PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Biological Control of Air-Borne Pathogens [and Discussion]

J. Rishbeth, R. D. Lumsden, J. N. Gibbs, W. D. Hamilton and R. J. Cook

Phil. Trans. R. Soc. Lond. B 1988 **318**, 265-281
doi: 10.1098/rstb.1988.0009

Phil. Trans. R. Soc. Lond. B **318**, 265–281 (1988)
Printed in Great Britain

Biological control of air-borne pathogens

By J. Rishbeth, F.R.S.

Botany School, University of Cambridge, Downing Street, Cambridge CB2 3EA, U.K.

Some pathogens are partly controlled by microorganisms that occur naturally on aerial surfaces of plants, and many attempts have been made to improve control by applying selected antagonists to such surfaces. Antagonists often compete for nutrients with the pathogen, and antibiotics may be formed that reduce germination of its spores and subsequent growth. Hyphae of fungal pathogens may be killed on contact with the antagonist or by direct penetration. The plant's defences may be stimulated before challenge by a pathogen. Apart from killing the pathogen, an antagonist may reduce its reproductive capacity. The examples given illustrate the operation of these different mechanisms in the control of a wide variety of diseases. For diseases of foliage, flowers or fruit, glasshouse crops offer more attractive possibilities for control than field crops because the population level of antagonists is easier to maintain. In some cases plants can be protected by inoculation before transplanting them to the field. Foliage and canker diseases of forest trees present problems too intractable for successful control, but in orchards the prospects are better; for example, methods are available for combining pruning with application of inoculum. Similarly, in some circumstances tree stumps can be inoculated to prevent colonization by a pathogen. Where biological methods are as effective as chemical ones and comparable in cost, they are to be preferred on environmental grounds. In some cases they can be combined with advantage; for example a lower concentration of fungicide may suffice if applied with an antagonist.

Introduction

Interest in biological control of pathogens that affect aerial parts of plants developed more slowly than in the case of pathogens affecting roots. This is probably because those of the former type tend to be more readily controlled by chemical methods, which are often very effective. As Baker & Cook (1974) point out, the two types of method are in direct competition. In addition, the ecology of microorganisms present on aerial surfaces, an understanding of which facilitates successful biological control, only became a popular field of investigation comparatively recently. However, when some pathogens developed resistance to fungicides and concern increased about toxic residues in plants, the advantages of biological methods became more evident. Some fungal diseases actually increased in severity as a result of fungicide treatment. Pathologists began to realize that some pathogens are partly controlled by microorganisms occurring naturally on aerial surfaces of plants, and that it is useful to look for ways of increasing this control.

In this account emphasis is laid upon the direct use of microorganisms. As the title implies, the majority of diseases considered affect aerial parts of plants, but there are a few anomalies. Thus air-borne fungi that are controlled by inoculating stumps, such as *Heterobasidion annosum*, are included even though they mainly attack roots. A pathogen regularly transmitted by insects, *Erwinia amylovora*, is mentioned because it is also dispersed by wind. *Agrobacterium radiobacter* var. *tumefaciens*, although soil-borne, is included because the symptoms it causes

appear predominantly on stems. The examples that follow have been selected to illustrate the different types of disease that are subject to biological control; methods range from interesting possibilities, which for various reasons may be unsuitable for wide application, to well established procedures employed on a large scale. In many cases the mechanism of control is only partly understood. Inevitably there is some overlap between categories of disease; for example some pathogens that enter stem wounds cause galls or cankers. Many aspects of biological control have been discussed at greater length than is possible here by Blakeman & Fokkema (1982) and Cook & Baker (1983), for example.

Foliage diseases

In recent years considerable attention has been paid to components of the microflora present on the leaf surface, which is a specialized habitat commonly known as the phylloplane. This is often subject to rapid and wide fluctuations of temperature, relative humidity and radiant flux. The nutrient status of water films is usually low but may be increased, especially as leaves grow older, by leakage from their cells and deposition of materials such as honey dew from aphids and pollen. The resident microflora and leaf pathogens compete for nutrients, and the results of such competition and antagonistic reactions between them may largely determine disease severity.

Several cases are known where the existence of natural biological control has been revealed by use of a fungicide. Such an effect was demonstrated experimentally by Fokkema *et al.* (1975) with rye; they monitored simultaneously colonization of the leaf surface by saprotrophic fungi and leaf infections by *Cochliobolus sativus*. Spraying leaves with benomyl reduced the population of saprotrophic fungi about tenfold by comparison with water-sprayed controls, and there was a corresponding increase in necrosis when such leaves were inoculated with a strain of *C. sativus* resistant to benomyl.

Naturally occurring saprotrophic fungi have been used in attempts to control leaf-infecting fungi. An early example is provided by the work of Wood (1951), who inoculated leaves of lettuce plants growing in a frame with selected antagonists and *Botrytis cinerea*. Subsequent leaf rot by *Botrytis* was reduced, the most effective fungal antagonists being *Penicillium clavariaeforme* and a species of *Fusarium*. Better control was obtained when antagonists were inoculated 3 days before *B. cinerea* than when they were inoculated simultaneously. Pace & Campbell (1974) made small wounds in leaves of cabbage and Brussels sprout seedlings and inoculated them with suspensions of spores of *Epicoccum nigrum* (1.6×10^7 ml^{-1}) or *Aureobasidium pullulans* (3.6×10^7 ml^{-1}) and also with spores of the leaf pathogen *Alternaria brassicicola* (1.6×10^7 ml^{-1}). Levels of infection were significantly lower ($p < 0.01$) with an antagonist present than with the pathogen alone. If antagonists were introduced into wounds 14 hours before inoculation with *A. brassicicola*, infection was generally about 50% lower than with simultaneous inoculation. There was evidence that *A. pullulans* produced an inhibitor.

There have been few reports of field applications. Fokkema *et al.* (1979) sprayed suspensions of *Sporobolomyces roseus* and *Cryptococcus laurentii* var. *flavescens*, together with nutrients, onto recently expanded wheat leaves and noted a hundredfold increase in their leaf-surface population within a few days. When leaves were subsequently inoculated with *Septoria nodorum* or *Cochliobolus sativus*, infection by both pathogens was about 50% less than on leaves sprayed with water. This effect lasted for 3 weeks but later disappeared as the population of inoculated

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

yeasts declined. *Chaetomium globosum* was found to be antagonistic to the apple-scab pathogen, *Venturia inaequalis*, under field conditions (Cullen *et al.* 1984). Suspensions of *C. globosum* ascospores at a concentration of $1–2 \times 10^6$ ml^{-1}, applied to young apple leaves at intervals of 1–2 weeks, reduced the incidence of scab by more than 20%. Ascospores germinated on scab-infected tissue more freely than on healthy tissue. However, the population of *C. globosum* declined from 314 to 36 propagules per unit area of leaf surface between applications. In the same investigation, *Aureobasidium pullulans* was found to have too low a survival rate after inoculation to exert any useful control. There was evidence that its decline was partly due to desiccation, and other factors such as removal by rain and competition with phylloplane microorganisms probably contributed. The majority of pathogens that are sensitive to antagonism normally grow over the leaf surface to some extent before penetrating it (Blakeman & Fokkema 1982). In many instances such antagonism probably results from competition for nutrients, which restricts surface growth of the pathogen and thus its opportunity to cause infection.

