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Biocontrol of Soil-Borne Plant Pathogens with Introduced Inocula [and Discussion]

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Biocontrol of soil-borne plant pathogens with introduced inocula

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Despite more than 60 years of research on biocontrol of plant pathogens, introduced inocula of only two control agents are used widely and successfully against soil-borne or root-infecting pathogens in current commercial practice. Several others are undergoing exploratory commercial development or are used on a limited or local commercial scale. This review adopts a critical approach to the strategies for control of soil-borne pathogens with applied antagonists, and identifies some areas in which rapid developments could occur. In most instances it will be necessary to combine the use of microbial inocula with management practices designed to minimize disease losses. Also, in most instances, biocontrol strategies should be targeted against small pathogen populations, to prevent or delay the build up of disease, rather than to control existing high levels of pathogens. Natural, rapid senescence of cereal root cortices, which is influenced by both genetic and environmental factors, offers prospects for developing the use of weak parasites for control of take-all disease, caused by *Gaeumannomyces graminis* var. *tritici*. Recent studies on biocontrol of take-all by the fungus *Microdochium bolleyi* are presented, and special emphasis is given to the strategies most likely to be of value for take-all control.

Introduction

Soil is an intensely competitive environment for pathogens. As early as 1931, Sanford & Broadfoot showed that a wide range of common soil fungi and bacteria could, individually, control the take-all disease of wheat when introduced with the pathogen *Gaeumannomyces graminis* var. *tritici*, into previously sterilized soil. Similar demonstrations have since been made for many diseases, and now are regarded as commonplace. Gerlagh (1968) described this phenomenon as 'general antagonism' and contrasted it with 'specific antagonism' which occurs in only some soils, affects specific diseases or types of disease, and occurs in addition to general antagonism. Examples of specific antagonism, now often termed 'suppressiveness', include take-all decline, discussed by Hornby (1979), and the failure of some cyst nematodes to increase in continuous cropping systems (Kerry 1981). Such naturally occurring biocontrol mechanisms operate in normal agricultural practice and have been exploited purposefully or by default for many centuries. More recently, our understanding of them has enabled biocontrol to be managed by manipulation of the crop, the environment or other factors (Cook, this symposium), and this must be seen as a direct product of the years of research into biocontrol. It is reflected in many books and reviews that have appeared over the years (Baker & Snyder 1965; Toussoun *et al.* 1970; Baker & Cook 1974; Bruehl 1975; Schippers & Gams 1979; Papavizas 1981; Cook & Baker 1983; Parker *et al.* 1985).

In contrast to biocontrol by disease management, however, the direct, purposeful application of organisms to achieve control of soil-borne pathogens has met with only limited success. One

of the main reasons for this is that the very complexity of the soil environment and the antagonisms that are exploited so successfully in disease management mediate also against introduced control agents. Table 1 lists some of the few biocontrol agents that have been used commercially, are said to be undergoing commercial development, or for which research results seem to be directly translatable into normal agricultural practice. It is difficult to appraise the

TABLE 1. EXAMPLES OF BIOCONTROL AGENTS USED COMMERCIALLY OR IN NEAR-COMMERCIAL CONDITIONS AGAINST SOIL-BORNE OR ROOT-INFECTING PATHOGENS

control agent	disease	crop	reference
Phlebia gigantea	*Heterobasidion* root rot	pine	Rishbeth (1975)
Agrobacterium radiobacter var. *radiobacter*	crown gall	rose, others	Kerr (1980)
Trichoderma harzianum	white rot	onion	Abd-El Moity (1983)
Bacillus subtilis	stem rot	carnation	Aldrich & Baker (1970)
Sporidesmium sclerotivorum	lettuce drop	lettuce	Adams & Ayers (1982)
Gaeumannomyces graminis (hypovirulent)	take all	wheat	Lemaire *et al.* (1977)
Talaromyces flavus	*Verticillium* wilt	aubergine	Marois *et al.* (1982)
Pythium oligandrum	damping-off	sugar beet	Veslý (1979)

degree of commercial interest in most of these agents, but to date only two of them have been adopted widely and with obvious success: the use of *Phlebia* (*Peniophora*) *gigantea* to control *Heterobasidion annosum* root rot of pines, and the use of *Agrobacterium radiobacter* var. *radiobacter* for control of crown gall (*A. radiobacter* var. *tumefaciens*). These methods were first reported in 1963 and 1972, respectively, and are discussed by Rishbeth (this symposium). The fact that no major advance in commercial exploitation of this biocontrol strategy has occurred over the past 15 years gives cause for serious concern and justifies (indeed, necessitates) a critical appraisal of the approaches that have been adopted to date.

In this paper I shall address the prospects for biocontrol of soil-borne pathogens by means of introduced antagonists, with particular emphasis on take-all disease of cereals. I shall avoid undue overlap with topics covered by other contributors to this symposium. My purpose is not to assess the academic advances of the past decades, except insofar as they relate directly to the application of biocontrol; rather, there is a more urgent need to consider the prospects for commercial realization of biocontrol with introduced antagonists and the strategies that we should adopt in future work.

