BIOLOGICAL CONTROL OF PLANT DISEASES - PRESENT AND FUTURE TRENDS

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ABSTRACT - Foliar pathogens are not easy to control using biological agents because of difficulties in ensuring their persistence. Species of *Bacillus* and *Pseudomonas* controlled some fungal pathogens and yeasts and *Trichoderma* spp. limited necrotrophic pathogens. Bacterial pathogens were controlled by related saprophytes. Root diseases offer greater potential for biocontrol. *Pythium* diseases were suppressed by bacteria. A *Chaetomium* and an unidentified basidiomycete significantly reduced wheat take-all. *Zygorrhynchus moelleri* inhibited the growth of *Rhizoctonia* and *Pythium* by producing β-1,3-glucanases and an antifungal indole compound, indole-3-ethanol. *Z. moelleri* enhanced root and shoot growth and caused earlier flowering and fruiting of horticultural plants. There are good prospects for biocontrol of many seed-borne diseases. Of a range of microorganisms applied to linseed seeds to control the pathogen *Alternaria linicola*, an isolate of *Bacillus* sp. and *Trichoderma harzianum* were the most effective. Various additives to alginate pellets can greatly extend the persistence of biocontrol organisms.

Index terms: biocontrol; foliar pathogens; root diseases; seed-borne diseases.

CONTROLE BIOLÓGICO DE DOENÇAS DAS PLANTAS - TENDÊNCIAS ATUAIS E FUTURAS

RESUMO - Patógenos foliares não são fáceis de controlar usando agentes biológicos devido às dificuldades em garantir sua sobrevivência. Espécies de *Bacillus* e *Pseudomonas* controlaram alguns fungos e leveduras, e *Trichoderma* spp. alguns patógenos necrotróficos. Patógenos bacterianos foram controlados pelos saprófitas mencionados. Doenças radiculares oferecem maior potencial para biocontrole. Doenças causadas por *Pythium* foram eliminadas por bactérias. *Chaetomium* e um basidiomiceto não identificado reduziram significativamente o mal-do-pé do trigo. *Zygorrhynchus moelleri* inibiu o crescimento de *Rhizoctonia* e *Pythium* ao produzir β-1, 3-glucanases e um composto antifúngico, o indol-3-etanol. *Z. moelleri* aumentou o crescimento da raiz e brotos e causou o florescimento e frutificação precoce de plantas hortícolas. Há boas perspectivas para o biocontrole de muitas doenças transmitidas por sementes. Dentre um conjunto de microorganismos que foi aplicado à sementes de linhaça para controlar *Alternaria linicola*, um isolado de *Bacillus* sp e *Trichoderma harzianum* foram os mais efetivos. Vários aditivos empregados em peletes de alginato podem prolongar a sobrevivência de organismos de biocontrole.

Termos para indexação: patógenos foliares, doenças radiculares, doenças de sementes.

INTRODUCTION

There is currently increasing interest in the development of biological techniques for the control of plant diseases. There are a number of reasons for this. Regulations governing the use of pesticides are restricting the choice and frequency of application of many previously widely-used fungicides. Concern about fungicide residues on surfaces of edible parts of crops, e.g. fruits, is now reducing their use for control of wound pathogens, skin spotting pathogens, etc. Certain pathogens have

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developed resistance to fungicides repeatedly used over many years and can no longer be effectively controlled by chemicals.

Development of new agrochemicals is becoming increasingly expensive, partly because of the cost of implementing the very extensive toxicity and other safety tests now required by law. Consequently it is often now too expensive to develop chemicals for use on so-called 'minor' crops where the smaller size of the market may be inadequate to recover development costs. Certain types of diseases have never been amenable to chemical control, e.g. root diseases such as take-all, because of the difficulty in ensuring that adequate amounts of the chemical are able to reach and protect roots which may spread out several metres from each plant.

To be effective fungicides must be applied at frequent intervals, even those which are systemic are not usually effective for more than two to three weeks. On the other hand, biological agents which are sufficiently adapted to the habitat in which they are to be released, may persist over periods of several months or more.

Certain plant pathogens, for example bacteria, cannot readily be controlled by chemicals. For this group of pathogens a biological system may provide an attractive method of control.

In this paper the operation of biological control agents will be discussed in the context of the environment of shoot, seed and root.

BIOLOGICAL CONTROL OF FOLIAR DISEASES

Shoot environment as a habitat for microorganisms

A saprophytic microflora consisting of bacteria, yeasts and filamentous fungi is normally present on the surfaces of aerial parts of green plants (Blakeman, 1985). This microflora is not randomly distributed but occupies specific niches on the plant surface which can provide an appropriate environment to support growth. Although the three main groups of organisms are present on leaves throughout the year there is normally an underlying seasonal succession in activity. Bacteria, which are often the sole initial colonists as young leaves open from buds, feed on amino acids and, to a lesser extent, soluble carbohydrates exuded from underlying leaf tissues. Basidiomycetous yeasts predominate in the middle of the growing season when the supply of carbohydrates is enriched by aphid honeydew which is partially converted to an abundance of extracellular polysaccharide which surrounds the yeast cells. Spores of filamentous fungi start to germinate as leaves begin to senesce towards the end of the growing season giving rise to sporulating colonies.