A very different mechanism of control is involved when the host defences are stimulated before challenge by a pathogen. An example of such a method, sometimes referred to as cross-protection (Cook & Baker 1983), is provided by control of tobacco brown spot, caused by *Alternaria alternata*. When a non-pathogenic strain of this species was applied at a concentration of 10^4 conidia ml^{-1} to tobacco leaves and a pathogenic strain of the same species was inoculated at the same dosage 3 days later, leaf spotting was reduced by 60% in laboratory experiments and 65% under field conditions (Spurr 1977). Similarly, when a cotyledon or the first leaf of a cucumber seedling was inoculated with *Colletotrichum lagenarium*, causing anthracnose, and the plant was again inoculated with the same pathogen 7 days later, the number and size of lesions were reduced (Kuć & Richmond 1977). The extent and persistence of this effect were proportional to the dosage used for the first inoculation. Even more interestingly from a practical viewpoint, appreciable protection of cucumber and watermelon was obtained by applying *C. lagenarium* spores to the first true leaf of seedlings about 7 weeks before transplanting them to the field (Caruso & Kuć 1977). This type of resistance can be induced by microorganisms other than *C. lagenarium* and is therefore not very specific. One of the defence mechanisms has been studied in melons by Esquerré-Tugayé *et al.* 1979), who showed that protection is associated with the appearance in cell walls of glycoproteins rich in hydroxyproline, production of which is triggered by ethylene. In other cases there is evidence that phytoalexins are produced.

By contrast with the fungi considered so far, hyperparasites have a different mode of action because they infect biotrophic pathogens such as powdery mildews and rusts. If used for biological control, hyperparasites must be applied after infection has occurred, rather than before as in the case of saprotrophs. Their potential for control is disputed (Krantz 1981), but some good results have been obtained in the glasshouse. Thus Jarvis & Slingsby (1977) found that infections of cucumber by *Sphaerotheca fuliginea* were significantly reduced by spraying them with a suspension of conidia of *Ampelomyces quisqualis* at a concentration of 10^5 ml^{-1}. This treatment was given at intervals of 7–10 days for about 1 month and was particularly effective when plants were sprayed with water between inoculations. When this was done the yield of cucumbers was increased by 56%. Sundheim (1982) also obtained an increase in yield after spraying infected cucumbers with *A. quisqualis*; this increase was about 60% despite the fact that development of powdery mildew was not retarded. Similar increases of yield were

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

obtained with the fungicide triforine, and also a combination of the hyperparasite with one third the normal concentration of triforine. Air-borne dispersal of *A. quisqualis* was indicated by its parasitism of *S. fuliginea* on unsprayed control plots. Grabski & Mendgen (1985) sprayed beans with a suspension of spores of *Verticillium lecanii*, a parasite both of rusts and insects, 10 days after they had become infected by *Uromyces appendiculatus* and found that spread of the rust to adjacent uninoculated plants was reduced by 68% by comparison with unsprayed controls.

Hyperparasitism is sometimes exhibited by relatively unspecialized phylloplane fungi. For example, cucumber powdery mildew has been controlled with *Tilletiopsis minor* (Hijwegen 1986). This fungus restricted growth of *S. fuliginea* when applied to plants growing in a Weiss climate cabinet or glasshouse at concentrations between 10^6 and 2×10^8 propagules ml^{-1}; applications were made 7–9 days after inoculating with the pathogen. The number of apparently healthy *S. fuliginea* conidiophores bearing conidia was reduced to 1% of those on untreated plants. After spraying twice with *T. minor* at an interval of 3 days, no secondary infections occurred and plants remained free from powdery mildew for 3 weeks. Preventative spraying 1 day before inoculation with *S. fuliginea* had little effect. A strain of *T. minor* resistant to the fungicide fenarimol was found that might be useful in a scheme of integrated control. *Cladosporium* spp. are believed to reduce epidemics of popular rust, caused by *Melampsora laricipopulina*, in the Canberra district of Australia partly because they are hyperparasitic on this rust. Sharma & Heather (1983) have demonstrated post-penetrative antagonism by *Cladosporium tenuissimum* to the rust, which produces fewer uredia; earlier work had shown that pre-penetration effects also occur.

Newhook (1951) showed that various bacteria gave protection against *Botrytis cinerea* when they were applied to wounded leaves of lettuce seedlings. He attributed this to production of antibiotics and increase of pH in lettuce tissue from 6.1 to 7.8–8.4, at which level growth of *B. cinerea* and activity of any pectinase it produced were minimal. Since then considerable interest has developed in the use of bacteria for controlling foliage diseases. As in the case of saprotrophic fungi, bacteria must be applied to plants in time and in sufficient numbers to antagonize pathogens (Spurr 1981). The search for suitable bacteria may be lengthy: thus Leben (1964) found that only one of 230 isolates obtained from cucumber leaves was active against *Colletotrichum lagenarium*. This bacterium, later identified as *Pseudomonas cepacia*, reduced disease incidence in the glasshouse but not in the field, where fewer than 1% of cells remained viable on sprayed leaves after 1 day. After investigation of factors that affect survival of bacteria and their adherence to leaves, field trials were done on crops of tobacco and peanut (Spurr 1981). *P. cepacia* was tested, as were *Bacillus cereus mycoides* and *B. thuringiensis*. This last species had shown activity in earlier bioassays and is of particular interest because it is widely used against insect pests and readily available in commercial preparations. Aqueous sprays containing about 10^8 bacterial propagules ml^{-1} were applied at intervals of 7 or 14 days. A significant reduction in the number of leaf-spot lesions in tobacco, caused by *Alternaria alternata*, was obtained with *P. cepacia* and *B. cereus mycoides*, and also with a commercial formulation of *B. thuringiensis*. Infection by *Mycosphaerella arachidis*, causing leaf-spot on peanut, was also reduced by these bacteria, but much better control was obtained with benomyl. A noteworthy feature of this experiment is that significant reductions in disease were obtained over three successive seasons, which differed in temperature and rainfall.

Work on bean rust, caused by *Uromyces phaseoli*, has shown that a biotroph can also be

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

controlled by bacteria (Baker *et al.* 1983). Under certain conditions in the glasshouse or cold frame, infection by *U. phaseoli* was reduced by *B. subtilis*, *B. cereus mycoides*, *B. thuringiensis* and *Erwinia ananas* pv. *uredovora*. One strain of *B. subtilis* was especially effective, giving 95–98% reduction in the number of rust pustules when suspensions were sprayed onto plants 2 hours – 5 days before inoculating with *U. phaseoli*. A heat-stable inhibitor was present in culture filtrates of *B. subtilis*. In later field experiments, in which *B. subtilis* was applied three times a week, severity of bean rust was reduced by at least 75% in two successive years (Baker *et al.* 1985). However, one of the two isolates tested reduced yield greatly; the other did not. In some tests, control by the bacterium was better than the once-weekly application of the fungicide mancozeb. Experiments have also been done with some tropical crops. Purkayastha & Bhattacharyya (1982) found *Bacillus megaterium* to be highly antagonistic towards *Colletotrichum corchori* on jute: spraying leaves with a bacterial suspension 1 day before inoculating with *C. corchori* greatly reduced the number of lesions after 2 days and the spread of lesions after 4 days. The same species was used by Islam & Nandi (1985) in an attempt to control brown spot of rice, caused by *Drechslera oryzae*. In pot experiments infection was prevented by spraying with a relatively low concentration of cells, 10^4 ml^{-1}, 8 hours – more than 15 days before inoculating with the fungus. In field experiments spraying with bacterial suspension at 15 day intervals until the grain was mature reduced disease incidence and improved crop growth and yield.

A remarkable instance of biological control is provided by the use of *Bdellovibrio bacteriovorus* for bacterial blight of soybeans, caused by *Pseudomonas syringae* pv. *glycinea* (Scherff 1973). In a glasshouse experiment, *B. bacteriovorus*, which was isolated from the rhizosphere of soybean roots, inhibited the development of local and systemic lesions when inoculated with the pathogen at ratios of 9:1 or 99:1. The mechanism of control is unusual, for the minute comma-shaped bacterium penetrates the host cell by a very rapid drilling action, and after entering it destroys the contents.

