under laboratory conditions may not be readily expressed under normal cropping environments (A'Court, personal communication). Growth and reproduction of fungi and bacteria may be significantly influenced by environment. As a result, small changes in factors such as temperature and humidity, may make major changes in the effectiveness of biological control. As a result, performance of apparently active control organisms under field conditions is variable for many plant pathogen interactions.

Dowding (1978) considered that activity under laboratory conditions may give a false impression of activity in 'real

life', although such techniques as agar plate antagonism have to be employed, at least in the early phases of any screening programme.

Whether antagonism in plate cultures can be expressed as an ability to control pathogens within crops may depend on the mode of action. In some cases, antagonism under laboratory conditions has been attributed to a change in substrate pH as a result of colonisation (Newhook, 1951), or to nutrient impoverishment (Skidmore, 1976).

The use of such antagonists as biological control agents in natural substrates is less likely to be successful than where antagonism results from production of antimicrobial substances. This is because composts, peats and soils generally have a buffering capacity which tends to mask the effect of localised pH or nutrient changes. The occurrence of antimicrobial substances has been widely reported and the role of these in biological control studied, in some cases (McGinty, McFadden, Rawlinson and Buck, 1984, Baker, 1987).

Various methods have been employed to select potential biological control agents under laboratory conditions. Typically, those organisms showing a statistically significant (>95%) ability to reduce or prevent growth of the pathogen has been employed. However, other parameters may be important, such as prevention of sporulation and consequent spread of the target.

Where such screening has provided organisms with better than 80% - 90% inhibition in the laboratory this may only be expressed as 10% - 20% disease control within crops (Rodgers, personal communication). Even where disease control is statistically significant, variability may be considerable. In the case of control of M.perniciosa, production of infected tissue was reduced by 83.1% (Szmidt 1985) but the range was approximately 46% to 100%. Fahy, Nair and Bradley (1981) showed that while some strains of Pseodomonas spp. bacteria may be able to increase production of healthy mushrooms in the presence of Ps. tolaasii, other isolates selected by the same procedure in the laboratory may be less effective. Furthermore, the relationship between production of healthy mushrooms and those showing disease symptoms is not direct under such conditions.

Where disease expression is quantitative, i.e. not 'all-or-nothing', symptoms may be only partially reduced by biological control agents. This was observed by Raffle (1990) studying control of Diehliomyces microsporus in which three distinct levels of infection and severity were observed. In such cases a numerical estimate of the degree of control may be difficult to determine.

In some instances, control may be difficult to achieve under natural conditions because of an inability to deliver sufficient inoculum. In such cases where a broad approach, perhaps by treating entire fields has failed, success may yet be achieved by altering the selection procedure for control organisms. In

particular, organisms which are reproductively dependent on pathogen propagules may be more successful (Adams and Fravel, 1990).

Mushroom environments are, by definition, more controlled than any other cropping regime employed in production horticulture or agriculture. Equally, the substrate on which the crop is grown generally meets exacting physical, nutritional and biological requirements. The implication is that if biological control is likely to be a viable proposition, then it has as great a chance of success for mushroom crops as for any.

7.2 Application technology

The requirements for successful adoption of biological control technology were reviewed by Beringer and Powell (1988). In order to be successfully commercialised, biological control organisms must be compatible with existing practice. Any requirement for novel machinery or equipment to apply organisms to crops is likely to be a disincentive to their use.

Preparations should be safe and easy to handle, have a long shelf-life with uniform viability and be able to be used in concert with other organisms or selected chemicals.

As with any new-product development, investment cost must be balanced against market size and possible returns. 1987 estimates put the average cost of each new agro-chemical at

approximately $80 million, with a ratio of 1 'active' : 20,000 screened. By contrast the costs of biological control agents may be considerably less and the chance of success, given closely regulated selection protocols, significantly higher (Lethbridge, 1988).

Various methods of application of organisms are cited in the literature, but none meet all of the above requirements. These include the preparation of peat or clay granules on which organisms have been cultivated, use of simple spore suspensions or freezing (Digat, 1988, Gianinazzi, Trouvelot and Gianinazzi-Pearson, 1990, Healey and Harvey, 1989).

Fungi or bacteria e.g. Bacillus spp., which form resistant resting spores appear currently most suited to long-term storage. Simple preparation of Pseudomonas spp. as control agents for cotton pathogens have proved unreliable in the U.S.A., and have been withdrawn (Rodgers, personal communication).

In order to maximise investment in biological control agents some form of protection of technology is desirable. Patenting of a living organisms is not possible unless some form of novelty can be demonstrated, (A'Court, personal communication). Although 'marking' may be possible by, for instance, breeding resistance to a particular antibiotic (Bochow, personal communication), this may be insufficient for patenting purposes. Manipulation of an organism to include a specific genetic marker may be possible, but this has legislative implications.

The most apparent route to protection of biological control technology is via methods of application rather than trying to attempt regulation of individual organism strains (A'Court, personal communication).

A range of potential 'carrier' substrates was previously submitted, in confidence, to the HDC mnushroom panel. (Szmidt, 1990). This included natural and sydnthetic materials currently available as 'pilot' products.

7.3 Legislation

In the U.K., all pesticides, including herbicides and fungicides must be approved for use (Iven, 1991).

The Food and Environment Protection Act (1985), (FEPA), includes statutory powers to control pesticides. Control of Approvals is achieved by the Control of Pesticides Regulations (1986), (COPR). Other legislation concerned with materials which may be applied to the environment includes the Control of Substances Hazardous to Health Regulations (1986), (COSHH) and The Food Safety Act, (1990).