Fungal spores and bacterial and yeast cells may be deposited as airborne inocula or by water splash onto leaves. Airborne propagules may be deposited on sites which are unfavorable for growth. Propagules splashed onto leaves may collect in films of water and be moved by drainage channels and be deposited over lines of junctions between epidermal cells where multiplication or growth may take place. In the case of air-borne fungal spores deposited onto a leaf, germ tubes may grow to favorable sites for penetration. When such sites coincide with those occupied by the saprophytic flora an interaction is likely to take place.

The microclimate of crop canopies may fluctuate rapidly due to changes in relative humidity, wetness, temperature and amount of sunlight. This in turn markedly affects the growth or survival of the leaf microflora. For example, viability of microorganisms is adversely affected during periods of low relative humidity causing desiccation. Prolonged sunlight results in UV damage to microbial cells. Leaves towards the centre of a canopy suffer less from environmental extremes than those at the periphery which are

most exposed to the influence of climatic changes.

In general the physical environment around roots tends to be much more constant than that around leaves. The variable nature of the environment surrounding shoots is responsible for the substantial short term microbial population changes. Adverse microclimatic conditions on foliar surfaces may often explain the failure of microorganisms applied as biocontrol agents to persist in this environment. The majority of bacterial cells applied to leaves may not survive for more than a few hours under conditions of bright sunlight. On the other hand yeasts can tolerate dry conditions over prolonged periods (Fokkema et al. 1979). Filamentous fungal spores have some ability to resist adverse environmental conditions until germination commences when the organism becomes more vulnerable.

Control of fungal pathogens

Although the control of foliar fungal pathogens by biological agents is not as yet widely practised on a commercial scale there have been a number of experimental approaches which show potential for further development. All three main groups of microorganisms, bacteria, yeasts and filamentous fungi, have been examined for their ability to control different types of pathogens and a number of different mechanisms have been identified.

Strains of microorganisms to be used as biocontrol agents, providing they possess sufficient activity against the pathogens, are ideally best obtained from the same foliar environment as the pathogen which they are required to control. The biocontrol agent is thus more likely to be adapted to the habitat in which it is required to work. Alternatively some investigators have preferred to use organisms of proven high activity against the pathogen even though they may originate from a different microbial habitat.

Use of bacteria – Probably the most widely used group of bacteria for biocontrol purposes, the *bacilli*, are not normally regarded as resident organisms on foliar surfaces, although they may be splashed onto leaves from soil and persist for some time in this habitat. Nevertheless there are a number of examples of the use of *Bacillus* spp. to control foliar fungal pathogens. *B. subtilis*, which has probably been the most effective species in this genus for biocontrol purposes, has been tested against a range of leaf and fruit pathogens. *B. subtilis* gave over 95% control of bean rust, *Uromyces phaseoli*, on snap and dry beans, when sprayed onto plants in a greenhouse (Baker et al. 1983) up to five days before uredospore inoculation. No control of disease occurred if the antagonist was applied after uredospore germ tubes had penetrated leaves. This was not unexpected since the bacterium interfered with uredospore germination.

B. subtilis has been shown to be a natural colonist of apple leaf scar tissue of Bramley's Seedling apples in Northern Ireland (Swinburne, 1973). Leaf scars are an important entry point for the apple canker pathogen, *Nectria galligena*. If scars become colonised by *B. subtilis*, development of *N. galligena* in the leaf scar tissue is prevented. A characteristic of Bramley's Seedling apples is that leaf fall occurs over a prolonged period in the autumn. Colonisation of scars by the canker pathogen can occur rapidly as soon as each leaf is abscissed. Attempts to enhance the protection of leaf scars by artificial application of *B. subtilis* cells failed because a single spray would only result in a small proportion of leaf scars being colonised by the antagonist, the bacterium having no capacity to survive on bark and subsequently migrate to other scars after the remaining leaves had fallen.

A potentially favorable situation for the operation of a biological control system is the application of antagonists to surfaces of fruits to prevent the development of wound

pathogens during storage. Ideal conditions are present in storage for the antagonist inoculum to multiply and persist on fruit surfaces under high relative humidities at constant temperatures and in the absence of ultraviolet light which has damaging effects on microbial cells. An isolate of *B. subtilis* has been shown to control brown fruit rot caused by *Monilinia fructicola* in storage when applied to peaches, nectarines, apricots and pears (Pusey & Wilson, 1984). The mode of action was similar to that found with bean rust uredospores, i.e. germination and germ tube growth of the *Monilinia* spores was inhibited.