Finally, a method of control that does not involve direct use of microorganisms should be mentioned. Schönbeck & Dehne (1986) describe a method by which resistance to biotrophic leaf pathogens that form haustoria can be induced by applying microbial metabolites. A strain of *Bacillus subtilis* that had no detectable ability to produce antifungal compounds was used. The reduction of powdery mildew on induced-resistant wheat plants was greater in field experiments than in the glasshouse, in marked contrast to results usually obtained with antagonists. In addition, far fewer conidia were produced. Resistance appears to be based on an impairment of fungal nutrition caused by reduced haustorial efficiency. This method may have good potential as an additional means of disease control.

Diseases of flowers and fruits

One line of research in this field developed from the observation that in wind-fall apples *Trichoderma pseudokoningii* often replaces *Botrytis cinerea* in lesions at the top of the fruit. Tronsmo & Raa (1977) found that although *T. pseudokoningii* partly controlled *B. cinerea* infection when they were both sprayed onto flowers, it did not control natural infection because of failure to grow at temperatures below 9 °C. Later it proved possible to control such infection by using cold-tolerant strains: for instance spraying apple blossom in an orchard three times with *T. harzianum* in 1% malt extract at a concentration of 10^7 spores ml^{-1} gave a reduction in fruit rot

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

of 41 % and was as effective as fungicides in current use (Tronsmo & Ystaas 1980). In the case of strawberries many fungicide applications may be required to control *B. cinerea* rot; the successful use of *Trichoderma* spp. for spraying during the flowering period (Tronsmo & Dennis 1977) is therefore encouraging, particularly because it was again as effective as using a fungicide. Hyphal interaction and production of non-volatile inhibitors by the antagonists were thought to be important in the control of rot.

Extensive trials in French vineyards have shown that *Trichoderma* sp. also has considerable potential for controlling *B. cinerea* rot of grapes. Dubos *et al.* (1978) applied homogenized cultures of *T. viride* containing 10^8 spores ml^{-1} to vines from the beginning of flowering until 3 weeks before harvest: the proportion of rotted grapes was reduced from 32 % in controls to 9 %. This method was nearly as effective as using the fungicide dichlofluanid. Treatment before and after flowering probably ensures that much of the senescent floral material is colonized by *T. viride*; this tends to restrict establishment of *Botrytis* and delays the appearance of fruit rot. Inoculum of various *Trichoderma* spp. has been produced in Europe for several years, especially in the form of pellets (Ricard 1981).

In cereals infected by *Claviceps purpurea*, the cause of ergot, several *Fusarium* spp. can colonize ovary tissue containing the parasite. Mower *et al.* (1975) tested a world-wide collection and discovered that certain strains of *F. roseum* were the most virulent hyperparasites of *C. purpurea*. Of these, they discovered that *F. roseum* f.sp. *sambucinum* was a very effective agent of control both in glasshouse and field trials. *F. roseum* spores were sprayed onto field-grown rye, irrigated by sprinkler, in which the disease was at the honey-dew stage. Spores produced on the sphacelial stage were dispersed by insects, thus providing secondary spread. The level of control over ergot was difficult to determine but was probably about 95 %. The isolate used was not pathogenic to cereals, unlike some others, and had the added advantage of breaking down the alkaloid ergotamine into less toxic compounds.

As with foliage diseases, bacteria can sometimes be used for control. *Glomerella camelliae* causes serious damage to an oil-producing tree in southern China, *Camellia oleifera*; one source of loss is the fruits, some 20–50 % of which may drop prematurely. D.-P. Zeng (personal communication) found that when a strain of *Bacillus subtilis* is applied to the wilting flowers the proportion of diseased fruits is reduced: in fifteen field experiments done at six locations, the mean reduction was 59 %. The mode of protection here seems similar to that occurring with *Trichoderma viride* on grapes. Attempts have been made to control fireblight of apples and pears, caused by *Erwinia amylovora*, by using selected bacteria. In this disease the blossom is commonly infected first, and later stages may cause severe dieback of branches. It is usually treated by frequent applications of bactericides such as streptomycin, but populations of *E. amylovora* resistant to this compound are widely present in the western U.S.A., for example. Riggle & Klos (1972) used *E. herbicola*, which is common on leaf surfaces, to inoculate pear blossom 1 day before further inoculation with *E. amylovora*; a suspension containing 10^8 cells ml^{-1} was applied in each case. The disease was partly controlled both in glasshouse and orchard trials, infection being reduced by up to 50 % in the latter. The antagonistic effect of *E. herbicola* was shown to occur in nectar, and evidence was obtained that the bacterium competes with *E. amylovora* for organic nitrogen and reduces the pH to an inhibitory level.

Stimulation of host resistance has been utilized to protect fruits. D.-P. Zeng (personal communication) discovered that when spores of *Glomerella cingulata*, isolated from poplar, were sprayed onto fruits of *Camellia oleifera*, the fungus penetrated them but caused no symptoms.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

When such fruits were subsequently inoculated with a suspension of spores of *Glomerella camelliae*, infection was reduced: in five field experiments done over a 3 year period, the mean reduction was 44%. The species used here is closely related to the pathogen.

Unlike most of the previous examples, a method described by Pusey & Wilson (1984) involves post-harvest treatment. Peaches, nectarines, apricots and plums were wounded, sprayed with bacterial suspension and inoculated 1–2 h later with spores of *Monilia fructicola*. Of the bacteria tested, one strain of *Bacillus subtilis* controlled brown rot on all types of fruit at temperatures ranging from 1–30 °C. Brown rot was partially controlled at bacterial concentrations of 10^6 and 10^7 propagules ml^{-1} and completely at 10^8 propagules ml^{-1}. An antifungal metabolite was probably involved in control because a culture filtrate also protected fruit from rot.

Diseases of woody tissues

Cankers and galls

Some attention has been given to the role of hyperparasites in controlling rusts that cause tree cankers. Kuhlman & Matthews (1976) made surveys in Florida and found that about 90% of uredial sori of *Cronartium strobilinum* on oak were parasitized by *Sphaerellopsis filum* (*Darluca filum*). Inoculation experiments were done on oak leaves infected by another rust, *C. quercuum* f.sp. *fusiforme*: conidia of *S. filum* were applied at a concentration of 10^6 ml^{-1}, which resulted in 74–90% colonization of telia 1–5 weeks later (Kuhlman *et al.* 1978). *Cladobotryum amazonense* is a newly described hyperparasite of *Crinipellis perniciosa*, which causes witches' broom disease of cocoa; it overgrows the basidiocarps and prevents spore dispersal (Bastos *et al.* 1981). *C. amazonense* produces a heat-stable metabolite which in field applications protected cocoa tissues, particularly pods, from infection by *C. perniciosa*. *Tuberculina virosa* (*T. maxima*) is not a hyperparasite in the strict sense but invades tissues infected by rusts, chiefly those causing cankers or galls on conifers. It degrades the host cells and probably suppresses the rust by destroying the food source vital to its survival (Wicker 1981). The extent to which it exerts natural control seems variable. Kimmey (1969) surveyed stands of white pine in the northwestern U.S.A. and found that 62% of all lethal-type cankers caused by *Cronartium ribicola* were inactivated, and that production of aecia on them was greatly reduced. There was evidence that most of this was due to *T. virosa*. However, Wicker (1981) considers that though the fungus has prolonged the life of infected trees, it has not controlled the disease. These fungi all suppress the formation of inoculum to some extent and, as Cook & Baker (1983) suggest, they may in so doing reduce disease from a potentially epidemic status to a largely endemic one. Little work has been reported on practical application. By contrast, the next disease to be considered provides an example of biological control that is applied very widely.