Whether organisms isolated for U.K. environments and reapplied to crops need to be controlled in this way is open to debate. The current view is that it is legally required (Maris, personal communication), but that it is a situation that needs further clarification, (Rodgers, personal communication). These requirements include all relevant toxicity and efficacy data and

may be a major stumbling-block for new developments in biological control, unless resolved.

Currently no formulation for biological control of any disease is approved for use in the U.K.. However, a number of preparations are marketed in other countries. For mushrooms control of blotch may be possible using 'Conquer' (Healey and Harvey 1989), or 'Concord' (Fletcher et.al. 1989). Legislative constraints may be less where products are marketed on the basis of crop promoting, rather than pesticidal properties. In particular, mycorrhizal fungal formulations are available as a range of proprietary products (Gianinazzi, et.al., 1990, Landis, Timms, McDonald and Barnett, 1990).

No organisms which have been genetically manipulated, have been considered for Approval. It is anticipated that such approved use would be extremely difficult to achieve under the current system and developments await clarification of the legislation (Rodgers, personal communication). This problem is likely to apply equally to those organisms into which a 'genetic marker' has been introduced as to hybrid strains.

However, marker organisms are already in use in the U.K. for tracing silage effluent in water courses. Hughes (1991).

8. CONCLUSIONS

Biological disease control encompasses techniques with broad application to the mushroom industry.

The principles of biological control are ecologically attractive. They offer a viable alternative to chemical disease control which has yet to be exploited.

The use of biological control is likely to be increasingly important where disease resistance occurs or where chemicals are either ineffective or unavailable.

All major fungal and bacterial pathogens of A.bisporus may be theoretically challenged using biological control. Organisms require further testing under both laboratory and commercial conditions.

No biological disease control agents are commercially available in the U.K.

The environment in which A.bisporus is commercially produced is generally well controlled and predictable. This implies that, for this crop, the objective of establishing reliable populations of control organisms is more likely to be achieved than in any other horticultural or agricultural environment.

The recommended strategy is to improve the total antibiosis of mushroom substrates by encouraging 'cocktails' of beneficial organisms, particularly bacteria, to become established.

Some organisms are available which, under laboratory conditions, may significantly increase healthy yield of, or reduce infection of, A.bisporus.

National culture collections and laboratories working with a range of fungal and plant pathogens may provide a further source of suitable control organisms.

Efficacy of organisms under laboratory conditions is not always consistently duplicated under commercial conditions.

Reasons for variability in results are not known, but are thought to be due to differences in microbial populations between substrates.

Current formulations of organisms do not meet all guidelines for successful commercialisation. In particular, shelf-life, repeatability and strain stability are not well defined.

Development and patenting of formulation method is a more viable route to protecting technology than is patenting of individual organism strains.

Current legislation is a disincentive to the development of genetically manipulated organisms. The current timetable for introduction of such organisms to any U.K. crop is probably in excess of year 2000.

Where organisms have been isolated from natural environments and contribute positively to crop growth, rather than acting

solely as antagonists to pathogens, legislation may be less problematic.

The dependence of A.bisporus on a beneficial microflora within substrates, particularly the casing layer, provides opportunities and risks. The use of cultivated bacteria in casing may add to cropping potential. However, the differences between strains of bacteria which are potentially beneficial and those which are pathogenic are slight. Any industrial application of bacteria, particularly Pseodomonas spp. must include monitoring of risks of pathogenic strains or interactions arising.

A.bisporus has an antimicrobial capacity which is not fully understood. Definition of the principles of antimicrobial activity of the crop may help understand population dynamics of beneficial organisms. It may also provide a means by which disease resistance could be improved in the course of breeding.

Virus infection of A.bisporus may respond to some form of control within the normal cycle of 'virus-break' regimes. However, there is no supporting experimental evidence of the use of ameliorated strains within fungi.

Modern biotechnology offers techniques such as genetic engineering and cell fussion which may be of future value in developing crop disease resistance.

9. RECOMMENDATIONS FOR FUTURE WORK

A range of development objectives are considered appropriate:

1) Legislation concerned with application of biological control organisms requires further clarification. In particular, the paradox of restricted use of biological control agents contrasting with the apparently uncontrolled use of crop promoting or benign organisms requires explanation.

Action: Public sector

2) Formulation technology is the key to exploitation of biological disease control. Methods of applying organisms to crops and ways in which biological systems can be integrated into normal husbandry requires further study. This can be pursued via SAC project proposal SACA/CRU/90.2. "Mushrooms : Application of biological control organisms".

Action: Industry

3) Further screening of existing isolates of potential biological control agents is required. In particular, assessment of long-term viability and efficacy against a range of diseases is required. Cross-protection and efficacy of 'cocktails' of organisms requires further study.

Action: Industry

4) The basic understanding of eco-physiological factors affecting microbial dynamics and establishment of individual organisms within substrates requires further study.

Action: Public sector

5) The biology of disease resistance, as opposed to antibiotic aggression, in fungi, is poorly understood. A study of interactions between defensive and aggressive fungi may provide a basis for future breeding developments.

Action: Public sector

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APPENDIX

CONVENTIONAL DISEASE CONTROL

Conventional disease control is a subject which is outwith the remit of this review. Nonetheless, any novel disease control strategies must be able to take account of existing technology, such as pesticide and disinfectant regimes and be compatible with current husbandry techniques.

In an ideal situation, cultivation of A.bisporus need not be affected by pathogens or pests. As technology improves, the potential for isolation of the crop from antagonists increases. Indeed, isolation (hygiene) measures are still likely to be the most secure defence against antagonists.

The attractions of biological control lie in an ability to integrate with conventional techniques, a potential for providing benefits to the crop in the absence of disease as well as in disease control per se.