PHASES OF THE PATHOGEN CYCLE AMENABLE TO BIOCONTROL

Figure 1 shows four main stages in a generalized cycle of the activities of a soil-borne pathogen. Each stage represents a potential target for biocontrol, and together the stages provide a conceptual framework within which to assess the prospects for development of commercial biocontrol strategies.

Soil-borne inoculum

Soil-borne pathogens survive in the absence of hosts either as dormant propagules or as active populations. The dormant propagules include spores, sclerotia and resting mycelia of

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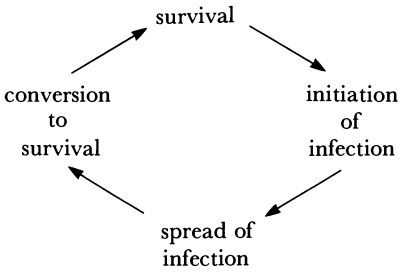

FIGURE 1. Phases in the pathogen cycle that are amenable to biocontrol.

fungi, cysts of some nematodes, and seeds of parasitic higher plants. The active populations may subsist on roots and residues of non-host plants or as declining populations in host residues that were colonized in the parasitic phase; very few important pathogens can increase their populations in the absence of an appropriate host by competitive colonization of soil organic matter.

The logistical problems of targeting biocontrol agents at this phase of the pathogen cycle are considerable, because of the volume of soil that may need to be treated and the non-uniform distribution of pathogen inoculum in soil, reflecting foci of disease in previous crops. Only one of the examples in table 1 is targeted primarily at this phase: the experimental use of *Sporidesmium sclerotivorum* to control soil-borne sclerotia of *Sclerotinia minor*.

S. sclerotivorum grows as a parasite on sclerotia but not on mycelia of its hosts, apparently destroying them by inducing autolysis of the central sclerotial tissues and by using the autolytic products to support its own growth and sporulation. Its spores germinate in response to substances released by sclerotia, and it can form radiating hyphae to infect sclerotia up to 1 cm away from those already infected in soil. Adams & Ayers (1982) found that a single application of *S. sclerotivorum* to field plots, dispersed thoroughly at a rate of 100 spores per gram of soil in the plough layer, resulted in 40–55 % control of lettuce drop caused by *S. minor* in three successive lettuce crops grown over the next two seasons; with 1000 spores per gram, control was increased to 63–83 %, but with 10 spores per g of soil, the degree of control was only 23–28 %. In this trial the inoculum density of the pathogen was 16–24 sclerotia per 100 g of soil, which is not unusual in some commercial fields (Adams *et al.* 1984). The results are thus encouraging and have led both to patenting of *S. sclerotivorum* as a biocontrol agent, and to the production of experimental commercial inocula on a vermiculite-based nutrient medium. From laboratory studies, Adams *et al.* (1984) suggested that the lowest application rate of the commercial product at which control of diseases caused by *S. minor* could be expected is about 22 kg per hectare†, equivalent to 5 spores per gram of soil. But this assessment apparently was based on soil samples supplemented with 600 sclerotia per gram, and the degree of control was much smaller at sclerotial densities equivalent to those usually found in the field. Also, and perhaps most importantly, the spores of the control agent were thoroughly mixed into the soil in a way that would be impractical on a commercial scale. So the rate of 22 kg inoculum per hectare is perhaps the theoretical lower limit, and the field experiments suggest that at least twenty times this amount may be needed to achieve 50 % disease control by incorporation into soil.

Even if biocontrol agents could be applied economically to the mass of soil in commercial fields to destroy much of the inoculum, they might not bring about significant disease control

† 1 hectare = 10^4 m^2.

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if the inoculum is present well in excess of amounts needed to cause economic crop losses. A recent example illustrating this problem involved the use of artificial stimulants to trigger germination of the sclerotia of *Sclerotium cepivorum* (white rot of onion), causing them to die in the absence of an appropriate host. In field plots heavily infested with sclerotia, the stimulant diallyl disulphide (a constituent of artificial onion flavouring) reduced sclerotial numbers by up to 40% but had no effect on disease levels because the remaining sclerotia (50–60 per kilogram of soil) were sufficient to cause severe disease (Merriman 1983). Even *S. sclerotivorum* is not so effective against lettuce drop caused by *Sclerotinia sclerotiorum* as against the similar disease caused by *S. minor*. The main reason is that sclerotia of *S. sclerotiorum* germinate to produce air-borne ascospores, so any surviving sclerotia produce a considerable number of infective propagules, whereas sclerotia of *S. minor* germinate to produce mycelia capable of only localized infection in normal field conditions.