Crown gall, caused by *Agrobacterium radiobacter* var. *tumefaciens*, affects a wide variety of cultivated plants, especially fruit trees. The bacterium, which is present in the soil and rhizosphere, enters through wounds such as those made during propagation or transplanting. It induces unregulated cell division, leading to the formation of large galls; these are formed in various positions and may reduce yield or kill the plant. During study of agrobacteria in the soil of a stone-fruit nursery in Australia, Kerr (1980) found strains that did not induce galls when inoculated into plants. Experiments with one of these, now known as *A. radiobacter* var. *radiobacter* strain 84, showed that it had a remarkable potential for controlling pathogenic strains. It produces a nucleotide bacteriocin, known as agrocin 84, which selectively inhibits

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

most pathogenic agrobacteria. The bacterium also prevents transfer of the tumour-inducing plasmid from the pathogen to the host and, most importantly, is an effective colonizer of roots.

In the control method devised by Kerr (1980), planting material is dipped in a cell suspension of strain 84 at a concentration of 10^6–10^7 propagules ml^{-1}: this gives nearly complete control of the disease in crops such as stone fruits and roses after planting. The treatment is very cheap, costing only a few cents for each tree or shrub. Commercial firms market strain 84 in Australia, New Zealand and the western U.S.A.; it is distributed mainly in the form of cultures on nutrient agar or in a peat formulation, by using techniques developed for *Rhizobium* spp. The practical aspects of using this method and successful applications to host plants in different locations have been reported by Moore (1979); it is the most widely employed method of controlling a plant pathogen by means of an antagonist. Recent examples of its use include application to Colt rootstocks in England (Garrett & Fletcher 1983) and stone fruits in Hungary (Süle 1983). There are some limitations: of the *A. radiobacter* var. *tumefaciens* biotypes recognized so far, 1 and 2 are sensitive to agrocin 84 whereas 3, which occurs on grapevines, is not. In addition, mutants resistant to agrocin 84 sometimes occur, although this has not been a problem in Australia so far.

Diseases originating from stem wounds

Wound-sealing methods are now believed to have little or no effect in preventing decay (Shigo 1971), and therefore increasing interest is being taken in biological control of pathogens that infect tree wounds. *Chondrostereum purpureum* often causes silver-leaf and branch die-back of fruit and ornamental trees after its basidiospores have entered wounds caused by pruning, for example. In plum tree nurseries Grosclaude (1970) showed that fresh pruning wounds could be completely protected from infection by applying a suspension of *Trichoderma viride* conidia at a concentration of 10^6 ml^{-1} 2 days before inoculation with *C. purpureum*. Simultaneous inoculation resulted in partial control. Later, pruning shears were designed that could apply a *T. viride* suspension to wounds at the time of pruning (Grosclaude *et al.* 1973). The protective effect of *T. viride* results from its production of antibiotics in the wood vessels and the formation of gum barriers by the host in response to the presence of the fungus (Grosclaude 1974). A curative treatment has been reported by Corke (1978), who inoculated pear trees severely affected by *C. purpureum* with *T. viride* and found that symptoms during the next 3 years were significantly reduced by 61 %, compared with equivalent untreated trees.

Eutypa armeniacae causes gummosis and branch die-back of apricots in Australia after infection of pruning wounds by ascospores. Carter (1971) produced evidence that in the past this disease had been aggravated by the use of copper fungicides to control the foliage pathogen *Clasterosporium carpophilum*, because this treatment reduced the population of potential antagonists on the leaf surface. Of several antagonists tested, the best results were given by *Fusarium lateritium*, which reduced the number of infections by *E. armeniacae* through pruning wounds if these were sprayed with a spore suspension 1 day before inoculating with the pathogen. A dosage of 4.4×10^3 spores ml^{-1} gave effective protection (Carter & Price 1974). *F. lateritium* produces a non-volatile fungitoxin which inhibits germination of *E. armeniacae* ascospores and mycelial growth of the fungus. Later research showed that inoculation gave better results when combined with a fungicide treatment; for example, a wound application

containing 10^4 spores ml^{-1} of *F. lateritium* and 125 μg ml^{-1} of benomyl protected 98% of shoots against *E. armeniacae* (Carter & Price 1975). *F. lateritium* is much more tolerant of benomyl than *E. armeniacae*. The method provides immediate protection by the fungicide and longer-lasting protection by the anatagonist. A simple pneumatic-powered control device was produced that delivers biocide to wounds made during pruning by a standard pneumatic secateur (Carter & Perrin 1985).

Large, broadleaved trees are often decayed by a variety of wood-rotting hymenomycetes after lopping, particularly in urban areas, or after damage caused by high winds. Pottle & Shigo (1975) made wounds in 45 year old red maples and inoculated them with suspensions of *Trichoderma viride* conidia. When isolations were made after 1 year, *T. viride* was obtained from all inoculated wounds, no hymenomycetes were found and non-hymenomycete fungi were less frequent than in uninoculated wounds. By contrast, hymenomycetes were often isolated from decayed wood associated with uninoculated wounds. Results of later sampling, reported by Pottle *et al.* (1977), showed that *T. viride* had delayed colonization by hymenomycetes for at least 21 months in wounds made during summer. Smith *et al.* (1981) suggested that control by *T. viride* can be partly explained by its replacement of pioneer wound-colonizing fungi, such as *Phialophora melinii*, because these render wood susceptible to decay by reducing the concentration of phenolic compounds that inhibit wood-rotting fungi. More recently, Mercer & Kirk (1984) did similar experiments on 40 year old beech and found that inoculating wounds with *T. viride* significantly reduced colonization by decay fungi over a period of 4 years: the level was only about 15% of that in controls. Inoculation with *T. viride* resulted in more extensive wood staining than in untreated wounds, and much of the stained area remained sterile during the first year. This may indicate that inoculation with *T. viride* promotes a strong reaction from the tree, thus assisting control of decay fungi.

Lesions on the bark of apple trees caused by *Nectria galligena* are due to infection through leaf scars as well as through pruning wounds. Swinburne (1973) isolated several microorganisms from leaf-scar tissue and found that *Bacillus subtilis* was the most antagonistic to *N. galligena* in culture. When shoots of apples growing in field plots were sprayed after leaf fall with a suspension of *B. subtilis* containing about 10^{10} cells ml^{-1}, some protection was given against *N. galligena*, which was inoculated 1 day later. The numbers of *B. subtilis* recovered from inoculated leaf scars remained fairly constant until the following spring, when the primary protective layer was shed. In later field trials, reported by Swinburne & Brown (1976), apple shoots that had been manually defoliated were inoculated with *N. galligena* and either sprayed with a suspension of *B. subtilis* or with the fungicide phenylmercuric nitrate; controls were not sprayed. During the following summer some of the shoots were further treated with the fungicide dithianon. The two fungicides alone gave some control over subsequent cankering, but *B. subtilis* was ineffective unless inoculated shoots were treated later with dithianon. However, such shoots had significantly less cankering than uninoculated shoots treated with this fungicide.

Corke & Hunter (1979) applied *B. subtilis* to pruning wounds on apple trees. In one experiment they found that if this was done 1 day before inoculation with *N. galligena* the number of shoots it colonized was reduced by 60% and the size of lesion that developed by 55%. By contrast, when *B. subtilis* was inoculated 1 day after *N. galligena*, no lesions were formed. Inoculation of fresh pruning wounds gave as good protection as treatment with

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

benomyl. Inoculation with *B. subtilis* also led to the release during the following year of 96% fewer conidia of *N. galligena* than uninoculated controls. It was considered much cheaper to reduce inoculum production in this way than by treating with fungicide.