B. subtilis appears to owe its success as a pathogen antagonist to peptide antibiotics active against yeasts, bacteria and filamentous fungi (McKeen et al. 1986; Loeffler et al. 1986) as well as an ability to colonise wound sites actively.

Some species of *Bacillus* possess lytic activity. Examples include the destruction of cereal rust uredospore germ tubes (Morgan 1963) and the lysis of rust fruiting structures, e.g. pycnidia, aecidia and uredia (Levine et al. 1936).

Of the naturally-occurring bacteria on leaves, pseudomonads predominate and have been shown to play a role in inhibiting germination and germ tube growth of necrotrophic pathogens such as *Botrytis cinerea,* cause of grey mould, and *Phoma betae,* leaf spot of beet crops (Blakeman & Brodie 1977). Such bacteria are attracted to fungal spores as a result of enhanced nutrient leakage and their continued presence in this situation prevents uptake by the fungal spore of exogenous nutrients derived from the leaf as well as the reabsorption of leaked products from the spore (Brodie & Blakeman, 1977). Strains of *Pseudomonas* which most actively competed for amino acids were found to be more effective in inhibiting the germination of pathogen spores.

Some pseudomonads on leaves can also produce antibiotics. *Ps. fluorescens* has been shown to produce at least two antifungal antibiotics active against plant pathogens (Howell & Stipanovic, 1980). *Ps. cepacia* has been used to control *Cercospora* leaf spot on peanut and Alternaria leaf spot on tobacco (Spurr, 1981).

Use of yeasts and filamentous fungi – Leaf yeasts antagonise fungal pathogens predominantly by nutrient competition. They have little or no capacity to produce antibiotics. Pathogens which can be most successfully antagonised by yeasts are those which are necrotrophic and possess a temporary saprophytic phase on the plant surface prior to penetration, e.g. *Cochliobolus sativus* on cereals (Fokkema, 1973). Nutrient-competing saprophytes such as yeasts and some filamentous fungi such as species of *Cladosporium* and *Aureobasidium pullulans* play an important role in reducing amounts of nutrients present on leaves resulting from deposition of pollen or aphid honeydew (Fokkema 1971). Where saprophytes are absent or present in reduced numbers such sources of additional nutrients greatly stimulate pre-penetration development of necrotrophic pathogens leading to increased infection levels.

Antagonism of foliar pathogens resulting from antibiotic production by leaf-inhabiting filamentous fungi has seldom been reported. However, antibiotic-producing fungi from habitats other than the foliar environment have been used to control pathogens. *Trichoderma* species which produce both volatile and non-volatile antibiotics as well as fungal cell-wall-degrading enzymes have been demonstrated to control *B. cinerea* causing both grey mould on leaves, berries and stems of vines (Dubos & Bulit 1981) and dry eye rot of apple fruits (Tronsmo 1986). Levels of control can approach that achieved by use of fungicides applied at similar frequencies to *Trichoderma* spore applications. A difficulty in the use of *Trichoderma* species for control of diseases in cool environments, for example dry eye rot of apples, is that of selection of a

strain that can germinate and grow at sufficiently low temperatures.

Andrews et al. (1983) showed that *Chaetomium globosum*, although isolated from apple leaf litter, was able to control scab (*Venturia inaequalis*) development when applied to leaves under experimental conditions. The most likely mechanism was antibiotic production.

Some fungi have become specialised to attack the cells of plant pathogens by developing the capacity to produce lytic enzymes such as chitinases and glucanases which degrade fungal cell walls. Cereal rusts are parasitised by several fungi, of which *Eudarluca caricis* is the best known. Germination of the hyperparasite is stimulated by the presence of rust uredospores on cereal leaves (Stahle & Kranz, 1984). Uredospores are penetrated both mechanically and enzymatically. Development of satisfactory biocontrol procedures using hyperparasites such as *E. caricis* has not proved easy, partly because conidia of the hyperparasite do not remain viable for long in the absence of the rust host (Swendsrud & Calpouzos, 1972). Other hyperparasites of cereal rusts include *Verticillium lecanii* and *Aphanocladium album*.

Control of bacterial pathogens

Attempts at biological control of bacterial plant pathogens have generally involved the use of saprophytic bacteria.

Bacteria (as opposed to the *filamentona* fungi) are able to multiply at a sufficiently rapid rate to compete with bacterial pathogens on the plant surface. Many saprophytic bacteria are taxonomically closely related to pathogens and thus possess similar site preferences on the plant surface so that cells of the two organisms may come into direct contact with each other.

Competition phenomena are believed to form the basis of a significant number of interactions between bacteria on leaves. For example, the preferential occupation of specific sites on leaves as a result of inoculation of young seedlings with saprophytic bacteria gives protection against subsequent colonisation by ice nucleation active (INA) bacteria, consequently reducing frost damage. This mechanism is one of 'exclusion' of the pathogen by the saprophyte. For example, when a non-INA strain of *Erwinia herbicola* was applied to corn leaves prior to inoculation with an INA strain of the same bacterium, colonisation of leaves by the INA strain was greatly reduced (Lindow et al. 1983). When a large population of INA *Ps. syringae* had colonised pear foliage, subsequent application of non-INA antagonists failed to reduce numbers of the INA bacteria demonstrating that when sites on the plant surface have already been occupied, displacement by competing cells cannot easily take place (Lindow, 1986). Nutrient competition may also be important in interactions between closely related bacteria since protection from frost damage can be achieved using artificially constructed INA-negative strains where nutrient requirements of pathogen and antagonist are likely to be identical.

The significance of antibiotic production by some leaf-inhabiting bacteria in interactions with pathogens on leaves is not clear. Although antibiotics can be demonstrated under conditions of agar culture, it is unlikely that in most situations adequate energy resources are available from the dilute solutions of leachates on leaves to support high enough levels of metabolite production. Some leaf bacteria produce iron-chelating compounds known as siderophores under low iron conditions. As well as removing iron, siderophores may also possess antibiotic activity.

Bacteriocins are protein-containing compounds with a highly specific action against strains or closely related species of the same genus. Amongst leaf-inhabiting bacteria they have been shown to be produced by *Erwinia* and *Pseudomonas*.

There appear to be no leaf-inhabiting bacteria reported as having biocontrol

potential through parasitising bacterial pathogens. However, a parasitic bacterium from the rhizosphere, *Bdellovibrio bacteriovorus*, has been shown to control *Ps. syringae* pv. *glycinea* on soybean leaves under experimental conditions in a glasshouse (Scherff 1973). It is doubtful whether effective control could be achieved in the field because of the need to maintain high populations of the parasite.

Biological control of fireblight

Fireblight, a destructive bacterial disease of certain rosaceous plants caused by the bacterium *Erwinia amylovora* has been extensively studied in relation to possibilities for biological control. The most widely used antagonist has been *Erwinia herbicola* which often occurs closely associated with *E. amylovora* on plant surfaces. Several mechanisms have been put forward to explain the antagonism between the two organisms. These include acid production by *E. herbicola*, which is readily demonstrated using culture media to inhibit *E. amylovora*. However, when the two organisms were present together in pear fruits pH changes were insufficient to prevent growth of *E. amylovora* (Beer & Rundle 1984).

Competition for organic nitrogen by *E. herbicola* in pear nectar, which created conditions of nitrogen deficiency, was believed to explain the inhibition of growth of *E. amylovora* (Riggle & Klos 1972).

Although some strains of *E. herbicola* produce bacteriocins active against *E. amylovora*, non bacteriocin-producing strains of *E. herbicola* were also equally effective in inhibiting *E. amylovora* in pear fruits (Beer & Rundle 1984).

A strain of *E. herbicola* was shown to interfere with chemotactic responses of *E. amylovora* which assist the pathogen in locating suitable sites for multiplication on foliar surfaces (Klopmeyer & Ries, 1987). Scanning electron micrographs showed that strains of *E. herbicola* which were most successful as antagonists of *E. amylovora* competed for the same site as the pathogen on stigmatic surfaces of apple (Hattingh et al. 1986).

When *Ps. syringae* was pre-inoculated prior to subsequent *E. amylovora* inoculation or applied simultaneously with the pathogen onto hawthorn twigs under varying relative humidity conditions, the pathogen failed to be inhibited. *E. amylovora* populations showed better recovery after exposure to low relative humidity conditions than *P. syringae*. This demonstrated that *Ps. syringae* was unlikely to prove an effective antagonist under such conditions. In experiments comparing antagonistic interactions between *Ps. syringae* and *E. amylovora* on a host (*Sorbus* sp.) and non-host (*Syringa vulgaris*), it was found that *P. syringae* could only effectively reduce *E. amylovora* populations on the non-host. The better adaptation of the pathogen to its host enabled high populations to be maintained despite the presence of a potential antagonist.

BIOLOGICAL CONTROL OF ROOT DISEASES

Root environment as a habitat for microorganisms

Many microorganisms which can become established in the environment surrounding living plant roots (the rhizosphere) fail to develop in soil away from roots. This is because plant roots influence the soil environment in a number of ways, especially by nutrient enrichment resulting from root exudates which provide a source of amino acids and carbohydrates to support microbial growth.

Bacterial populations around roots comprise similar Gram-negative genera to those associated with leaves. Some of these such as the pseudomonads have been shown to be effective biocontrol agents against root diseases.

Because of the more favorable environment around roots, with fewer extremes than on leaves, biocontrol organisms can persist for longer periods and spread over developing root systems providing a degree of protection against pathogens.

In this section examples of research on the control of root diseases by bacteria and filamentous fungi are taken from work currently in progress in The Department of Mycology and Plant Pathology and The Plant Pathology Research Division, Belfast.

Suppression of *Pythium* by bacteria

In a study of the potential of a large number of isolates of bacteria from apple roots, orchard soil and soil from a parsley field to inhibit *Pythium* spp., Ward (1989) found that less than 10% of the isolates suppressed colony growth of *Pythium* on agar by more than 50% in a ring-plate assay. Effective bacterial isolates produced inhibition zones indicating antibiotic production.

Growth of apple and parsley seedlings, over an eight week period, in compost contamined with *Pythium* spp. was stunted; mean seedling weights were reduced by 144% and 107% respectively compared with growth in pathogen-free compost. Of the 40 bacteria antagonistic in the ring-plate assay, applied as drenches to compost, 20 isolates significantly reduced the severity of *Pythium*-induced disease in apple seedlings, while in the presence of 15 other isolates, disease severity was significantly increased. Eleven of the isolates which reduced disease severity in apple seedlings also significantly reduced root disease in parsley. When any one of six of these biocontrol bacteria was used to drench *Pythium*-contaminated compost, growth of apple seedlings was significantly greater than in undrenched, pathogen-free compost. Only one of these, an isolate of *Pseudomonas putida*, similarly enhanced growth of parsley seedlings.

Control of take-all of wheat by antagonistic fungi

Take-all (*Gaeumannomyces graminis*) has been recognised in N. Ireland for many years, but with the rise in popularity of winter crops it has become a significant problem. It is now one of the major factors in preventing continuous wheat-growing in the area. The pathogen attacks the roots and causes clogging and disruption of the vascular system, which results in light coloured and light weight heads. Although there are claims of partial control with the seed-dressing Baytan, these are not always very consistent and rotation appears to be the most rewarding method of control at present (Jones & Clifford 1983).

Natural biological control exists to some extent, in that disease levels increase in continuous wheat up to a maximum after about five years and thereafter decline. This decline has been attributed to various antagonistic fungi in the soil, such as *Phialophora* spp. (Deacon 1981). However, to date, no successful commercial biological control preparation has been available. Work in Belfast has concentrated on two possible agents, both isolated from wheat, a *Chaetomium* sp. from seed, and a white basidiomycete isolated from roots.

Effect of Chaetomium – Take-all was introduced to the soil of 0.5 m diameter concrete pots (placed in the open air during the growing season) as autoclaved and inoculated seed. The *Chaetomium* isolate was inoculated into the same soil in three ways – with autoclaved and inoculated straw, with autoclaved and inoculated seed, or with seed first treated in this manner, but subsequently air-dried, ground up and used as a seed-dressing. The effect of the *Chaetomium* isolate was compared with that of isolates of *Zygorrhynchus moelleri* and *Microdochium bolleyi*, the first applied as a drench of spores and the second as inoculated straw. There was also an uninoculated control. Wheat seeds were added at the same time as the inoculants.

Subsequent levels of take-all in plants from *Chaetomium*-treated pots were considerably lower than in plants from control pots. There was a lesser effect with *M bolleyi* and no effect with *Z. moelleri*. There appeared to be no differences due to methods of inoculation. Levels of take-all were clearly related to amounts of inoculum. Plants from *Chaetomium* treatments had fresh weights greater than those of control or *Zygorrhynchus*-treated plants. On the other hand *Zygorrhynchus*-treated plants without take-all showed evidence of growth-stimulation. Although these promising results were obtained in outdoor pots, tranference of the system to field conditions has so far proved disappointing.

Effect of white basidiomycete – This was also highly effective at reducing the level of take-all of plants in pots and increasing their fresh weight. As with *Zygorrhynchus*, there was some evidence of growth-promotion in the absence of take-all.

Pathogen suppression and enhancement of plant growth by *Zygorrhynchus moelleri*

Zygorrhynchus moelleri is frequently isolated from agricultural soils. When inoculated into soil it extensively colonises roots where it may occupy potential infection sites, antagonise root pathogens and produce hormones for plant growth enhancement.

Pathogen suppression – When paired with *Rhizoctonia solani* and *Pythium* spp. on agar, *Z. moelleri* inhibited growth of the opposing fungi (Brown 1987). *R. solani* either ceased growth or advanced very slowly for a few millimetres; hyphae of the antagonist intermingled with those of *Pythium* spp. Microscopic examination of dual cultures with *Pythium* spp. revealed that many of the pathogen hyphae were lysed and devoid of contents. Lysis of *R. solani* hyphae was observed at the boundary between colonies of pathogen and antagonist. Inhibition of growth of *R. solani* by *Z. moelleri* was also demonstrated by reduced cellulolysis of the pathogen on filter paper (Brown, 1988). Neither *R. solani* nor *Pythium* spp. could be reisolated from composts 10 days after dual infestation with *Z. moelleri* (Brown 1987).

When grown in liquid cultures containing laminarin or hyphal walls prepared from *R. solani* or a *Pythium* sp. as the sole carbon source, *Z. moelleri* produced the lytic enzymes β-1, 3-glucanase and β-1, 3(4)-glucanase (Brown 1987). Maximum enzyme production was detected in 4-day old cultures. Neither chitinase nor cellulase was produced by this fungus. Cell-free culture filtrates containing these enzymes lysed, to varying degrees, hyphal walls of a number of plant pathogenic fungi.

On agar *Z. moelleri* produced substance(s) which inhibited colony growth of *R. solani* and *Pythium* spp. as well as other soil-borne fungi. A compound with antifungal activity was extracted with diethyl ether from liquid cultures of *Z. moelleri*. The compound was located on thin-layer chromatograms oversprayed with *Cladosporium cladosporioides*, and was believed to be indolic in nature.

The presence of *Z. moelleri* in composts gave reductions in the severity of a number of root diseases. After six weeks in compost contaminated with *R. solani*, flax seedlings were stunted, while in compost contaminated with *R. solani*, but also containing 10^6 propagules/g of *Z. moelleri*, the pathogen had no significant effect on plant growth (Brown 1987). Pre-emergence damping-off in flax seedlings caused by *Pythium intermedium* was also suppressed by the inclusion of *Z. moelleri* in compost. Suppression of *Pythium* root disease on parsley, radish and *Zinnia* plants was reduced by more than 70% in composts containing the antagonist (Brown 1987) (Table 1). The dry weight of cucumber plants 50 days after sowing seed in compost contaminated with *Pythium* sp. was enhanced by almost 300%, and mature fruits were produced 13 days earlier, when *Z. moelleri* was incorporated into the compost.

TABLE 1. Influence of *Zygorrhynchus moelleri* on disease severity in seedlings of three host plants induced by *Pythium* spp. (Data of Brown, 1987).

Treatment	Parsley[ac]	Radish[bd]	Zinnia[bd]
None	0	0	3
Pathogen only	59	80	88
Z. moelleri only	5	0	7
Pathogen + *Z. moelleri*	11	17	23
S.E.M.[e]	3.7	5.1	4.4

[a] = contaminated with *P. paroecandrum.*
[b] = contaminated with *P. intermedium.*
[c] = assessed as reduction (%) in plant dry weight.
[d] = assessed as percentage damping-off.
[e] = S.E.M. = standard error of means.

Plant growth enhancement – *Z. moelleri* can also enhance the growth of plants in the absence of pathogens. Two flowering annuals, *Mesembryanthemum* and *Calendula*, produced plants of *c.* 40% greater dry weight in pathogen-free soilless compost infested with *Z. moelleri* than in uninfested compost (Brown & Surgeoner 1990). The flowering perennial *Viola* showed 180% increase in dry weight in *Z. moelleri*-infested compost. This greater growth response in *Viola* compared with the other two species could have been expected from its more bushy growth habit. Plants of all three species also produced flowers several days earlier in the infested compost. Cucumber and tomato plants similarly showed a growth response of 35 to 40% increase in plant dry weight 50 days after sowing seed on pathogen-free *Z. moelleri*-infested compost compared with plants in pathogen-free *Z. moelleri*-infested compost compared with plants in pathogen-free uninfested compost (Brown & Surgeoner 1991). Mature cucumber fruits were produced five days earlier and *c.* 25% more fruit was harvested from 15-wk-old tomato plants in the infested compost. Significantly more chlorophyll was also extracted from leaves of tomato plants of this age in the infested compost than in the uninfested compost.

Seedlings of tomato and cucumber grown on agar in close proximity to a colony of *Z. moelleri* developed more lateral roots than seedlings grown in the absence of the fungus. Tomato seedlings, from which the roots had been removed, similarly developed more roots when suspended in a spore suspension of *Z. moelleri* than when suspended in water. Spore germination occurred in the suspension and fine hyphae were observed growing from the developing roots.

The mechanism of growth enhancement is believed to be related to the production by *Z. moelleri* of indole-3-ethanol (IEt), an analogue of indole-3-acetic acid (IAA). In casamino acid medium, growth of *Z. moelleri* reached the stationary phase within 96 h. IEt production commenced within 24 h incubation and peak production (*c.* 30.0 μg 100 ml^{-1} medium) occurred after 108 h growth. Very small amounts of IAA (<0.5 μg 100 ml^{-1} medium) were also detected in culture filtrates. *Z. moelleri* metabolises IEt but not to IAA. When culture filtrates and extracts of mycelium from *Z. moelleri*, obtained from tryptophan-containing medium, were assayed for IET oxidase activity, neither indole-3-acetaldehyde nor IAA was detected in reaction mixtures, indicating an absence of activity of this enzyme. Evidence from a mycorrhizal interaction suggests that *Z. moelleri* may

transfer IET to plant roots where it may be utilised as a precursor for IAA synthesis (Barroso et al. 1986). IEt induces a root growth response similar to that of IAA as a result of its regulated conversion to IAA by the plant (Percival et al. 1973).

When separated on TLC, IEt was shown to be the compound produced by *Z. moelleri* in liquid culture which showed antifungal activity. IEt is, however, relatively weakly fungitoxic, having an ED_{50} value of *c.* 200 ppm against *Pythium* spp. and *R. solani* in poison-plate assays.

BIOLOGICAL CONTROL OF SEED-BORNE PATHOGENS

Seed environment as a habitat for microorganisms

The seed provides a very suitable medium for the maintenance of pathogens, enabling them to be associated with the host right from germination. In some cases, such as smuts (*Ustilago* spp.) and bunts (*Tilletia* spp.) and ergots (*Claviceps* spp.), the association between host and pathogen is a very intimate one and the whole seed becomes converted either into a mass of spores or fungal mycelium. In the majority of cases of seed-borne diseases, however, the association is less intimate. Neergaard (1977) stated that the seed coat is the commonest site for most seed-transmitted Fungi Imperfecti. In many instances while the seed-coat may be infected the underlying endosperm is completely unaffected.

This can be clearly seen in the case of flax/linseed (*Linum usitatissimum*). Seed-borne fungi enter the capsule as it begins to mature and colonise the outer layer of the seed-coat and become dormant. The outer layer is shiny and hard when dry, but when water is imbibed as the seed germinates, the layer becomes mucilaginous. At this stage the dormancy of the hyphae is broken and they start to grow very rapidly. In the case of the main linseed pathogen in the U.K., *Alternaria linicola*, hyphae can begin to emerge from the seeds as little as 2 h after the seed is placed in a moist environment. A germinating seed may have hyphae growing out of, and over, the seed coat, while the emerging radicle is completely clear of hyphae. If conditions are favorable for the growth of the seedling it may escape with relatively little damage, but if the weather is cold and wet and germination is delayed, hyphae will infect the seedling and may cause its death.

Biological control of seed-borne pathogens of linseed

The role of a disease control treatment on seeds is to contain the pathogen to the area of the seed and allow the emerging seedling to remain uninfected. This may be difficult for pathogens which have an intimate relationship with the seed, such as those mentioned earlier. However, with a situation, such as that described for linseed, control of pathogens would appear to be relatively easy and, indeed, there are fungicidal treatments which are very effective and can reduce a level of contamination with *A. linicola* of 100% to practically zero. On the other hand, there have been problems with resistance of *A. linicola* to the main fungicide used for this purpose, iprodione (Mercer et al., 1989). For that reason the possibility of biological control was investigated.

Choice of isolates – Thousands of seed samples are examined at the Plant Pathology laboratory in Belfast annually and from these samples isolates were made of the various microorganisms present. Unfortunately the great majority had at least some measure of pathogenicity. Amongst the least pathogenic fungi were another *Alternaria* sp. (*A. alternata*) and the ubiquitous *Epicoccum nigrum*. There were also a number of *Pseudomonas* spp. and the occasional *Bacillus* sp. As well as these naturally-occurring microorganisms, a range of isolates *of*

Trichoderma spp. was also examined. Some of these performed very well on contaminated seed plates. Screening against *A. linicola* was carried out (on paired plates in the case of fungi and ring plates in the case of bacteria) with 15 isolates of *Trichoderma* spp., one isolate each of *A. alternata*, *E. nigrum* and a *Bacillus* sp., and three isolates of *Pseudomonas* spp. Further screening was carried out on seed with a very high level (85%) of natural contamination with *A. linicola*. This was performed either on 1/16% malt agar in Petri dishes, or in compost in pots. Results were largely similar in both pots and dishes.

Effect on percentage seed germination – There was an overall reduction in percentage germination, although there was substantial variation between isolates. Some, like T12 (*T. viride*), had little effect while others like T5 (*T. harzianum*), T7 (*T. hamatum*) and T14 (*T. viride*), were quite severe.

Disease control – There was a substantial overall reduction in levels of the pathogen *A. linicola*, although with considerable variation between isolates. There was a tendency for the most effective antagonistic organisms such as T5 (*T. harzianum*) and T7 (*T. hamatum*) also to be the most phytotoxic. The most practical choice would be an organism such as T1 (*T. harzianum*) which gave acceptable disease control with little phytotoxicity.

There was an overall improvement (average 127% for all isolates tested) in percentage of healthy plants over the control, although from a very low level. The most effective isolates were B1 (*Bacillus* sp.) and T1 (*T. harzianum*), giving three to four times as many healthy plants as the average of all isolates. Seedling emergence was very poor after treatment with T5 (*T. harzianum*) and T7 (*T. hamatum*).

A measure of disease control was thus achieved with most isolates. In some cases this effect was cancelled out by a high level of phytotoxicity. An isolate of *Bacillus* sp. and *T. harzianum* were the most promising biocontrol agents for use on seed of flax and linseed.

PELLETISATION OF BIOCONTROL AGENTS

Various materials incorporated into alginate pellets have been examined for efficacy in enhancing survival of biocontrol bacteria and fungi (Ward 1989). Survival of biocontrol microorganisms in alginate pellet formulations was highly variable. Spore-forming bacteria were pelleted much more successfully than non-spore-forming genera, and melanised spores of fungi, e.g. zygospores of *Z. moelleri* survived pelletising processes more successfully than non-melanised spores. The addition of carboxymethyl cellulose and glycerol to pellet formulations generally improved the duration of viability of bacteria (Fig. 1). The inclusion of gum arabic or gum guar improved the pelletisation of fungal spores.

The release of bacteria from alginate pellets varied with soil type, larger numbers of bacteria being released more quickly in less acidic substrates such as limed John Innes No. 2 compost than in peat. Larger numbers of bacteria were found associated with parsley roots, after release from pellets, than with soil particles, and soil type had less influence on bacterial numbers at root surfaces than on soil particles. Application of an antagonistic isolate of *P. putida* in an alginate pellet formulation to *Pythium*-contaminated composts reduced disease severity. In some soils, however, bacteria-free pellets, included in experiments as controls, exacerbated the disease.

P. putida was successfully incorporated into and released from alginate pellets which also contained the fungicides Ridomil mbc 60WP, Aaterra 35WP, Dithane 75WP, Fubol 75WP or Benlate 50WP at concentrations equivalent to manufacturers' rates. These pellets were more efficacious when used to control *Pythium*-induced root disease in parsley than when pellets containing fungicide alone were used.

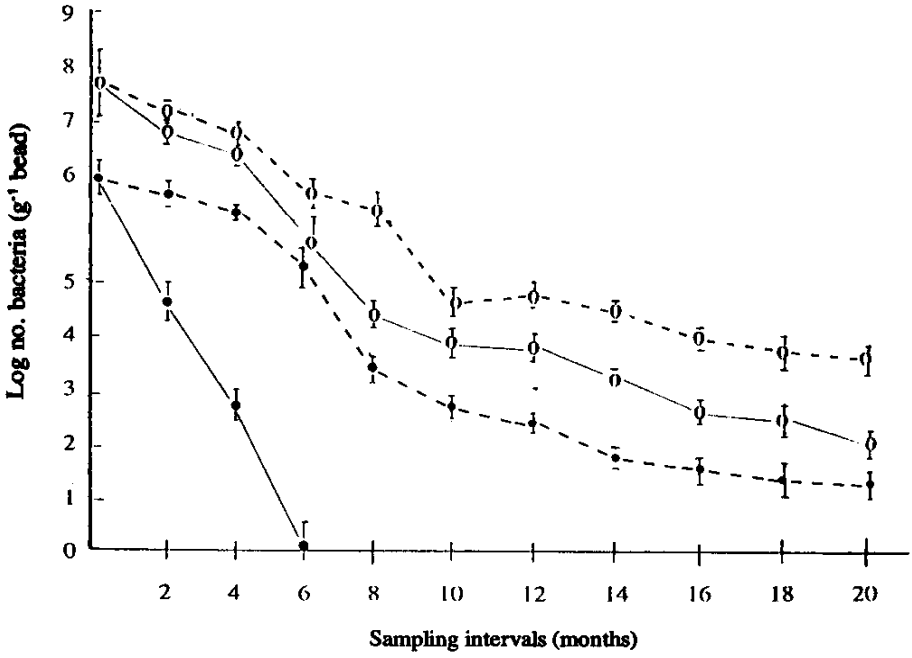

FIG. 1. Survival of biocontrol bacteria *Pseudomonas putida*, KK-EFR1a rif (–●–) and *P. fluorescens/ Bacillus cereus* subsp. *mycoides*, LK – AS4 rif (–0–) in freeze-dried alginate beads (solid line) and in alginate beads containing 0.2% w/w carboxymethylcellulose + 5% w/w glycerol (dashed line). Bars represent standard error P = 0.05. (Data of Ward 1989).

FUTURE PROSPECTS

The use of biological control methods is likely to become more widespread in the future as increasing pressure develops to limit both environmental damage from the use of chemicals and resistance of pathogens to pesticides.

Systems for the control of root pathogens are likely to be developed first because the soil environment provides a more favorable environment for the persistence of antagonists. There will be increasing attention given to enhancing the persistence of biocontrol agents by improving methods of pelletisation of inoculum.

At present it is only feasible to prepare industrial quantities of inoculum of biocontrol agents by using organisms which produce abundant propagules in liquid culture, e.g. bacterial cells and fungi that form spores under submerged conditions. Methods will need to be found for improving the ability of otherwise useful biocontrol agents to form large numbers of spores under conditions which will enable bulk inoculum production.

It is likely that genetically engineered biocontrol agents will be increasingly used in the future because it is often difficult to select from the natural microflora an organism that is both well adapted to persist in the environment of roots or shoots of crop plants and which possesses a high level of

antagonistic activity against pathogens. Introducing an antagonistic capability, such as antibiotic or lytic enzyme production, into an organism which is both persistent and an effective colonist on roots or shoots may be the way in which such difficulties can be overcome. Such developments will need to be associated with risk assessment studies to ensure the safety of released engineered biocontrol agents.

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