There is, nevertheless, much scope for 'introductions' of control agents such as *S. sclerotivorum* in sites where they do not occur, to achieve a degree of 'natural' disease control that may be sufficient in itself or may facilitate other control methods. This approach has been used repeatedly by entomologists but seldom by plant pathologists, who have tended to assume (despite evidence to the contrary for pathogenic species) that most microorganisms occur wherever ecological conditions suit them. In nature, *S. sclerotivorum* grows only as a sclerotial parasite of *Sclerotinia* spp., *Sclerotium cepivorum* and *Botrytis* spp. It may not be generally distributed, although it occurs quite widely in the U.S.A. The sclerotial mycoparasite *Coniothyrium minitans* might similarly be used for introductions; it too is an effective biocontrol agent in field conditions, being able to parasitize both the sclerotia and mycelia of its hosts (references in Cook & Baker (1983)). Parasites of the thick-walled resting spores of other pathogens such as *Pythium*, *Phytophthora* and *Aphanomyces* spp. have been known but largely neglected for some decades; they are attracting attention once again as potential biocontrol agents (Ayers & Lumsden 1977; Sneh *et al.* 1977; Wynn & Epton 1979; Humble & Lockwood 1981). Several of these control agents are host-dependent; so they would need to be introduced where the population levels of their hosts (the pathogens) are uniformly high, but they could subsequently help to maintain lower pathogen populations.

A further possibility for control of soil-borne inoculum is to combine the use of antagonists with soil treatments such as organic amendment or partial sterilization with heat or fumigants. This has attracted much experimental attention and has been reviewed by Papavizas (1981) among others. The antagonists most suited to this approach are mentioned in a later section, but to date the method has not been used widely, if at all, in commercial practice.

Initiation of infection

For most root-infecting pathogens, soil inoculum is triggered into activity by a nearby root. In a few instances the effect is host-specific, as for *Sclerotium cepivorum* on *Allium* spp. (Coley-Smith & King 1970), some cyst nematodes (Clarke & Perry 1977) and some parasitic higher plants (Musselman 1980). In most instances, however, the effect is not host-specific. Other soil-borne pathogens are induced to develop infective stages not by the host but by seasonal factors such as low temperatures, which stimulate sporulation by the eyespot fungus (*Pseudocercosporella herpotrichoides*) of cereal stem bases.

Initiation of infection is an especially vulnerable stage for a pathogen, which has committed all or part of its inoculum resources for infection and finds itself in a zone of intense microbial

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activity supported by host-derived nutrients in the rhizosphere or spermosphere. Targeting of biocontrol against this stage is particularly attractive if the control agent can be applied to seeds or other planting material or can be introduced with the seed in drill rows. Relatively small amounts of inoculum are then required if the control agent can proliferate on plant-derived nutrients. In fact, all of the commercially successful examples of biocontrol with introduced antagonists are of this type. Spores of *Phlebia gigantea* are applied to freshly exposed surfaces of pine stumps during forest thinning or felling operations, to protect the stumps against colonization by *Heterobasidion annosum* (Rishbeth 1975). Strain K84 of *Agrobacterium radiobacter* var. *radiobacter* is applied to roses and other horticultural crops during transplanting from nurseries to field sites, to protect wounds against invasion by *A. radiobacter* var. *tumefaciens* (Kerr 1980). Commercial control of white rot of onion (*Sclerotium cepivorum*) was achieved on 230 ha in Egypt by adding to drill rows (at 425 kg per hectare) barley grain colonized by *Trichoderma harzianum* (Abd-El Moity 1983). Carnation cuttings are dipped in inoculum of *Bacillus subtilis* (Aldrich & Baker 1970) or other bacteria to protect the exposed wounds from invasion by *Fusarium* spp., a method used on a limited scale in the Colorado glasshouse industry.

The success of the two most widely used biocontrol agents, *P. gigantea* and *A. radiobacter* var. *radiobacter*, depends in large part on the fact that they gain prior occupancy of the infection court and also that their populations exceed those of the pathogens. Control of the initiation of infection, therefore, might often be most effective against relatively small pathogen populations.

We should consider applying microbial inocula to all transplanted crops, not only because root wounds could be protected, as above, but also because a considerable volume of root material is exposed for inoculation at this time, and in many instances the plants will be transferred from pathogen-free nurseries to potentially contaminated field sites. Marois *et al.* (1982) obtained impressive control of *Verticillium dahliae* wilt of aubergine in field experiments, by inoculating potting composts or field planting holes with spores of *Talaromyces flavus*. It was stated that the inoculum from one two week old colony on potato-dextrose agar is enough to treat 100 plants and that the methods can be implemented readily in present production systems; based on this report the method is included in table 1. The technology for inoculation of bare-rooted plants is easily developed, particularly now that a range of suitable gels is available. This year some three million one year old Sitka-spruce seedlings will be inoculated with ectomycorrhizal fungi, with a gel-based procedure, during transplanting of the seedlings to 'lining-out' beds in a commercial forest nursery; the total cost of this procedure is currently £2 per 1000 seedlings (F. M. Fox & J. W. Deacon, unpublished data) but could be reduced considerably. For larger plants such as orchard crops it may be more convenient to place biocontrol inocula or even disease-suppressive soil into planting holes. Indeed, the papaya replant problem caused by *Phytophthora parasitica* in Hawaii has been overcome simply by filling planting holes with pathogen-free soil (Ko 1982). Replant problems of orchard crops such as apple (Sewell 1981) might be amenable to similar control strategies. It is remarkable that the potential for applying microbial inocula to roots of transplanted crops has been exploited so little; this could become one of the main areas of commercial development in the coming years.