Diseases originating from stumps

Heterobasidion annosum is a wood-rotting fungus that causes serious damage to conifers, mainly through butt-rot and killing. Freshly cut stumps often become colonized by wind-borne basidiospores and infections occur on adjacent trees when the fungus passes to their roots from stump roots in contact with them. Subsequent radial spread may result in the formation of large disease foci. Of several fungi tested as possible antagonists for use on pine stumps, *Peniophora gigantea* gave the best results (Rishbeth 1963). With the largest inoculum of *H. annosum* spores likely to be deposited naturally, a dosage of 10^4 *P. gigantea* spores gave sufficient control on stumps having a wood diameter of 16 cm. *P. gigantea* competes successfully with *H. annosum* at the cut surface, enters the lateral roots of stumps and usually checks advance of the pathogen in any tissues infected at the time of felling; it also replaces the pathogen to some extent. This latter process is probably due to hyphal interference by *P. gigantea* (Ikediugwu *et al.* 1970). The control method supplements a process that occurs naturally but is too erratic to be reliable.

Inoculum is produced commercially in fluid form and distributed in sachets. Each sachet contains 1 ml suspension in which there are at least 5×10^6 viable oidia (Greig 1976); these are obtained from cultures of *P. gigantea* growing on malt agar. The contents of a sachet are added to 5 l water and 5 g of dye is added to colour the stumps. At least 100 stumps of 20 cm diameter can be treated with 5 l suspension and the cost, including labour, is from 0.6–1.2 p per stump. In Britain the method is used during thinning of pine plantations over an area of about 62000 ha† (Webb 1973). It has been introduced on a small scale during mechanical harvesting of pines in the southern U.S.A. In an interesting variation of the method, Artman (1972) added oidia of *P. gigantea* to lubrication oil of the chain saw to inoculate the cut. *P. gigantea* is also used fairly extensively in Poland, where only the rate at which inoculum can be produced limits its wider use (Sierota 1981). The method does not adequately protect young pines replanted at sites having extensive root infection at the time of felling. This failure is mainly due to the inability of *P. gigantea* to replace *H. annosum* is very resinous roots. The use of *P. gigantea* for stumps of conifers other than pines has not progressed beyond an experimental stage, although the fungus provides good protection for stumps of Norway spruce (Rishbeth 1970; Kallio & Hallaksela 1979).

Attempts have been made to control other stump-colonizing fungi by means of inoculation. Nelson & Thies (1985) drilled holes in stumps of Douglas fir containing *Phellinus weirii* and introduced inoculum of *Trichoderma viride*. Colonization by this species 1 year later was greatest in the upper, more heavily decayed region of the stump, whereas it was least in the lower region; it had not grown well in sound wood. Stumps of broadleaved trees are sometimes colonized by *Armillaria mellea* and may then become sources of infection for a much wider variety of trees than in the case of *H. annosum*. To enable fungi potentially antagonistic to *A. mellea* to grow, such stumps need to be killed by treating the surface with 40% aqueous

† 1 hectare = 10^4 m^2.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

ammonium sulphamate directly after cutting. In birch stumps so treated and then inoculated simultaneously with *Coriolus versicolor* and *A. mellea*, the amount of wood occupied by the latter fungus 4 years later was greatly reduced compared with controls inoculated with *A. mellea* alone (Rishbeth 1976, 1979). It was shown that wood-decay fungi differ considerably in their ability to reduce the size of potential food-base available to *A. mellea* in stumps.

Discussion

The investigations outlined here provide useful information about the conditions necessary for successful biological control. Although most of the methods described are unlikely to be applied on a large scale at present, a few seem sufficiently promising to be employed at once, and it is certainly possible that circumstances will change sufficiently to make others attractive in the future.

There is a fairly strong presumption that the most appropriate microbial antagonists are to be found in or near the natural infection courts of the pathogen, as in the case of *Peniophora gigantea*, isolated from decaying pine stumps. Because *Bacillus thuringiensis* gave some control over infection by *Alternaria alternata* and *Uromyces phaseoli*, it might be useful to test potential antagonists from other sources. However, as Blakeman & Fokkema (1982) point out, microorganisms naturally resident on aerial plant surfaces have become adapted to survive and grow in this habitat, and should therefore be preferred to microorganisms from other habitats which may be equally antagonistic. It is perhaps significant that *Trichoderma* spp., which are only occasionally found in the phyllosphere, have not been used successfully as antagonists on leaf surfaces but are suitable for other purposes such as colonization of withered flowers and some woody tissues. It is interesting that the ubiquitous *Bacillus subtilis* can be used to control such a wide variety of pathogens. Care is needed in the selection of strains, because some otherwise effective antagonists have undesirable side effects, such as reducing crop yield. Field tests are essential for selecting effective strains of some antagonists, such as *Trichoderma* spp. and *P. gigantea*. In addition to providing adequate control, an antagonist must be safe to use. An antagonist that is appropriate for one type of infection court may be unsuitable for another: thus *Trichoderma viride* controls *Chondrostereum purpureum* well in pruning wounds on plum but protects pine stumps relatively poorly from infection by *Heterobasidion annosum*.

The dosage of an antagonist reported as achieving control is often high; in the investigations mentioned here the concentration of propagules ranged from 4×10^3–10^{10} ml^{-1}, with a mean of about 10^6 ml^{-1}. The effect of using different concentrations is not always tested, but the results may be instructive. Thus Doherty & Preece (1978) attempted to control *Puccinia allii* on leek growing in controlled environments by spraying leaves with suspensions of *Bacillus cereus*. A concentration of 2.5×10^7 bacteria ml^{-1} gave no control, 4.5×10^8 bacteria ml^{-1} reduced the frequency of rust pustules by 41%, whereas 6×10^9 bacteria ml^{-1} reduced it by 93–99%. Particularly for applications on arable crops, the need for such dosages would involve a massive production of inoculum. For most of the potentially useful antagonists so far considered, this would be difficult but not impossible, as judged by the vast scale on which biological agents are used to control pests of field crops in China. Experience with preparing inoculum of *Bacillus thuringiensis* suggests that this could be done for other potentially useful species of *Bacillus*. Inoculum of various *Trichoderma* species is already produced on a fairly large scale. A wide

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

range of antagonistic fungi produce asexual spores freely in culture, and even non-sporing fungi could probably be used on a smaller scale, homogenized mycelium serving as inoculum for stumps, for example (Rishbeth 1963).

The timing of protective treatments is clearly important. In experiments the commonest reported period for applying saprotrophic fungi or bacteria is 1–3 days before inoculating with the pathogen, although in one instance a better result was obtained by application soon afterwards. Hyperparasites are often applied 7–10 days after a biotrophic pathogen has become well established; such a delay would be critical if the pathogen had already caused serious damage. As Tronsmo (1986) states, the epidemiology of a disease must be taken into account in order to obtain the maximal effect of the treatment. In certain cases control is more effective when nutrients are added with the antagonist, but in others addition of nutrients might well stimulate growth of the pathogen.

Survival of the antagonist for long enough to control the pathogen is crucial. In this respect less difficulty is likely to arise with relatively protected infection courts such as leaf scars, pruning wounds or stump tissues than with exposed surfaces. Many potential antagonists that seem promising in glasshouse trials fail in subsequent field tests because they are killed by desiccation or exposure to intense sunlight, for instance. Even if an antagonist is shown to reduce infection to an acceptable level under such conditions, frequent applications may be needed to maintain its population at an effective level. Repeated treatment may also be required because tissues susceptible to infection, such as young leaves, are produced over a considerable period; although this also applies to treatment with most fungicides.

The precise circumstances in which air-borne pathogens are best controlled by biological agents is a controversial matter. In the case of diseases that affect foliage, flowers or fruit, glasshouse crops offer more attractive possibilities than field crops because conditions are to some extent controllable and the population level of antagonists is more easily maintained. Procedures such as supplementary spraying with water are more likely to succeed in the glasshouse. Even so, care is needed not to create conditions that favour the pathogen. Inoculation of plants such as cucumbers in the glasshouse with non-pathogenic fungi, to confer protection after transplanting them to the field, is potentially very useful. For most arable crops the problem of antagonist survival seems too formidable in our present state of knowledge to permit application on a really large scale.

In the case of diseases affecting trees, their longevity and the large surface area needing protection create extra difficulties. It seems most unlikely that regular spraying with an antagonist could be used for controlling pathogens such as those causing stem cankers on forest trees. It is not so difficult to envisage biological control of leaf pathogens in orchards, but more effective methods would be needed. However, when the target area for protection is much smaller and the need arises only once, as for instance when a stump is created, the prospect for effective biological control is much better. Similar opportunities arise when orchard trees are pruned, and successful methods are available of combining this operation with introduction of inoculum. Experiments involving larger wounds on trees require many years for adequate assessment, but the preliminary results obtained by inoculation with *Trichoderma viride* are promising.

As mentioned at the outset, biological methods compete directly with chemical ones. In many of the investigations mentioned earlier no comparison was made between the two types of method, but in some the level of control obtained with an antagonist was too low to be of

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

interest to a commercial grower. Nevertheless, such work is valuable in preparing the way for development of more effective methods. Research projects in which biological and chemical methods were compared are particularly useful. Often treatment with a fungicide gave better results than spraying with an antagonist, or fewer fungicide applications were needed to give a similar amount of protection. Sometimes, however, the biological method was as good as the chemical one. On environmental grounds there is good reason for believing that a biological method is preferable if it gives a similar level of control at a comparable cost. In the case of fruits and other edible crops the risk of detrimental effects from metabolites produced by antagonists may well be less than those associated with use of fungicides, but must be taken into account.

Biological and chemical methods are by no means strict alternatives; enough evidence already exists to show that they can sometimes be combined with advantage. In the control of *Eutypa armeniacae*, for example, the two components provide shorter- and longer-term protection. Research with powdery mildew of cucumber indicates that a lower concentration of fungicide may suffice if applied with a suspension of an antagonist. Because sensitivity of an antagonist to a fungicide rules out such a combination, it is encouraging to find awareness in some recent work, for example on *Tilletiopsis minor*, of the potential value of fungicide-resistant strains. However, as Krantz (1981) observes, several biological and technical problems must be solved before hyperparasites can be used in integrated control.

The same need arises throughout the whole field of biological control of air-borne pathogens: thus more research is required to determine the conditions that favour growth and survival of antagonists on plant surfaces, for instance. Studies on the colonization of leaves after inoculation with bacteria and yeasts (Blakeman 1985) provide a useful example. Considerable progress has been made in selecting antagonists for particular purposes. It is tempting to continue testing species such as *Trichoderma viride* and *Bacillus subtilis* in yet further situations, but opportunities abound for finding new antagonists. Within species there is much scope for selecting, and creating by genetic manipulation, new strains capable of more effective antagonism and better survival, for example. Further work on the mechanisms by which control is achieved should assist in the development of better techniques. Continuation of such basic research is undoubtedly necessary, but of at least equal importance are practical aspects, such as the need to test existing methods more fully and to develop new ones. To facilitate sensible choices, adequate comparison with methods of chemical or integrated control is important, as are estimates of cost.

I thank Dr H. J. Hudson for helpful discussion.

References

Artman, J. D. 1972 Further tests in Virginia using chain saw-applied *Peniophora gigantea* in loblolly pine stump inoculation. *Pl. Dis. Reptr* **56**, 958–960.

Baker, C. J., Stavely, J. R., Thomas, C. A., Sasser, M. & MacFall, J. S. 1983 Inhibitory effect of *Bacillus subtilis* on *Uromyces phaseoli* and on development of rust pustules on bean leaves. *Phytopathology* **73**, 1148–1152.

Baker, C. J., Stavely, J. R. & Mock, N. 1985 Biocontrol of bean rust by *Bacillus subtilis* under field conditions. *Pl. Dis.* **69**, 770–772.

Baker, K. F. & Cook, R. J. 1974 *Biological control of plant pathogens*. San Francisco: W. H. Freeman.

Bastos, C. N., Evans, H. C. & Samson, R. A. 1981 A new hyperparasitic fungus, *Cladobotryum amazonense*, with potential for control of fungal pathogens of cocoa. *Trans. Br. mycol. Soc.* **77**, 273–278.

Blakeman, J. P. 1985 Ecological succession of leaf surface microorganisms in relation to biological control. In *Biological control on the phylloplane* (ed. C. E. Windels & S. E. Lindow), pp. 6–30. St Paul, Minnesota: American Phytopathological Society.

Blakeman, J. P. & Fokkema, N. J. 1982 Potential for biological control of plant diseases on the phylloplane. *A. Rev. Phytopath.* **20**, 167–192.

Carter, M. V. 1971 Biological control of *Eutypa armeniacae*. *Aust. J. exp. Agric. Anim. Hus.* **11**, 687–692.

Carter, M. V. & Price, T. V. 1974 Biological control of *Eutypa armeniacae*. II. Studies of the interaction between *E. armeniacae* and *Fusarium lateritium*, and their relative sensitivities to benzimidazole chemicals. *Aust. J. agric. Res.* **25**, 105–119.

Carter, M. V. & Price, T. V. 1975 Biological control of *Eutypa armeniacae*. III. A comparison of chemical, biological and integrated control. *Aust. J. agric. Res.* **26**, 537–543.

Carter, M. V. & Perrin, E. 1985 A pneumatic-powered spraying secateur for use in commercial orchards and vineyards. *Aust. J. exp. Agric.* **25**, 939–942.

Caruso, F. L. & Kuć, J. 1977 Field protection of cucumber, watermelon and muskmelon against *Colletotrichum lagenarium* by *Colletotrichum lagenarium*. *Phytopathology* **67**, 1290–1292.

Cook, R. J. & Baker, K. F. 1983 *The nature and practice of biological control of plant pathogens*. St Paul, Minnesota: American Phytopathological Society.

Corke, A. T. K. 1978 Microbial antagonisms affecting tree diseases. *Ann. appl. Biol.* **89**, 89–93.

Corke, A. T. K. & Hunter, T. 1979 Biocontrol of *Nectria galligena* infection of pruning wounds on apple shoots. *J. hort. Sci.* **54**, 47–55.

Cullen, D., Berbee, F. M. & Andrews, J. H. 1984 *Chaetomium globosum* antagonizes the apple scab pathogen, *Venturia inaequalis*, under field conditions. *Can. J. Bot.* **62**, 1814–1818.

Doherty, M. A. & Preece, T. F. 1978 *Bacillus cereus* prevents germination of uredospores of *Puccinia allii* and the development of rust disease of leek, *Allium porrum*, in controlled environments. *Physiol. Pl. Path.* **12**, 123–132.

Dubos, B., Bulit, J., Bugaret, Y. & Verdu, D. 1978 Possibilités d'utilisation du *Trichoderma viride* Pers. comme moyen biologique de lutte contre la pourriture gris (*Botrytis cinerea* Pers.) et l'excoriose (*Phomopsis viticola* Sacc.) de la vigne. *C. r. hebd. Séanc. Acad. Agric. Fr.* **64**, 1159–1168.