Ironically, numerous biocontrol agents have been applied experimentally to seeds or other planting material, to give short-term protection against seed rots and seedling diseases, for

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which there is, in general, adequate, cheap and environmentally safe fungicidal control at present. In several cases the biocontrol agents were as effective as conventional fungicide treatments, though somewhat less predictable. This topic was first reviewed by Wood & Tveit (1955) and more recently by Kommedahl & Windels (1981). There has, however, been little commercial uptake of this biocontrol strategy. Al-Hamdani *et al.* (1983) reported that commercial seed-coating with *Pythium oligandrum* for protection against damping off would be more expensive than conventional chemical treatment. The economics might be transformed if biocontrol agents can be shown consistently to control disease and also to enhance plant growth in the absence of pathogens. Such growth enhancement is reported for fluorescent pseudomonads (see Schippers, this symposium), some bacilli (Merriman *et al.* 1974), and most recently for *Trichoderma harzianum* (Chang *et al.* 1986). Irrespective of this, national, state and self-regulatory bodies might consider encouraging the use of biocontrol strategies when these are proved to be as effective or nearly as effective as current chemical control methods. Is there a case to be made for positive discrimination in agricultural legislation?

Spread of infection

Biocontrol in this and in the previous phase of the pathogen cycle might operate through induced host resistance. This is the suggested mechanism whereby some vascular wilt fusaria that are not pathogenic to particular hosts protect those hosts against pathogenic strains (Wymore & Baker 1982; Ogawa & Komada 1985). It is implicated in the protection of carnation cuttings against stem rot (*Fusarium avenaceum*) when they are pre-dipped in suspensions of *Bacillus subtilis* or other non-pathogens (Baker *et al.* 1978). Biocontrol of take-all of wheat by prior inoculation with *Phialophora graminicola* may operate in part by induced endodermal and stelar lignification (Speakman & Lewis 1978), although this could not be confirmed in the case of grasses and maize (Speakman *et al.* 1978). Immersion of potato tuber seed pieces in suspensions of non-pathogenic pseudomonads gave some protection of the plants when they were challenged 3–4 weeks later by stem or root inoculation with a highly pathogenic strain of *Pseudomonas solanacearum* (Kempe & Sequeira 1983). Induced systemic resistance also has been demonstrated experimentally for pathogens of shoots (Kuć 1982). Interest in the practical application of these phenomena is heightened by evidence that the systemic fungicides metalaxyl and especially fosetyl-Al may act at least partly by induction of host-resistance (Guest 1984; Ward 1984; but see Cohen & Coffey 1986).

Control of the spread of infection can operate also by direct antagonism in the root zone, because many root pathogens produce only limited lesions (for example, *Pythium* and *Phytophthora* spp. in individual root tips) and thus undergo several cycles of infection on a single host, whereas other pathogens characteristically spread along the root surface. Lesions caused by necrotrophic pathogens are invariably colonized by secondary invaders, which exploit the food base that could otherwise be used by pathogens for spread of infection. Some types of fluorescent pseudomonad, for example, show a predilection for lesions caused by the take-all fungus on cereal roots. The ratio of their numbers on diseased compared with healthy roots greatly exceeds that for other soil bacteria (Weller 1983), a fact that implicates fluorescent pseudomonads in take-all decline (Cook & Rovira 1976; Rovira & Wildermuth 1981).

Despite all these examples, we still lack a commercial biocontrol agent that can be applied economically to any of a wide range of field crops such as cereals, legumes, potatoes or crucifers, and that will limit the spread of any of their root diseases. The practical difficulties centre

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around the ability of biocontrol agents to colonize roots from inocula applied to seeds or other planting material. Roots release substantial amounts of nutrients that support microbial growth, but on any one root region the microbial population increases to a maximum at which its energy requirements match the rate of nutrient supply (Newman 1985). So a biocontrol agent applied to seeds, for example, would need to keep pace with the rate of root growth, unless it can utilize nutrients unavailable to other microbes, increase the rate of nutrient release, or antagonize the existing population and progressively replace it. In general, a microorganism is excluded from a root region once this has been colonized by others.

Much remains to be learned about the dynamics of colonization of plant roots. *Rhizobium* spp. and *Agrobacterium* spp. are very effective colonizers of roots, including those of non-hosts such as grasses (Schroth *et al.* 1979). Fluorescent pseudomonads also are good rhizosphere colonizers (Schippers, this symposium). Several other organisms have been detected on roots at some distance from their sites of application: for example, the biocontrol agents *Talaromyces flavus* (Fravel *et al.* 1985), *Penicillium oxalicum* (Windels & Kommedahl 1982) and *Enterobacter cloacae* (Chao *et al.* 1986). But *Trichoderma* spp., despite their often highly antagonistic properties, are relatively poor root colonizers (Papavizas 1985). Chao *et al.* (1986) recently demonstrated the importance of percolating water in carrying spores or bacterial cells down roots. This may help to explain the poor performance of *Trichoderma* spp., which require light for sporulation and thus may be able to spread only as hyphae along roots.

Cereal roots, with special reference to take-all

The work of Holden (1975, 1976) opened new avenues for the study of root colonization, especially for cereals and grasses. By means of nuclear and cytoplasmic stains, the root cortex can be shown to senesce early in the lives of these plants, even in the absence of pathogens or other microorganisms and long before the roots show evidence of cortical browning or sloughing. It is not unusual for several cortical cell layers to be anucleate and evidently incapable of defence in only 6–10 day old regions of wheat roots in glasshouse conditions. Data on the rates of natural cortical senescence in various glasshouse and field conditions, and differences between species and cultivars in this respect, are given in Henry & Deacon (1981), Deacon & Henry (1981), Deacon & Lewis (1982), Deacon & Mitchell (1985) and Kirk & Deacon (1986).

Cortical senescence seems to provide a major source of nutrients for rhizosphere bacteria (Van Vuurde & Schippers 1980; Deacon & Lewis 1982). Equally important, it enables weakly parasitic fungi to invade the cortex as resistance of the cells declines. Some of these weak parasites might contribute to disease complexes, but others are known to control major root pathogens. *Phialophora graminicola* is one such example, a non-pathogenic parasite that depends on root cortical senescence (Holden 1976; Deacon 1980; Deacon & Lewis 1986). Natural populations of this fungus in British grasslands are thought to delay the establishment of severe take-all in subsequent cereal crops (Deacon 1973; Slope *et al.* 1978), and also to restrict the occurrence of take-all patch disease of sports turf (Deacon 1974). In field trials in Australia, inoculum of weak parasites closely related to *P. graminicola*, namely *Gaeumannomyces graminis* var. *graminis* and a *Phialophora* sp., gave significant control of take-all of wheat (Wong & Southwell 1980). Take-all patch of turf grass was similarly controlled by these fungi in glasshouse trials (Wong & Siviour 1979). Hypovirulent isolates of the take-all fungus itself have been shown to control take-all of wheat in field trials in France (Lemaire *et al.* 1977). Fungal

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viruses were suggested to be involved in this case (discussed by Buck, this symposium) but the similar behaviour of all these control agents on roots suggests a common biocontrol mechanism based on exclusion of the pathogen from senescing cortical tissues (Deacon & Henry 1980; Kirk 1984) and perhaps accompanied by induced endodermal resistance (Speakman & Lewis 1978).

From the above, it is seen that we have some understanding of the ecology of the cereal root zone, as well as field demonstrations of the effectiveness of biocontrol inocula and evidence for a role of some weak parasites in natural control of take-all. So how close are we to achieving a commercially viable biocontrol strategy for this disease? There are still two major limitations. Firstly, although significant disease control has been obtained in field trials, in most instances it has been insufficient to give economically worthwhile grain yields; this is true not only for control by weak parasites but also for control by fluorescent pseudomonads and *Bacillus* spp., as summarized in table 2. The reason seems clear: our efforts have been directed towards

TABLE 2. REPORTED YIELD RESPONSES OF WHEAT TO BIOCONTROL AGENTS IN FIELD SITES NATURALLY OR ARTIFICIALLY INFESTED WITH THE TAKE-ALL FUNGUS, *GAEUMANNOMYCES GRAMINIS* VAR. *TRITICI*

	grain yield as the percentage of yield in healthy (uninfested) sites	
biocontrol agent (source of data)	no treatment	with biocontrol agent
G. graminis var. *graminis* (Wong & Southwell 1980)	92	100
	68	80
	68	82
	68	80
	67	89
	55	70
	53	61
	32	45
Pseudomonas fluorescens (Weller & Cook 1983)	88	90–93
	16	19–40
Bacillus pumilus (Capper & Campbell 1986)	13†	29†

† Calculated from the presented data as the percentage of mean grain yield (4.99 t ha^{-1}) for healthy crops on the farm.

control of the disease at or near its maximum levels, whereas the weak parasites, at least, give best control at much lower inoculum levels of the pathogen (Wong & Southwell 1980; Lemaire *et al.* 1977) when the take-all fungus itself may depend on senescing root cortical tissues as a food base for infection (Deacon & Henry 1980; Kirk 1984). It may be more appropriate, therefore, to use biocontrol agents to delay the development of severe disease in a sequence of cereals, as *P. graminicola* seems to do naturally after grass crops. The second limitation is that all the field demonstrations of control by weak parasites have involved inocula of biocontrol agents produced on solid substrates, usually sterilized oat grains. Cheap liquid fermentation methods will be needed, preferably for production of spore inocula that can be applied to seeds.

With this last point in mind, Kirk & Deacon (1987*a*, *b*) investigated other weak parasites of cereal and grass roots, especially those that readily produce spores in culture. The fungus

Microdochium bolleyi was found to colonize senescing root cortices, with no detrimental effect on its hosts, and in glasshouse conditions it gave as good control of take-all as did *P. graminicola* at equivalent inoculum levels. *M. bolleyi* has been implicated in control of other pathogens of cereal roots and stem bases, such as *Pythium graminicola*, *Fusarium* spp. and *Pseudocercosporella herpotrichoides*, and is known to be a common inhabitant of these parts of plants in field conditions. So *M. bolleyi* would be attractive for use in biocontrol if naturally occurring levels of its population could be augmented sufficiently. It differs from *P. graminicola* and similar fungi in this respect, because the populations of these decline progressively in cereal monocultures, a point that may help to explain their temporary but not lasting ability to control the development of take-all disease.

M. bolleyi produces abundant spores by a budding process in liquid culture. In preliminary studies (J. W. Deacon, unpublished data) after spores have been applied to wheat seeds in water or carboxymethylcellulose gels, *M. bolleyi* was found to colonize both the seed coat and the root cortex when the seeds were sown in soil. Viable counts of up to 1.5×10^5 spores per seed were detected on seeds after brief immersion in water- or gel-based spore suspensions containing 1.8×10^6 spores per millilitre, and in the best treatments viable counts of 2.7×10^4 per seed were obtained after the seeds had been air-dried overnight by exposure on a laminar-flow bench. In addition, spores of *M. bolleyi* applied to seeds, without drying, significantly reduced the spread of take-all lesions up roots from an inoculum layer positioned 2 cm below seeding level in pots of soil in a glasshouse (J. W. Deacon, unpublished data). *M. bolleyi* thus joins the increasing list of potentially usable biocontrol agents for take-all. It has yet to be tested in field conditions, but it has the attributes required of a potentially commercial control agent and its development for this can be based on an understanding of its ability to colonize roots in soil.

Before leaving this subject, I will attempt to identify strategies for biocontrol of the spread of infection. Take-all will again be used to illustrate the sorts of factors that need to be considered. First, the overwhelming weight of evidence suggests that economically feasible biocontrol strategies should not be directed against massive disease levels, as illustrated for take-all in table 2 and mentioned earlier for control of the initiation of infection by *Phlebia gigantea* and *Agrobacterium radiobacter* var. *radiobacter*. Cross-protection involving induced host resistance also is usually most effective against relatively low inoculum levels of pathogens. Indeed, the only case in which biocontrol might logically be directed against high pathogen levels is when the control agent depends on, or is favoured by, the pathogen. Secondly, it is probably unrealistic to expect that an applied antagonist could colonize and dominate all or even most of the root system. So we should attempt to identify key protectable sites. In the case of take-all, which spreads along roots by mycelial growth, such key sites are the proximal regions of roots, close to the stem base, and also the basal stem tissues themselves. If the pathogen can be excluded from these regions, then it must infect each root separately rather than grow from one to all others near their points of origin. Fortunately, this key region for the pathogen is also the region most easily protectable with seed-applied inocula. On the other hand, there are circumstances in which biocontrol of take-all might be unachievable by any practical method. An example is when relatively few roots penetrate deeply into soil, because of impedance, and when there is little rainfall during the later part of the growing season, so the crop depends increasingly on water stored in the lower part of the soil profile. Then only the few roots that have penetrated deeply would need to sustain local vascular lesions at some

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point along their lengths for the crop to fail. The crater disease of wheat in South Africa occurs in conditions such as these, where dramatic crop losses are associated with only localized root lesions caused by *Rhizoctonia solani* (Deacon & Scott 1985). A third and vital part of a biocontrol strategy involves manipulation of the crop or its environment, either to promote growth and activity of the control agent or to minimize pathogen activities and their effects on the crop. The opportunities here are many and varied (Cook, this symposium) but one example will suffice. Of the many factors now known to affect the rate of cortical senescence in cereal roots, the availability of nitrogen seems to be of over-riding importance. I. M. M. Gillespie (personal communication) has shown that the rate of senescence in both attached seedling roots and excised root pieces is greatly increased if nitrogen is present at sub-optimal levels, whereas phosphorus and potassium supply have much lesser effects. Because several biocontrol agents that decrease take-all exploit and even depend on naturally senescing root cortices, it might be possible to manipulate the rate of senescence to aid their establishment from inocula applied to seeds or in the drill rows.

Conversion to the survival phase

The importance of this relatively short phase in the pathogen cycle is illustrated by the finding of Christias & Lockwood (1973) that 39–52% of mycelial carbon content can be remobilized and converted into newly formed sclerotia within 4 days when mycelial mats are subjected to stress conditions, as in soil. Pathogens that survive by slow saprophytic growth also depend on efficient conversion to the survival phase; in most instances they lack the competitive saprophytic ability necessary to colonize organic substrates in soil and so can persist only by exploiting host residues that they colonized as parasites or shortly after the host died. Such 'residue possession' (Bruehl 1975) is typical of the take-all fungus, the eyespot fungus and *Cephalosporium gramineum* (leaf stripe) of cereals, as also of *Armillaria mellea* in tree roots.

Any factor that reduces pathogenic activity in the living host will reduce the amount of inoculum available for infection of subsequent crops. For example, the take-all fungus survives less well in the rather ephemeral dead roots than in the more massive stem base tissues of cereals, so biocontrol agents that restrict or prevent invasion of the crown at the end of the growing season will have a corresponding effect on pathogen survival. The low level of damage by cereal cyst nematode (*Heterodera avenae*) in some continuous cereal cropping systems is associated with a low population of eggs in the soil. This is due in part to the activities of egg-parasitic fungi such as *Verticillium chlamydosporium*, but in largest part to parasitism of the female cyst nematodes by the zoosporic fungus *Nematophthora gynophila* (Kerry 1981). Unfortunately, *N. gynophila* has not been grown in axenic culture, so it cannot easily be produced as a biocontrol agent, but it might be a good candidate for introductions.

There may well be scope for applying biocontrol agents to crop residues before these are incorporated into soil, the objective being to limit substrate possession by pathogens. This phenomenon probably contributes to the success of *Phlebia gigantea* in controlling *Heterobasidion annosum* in pine plantations; *P. gigantea* not only restricts colonization of the stump surface from air-borne spores of the pathogen but also can prevent colonization of the stump tissues by the pathogen from infected roots (Rishbeth 1975). In general, applications of biocontrol agents to crop residues may be preferable to direct soil-application (as discussed earlier), even when the control agents are targeted primarily at the survival phase.

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Several organisms seem to be good candidates for inoculation of crop residues but at present are among the ranks of the unemployed or, at least, under-employed. They include *Trichoderma* spp., *Gliocladium virens*, *G. roseum*, *Talaromyces flavus* (*Penicillium vermiculatum*), *Pythium oligandrum*, *P. acanthicum* and *P. periplocum*. These fungi share the interesting and rather unusual property of being able to overgrow colonies of other fungi in culture, often parasitizing or otherwise antagonizing their 'hosts'. They can be selectively isolated merely by placing soil organic matter on agar plates precolonized by susceptible fungi (Deacon & Henry 1978; Jager *et al.* 1979; Foley & Deacon 1985) or by burying hyphal mats of these in soil (Liu & Baker 1980). In many instances they have been recorded as secondary invaders of diseased plant tissues. Of all these fungi, *Trichoderma* spp. merit special comment because they have been implicated in numerous examples of natural biocontrol and have been shown repeatedly to control soil-borne pathogens in experimental conditions (reviewed by Papavizas (1985)). Commercial preparations based on *T. viride* and *T. polysporum* (Ricard 1981) are now available for control of silver leaf disease of fruit trees and for disease of mushrooms caused by *Verticillium malthousei* but have yet to find a major commercial role against diseases of crop plants caused by soil-borne pathogens.

Conclusion

The scope for biocontrol of soil-borne pathogens with directly applied antagonists is considerable. But so it has been for at least the past decade because, despite substantial advances in our knowledge, little has changed that alters our ability to put the knowledge into practice. I think we must concede, therefore, that our approaches have been wrong, and that we have failed (or have not tried) to adopt appropriate strategies. The most apposite definition of strategy that I can find is in the Oxford Pocket Dictionary: 'The art of war, especially the part of it concerned with the conduct of campaigns, choice of operations to be attempted, and getting of forces into favourable positions for attempting them.' Every aspect of this definition applies directly to our subject. In terms of the 'choice of operations to be attempted' one is forced reluctantly to ask if the work of the past 30 years on biocontrol of seed and seedling diseases has been strategic. It has led to some advances in basic knowledge, of which the recent work by Nelson *et al.* (1986) is among the most exciting. But we seem to be no closer to applying biocontrol against seedling diseases than when Wood & Tveit (1955) first reviewed this topic. We could do so, and perhaps we should, but still there is no pressing need for it in the agricultural industry. Similarly, despite a wealth of research on massive augmentation of soil with biocontrol agents (reviewed by Papavizas (1984)), there are few, if any, instances in which the economic and practical feasibility of this has been tested on a large scale.

On the other hand, there are now many practical opportunities to be exploited. Disease control by classical introductions remains to be explored in many cases, and new technologies offer exciting prospects for inoculation of transplanted crops and containerized plants, and for biocontrol in glasshouse and nursery situations in general. The prospects for obtaining season-long protection against root- and stem-base pathogens of common field crops seem more distant, unless use can be made of induced resistance. There will be no short cuts in these respects (Garrett 1965), so it is important to ensure that we are travelling along the right paths. To date, we have attempted to control the initiation and spread of infection in field sites heavily infested with the target pathogens, but this approach must now seriously be questioned because

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the evidence suggests that it is wrong. The corollary is that we should start to apply control agents before a problem develops, a recommendation that will require considerable confidence on the grower's part but will commend itself to the producer of commercial inocula. We need also precisely to define our objectives, whether they be to delay disease long enough for the crop to come through to maturity, to protect specific regions of the host, or even to reduce disease in one crop such that less pathogen inoculum is available for the next. And perhaps most importantly, we should not regard biocontrol necessarily as an alternative to other control strategies, even chemical control. Instead, our attempts to develop biocontrol methods should incorporate any other factors that can minimize disease or its effects on yield. For example, yield losses in the peak take-all years of a cereal sequence seem to be minimized in late-sown crops in southern Britain (Yarham 1986), whereas early sowing is desirable in the absence of take-all. If biocontrol for field crops is to become a practical reality, then we may have to offer growers a strategy, a package of simple and economic measures, not just a packet of inoculum. Further research must reflect this need if the pace of commercialization of biocontrol is to be increased.