Esquerré-Tugayé, M.-T., Lafitte, C., Mazau, D., Toppan, A. & Touzé, A. 1979 Cell surfaces in plant-microorganism interactions. II. Evidence for the accumulation of hydroxyproline-rich glycoproteins in the cell wall of diseased plants as a defence mechanism. *Pl. Physiol.* **64**, 320–326.

Fokkema, N. J., van de Laar, J. A. J., Nelis-Blomberg, A. L. & Schippers, B. 1975 The buffering capacity of the natural mycoflora of rye leaves to infection by *Cochliobolus sativus*, and its susceptibility to benomyl. *Neth. J. Pl. Path.* **81**, 176–186.

Fokkema, N. J., den Houter, J. G., Kosterman, Y. J. C. & Nelis, A. L. 1979 Manipulation of yeasts on field-grown wheat leaves and their antagonistic effect on *Cochliobolus sativus* and *Septoria nodorum*. *Trans. Br. mycol. Soc.* **72**, 19–29.

Garrett, C. M. E. & Fletcher, D. A. 1983 Crown gall (*Agrobacterium tumefaciens*). *Rep. E. Malling Res. Stn 1982*, 79.

Grabski, G. C. & Mendgen, K. 1985 Einsatz von *V. lecanii* als biologisches Schadlingsbekämpfungsmittel gegen den Bohnenrostpilz *U. appendiculatus* var. *appendiculatus* im Feld und in Gewächschaus. *Phytopath. Z.* **113**, 243–251.

Greig, B. J. W. 1976 Biological control of *Fomes annosus* by *Peniophora gigantea*. *Eur. J. For. Path.* **6**, 65–71.

Grosclaude, C. 1970 Premiers essais de protection biologique des blessures de taille vis-à-vis du *Stereum purpureum* Pers. *Annls. Phytopath.* **2**, 507–516.

Grosclaude, C. 1974 Le pénétration des spores de champignons dans les blessures de taille des arbres fruitiers. Application au cas de la protection biologique vis-à-vis du *Stereum purpureum*. *Revue Zool. Agric. Path. Veg.* **73**, 1–21.

Grosclaude, C., Ricard, J. & Dubos, B. 1973 Inoculation of *Trichoderma viride* spores via pruning shears for biological control of *Stereum purpureum* on plum tree wounds. *Pl. Dis. Reptr* **57**, 25–28.

Hijwegen, T. 1986 Biological control of cucumber powdery mildew by *Tilletiopsis minor*. *Neth. J. Pl. Path.* **92**, 93–95.

Ikediugwu, F. E. O., Dennis, C. & Webster, J. 1970 Hyphal interference by *Peniophora gigantea* against *Heterobasidion annosum*. *Trans. Br. mycol. Soc.* **54**, 307–309.

Islam, K. Z. & Nandi, B. 1985 Control of brown spot of rice by *Bacillus megaterium*. *Z. PflKrankh. PflPath. PflSchutz.* **92**, 241–246.

Jarvis, W. R. & Slingsby, K. 1977 The control of powdery mildew of greenhouse cucumber by water sprays and *Ampelomyces quisqualis*. *Pl. Dis. Reptr* **61**, 728–730.

Kallio, T. & Hallaksela, A.-M. 1979 Biological control of *Heterobasidion annosum* (Fr.) Bref. (*Fomes annosus*) in Finland. *Eur. J. For. Path.* **9**, 298–308.

Kerr, A. 1980 Biological control of crown gall through production of agrocin 84. *Pl. Dis.* **64**, 25–30.

Kimmey, J. W. 1969 Inactivation of lethal-type blister rust cankers on western white pine. *J. For.* **67**, 296–299.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Krantz, J. 1981 Hyperparasitism of biotrophic fungi. In *Microbial ecology of the phylloplane* (ed. J. P. Blakeman), pp. 327–352. London: Academic Press.

Kuć, J. & Richmond, S. 1977 Aspects of the protection of cucumber against *Colletotrichum lagenarium* by *Colletotrichum lagenarium*. *Phytopathology* **67**, 533–536.

Kuhlman, E. G. & Matthews, F. R. 1976 Occurrence of *Darluca filum* on *Cronartium strobilinum* and *C. fusiforme* infecting oak. *Phytopathology* **66**, 1195–1197.

Kuhlman, E. G., Matthews, F. R. & Tillerson, H. P. 1978 Efficacy of *Darluca filum* for biological control of *Cronartium fusiforme* and *C. strobilinum*. *Phytopathology* **68**, 507–511.

Leben, C. 1964 Influence of bacteria isolated from healthy cucumber leaves on two leaf diseases of cucumber. *Phytopathology* **54**, 405–408.

Mercer, P. C. & Kirk, S. A. 1984 Biological treatments for the control of decay in tree wounds. II. Field tests. *Ann. appl. Biol.* **104**, 221–229.

Moore, L. W. 1979 Practical use and success of *Agrobacterium radiobacter* strain 84 for crown gall control. In *Soil-borne plant pathogens* (ed. B. Schippers & W. Gams), pp. 553–568. London: Academic Press.

Mower, R. L., Snyder, W. C. & Hancock, J. G. 1975 Biological control of ergot by *Fusarium*. *Phytopathology* **65**, 5–10.

Nelson, E. E. & Thies, W. G. 1985 Colonization of *Phellinus weirii*-infected stumps by *Trichoderma viride*. I. Effect of isolate and inoculum base. *Eur. J. For. Path.* **15**, 425–431.

Newhook, F. J. 1951 Microbiological control of *Botrytis cinerea* Pers. I. The role of pH changes and bacterial antagonism. *Ann. appl. Biol.* **38**, 169–184.

Pace, M. A. & Campbell, R. 1974 The effect of saprophytes on infection of leaves of *Brassica* spp. by *Alternaria brassicicola*. *Trans. Br. mycol. Soc.* **63**, 193–196.

Pottle, H. W. & Shigo, A. L. 1975 Treatment of wounds on *Acer rubrum* with *Trichoderma viride*. *Eur. J. For. Path.* **5**, 274–279.

Pottle, H. W., Shigo, A. L. & Blanchard, R. O. 1977 Biological control of wound hymenomycetes by *Trichoderma harzianum*. *Pl. Dis. Reptr* **61**, 687–690.

Purkayastha, R. P. & Bhattacharyya, B. 1982 Antagonism of microorganisms from jute phyllosphere towards *Colletotrichum corchori*. *Trans. Br. mycol. Soc.* **78**, 509–513.

Pusey, P. L. & Wilson, C. L. 1984 Postharvest biological control of stone fruit brown rot by *Bacillus subtilis*. *Pl. Dis.* **68**, 753–756.

Ricard, J. L. 1981 Commercialization of a *Trichoderma* based mycofungicide: some problems and solutions. *Biocontrol News Inf.* **2**, 95–98.

Riggle, J. H. & Klos, E. J. 1972 Relationship of *Erwinia herbicola* to *Erwinia amylovora*. *Can. J. Bot.* **50**, 1077–1083.

Rishbeth, J. 1963 Stump protection against *Fomes annosus*. III. Inoculation with *Peniophora gigantea*. *Ann. appl. Biol.* **52**, 63–77.

Rishbeth, J. 1970 The possibility of stump inoculation for conifers other than pines. In *Proceedings of the 3rd International Conference on Fomes annosus*. Section 24, pp. 110–120. International Union of Forest Research Organizations. Washington: United States Department of Agriculture Forest Service.

Rishbeth, J. 1976 Chemical treatment and inoculation of hardwood stumps for control of *Armillaria mellea*. *Ann. appl. Biol.* **82**, 57–70.

Rishbeth, J. 1979 Modern aspects of biological control of *Fomes* and *Armillaria*. *Eur. J. For. Path.* **9**, 331–340.