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Discussion

A. R. Entwistle (*Institute of Horticultural Research, Wellesbourne, U.K.*). There are considerable practical difficulties associated with the screening of organisms for the biological control of soil-borne plant pathogens. These difficulties are often avoided by the use of *in vitro* screening, e.g. by culturing the potential biological agent and the plant pathogen on agar. Organisms with biological control activity *in vitro* may, however, prove to be less promising when tested in field conditions.

Would Dr Deacon advise how biological control agents should be tested against soil-borne plant pathogens?

J. W. Deacon. One of the most fruitful approaches to the selection of biocontrol agents has been to investigate organisms associated with natural reductions in pathogen populations or associated with relatively healthy plants in the midst of diseased ones. This should perhaps be the first line of approach, not least because it will identify control agents likely to be effective in the situations for which we wish to use them. Simple *in vitro* screening methods doubtless have some role to play, even if their results are not always matched by performance in practice. What must be avoided, however, is the mistake so often made in the past: perseverance in research with 'promising' control agents just because they are so promising *in vitro* and despite the sometimes obviously insuperable difficulties attending their use in commercial conditions. Some of the work on soil-augmentation with biocontrol agents such as *Trichoderma* spp. falls into this category: logic dictates that such methods would be impracticable because of the amounts of inoculum required. In short, small field trials in simulated or actual commercial conditions should be done early in the course of development of a biocontrol strategy, and the research should be redirected if the likely costs of inoculum production and application could not reasonably be matched by the benefits likely to accrue.

R. R. M. Paterson (*C.A.B. International Mycological Institute, Kew, U.K.*). Have the metabolites produced by all the biocontrol fungi been determined, and has it been established if any of the compounds might be responsible for the antagonistic activity?

J. W. Deacon. The modes of action of biocontrol fungi are many and varied, although in several instances I suspect that competition (e.g. for host-derived nutrients or senescing cereal root cortices) is a contributory factor. Among the mechanisms thought to be involved are mycoparasitism (e.g. by *Pythium oligandrum* and *Sporidesmium sclerotivorum*), contact inhibition termed hyphal interference (e.g. by *Phlebia gigantea*) and hyphal lysis (e.g. by *Pythium nunn*).

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Trichoderma spp. are among the few biocontrol fungi known to produce antifungal agents, but it is unclear if these compounds are responsible for biocontrol in nature. Metabolite production requires access to an available food source, so I suspect that, at best, it is secondary in importance to the attributes that enable a successful biocontrol agent to obtain its nutrients.

J. M. Lynch (*Glasshouse Crops Research Institute, Littlehampton, U.K.*). I was pleased to hear Dr Deacon's suggestion that the battlefield for the antagonist against the pathogen should be treated at the earliest possible stages of crop growth; unlike biological pest control, disease control should be preventative. However, with pathogens that are borne on crop residues I wonder whether the primary battle should take place on the residue. With colleagues Naresh Magan and Paul Hand, I have found that *Fusarium* can be totally suppressed on stand by a *Trichoderma* spray.

J. W. Deacon. The application of biocontrol agents to crop residues seems to be a profitable area for research and I am pleased to learn of your success with *Trichoderma*. Many types of residue are accessible for inoculation before they are ploughed into the soil, and control agents might be selected for their abilities to utilize and increase their population levels on residues. Disease control then could be effected by (1) interference with conversion of the pathogen to its resting or saprophytic survival phase, (2) antagonism during survival, and (3) interference with the initiation of infection in the next crop (see figure 1).

J. Irvine (*University College London, U.K.*). In the case of soil-borne problems, where re-infection and inoculum build-up is slow, controlling propagation of a pest may offer little protection in the season of application. Such measures may require government subsidy over a long-term. What are Dr Deacon's views on implementing this kind of strategy?

J. W. Deacon. The type of biocontrol that might attract or even require government subsidy is the classical type introduction of an agent to control an exotic pathogen, as is sometimes practised against insects and weeds. It applies mainly to cases in which control on a single farm would be fruitless because of the threat of re-invasion of the target species from neighbouring areas. We have paid too little attention to the prospects for introductions of control agents against soil-borne pathogens. But, as Mr Irvine says, the rate of re-infestation and inoculum build-up by these pathogens is generally slow, so individual landowners could employ introductions and benefit from them without the need for subsidy.

R. N. Strange (*Department of Botany and Microbiology, University College London, U.K.*). I was interested to see Dr Deacon's slides of enucleate cortical cells of wheat. Could he explain why these cells are not rapidly destroyed by the soil microflora?

J. W. Deacon. I cannot explain this, but I should say that we remove our roots very carefully from the soil because we are as much interested in the senescent parts as in the living ones. Even from field plots, we find that one or, usually, two dead cortical cell layers remain attached to cereal roots if these are sampled carefully by collecting soil blocks and washing soil from the roots in the laboratory.

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