Scherff, R. H. 1973 Control of bacterial blight of soybean by *Bdellovibrio bacteriovorus*. *Phytopathology* **63**, 400–402.

Schönbeck, F. & Dehne, H.-W. 1986 Use of microbial metabolites inducing resistance against plant pathogens. In *Microbiology of the phyllosphere* (ed. N. J. Fokkema & J. Van den Heuvel), pp. 363–375. Cambridge University Press.

Sharma, I. K. & Heather, W. A. 1983 Post-penetration antagonism by *Cladosporium tenuissimum* to uredinial induction by *Melampsora larici-populina*. *Trans. Br. mycol. Soc.* **80**, 373–374.

Shigo, A. L. 1971 Wound dressing research on red maples. *Int. Shade Tree Conf. Proc.* **47**, 97–98.

Sierota, Z. H. 1981 Chances of practical control of *Fomes annosus* (Fr.) Cke. infection using biological methods. In *Root and butt rots in Scotch pine stands* (International Union of Forest Research Organizations Working Party S2.06.01), pp. 62–68. Poznań: Polish Academy of Sciences.

Smith, K. T., Blanchard, R. O. & Shortle, W. C. 1981 Postulated mechanism of biological control of decay fungi in red maple wounds treated with *Trichoderma harzianum*. *Phytopathology* **71**, 496–498.

Spurr, H. W., Jr. 1977 Protective applications of conidia of nonpathogenic *Alternaria* sp. isolates for control of tobacco brown spot disease. *Phytopathology* **67**, 128–132.

Spurr, H. W., Jr. 1981 Experiments on foliar disease control using bacterial antagonists. In *Microbial ecology of the phylloplane* (ed. J. P. Blakeman), pp. 369–381. London: Academic Press.

Süle, S. 1983 Biologiai vedekezes a gyumolcsfak agrobakteriumos gyokergolyvaja ellen. *Novenyvedelem* **19**, 337–340.

Sundheim, L. 1982 Control of cucumber powdery mildew by the hyperparasite *Ampelomyces quisqualis* and fungicides. *Pl. Path.* **31**, 209–214.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Swinburne, T. R. 1973 Microflora of apple leaf scars in relation to infection by *Nectria galligena*. *Trans. Br. mycol. Soc.* **60**, 389–403.
Swinburne, T. R. & Brown, A. E. 1976 A comparison of the use of *Bacillus subtilis* with conventional fungicides for the control of apple canker (*Nectria galligena*). *Ann. appl. Biol.* **82**, 365–368.
Tronsmo, A. 1986 Use of *Trichoderma* spp. in biological control of necrotrophic pathogens. In *Microbiology of the phyllosphere* (ed. N. J. Fokkema & J. Van den Heuvel), pp. 348–362. Cambridge University Press.
Tronsmo, A. & Dennis, C. 1977 The use of *Trichoderma* species to control strawberry fruit rots. *Neth. J. Pl. Path.* **83** (suppl. 1), 449–455.
Tronsmo, A. & Raa, J. 1977 Antagonistic action of *Trichoderma pseudokoningii* against the apple pathogen *Botrytis cinerea*. *Phytopath. Z.* **89**, 216–220.
Tronsmo, A. & Ystaas, J. 1980 Biological control of *Botrytis cinerea* on apple. *Pl. Dis.* **64**, 1009.
Webb, P. J. 1973 An alternative to chemical stump protection against *Fomes annosus* on pines in state and private forestry. *Scott. For.* **27**, 24–25.
Wicker, E. F. 1981 Natural control of white pine blister rust by *Tuberculina maxima*. *Phytopathology* **71**, 997–1000.
Wood, R. K. S. 1951 The control of diseases of lettuce by the use of antagonistic organisms. I. The control of *Botrytis cinerea* Pers. *Ann. appl. Biol.* **38**, 203–216.

Discussion

R. D. Lumsden (*United States Department of Agriculture Agricultural Research Service, Beltsville, U.S.A.*). Dr Rishbeth, from your extensive knowledge and experience with the discovery and development of the one major example of successful biological control that we have in our own discipline, what are the factor or factors that you judge to be responsible for the success of *Peniophora gigantea* as a biological control agent against *Heterobasidion annosum*?

J. Rishbeth. *Peniophora gigantea* is one of the few fungi that can colonize the surface of freshly cut pine stumps. It is very competitive, preventing establishment of *Heterobasidion annosum* and often replacing this pathogen if it is already present. *Peniophora* grows extensively in stump roots and rapidly decays the wood, but does not invade living roots. Fortunately it is easy to grow in culture and produces abundant asexual spores which are effective for inoculation.

J. N. Gibbs (*Forest Research Station, Farnham, U.K.*). I wish to emphasize the competitive mode of action of *Peniophora*; its success in practice lies in its ability to grow rapidly into the fresh stump regardless of whether the pathogen (*Heterobasidion*) is present or not.

W. D. Hamilton, F.R.S. (*Department of Zoology, Oxford University, U.K.*). Is it a possible generalization that the antagonists of air-borne plant pathogens are less air-borne themselves? This might be expected from theory in that species that have poor chances of moving from their present host to another should evolve properties that tend to keep their present host alive. In contrast, species with excellent dispersal may evolve courses of rapid exploitation that result in pathogenesis. Of the antagonists of pathogenic fungi that you mention, I think that the two slightly familiar to me, *Trichoderma viride* and *Peniophora gigantea*, may illustrate poor dispersal. *T. viride*, for example, sporulates under bark and may rely on dispersal by rather loosely associated insects rather than on wind. Do you see such a general contrast between the antagonists and the pathogens?

J. Rishbeth. I doubt whether such a contrast is general, although Professor Hamilton may well be correct about *T. viride*, which I have seldom detected when trapping air-borne spores. On the other hand, spores of *P. gigantea* are at least as common in the air as those of *Heterobasidion*

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

annosum unless conditions are very dry. Of other microorganisms used for biological control, spores of *Bacillus* spp. also occur commonly in the air, whereas ballistospores of *Sporobolomyces* are often extremely abundant in moist air. Hyperparasites such as *Ampelomyces* are probably dispersed fairly effectively in spores of their fungal hosts.

R. J. Cook (*United States Department of Agriculture Agricultural Reseach Service, Washington State University, Pullman, U.S.A.*). In response to the question from Dr Neuenschwander [not printed] of whether or not plant pathologists make use of theory and models of population dynamics in our research on biological control, I think the answer is a qualified no. Modern epidemiology in plant pathology makes considerable use of this approach in describing the increase of a pathogen population or frequency of virulence genes in a pathogen population on a susceptible host crop. This is used to some advantage in biological control systems where the strategy is some kind of gene deployment achieved by a network, sequence, or mixture of plant cultivars designed to control the frequency of a particular race or pathovar of the pathogen. As to a counterpart of the efforts in entomology to describe or model population dynamics of a plant pathogen interacting with its natural enemies, we have done little or nothing along this line. A major reason is that many if not most of our successful biological controls involving introduced or managed populations of antagonists of plant pathogens do not involve the strategy of regulating the pathogen population directly by predatory or hyperparasitic agents. The biological controls of plant pathogens with introduced agents described at this meeting, and in use under study around the world (systems that seem to work best for us), are aimed mostly at defence against the processes of infection, disease development, or reproduction of the pathogen at the end of the disease cycle. Examples exist of hyperparasites or hyperpathogens with ability to lower the inoculum density of a target plant pathogen, but generally these are too inefficient for effective biocontrol. The best use of natural enemies of the inoculum of plant pathogens are those that prevent inoculum from ever forming, e.g. hyperparasites in rust pustules, or those that destroy pathogen propagules of plant parasitic nematodes as they form on the host plant.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES