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CONTROL OF PLANT DISEASES BY USE OF ANTAGONISTIC ORGANISMS

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INTRODUCTION

The phenomenon of antagonism between micro-organisms has received much attention during the past few decades. It commonly occurs in plate cultures of pathogens which have become contaminated or in deliberately mixed cultures of pathogens and saprophytes. Many studies have shown that a significant proportion of the organisms found in the environment in which certain diseases develop profoundly affect the growth of the pathogen in pure culture. Inhibition or even complete suppression of growth has often been obtained in these conditions, and indeed in isolated cases active parasitism of the pathogen by a commonly occurring saprophyte has also been reported. The part played by secondary organisms in the development of plant diseases under natural conditions has been increasingly studied. For example, reduction of the severity of certain root diseases by cultural practices, such as green-manuring, has been attributed to changes produced in the nature and activity of the soil micro-flora. Variations in the incidence of disease which are associated with soil differences or seasonal fluctuations in the climate have been similarly explained in some instances. In the light of such evidence it was natural that the use of organisms antagonistic to specific pathogens should be considered as a practical control measure. This review deals with certain aspects of such work and will be confined to diseases caused by fungi; while no attempt has been made to cover the literature completely, it is hoped that no major work has been excluded. No reference will be made to virus diseases, as this would involve a series of special considerations. Neither will the

direct use of specific antibiotics as chemical substances be considered, since the principles governing their use in disease control will probably not differ materially from those applicable to more orthodox fungicides. Antagonism will be interpreted in the widest possible sense to include any activity of one organism which in some way adversely affects another growing in association with it so that the problem of disease control by antagonistic organisms becomes essentially a study of the competition between pathogens and saprophytes and may be approached in the following ways. In the first place, organisms selected for their antagonism to a particular pathogen may be introduced into and maintained in the environment, and so prevent establishment of the pathogen on the host plant or eliminate it as a causal agent. Secondly, conditions within the environment may be altered to produce a similar effect by modifying the nature and activity of the microflora already present. The first approach requires the isolation and selection of appropriate organisms; it is this aspect of the work which will be considered first.

ISOLATION AND SELECTION OF ANTAGONISTS

A series of surveys as well as many isolated reports give information on the types and numbers of organisms antagonistic to particular plant pathogens in pure culture on solid or in liquid media (2–4, 28, 34–36, 39, 110, 115, 119, 133, 138–140, 148, 149). The media used have varied widely in composition; they have usually been those commonly used in mycology laboratories. Antagonistic effects are most readily observed on solid media, and various techniques have been described for their estimation. The majority involve inoculation of the pathogen and test organism on the same plate, followed by observation and measurement of the mutual effects of their growth. Inoculations are generally made so as to permit some growth of the test organism before this is affected by growth of the pathogen. This may be accomplished by inoculating both organisms simultaneously at a distance from each other or by inoculating the test organism some time before the pathogen. The effects of their subsequent growth have been well described by various workers (2–4, 138, 139) and will not be given in detail here. Commonly there is a zone around the antagonist into which the hyphae of the pathogen do not penetrate;

the width of this zone has usually been taken as a measure of the activity of the antagonist. At the edge of such zones various morphogenic effects are frequently seen, distortion or lysis of the hyphae of the pathogen being commonly observed. Behind the edge the hyphae often branch much more profusely than in normal growth and may accumulate to form a distinct ridge, this being sometimes associated with more profuse sporulation. Occasionally isolated hyphae grow into the intervening zone, but with these exceptions the production of a zone means that the growth of both pathogen and antagonist across the surface of the agar sooner or later stops, two distinct colonies being formed.

In other cases no distinct zones are produced; growth of the pathogen stops, its colony then being penetrated and overgrown by hyphae of the antagonist. This is sometimes accompanied by active parasitism of the pathogen. Yet again both antagonist and pathogen may be mutually antagonistic to the extent that linear extension of the colonies takes place until they meet, but no penetration of either colony occurs. An organism showing any of these effects would be potentially useful but in the past attention has been directed to those producing wide zones of inhibition. It is probable, however, that in screening tests with plate cultures a better measure of the antagonistic properties would probably be the extent to which the total growth of the pathogen is inhibited. It is apparent that a slowly growing antagonist may well produce a striking zone of inhibition but competitively may not be so efficient as a very rapidly growing organism which, if its colony is not penetrated by that of the pathogen, limits the latter to a small area, even though the actual zone of inhibition may be absent or very narrow.

Studies such as the above illustrate competition between the antagonist and active hyphae of established colonies of the pathogen. Correlated studies have also been made of the ability of organisms to prevent establishment of a pathogen on an agar surface. This may be done by inoculating a limited area of plates with a suspension of spores or hyphal fragments of the test organism and then adding the pathogen to the same area, immediately or after various intervals. In a modification of this method the antagonist is sown on films of cellophane covering an agar surface. After the antagonist has grown for some time, the

cellophane together with the antagonist is removed and the surface of the agar previously below the colony sown with the pathogen. Curtailment of growth following these treatments is an indication that the pathogen is unable to compete with these organisms or that its growth is adversely affected by one of their metabolic products.

Tests of antagonism in pure culture have generally been made at one temperature only, and this has not always fallen within the range in which the antagonist would be expected to operate naturally. It would seem to be particularly important that a range of temperatures be used in screening tests and that organisms selected for further tests in disease control should have growth-temperature relations similar to those of the pathogen. This is perhaps more important for those diseases able to develop at intermediate or low temperatures, since under normal growing conditions it is unlikely that temperatures high enough to curtail growth of the antagonist would often be experienced.

In most surveys for potential antagonists, media of high nutrient content have been used, the composition probably having little relation to that of the substrate on which antagonistic effects would need to be produced to control disease. Use of such media, whether synthetic or from natural substances, might well give misleading information, since the antagonistic effects in many cases will depend on the production of antibiotic substances, and this is known to be particularly affected by the composition of the medium on which the antagonist is growing. It is always difficult to assess the nutrient conditions under which plant pathogens are active naturally, particularly when dealing with soil or seed-borne diseases. It would, however, always seem desirable to use media as close as possible to the natural substrate in screening tests where the main object is isolation of organisms potentially useful in field conditions. Thus in the case of a soil-borne pathogen a soil extract, and for a plant pathogen a tissue extract of the host, would be more appropriate than a synthetic medium such as Czapek's solution.

Against this view may be put the argument that even in soils the breakdown of fresh plant debris by saprophytes may well lead to a high local concentration of nutrients, even though the general level throughout the soil is quite low.

Some surveys have used liquid in place of solid media. Test organisms have been cultured for a period and removed aseptically. Various dilutions of the culture fluid are then inoculated with the pathogen and the dilution level at which growth is inhibited taken as a measure of the antagonism of the organism. This method has the advantage of precision in operation and of giving a numerical figure of activity but is otherwise less useful for the purpose at present under consideration. In such tests conditions for growth of the antagonist are even further removed from those obtaining naturally than are those of plate culture, particularly when the culture solutions are continuously agitated. Use of these methods is justified only when the main aim is the detection and isolation of antibiotic substances. Their use would not detect, for instance, antagonism by active parasitism of the pathogen or its exclusion by rapid growth of the antagonist.

Tests of antagonism in pure culture are of greatest value when conditions approach, as closely as is practicable, those at the site of the disease. The temperatures for screening tests should fall within the range in which the disease could be expected to develop, and nutrient levels should be those of the natural substrate wherever possible. It is also important that organisms selected for further trial should be types normally occurring in the disease environment and preferably obtained in the first place from it. Selected organisms should of course be non-pathogenic (this rather obvious point has not always been observed) and have properties making them suitable for easy application to plant surfaces or soil, and for rapid establishment. Thus spores or resting bodies should germinate well and quickly, and the organisms should have high growth rates and reproductive capacities, especially of relatively resistant spores. They should be easily cultured on readily available media and be non-exacting in their nutritional requirements; in this way large quantities of inocula could be easily and cheaply prepared.

The results of the many surveys which have been made will not be referred to in detail. It may be stated, however, that in general no difficulty has been experienced in obtaining a large number of species antagonistic to various plant pathogens in pure culture. A good proportion of these occur naturally with the pathogen, and prominent among them are the spore-forming bacteria, actino-

mycetes, species of *Penicillium* and *Trichoderma viride.* This is to be expected in view of the ubiquity of these organisms and the frequency with which they are isolated from soil by dilution methods. In this connection, however, it is to be noted that some of the species, in particular those of *Penicillium,* may not occur as actively growing mycelium in soils so frequently as has been supposed, and their importance as antagonists in this medium may well have been overemphasized. It is also interesting to note that few of the Phycomycetes, generally considered one of the dominant groups of soil-inhabiting fungi, exhibit marked antagonism in pure culture in the narrower sense of producing wide zones of inhibition. This class includes many rapidly growing species and may therefore be of considerable importance in the wider competitive sense under normal soil conditions.

This section concludes on a note of caution. Methods generally used for isolating organisms from the environment in which disease is initiated are very selective. This is particularly true for soil organisms. It may well happen that the methods used fail to isolate organisms which under natural conditions play an important part in controlling growth of pathogens. Thus ordinary methods of isolation from soil rarely produce representatives of the higher Basidiomycetes, Discomycetes or Pyrenomycetes. There is no easy solution to this particular problem. New methods of isolation are required which give a more balanced picture of the soil microflora as it occurs naturally.

DISEASE CONTROL—GENERAL CONSIDERATIONS

Where direct use of antagonists is envisaged, isolation and selection of organisms have generally been followed by an investigation of their ability to control disease in the absence of competition from organisms other than the pathogen. The methods which have been used necessarily vary with the type of disease under study. Much of the work along these lines has been done with soil-borne diseases, and here the technique has been to sterilize the soil, to inoculate it with the antagonist either simultaneously or some time before the pathogen, and after suitable intervals to test for the presence of the pathogen by growing susceptible hosts in it. With pathogens affecting aerial parts of plants, the plant tissue has been surface sterilized, damaged in some way when the

pathogen is a wound parasite, and inoculated with the pathogen simultaneously or after earlier inoculation with the antagonist. Under such conditions, control of disease has been obtained in a considerable number of cases and might well have been anticipated for organisms antagonistic under the conditions outlined in the previous section. Such experiments are in effect studies of antagonism in pure culture using natural substrates. They are of value in demonstrating that the selected antagonists are able or unable to grow and act antagonistically under such conditions and that their activity was not dependent upon the even more abnormal conditions of Petri dish or flask culture. The final stage is reached when organisms are tested for their ability to control disease under natural conditions and therefore in the presence of the normal microflora. Complications now arise, owing to the introduction into the disease complex of a variable factor, the importance of which is assessed only with difficulty, viz., competition between the antagonist and the saprophytes commonly associated with the pathogen. When disease does develop, the pathogen has obviously survived similar competition; the frequent inability of the introduced antagonist to become established and to multiply in the face of this competition probably accounts for the fact that there are few reports of disease control under these conditions and that conflicting results have been reported for similar experiments, even by the same worker. Nevertheless this is the stage of greatest importance; earlier ones can be regarded as essential preliminaries in all but a small minority of diseases.

Indirect use of antagonists is suggested by the fact that in many cases the microflora normally associated with the pathogen includes a number highly antagonistic in pure culture. Furthermore, certain treatments leading to a reduction in the incidence of disease have been shown to produce, at the same time, changes in the number and composition of this microflora. Indeed, it seems likely that some natural fluctuations in disease are caused by environmental variations affecting the pathogen not directly but indirectly by changing the activity of this microflora. Precise analysis of such changes presents great difficulties; it is only within broad limits that the effect of particular treatments or conditions can be predicted. It was therefore inevitable that the approach to this aspect of disease control has been largely em-

pirical and indeed is likely to remain so for some time in the future. Nevertheless, some encouraging results have already been obtained.

An account now follows of the work done in this field by the direct or indirect use of antagonists. No attempt will be made to consider the various diseases on a taxonomic basis; they will be dealt with in the first place in arbitrary groups, but subsequently the general problems involved in diseases of aerial parts of the plant, on the one hand, and of subterranean parts, on the other, will be discussed separately.

CONTROL OF SPECIFIC DISEASES

POTATO SCAB CAUSED BY *Actinomyces* (*Streptomyces*) *scabies.* This was the subject of one of the earliest comprehensive investigations of disease control by microbiological methods and is one of the few diseases for which some success has been reported under field conditions. It was shown (122, 123) that application of green manure to heavily infested soil gave substantial control of scab and also partially offset the increased severity of disease which generally followed heavy applications of lime. A series of pot experiments demonstrated that addition of green manure to sterile soil heavily infested with the pathogen was ineffective but that simultaneous addition of a saprophytic actinomycete, *Actinomyces praecox,* gave substantial control of the disease. This was associated with an almost complete suppression of the pathogen in the early stages of the experiment and a very considerable reduction later on. The control exercised by the addition of green manure was attributed to the fact that soils in which scab normally develops are generally low in organic matter; addition of a readily available organic food material might be expected, therefore, to lead to an increased growth of both saprophytic and parasitic actinomycetes. It was then postulated that under such conditions obligate saprophytes became dominant and, by appropriation of the available food supply and secretion of toxins, prevented multiplication of or even eliminated the parasitic types.

The failure of green manuring to control scab in a Canadian soil was attributed to the low pH (5.0–5.4) of this soil and the possibility that suitable antagonistic actinomycetes were either absent from this soil or were unable to multiply under acid conditions (147).

Work along similar lines has demonstrated control of this disease in sterile soil by the addition of an extract of unsterilized soil or manure, but, in contrast, addition of certain penicillia, bacteria or actinomycetes, including *A. praecox*, had no effect. In the field the addition of unsterile or sterile manure reduced scab; green manure was, however, ineffective (63).

Yet another antagonist has been studied in this respect. A species of *Trichoderma* was shown to be antagonistic to the pathogen in pure culture and also to give some reduction of disease when added as a suspension in furrows about the developing tubers (38). Further work on the effect of green manuring has shown in pot experiments with naturally infested soil that addition of two successive six-week-old crops of soy-bean reduced the percentage of the tuber surface covered by lesions from 48.1 to 10.3. Similar treatment with rye or clover, however, had little effect. Parallel studies on the effect of these treatments on the soil microflora showed that addition of rye had no appreciable effect on the number of fungi or actinomycetes but increased the bacterial population. Clover and soy-bean both caused large increases in all three groups, soy-bean being particularly effective in increasing the fungal population. It was also the only one to lower the pH significantly, reducing it from 6.4 to 5.0 (11, 145).

Some conflicting results have therefore been reported for this disease. While there is a measure of agreement that certain types of manuring are followed by a reduction in the incidence of the disease and that this reduction is attributable to a direct effect of the amendment on the numbers and activity of the soil microflora, there is no unanimity as to the group of organisms primarily concerned. Here may be quoted the results of recent work which demonstrated a wide range of cross-antagonisms among actinomycetes which were all antagonistic to *Streptomyces scabies*, making it unlikely that any one type would become dominant in a mixed population (108). The actual mechanism of the control which has been reported by such simple cultural methods has therefore still to be elucidated. It may well be different with different soils and climatic conditions.

DISEASES CAUSED BY *Rhizoctonia solani*. Investigation of this group of diseases was stimulated by the discovery of the active parasitism of the pathogen by *Trichoderma viride* in pure culture and that this fungus secreted into liquid media a substance later

identified as gliotoxin, highly toxic to this and other organisms in high dilution (177–180). The ability of this antagonist to control damping-off of citrus seedlings has been extensively studied. In a series of pot experiments the bottom layer of soil was inoculated with the pathogen and overlaid by peat in which were sown seeds of citrus plants. Addition of a suspension of spores of *T. viride* to the peat layer was effective in controlling damping-off when the pH was lower than 4.5, less effective at pH 5.7–6.1 and ineffective at pH 7.0. Addition of sand or soil to the peat layer also reduced the efficacy of this treatment. Control of disease affecting six- to eight-week-old seedlings was also obtained when the pathogen was added to the sub-soil and the upper layer of soil replaced with peat containing the antagonist or with soil acidified to pH 4.9. When seed was sown in soil at different pH values there was complete loss of seedlings at pH 7.3, partial control of damping-off at pH 4.9 and complete control at pH 4.0. Addition of the antagonist to soil at pH 6.4 to 6.7 or sand at pH 7.3 to 7.8 was quite ineffective. A parallel series of seed-bed experiments substantially confirmed these results. Here there was a considerable reduction in the area of seed-beds with damped-off seedlings after acidifying the upper layer of the soil or sowing the seed between layers of peat inoculated with the antagonist (181). These findings with acidified soil confirmed earlier work in which a series of soil treatments were tested for their ability to reduce losses of conifer seedlings caused by a number of pathogens, of which *R. solani* was one of the most important. Acidification of the seed-bed soil with aluminum sulphate or sulphuric acid was among the most effective and persistent treatments, and its practical use was recommended (169, 183). While the part played by micro-organisms in this acid treatment is by no means certain, that it is of some significance is suggested by the fact that *T. viride* is commonly found in such soil and that gliotoxin, one of the potent antibiotics secreted by this fungus, is stable only in relatively acid solutions. However, while this fungus produces gliotoxin in sterile soil, this material can not be detected in unsterile soil, even with very sensitive methods of assay (51).

A number of later investigations have emphasized the interaction between the soil microflora and this pathogen, and in particular have dealt with the activity of *T. viride* or related forms.

Cultures of this fungus grown on sterilized soil and added to sterile or unsterile potting soil which had been inoculated with the pathogen produced significant reductions of the numbers of diseased pea or cucumber seedlings (5, 70). Further experiments with a strain of *Bacillus simplex* which was active against the pathogen in vitro also produced similar results. In these tests increased stands of seedlings were obtained by adding to greenhouse soil (presumably unsterile) containing the pathogen, a water suspension of the bacteria or autoclaved broth cultures. It is to be noted, however, that equally significant results were obtained when an uninoculated medium was added (37). In another extensive series of studies with this pathogen, a special point was made of working under sterile conditions, thus avoiding many of the uncertainties which are inevitable when organisms are introduced into unsterile soil. Sterile seedlings of the Chinese cabbage were raised in culture tubes sealed with cotton wool. These were inoculated with the pathogen and one or more of a number of antagonists. The strongest antagonism was produced by two strains of *Trichoderma lignorum* followed by *Pyronema confluens, Cylindrocarpon didymum, Penicillium expansum, Cladosporium herbarum* and *Absidia spinosa.* The effect of more than one antagonist was additive except with *C. didymum* which reduced the antagonism of others. Generally similar results were obtained in pot experiments (79). In comparable work with lettuce seedlings, using sterilized sand or soil inoculated with the pathogen, almost complete control of disease was obtained by adding in a variety of ways organisms selected for their antagonism on a soil-extract agar. Those used included *T. viride, Penicillium clavariaeforme* and various species of actinomycetes and spore-forming bacteria. Parallel experiments with unsterile soil produced quite different results. Although the majority of organisms tested were somewhat effective in the early stages, this result did not persist, although two of the antagonists could be seen growing freely in the soil interstices (187).

An investigation of the black-root disease of beet led to the isolation of a number of strains of this pathogen and also to that of a new species, *Papulospora stoveri,* which was shown to be parasitic on the pathogen in pure culture. Addition of cultures of this antagonist to infested soil led to increased stands of seedlings

in two of three sowings over a period of 55 days (176). This disease was also controlled in sterile soil by pre-inoculation with a strain of *Bacillus subtilis* (46).

Other investigations have dealt with the effect of soil amendments on this pathogen with and without further addition of antagonists. Thus in one series of experiments an amendment of dried grass in unsterile soil caused a very significant reduction of disease of lettuce seedlings which was not increased by further addition of antagonists. Maize meal, however, was effective only after inoculation with antagonists (187). Similarly, with radish seedlings reduction of disease was obtained with a variety of organic amendments in a number of soil-types. This control was associated with a profuse development of other micro-organisms and was not observed in sterile soil. The effect of adding groups of these antagonists with and without organic supplements was also studied. In sterile soil the supplements alone did not reduce disease, but they did give some, though not much, control in the presence of the antagonists. In unsterile soil, substantial reduction of disease was obtained by the amendments; this was not further increased by adding antagonists. The positive results were attributed to an increase in the activity of the soil micro-flora able to utilize the amendments, leading to a nitrogen starvation of the mycelium of the pathogen and inhibition of its growth by an increase in the concentration of carbon dioxide in the soil (17, 18).

A comprehensive series of experiments extending over eight years has dealt with the effects of a variety of inorganic and organic amendments on the severity with which sprouts of potato tubers are attacked by this pathogen. The treatments were applied to unsterile soil inoculated under natural conditions in a standard manner with cultures of the fungus grown on sterile soil. A feature of the results obtained was the wide variation in the amount of disease recorded for replicates of the same planting and between corresponding replicates of different plantings. In view of this the results are not easy to summarize, but in general terms it may be said that nitrogenous salts and maize meal caused the most significant reduction of disease and that sucrose, lime, sulphur and magnesium sulphate increased the virulence and persistence of the pathogen. The basis of these differences was not established but they were not connected with pH changes; while the addition of

maize meal caused profuse fungal growth, this did not occur with the nitrogen salts (151, 152). In a continuation of this work it was shown that the pathogen disappeared from natural soil within 120 days in the absence of a suitable host, in this case potato plants. In the presence of the host plants, the pathogen persisted as well in untreated soil as in soils supplemented with maize meal, sodium nitrate or calcium hydroxide (153).

There seems to have been little work done on the extension to the field of results obtained in pot experiments. In one such series of experiments in naturally infested soil no positive results were obtained by addition of cultures of antagonists to lettuce seed-beds, although the organisms used were highly active in vitro and one had been selected for its ability to grow and act antagonistically at low temperatures. However, certain other treatments under the same conditions did succeed in significantly increasing the stand of plants in winter seed-beds. There was again in this work a wide variation between replicates of different treatments. Three of these, application of green manure or maize meal or keeping the soil wet some months before sowing, reduced the incidence of damping-off. Rather surprisingly, plots in which the upper layer of soil had been kept dry throughout the summer produced considerably fewer seedlings than those which were untreated. These results confirmed field observations that damping-off in winter seed-beds was generally more severe following a warm dry summer (187).

The general conclusions from the above investigations are that many soil organisms are antagonistic to this pathogen, some indeed actually parasitic, and that certain are able to control disease in soil in the absence of other saprophytes. Less frequently, similar control has been obtained in pot experiments with unsterile soil. Further application of these methods under field conditions seems to have been attempted in relatively few cases and then with negative or uncertain results. More promising as a practical measure is the use of soil amendments. Here, as good or better results have been obtained than by use of the most potent antagonists selected by pure culture screening tests. A point in favor of such methods is of course that they are effective only in unsterile soil and compared with any addition of fungal or bacterial inocula, would be relatively cheap. However, even with

these treatments under field conditions erratic results have been obtained. Since variation in disease occurs naturally between different soils and at different times, some natural biological control of the pathogen is indicated. It is important to note here that conditions favorable for vegetative growth of the pathogen sometimes depresses its virulence and that virulence is often greater in natural than in sterilized soil (150). A profitable approach to the control of diseases caused by this and other soil-borne fungi might be to analyze the conditions under which the pathogen disappears from the soil or becomes virulent in order to provide data on which to base the development of special cultural operations aimed at creating these conditions artificially.

DISEASES CAUSED BY *Phytophthora and Pythium* spp. These diseases rather closely resemble in their development those caused by *Rhizoctonia solani,* and in general the studies made with them and the results obtained have been similar. An early investigation dealt with the damping-off of seedlings in forest nurseries caused by *Pythium debaryanum.* Sterilized soil in pots was inoculated with the pathogen and then with cultures of a variety of organisms, including *Trichoderma koningi, Phoma* spp., *Chaetomium* sp., *Rhizopus nigricans, Trichothecium roseum, Aspergillus* spp., *Penicillium* spp., *Bacterium* sp. and unidentified fungi. Pots inoculated with the saprophytes gave increased emergence and increased final stand. Application of the saprophytes to seed-beds was, however, ineffective (72).

Damping-off of tomato seedlings by *Phytophthora parasitica* and *P. cryptogea* and its control by microbiological methods have been the subjects of an extensive series of investigations containing much information on the formation of antibiotics by selected antagonists in soil amended in various ways (68, 69). These aspects of the problem will not be described here, but the implications of this and similar work will be referred to later. It will be noted, however, that in these experiments some control of disease was obtained in sterile soil by the addition of cultures of *Aspergillus clavatus* or *Penicillium clavatum* on suitable organic media. Here the technique was to incubate the soils after addition of the antagonists and then to inoculate with the pathogen. Control was substantially increased when, in addition, the soil was sprayed with a glucose solution (68, 69). In greenhouse tests with *P. parasitica*

inoculation of the soil of pots or of hotbeds with *T. koningi* led to increased stands of seedlings. In two such experiments under these conditions increases from 207 to 557 and from 98 to 287 compared with total stands of just over 800 in the untreated plots were obtained (90). Results of the work with *Pythium* spp. parasitizing various grasses or lucerne conform to the same general pattern, a wide range of organisms and a soil suspension increasing the number of healthy seedlings grown in sterile conditions in tubes. In this work it was shown, too, that weak or non-pathogenic species of *Pythium* also acted as antagonists to virulent strains and led to a reduction of disease caused by them (110). This effect was also observed with isolates from strawberry plants affected with root-rot (136).

A survey of the actinomycetes isolated from certain Iowa soils showed that 21 per cent were antagonistic to *Pythium graminicola* which causes a root-rot of corn plants. Addition of crude culture filtrates of one of the most antagonistic of the isolates to soil infested with the pathogen increased both the height and root systems of corn plants (121).

The influence of the soil microflora on the development of root-rot in sugar cane and corn caused by *Pythium arrhenomanes* has been very extensively studied, particularly in recent years. An earlier investigation had shown that a number of actinomycetes from sugar cane soils were strikingly antagonistic to the pathogen in pure culture. Root-rot of both cane and corn was reduced in sterile soil by adding a culture of one of the most active forms (168). Later, extensive surveys of the actinomycete, bacterial and fungal flora of a variety of soil types in Louisiana was undertaken (34–36, 111, 112). At intervals throughout the year, thousands of isolates from these major groups were tested in plate culture for their antagonism to this pathogen, and an attempt was made to estimate the microbiological activity of a soil against *P. arrhenomanes* by calculating first the "Antibiotic Index", this being the average distance in millimeters of the inhibition zone between the various isolates and the pathogen on agar plates, and then the "Antibiotic Value" which is the Antibiotic Index × number of organisms per gram of soil.

The percentage of actinomycete isolates showing antagonism varied from 18.5 to 31.5 in different soils, the average inhibitory

activity increasing with soil pH, although this factor did not increase the size of the actinomycete population. It was shown, however, that the population of antibiotic forms was higher in the heavy soils generally favoring the disease and lower in the lighter soils where disease was normally less severe (34, 35). Similar results were obtained from analysis of the fungal population of heavy clay and light sandy soils, the antibiotic values being higher in the former. Approximately 15 per cent of the isolates tested were antagonistic in pure culture, the majority occurring in the genera *Penicillium, Aspergillus* and *Spicaria* (111, 112). Considerably lower values were obtained for the 5,638 bacterial isolates tested, only some 3.6 per cent on a sample basis showing antagonism (36).

While these studies have contributed much information on the activity of the microflora in different soils and at different seasons and have shown that relatively high proportions of isolates from these floras are antagonistic to this pathogen, so far no correlation between the antibiotic activity of different soils and the prevalence of root-rot has been demonstrated. More promising results were obtained with certain experiments under controlled conditions. Here, autoclaved soil artificially infested with *P. arrhenomanes* was exposed to the atmosphere. The populations of fungi, actinomycetes and bacteria on recolonization were assessed by dilution methods and the disease potential of the soil by measurement of the amount of root-rot of corn plants. No correlation was found between the Antibiotic Index for any of the groups of organisms and the amount of root-rot, but a definite relation was revealed between the Antibiotic Value of the actinomycete population and the amount of root damage. The effect of selected fungal and actinomycete antagonists in reducing disease in sterile soil subsequently infested with the pathogen was also studied. While some forms which were highly antagonistic in pure culture gave control of root-rot in these tests, others were quite ineffective. It can fairly safely be assumed that a larger proportion still of such forms showing in vitro activity would have no effect on the disease when introduced into unsterile soil directly as cultures (83–86). This difficulty of extending results obtained in pure culture or sterile soil to unsterile soil is well illustrated in recent work on the control of damping-off of alfalfa

caused by *P. debaryanum* and *P. ultimum.* In sterile loam both *T. lignorum* and *Streptomyces* sp. gave good control of disease when the soil was amended with 1% glucose. Similarly, good results were obtained when seed pelleted with *T. lignorum* was used. No control was obtained, however, when this antagonist was added to natural soil artificially or naturally infested with the pathogens. More promising results were obtained in unsterile soil amended with 1% ground oat-straw when one of a number of antagonists was added to the covering soil. At high greenhouse temperatures there was practically no damping-off, but at lower temperatures, although a similar protection was obtained in the early stages, the effect was only temporary, the treatment finally being ineffective (67).

In concluding this section mention must be made of the reports of active parasitism of certain pythiaceous root-rotting fungi by a variety of soil organisms, including other species of the genus *Pythium* (42–45). The possible significance of such parasitism in the natural fluctuation of populations of these pathogens, both in different soils and at different times, has been pointed out; the real importance has still to be assessed.

COTTON ROOT-ROT. The sequence of studies with this disease caused by *Phymatotrichum omnivorum* has been rather different from those made with other soil fungi. The efficacy of certain cultural treatments in reducing disease was first established by ad hoc methods and only subsequently were the mechanisms of the control investigated. An early report stated that addition of stable manure reduced the disease in infested fields (135). This and further investigations of the disease in Arizona showed that over many years substantial control had been obtained by applying organic manures along deep furrows during the fall and winter months. A definite reduction of disease followed the first application to infested soil, and in succeeding years the disease was never severe on plots treated in this way (95–97). These results were obtained within the irrigated zone; the method was not so successful elsewhere (157, 165). Analysis of the microbiological activity of treated and untreated soils showed that plots manured over periods of ten to twelve years had a considerably higher carbon dioxide output, higher numbers of bacteria, actinomycetes and

fungi, and a lower infestation by the pathogen than had untreated plots. The pathogen was, however, still relatively abundant in the manured plots. It was thought unlikely that the activity of the larger microbial population would produce carbon dioxide at rates high enough to build up concentrations inhibitory to the growth of the pathogen. As an alternative explanation an increased production of antibiotic substances under the conditions of heavy manuring was proposed (96). In further work on the seasonal fluctuations in the soil flora and the effects of ploughing in crops of sorghum or cowpea, it was found that a sharp increase in the population of bacteria, actinomycetes and fungi generally occurred early in the year. This was followed in the succeeding months by a fall in number which was continuous except for actinomycetes where a second but smaller increase occurred in November. The treatments had no great effect on the fungal population but gave very large increases in the bacterial and actinomycete populations (124). Detailed studies on the effect of similar treatments on the pathogen itself showed that, while it developed quickly and persisted for 16 days in untreated soil, in soil to which three per cent barnyard manure or chopped sorghum was added, it either failed to grow or the limited growth which did occur quickly disintegrated. Cotton seed meal, chopped hulls or cotton roots behaved similarly. In laboratory experiments on the survival of sclerotia, 19.6 per cent were eliminated in unamended soils, corresponding figures of 64.9 and 69.7 being obtained when barnyard manure and sorghum, respectively, were added. The major reduction in numbers occurred during the period of greatest microbiological activity following the addition of the amendments (125). In experiments on a field scale a substantial reduction in the number of diseased plants in infested fields was obtained by incorporating the amendments into soil by deep rotary ploughing (88, 89, 125). Similar effects have been produced by ploughing in cover crops of various legumes (113, 144).

In parallel work on the persistence of sclerotia, no difference in the survival rate was found in amended or untreated sterilized soils. In unsterilized soils, however, it was found that incubation temperatures favoring general microbial activity caused greatest loss of viability of sclerotia; 12, 32, 72 and 90 per cent of these were destroyed at soil temperatures of 2, 12, 28 and 35° C., while

soil moisture contents were found effective in the order 35, 58 and 80 per cent of water-holding capacity (32, 33).

These and similar results leave little doubt as to the efficiency of organic amendments in reducing losses caused by this pathogen, and indeed substantial results have been obtained on a field scale. Although there is a certain amount of circumstantial evidence that the activity of the soil microflora plays a part in this control, no direct proof is yet available and will be very difficult to obtain. It is known, however, that treatments which altered the carbohydrate content of the root bark of plants of fruiting age and which had a marked influence on the susceptibility of the plants to attack were also associated with marked changes in the bacterial flora at the root surface. High carbohydrate levels which increased the resistance of the plants also led to increased numbers of blue-green fluorescent bacteria. At the same time, however, there was a reduction in the total number of bacteria. Additional evidence that microbiological factors are involved was provided by the observation that maize plants, normally highly resistant to this pathogen, were severely attacked when grown in an infested sand and bentonite mixture previously sterilized, while in untreated soil, plants were unaffected, although strands of the pathogen could be seen growing in close proximity to the roots of the host. The nature and activity of the microflora at the root surface was therefore considered to be important in determining whether or not an attack of the roots by the pathogen developed (47). In this connection it is interesting to note that it has been found possible to modify this flora by direct inoculation. The organisms, *Aspergillus luchiensis, Penicillium luteum* and *T. lignorum,* antagonistic to the pathogen in pure culture, became established and multiplied in the rhizosphere of cotton roots after introduction either by seed inoculation or by direct addition to exposed root crowns. In each case the above organisms were recovered at considerable distances from the point of introduction in considerably greater numbers than in the control series (127). The direct parasitism of hyphae of the pathogen by the common saphrophyte *T. lignorum* has also been demonstrated (26, 27).

While much work remains to be done to elucidate the microbiological aspects of the effect of organic amendments and the factors involved in the comparative failure of these treatments in

certain areas, the successful control by such cultural methods in irrigated soils is particularly encouraging when one considers the very wide host-range of this pathogen and the absence of alternative methods.

CEREAL DISEASES CAUSED BY *Ophiobolus graminis, Fusarium* spp. AND *Helminthosporium* spp. The occurrence and development of diseases in relation to the activity of an associated microflora have probably been more extensively studied for this than for any other group of diseases. Particularly prominent is the work which has been done in Canada where the complex of diseases caused by these pathogens is of major importance. Infection of young seedlings arises either from soil-borne mycelium which may be closely associated with crop residues or from fungal material carried in or on the seed. The work which has been done on the microbiological control of these diseases may be conveniently grouped on this basis, and in this section the influence of the soil microflora on the survival and activity of the pathogens in soil will be considered. In this respect the studies with *O. graminis* are probably the most extensive and significant. No difficulty has been experienced in isolating from soils a wide variety of organisms antagonistic in pure culture to this fungus, and a considerable number of experiments have shown that, while inocula of the pathogen retain full virulence in sterile soil, their pathogenicity is reduced or even lost in unsterile soil (24, 54, 55, 109, 126, 134, 148, 151, 158, 184, 185). This is particularly marked under conditions which might be expected to favor a high level of microbial activity, e.g., high temperatures and relatively high soil moistures (56, 74). Similarly it has been repeatedly shown that a wide variety of organisms is able to give some control of disease in soil previously sterilized, but the results obtained under such conditions are poorly correlated with expectations based on observations of antagonism in pure culture (24, 102, 148, 160). Thus in one series of experiments of 45 organisms more or less ineffective in sterilized soil, 28 showed considerable antagonism to the pathogen on nutrient agar. In contrast, one species, *Typhula graminum,* which gave moderate control in soil and showed some antagonism on one type of agar, was compatible with the fungus on another (24). A decrease in virulence in sterilized soil has been observed generally when this is allowed to be recolonized by a saprophytic

flora; indeed it has been shown that in sterilized soil there may be a greater suppression of the pathogen than in natural soil, indicating that the original flora has been replaced, perhaps temporarily, by one more antagonistic (109).

Evidence that microbiological activity may be of importance under natural conditions is provided by experiments in which the severity of the disease was investigated in sterile and unsterile soil artificially infested and held at various temperatures. There was little difference at 13° and 18° C., but at higher temperatures the virulence of the pathogen was much reduced in the unsterile but not in the sterile series. This effect was attributed to increased activity of the soil microflora at the higher temperatures (74). However, alternative explanations of similar results in other experiments have been proposed. Thus in an investigation of the effect of organic amendments in natural soil, no correlation was found between the amount by which disease was reduced and the bacterial populations when these were estimated over the succeeding seven weeks (32). With this may be associated the observation that there were no major differences in the kind and number of microorganisms in naturally infested and clean prairie soils (32) and that while manuring may induce large increases in the microbial population of the soil generally, similar effects were not produced in the microflora of the root surface (31). Amendments reducing disease did, however, produce relatively large increases in the amounts of available phosphorus and nitrate nitrogen. Furthermore, the onset of disease in treated soils coincided with the disappearance of nitrate nitrogen from the soil (32). In further studies along these lines no correlation could be demonstrated between the severity of disease and the level of available phosphorus in infested and clean prairie soils, but it was shown in pot experiments with infested soil that the disease could be checked if superphosphate were added at planting time and a high level of nitrogen maintained throughout the growing season by repeated application of small quantities. The failure of other greenhouse experiments to demonstrate control by addition of inorganic materials was attributed to the fact that the amounts normally applied were considerably below those which would be available to individual wheat plants in heavily manured fields. This work therefore stresses the beneficial effect of certain organic and in-

organic additions to infested soil, and, while not discounting the part played by the microflora in indirectly influencing the activity of the pathogen, the results suggest that the principal effects of these treatments is to produce through the increased availability of essential nutrients plants less susceptible to attack (164). The results of another series of investigations on the effect of soil conditions on the development of this desease have suggested two further hypotheses to explain the way in which these and other soil amendments act and also the action of certain other factors known to influence attack of the host plant. The pathogen is considered in the first place to spread through the soil only on the roots of host plants and there to have an ascendant parasitic phase which is followed later by a pseudosaprophytic phase in which it persists on dead host tissue. Studies were made of the rate at which hyphae of the pathogen grew along the roots of wheat plants under a wide variety of soil conditions. The treatments tested included different soil types, soils at different pH values and moisture contents, soils sterilized by steam or various chemicals and soil artificially aerated. From the results obtained and from field observations on the occurrence of the disease it was suggested that the main factor retarding the growth of the pathogen along the roots of host plants was its susceptibility to high concentrations of carbon dioxide. Soil conditions favoring high carbon dioxide formation or low absorption would therefore adversely affect the pathogen and give a reduction of disease. They would include stimulation of microbial activity by addition of relatively large quantities of readily available organic amendments or suitable inorganic fertilizers (57, 58). In the saprophytic phase other factors were considered of importance. It was shown that the loss of viability of the fungus after it had colonized stubble was hastened by the addition of certain materials rich in carbohydrate but poor in nitrogen, e.g., starch or rye-grass meal, but was delayed by adding dried blood containing 13 per cent nitrogen, although this was followed by a more rapid decomposition of the straw. It was also shown that assimilable nitrogen prolonged the life of the fungus on the straw when added to the straw itself or to the surrounding soil (59, 60). Under field conditions the disease rating was reduced by growing an undersown trefoil crop on the stubble of the autumn crop and ploughing in this crop the following spring.

In this case the effect was, in part at least, thought to be produced by competition for available nitrogen between the crop and the pathogen (60).

No clear picture emerges from the very considerable work which has been done with this pathogen. It is clear, however, that the virulence and survival of the fungus are particularly sensitive to soil conditions and that a large measure of control may be obtained in the field by relatively simple cultural operations. The importance of the soil microflora in these experiments is, however, still a matter for conjecture; much of the effect obtained may depend on alterations in the susceptibility of the host to attack and on its ability to counter attacks of the pathogen by producing new roots under suitable nutrient conditions (7). In considering the persistence of the fungus in the absence of the host, such considerations do not apply and here microbiological factors are obviously at work; various mechanisms of control have been suggested and it is quite possible that several are involved under natural conditions.

Very similar work has been done with the other fungi associated with the "foot-rot" complex, particularly with *F. culmorum* and *H. sativum*. Again, the soil has been shown to contain a large number of organisms antagonistic to the pathogens in pure culture, some of which also gave a good measure of control when added to infested soil previously sterilized (8, 16, 66, 87, 91, 99, 103, 149, 151, 161). The influence of one pathogen in reducing infection by another has also been demonstrated (6, 104). Inocula of *H. sativum* and *F. culmorum* added to sterile soil and tested by sowing wheat seed at ten-day intervals gradually lose their virulence, the reduction being most marked in the first 40 days. In unsterilized soil virulence is much less than in sterilized soil, and here, too, it rapidly declines, being almost lost after ten days (23). The susceptibility of *H. sativum* to soil saprophytes was further shown by the failure of the pathogen to colonize unsterilized wheat stubble, although it grew readily on this substrate after sterilization. Similarly its growth was inhibited on green culms which had been moistened and incubated, although it grew and sporulated on culms before this treatment (159). Relatively little work has been done with these pathogens in natural soil; this is understandable in view of the difficulty generally experienced in establishing them under

these conditions. However, one such series has included experiments with natural soil. In preliminary pot trials one pint of an oat and wheat inoculum of an antagonist was added to four six-inch pots of sterilized soil. Infestation by the pathogen, *H. sativum,* was at a similar rate. In the field one pint of an inoculum of the antagonist was mixed with one pint of a culture of the pathogen and the mixture distributed along an 18-foot row. Substantial reduction of disease was obtained in both series of experiments by using separately, each of the following antagonists, *B. subtilis, T. viride* and *Penicillium* sp. It should be noted, however, that particularly large amounts of the antagonists were used in the pot tests and that in the field the method of inoculation established an association between pathogen and antagonist much more intimate than would have been obtained had the antagonist been added directly to infested soil in its natural state (8).

No comprehensive surveys on the effects of soil amendments on the virulence and persistence of these pathogens seem to have been made. The influence of micro-organisms on the seed-borne phases of diseases caused by these organisms will be considered in the following section.

SEED-BORNE DISEASES. Most of the work to be considered in this section relates to the seed-borne phases of attack of cereals by species of *Fusarium* and *Helminthosporium.* Other seed-borne diseases will also be included in this section, since the general problems involved for all are the same. *Prima facie,* this group of diseases would be considered the one most amenable to control by antagonists, especially in those cases where the pathogen is carried superficially, since no great problem would be involved in applying relatively large quantities of the antagonists to the limited area in which the pathogen is concentrated and from which it must grow. Some success along these lines has in fact been reported; either antagonists have been introduced into the local environment about the seed or use has been made of the saprophytic flora already present in a dormant state on most seed used commercially. Russian workers have been active in this field and have shown that inoculation of flax seed carrying the pathogens *Fusarium lini* or *Colletotrichum linicola* with certain soil bacteria capable of lysing these fungi, caused an increase in the number of healthy seedlings of certain varieties, although not of others (132). In

similar experiments with wheat artificially infested with *F. graminearum* large increases in the yield of grain were obtained when suspensions of a mycolytic bacterium or filtrates from liquid cultures of it were added to the suspension of the pathogen used for contaminating the seed before sowing (93, 94). Further experiments with flax wilt showed that treatment of seed with mycolytic bacteria produced a ten per cent increase in germinability, a 15 per cent reduction in the incidence of wilt at harvest and a ten per cent increase in yield. Similarly seed treatment of wheat with isolates of *Pseudomonas* or *Achromobacter* increased the percentage of healthy seedlings from 87 to 93 in clean samples and from 41 to 48 in samples infested with *Fusarium.* Corresponding increases in grain yield of some 30 per cent were obtained in pot trials (15). Damping-off of seedlings of *Pinus sylvestris* caused by seed or soil-borne species of *Fusarium* has also been controlled by treating the seed with a suspension of bacteria known from pure culture studies to cause relatively rapid lysis of the hyphae of the pathogen. Isolates of the genera *Pseudomonas* and *Achromobacter* were the most effective and under selected conditions reduced the amount of disease by 70 to 90 per cent (100). The results obtained in rather similar studies with barley seed naturally infested with *Fusarium* spp. and *Helminthosporium* sp. were less conclusive. A variety of organisms isolated from diseased barley seed or from soil continuously cropped with barley for 15 years were tested for their ability to reduce disease by these pathogens under a variety of conditions, e.g., treatments of seed with suspensions of spores and mycelial fragments of antagonists before sowing in the field, applying large quantities of cultures of antagonists to soil in the field or to sterilized sand in the green-house. Infected seed was also soaked in cultures of certain saprophytes for four hours before sowing, and with this treatment some reduction of disease was produced by cultures of *A. scabies* and *T. lignorum* used on seed infected with *Fusarium* spp. The other treatments were, however, largely ineffective. At least one of the antagonists used, *T. lignorum,* was antagonistic to *H. sativum.* Similar results were obtained when clean seed was superficially contaminated with a spore suspension of this pathogen and certain other saprophytes, and then sown in sterilized soil. A bacterium and also *Chaetomium spirochaete* re-

duced the number of deformed or stunted seedlings under these conditions. It was concluded from this work that the soil microflora did not have a marked effect on the incidence of seedling blight arising from barley seed infected with these pathogens; indeed it was noted that there were no differences in germination and amount of seedling injury when infected seed was planted in sterilized or unsterilized soil (30). In some contrast to these conclusions are those from work in which the influence of the saprophytic flora on the seed surface has been studied in relation to seedling blight caused by *H. sativum*. Here it was found that wheat seed incubated in a moist chamber for 24 hours before inoculation with the pathogen produced fewer diseased seedlings than a non-incubated series. A similar reduction was obtained when the seed was first soaked in a suspension of an antagonistic bacterium. These tests were carried out on the surface of blotting-paper in test tubes so that neither pathogen nor saprophyte was subjected to the competition of the soil flora (159). Complementary effects were produced when seed was treated with formaldehyde and lactic acid which almost completely removed the bacterial flora of the seed. Subsequent inoculation with *H. sativum* then led to heavier infection of seedlings, particularly when the seed was sown in sterilized soil. It was therefore concluded that certain seed samples carry a bacterial surface flora capable of materially reducing infection by this pathogen under certain conditions (105, 146). This work has been carried a stage further in the following experiments which included some under field conditions. In the first place, it was shown by Petri-dish tests, in which seed were inoculated with a spore suspension of the pathogen, that the surface flora of a sample of the variety Thatcher was more effective in reducing infection than was that of samples of the variety Reward, although evidence was produced which suggested that the difference lay in the greater number of organisms present instead of in their type. Incubation of moistened seed prior to inoculation and sowing in sterilized soil produced similar results, although here the difference between the varieties was not pronounced. In tests in unsterile soil seed were treated with one of the following: (*a*) suspension of bacteria from Marquis wheat incubated for 24 hours, (*b*) suspension of a bacterial culture increased on potato dextrose agar, (*c*) *a* and *b* together. The seed

were also inoculated with the pathogen. After ten days disease ratings of eleven, nine and five were obtained for treatments (*a*), (*b*) and (*c*), respectively, compared with that of 31 for seeds not treated with these bacteria. Further evidence of the importance of the seed flora was provided by observations that seed from heads kept moist for three days before harvest gave seedlings showing far less disease in Petri-dish tests than untreated seed of the same crop. Further treatment of such "weathered" seed with formalin destroyed this effect (106).

It will be noted that the above experiments were carried out with seed artificially contaminated with spores and mycelium of *H. sativum,* and it is obviously pertinent to enquire whether similar control of disease would have been obtained with naturally infested seed or with seed carrying a deep-seated infection. Control under these conditions has in fact been obtained by using selected strains of *Chaetomium* spp. against *Helminthosporium victoriae* and *Fusarium nivale.* This work originated from the observation that certain Brazilian varieties of oats were not susceptible to the virulent pathogen *H. victoriae.* Resistance was shown both to the strains naturally occurring on these seeds and to the strains isolated from the susceptible variety Vicland. The resistance of the Brazilian varieties disappeared, however, when seed was disinfected by a hot water treatment. An investigation of the saprophytic flora of such seed led to the isolation of a number of strains of *Chaetomium cochlioides* and *C. globosum* which were antagonistic to the pathogen in pure culture. Certain of these isolates also controlled disease on Vicland oats when added together with the pathogen in sterilized or unsterile soil. It was therefore postulated that the resistance shown by the Brazilian varieties came from the antagonistic activity of the surface flora and in particular of certain species of *Chaetomium* (172). In an extension of this work, oat-seed naturally infected with *F. nivale* was used. Forty-eight isolates of various species of *Chaetomium* were tested in pot experiments for their ability to control disease when added as oat-straw cultures to unsterile soil. While the majority of the isolates were quite ineffective, some gave partial control and one gave almost complete control of disease. In field experiments good control of disease was obtained when a weight of an oat-straw culture of a suitable isolate of *C. cochlioides* equal

to the weight of seed was added to the drills at the time of sowing. Another isolate of the same species gave little or no control under these conditions. A large measure of control was also obtained by dipping infected seed in a washed suspension of ascospores and perithecia of the above isolate and air drying, or by soaking infected seed in a cell-free filtrate of a 96-hour shake culture of the antagonist for three hours before sowing. In terms of emergence and seedling injury the control was of the same order as that obtained by the use of a standard organo-mercurial seed dust. It is interesting to note that the isolate giving this result was not strikingly antagonistic to the pathogen in pure culture.

The antagonist, introduced as an oat-straw culture, persisted in air dried unsterile soil for at least six months. When infected seed was sown in such soil adjusted to a suitable moisture content, the control of disease, while less than at the beginning of the storage period, was still substantial. In this and other experiments the antagonist was frequently observed as perithecia on seed coats and on the surface of the roots of oat seedlings. The notable features of this work are therefore that very significant control of disease was obtained using naturally infected seed grown in unsterile soil under normal field conditions, that only one strain of one species of many tested was effective and that the antagonist apparently became established and persisted for some time in the soil into which it was introduced (173).

As further examples of the influence of microbiological factors on disease developing from seed-borne pathogens, the following may be quoted: the observations that plants from flax seed naturally or artificially contaminated with *Polyspora lini* or artificially infested with *Colletotrichum linicola* consistently showed more disease in sterilized than in unsterile soil (75); the control of disease caused by *C. linicola* in sterilized soil by various soil organisms (101); the reduction of damage to maize seedlings caused by *Penicillium oxalicum* when artificially contaminated seed were sown in very wet soil or inoculated with other species of *Penicillium* (41); the peculiar effect obtained by growing at high temperatures plants from maize seed infected with species of *Gibberella* when the seed-borne pathogen, now relatively inactive, caused a reduction in the damage caused by *Trichoderma viride,*

not normally considered pathogenic (48); the protection of wheat seedlings from attack by a species of *Helminthosporium* and of flax seedlings from attack by *Fusarium lini* by treating seedlings or the soil in which they were growing with a broth culture of an antagonistic bacterium (138).

MISCELLANEOUS DISEASES CAUSED BY SOIL-BORNE PATHOGENS. A number of diseases upon which a more limited amount of work has been done compared with those dealt with above will now be considered. They are all caused by soil-borne pathogens, and with one or two exceptions the methods of approach have been those already described. The Panama wilt disease of bananas caused by *Fusarium oxysporum cubense* has received some attention, possibly because it is very difficult to control by more orthodox methods and because the host is particularly difficult to work with genetically. A survey of Jamaican soils for the prevalence of actinomycetes antagonistic to this pathogen on soil solution agar showed they were unevenly distributed. From 66 samples 122 were isolated which showed antagonism in pure culture; of these, 66 were slightly active, 39 active and 17 very active. A somewhat unexpected result was that antagonism was frequently more pronounced on solutions of soil from which the organism was isolated. Field experiments with naturally infested soil tested the ability of five of the above very antagonistic organisms and four actinomycetes isolated from infested soil to control disease when added to the soil at planting time. Although no striking results were obtained, there was some evidence that these treatments either produced a small but significant increase in growth of the plant or a decrease in the number of diseased plants (119, 120). In a study of the rhizosphere flora of susceptible and resistant varieties of banana it was noted that a bacterium, strongly antagonistic to the pathogen, was isolated in high numbers from an immune variety but was virtually absent from the very susceptible Gros Michel variety (71).

In similar work with root-rot (*Fusarium culmorum* and *F. dianthi*) and wilt (*Phytomomas caryophylli*) of carnation, mass cultures of ten soil fungi selected for their antagonism to these pathogens were added to soil in a green-house bench, and a month later the pathogens were also added. After nine months growth two isolates of *A. scabies* had significantly reduced the amount of

infection by *F. culmorum* and *P. caryophylli*. *Aspergillus niger* was also particularly effective against the bacterium, but no control of *F. dianthi* was obtained with this or the other organisms. Where some control was obtained the active organisms could be isolated from the treated soil (167).

It has been noted that *Fusarium udum*, the organism causing wilt of the pigeon-pea, may be relatively sensitive to the activity of the soil micro-flora, since considerably more disease developed in sterilized than in natural soil when these were artificially infested with the pathogen (175). A related organism, *Fusarium lini*, causing flax wilt, has, however, been shown to be much less affected by biotic factors in the soil. Thus it persists in the soil for many years and rapidly accumulates under continuous cropping with the host plant. Of 88 isolates of bacteria, actinomycetes and fungi, 12 were antagonistic to this pathogen, but none strikingly so, and no control of disease was obtained in field plots when large quantities of cultures of *Bacillus subtilis*, *T. lignorum* or *Penicillium* sp., all antagonistic in pure culture, were added to the rows in which the seeds were sown. These antagonists were also ineffective against *F. lini* in steamed soil. Under the same conditions, consistently better control of disease of barley caused by *H. sativum* was obtained (8). In contrast with these results, when cultures of a species of *Chaetomium* were added to steamed soil infested with *F. lini*, a third of the plants survived at flowering, while plants in the control series were killed one week after emergence (166).

Turning now to rather different groups of fungi, one may mention the antagonism of a number of bacteria isolated from dead or dying leaflets of clover to the clover rot fungus, *Sclerotinia trifoliorum*, and the parasitism of sclerotia by species of *Fusarium* and *Mucor* (137); also the series of investigations with the sclerotium-forming basidiomycetes, *Hypochnus centrifugus*, *H. sasakii* and with the imperfect fungus *Sclerotium oryzae-sativae*, all soil organisms which may persist as sclerotia in soils for long periods. Some control of diseases of rice caused by these pathogens was obtained by adding to sand, organisms known to be antagonistic in pure culture or by inoculating sclerotia with these organisms before placing them on the leaf sheath of the host plant. In each case competition from organisms other than those deliber-

ately introduced was absent (50). Later reports mention active parasitism of sclerotia and mycelium of two of these fungi by *T. viride* (76, 77).

Another disease of rice, leaf blast, caused by *Piricularia oryzae,* has been reduced by immersing roots of seedlings in 0.05–0.1% suspensions of powdered mycelium of *Cephalothecum* spp. in water. These treatments improved seedling growth and reduced the numbers of leaf spots by about 50 per cent (190).

In concluding this section, reference will be made once again to the activity of the fungus *T. viride* in two somewhat different connections. Firstly, a series of papers have referred to the importance of this organism as a natural antagonist of the tree parasites *Fomes* (*Polyporus*) *annosus, Armillaria mellea* and *Polyporus schweinitzii* (12, 141–143). It has been shown to be of significance in preventing colonization of tree stumps by *F. annosus,* increased severity of this disease on alkaline soils being attributed to the absence of the antagonist under these conditions (143). More directly, it has been demonstrated that the pathogen failed to grow on acid humus (pH 4.2) underneath spruce trees but developed satisfactorily on this medium after it had been sterilized. No growth occurred, however, if at the same time *Trichoderma* sp. was introduced (170). In an entirely different connection this antagonist has been shown to be active against *Armillaria mellea* (19) which is a destructive parasite of the roots of a large number of plants. The work to be reported deals with the interaction of fumigation treatments and activity of the antagonist in eliminating the pathogen from soils of citrus groves in California. A variety of chemical treatments were effective, carbon disulphide particularly so. Field observations showed in the first place that the pathogen, established on roots or trunks of a suitable host and protected by a pseudosclerotial layer, is able to persist in the soil for many years and obviously therefore resists attack by soil organisms, including *T. viride.* It was also noted that this fungus was almost always associated with roots in which the pathogen had become non-viable as a result of fumigation. At first it was considered that this was due to the resistance of *T. viride* to the fumigant; it would therefore be the first fungus to colonize the mycelium of the pathogen after it had been killed by the chemical treatment. Further experiments under controlled conditions

clearly indicated, however, a more active role for this antagonist. The pathogen did not grow in pure culture in its presence nor could it be isolated from mixed cultures of both. *T. viride,* when added to infested soil, killed the fungus with no additional treatment; the fumigant, carbon disulphide, was, however, ineffective against the pathogen in the same infested soil in the absence of the antagonist, even when applied at rates normally effective under field conditions. The conclusions drawn from these experiments were that, while in natural soil *T. viride* does not become sufficiently dominant to destroy *A. mellea,* once the equilibrium of the soil microflora has been upset by treatment with fumigants, this antagonist rapidly dominates the partially sterilized soil and in this condition is able to colonize structures of the pathogen previously unaffected (20).

DISEASES CAUSED BY RUSTS AND POWDERY MILDEWS. As might be expected from their nature, there are relatively few records of the influence of saprophytes on the occurrence and development of these diseases. A number of workers have, however, studied the active parasitism of rust fungi by other organisms and have considered its significance in the natural control of certain diseases (1, 78, 92, 107, 118, 129, 162, 163, 171). There is little doubt that these hyperparasites are not uncommon. Thus an early reference records that *Darluca filum* had been found in association with 24 per cent of the rust species then recorded (1906) for Australia (118) and, more recently, that a bacterium attacking the fructifications of *Puccinia graminis* was obtained from many field specimens of plants infected with this pathogen (107). *Tuberculina maxima* has often been found parasitizing the fructifications of *Cronartium ribicola* in nature and is probably the organism which has been most extensively studied in relation to disease control. The bark cankers caused by the rust may be actively colonized by this fungus; this prevents formation of aecedia and pycnidia the following year. Some control of disease by introducing the saprophyte under natural conditions has been claimed in Germany, but similar attempts in the Pacific North-West of the U.S.A. failed, although it was noted that the parasite was established naturally in small areas (78, 163, 171).

The bacterium already referred to (107) was found to actively parasitize the fructifications of a number of other rusts under

green-house conditions and also inhibited the development of diseases on both adult plants and seedlings. A species of *Trichoderma* which did not prevent infection, did, however, curtail subsequent development of disease. A similar result has been reported for a species of *Alternaria* which colonized lesions on the leaves of *Rhus villosa* caused by *Hemileia vastatrix,* and, acting as a weak parasite, produced barriers of dead tissue which could not be penetrated by the rust (29).

Use of hyperparasites in the control of powdery mildew diseases would seem a little more promising, since the mycelium of these pathogens is almost entirely superficial in most species. *Cincinnobolus cesatii* is well known as a parasite of members of this group (49, 188), but its use in disease control does not seem to have been considered. An infusion obtained by incubating a suspension of manure in water and used as a spray has, however, been applied with some success in localizing infection caused by the powdery mildews *Sphaerotheca mors-uvae, S. humuli* and *Erysiphe polygoni.* A suspension of mycolytic bacteria isolated from rotting hay was also partially effective (40).

No results of practical importance emerge from the above; further work is likely to be confined to the part played by other organisms in the natural fluctuation of disease caused by these pathogens.

SMUT DISEASES. Here, in isolated cases, rather more significant results have been obtained. A number of bacteria have been shown to influence markedly the growth of smut fungi in culture and to retard the progress of infection (13, 82).

In one series of experiments, significant reduction of disease of maize plants was obtained when certain bacteria were added to the inoculum of the pathogen. Pre-inoculation of the plant with the bacteria, while still reducing the infection rating, was less effective under these conditions. Cell-free filtrates from cultures of the bacteria were ineffective. The bacteria used were originally isolated either from lesions which failed to produce galls after inoculation or as contaminants of plate cultures of the pathogen. One such isolate rapidly disintegrated detached smut galls placed in Petri dishes and behaved similarly with large galls which had formed in infected plants. In both cases chlamydospore formation was prevented. The activity of these or similar bacteria was

thought to account for the failure of some inoculations and for the disappearance of galls which is occasionally observed in the field (13). These results have been confirmed in work in which other organisms as well as bacteria were used. A species of *Trichoderma* and various bacteria inhibited chlamydospore germination; other bacteria caused considerable reduction. Three methods of inoculation were used in tests with living plants: in field trials by injecting a sporidial suspension into the base of the stem of maize seedlings or dropping a sporidial suspension into the leaf spiral, in pot trials by adding a sporidial suspension to the seeds before sowing. In each case the sporidia were added with and without one of the following antagonists, a species of *Trichoderma,* an actinomycete and two bacteria. While the addition of each caused some reduction of infection, only the *Trichoderma* sp. produced a substantial effect. It is interesting to note that this organism was partially effective when used in conjunction with a chlamydospore suspension on seeds sown in unsterilized soil (189).

Although not strictly relevant to the problem of disease control, the interaction between *Tilletia foetida* and *Tilletia caries* in the infection of wheat plants provides another example of the effect of one fungus on the growth of another. While plants infected with these pathogens commonly occur in the same field, the pathogens are rarely found on the same plant. This interaction was demonstrated experimentally by sowing seed free of or artificially contaminated with *T. foetida* in a field naturally contaminated with *T. caries.* The presence of *T. foetida* on the seed markedly depressed the development of disease caused by *T. caries* (14).

The further significance of the above work on the control and natural occurrence of these pathogens can not at present be properly assessed. As with the rusts, however, the possibilities of practical control seem rather remote.

STORAGE DISEASES OF FRUIT AND VEGETABLES. Organisms causing these diseases commonly enter through wounds. Once established, the primary pathogen may grow rapidly through the host tissue. The initial infection may be followed by a series of secondary infections, the rotted tissue finally containing a variety of organisms. In such circumstances it is not always easy to determine which organism, or group of organisms, is primarily responsible for the final damage, and it is therefore not surprising

that a number of investigations have dealt with the ways in which invasion of fruit and vegetable tissue by one organism may be influenced by others. In one such series of investigations a number of pathogens responsible for rots of fruit or vegetables were studied. In general terms the following types of association were found; predominance of one organism excluding others, free admixture of two or more organisms, the primary parasite followed by secondary saprophytes. Temperature was found to play an important part in the development or suppression of organisms in mixed culture as was also the order in which the organisms were introduced into healthy tissue (114). Similar results were obtained with citrus fruit, complex interactions following the introduction of more than one pathogen into susceptible tissue. An increased rot was obtained with certain combinations, with others a reduction. Again, temperature played an important part in deciding which organism of mixed inocula became dominant (53, 154, 155). Further work along these lines has been reported for a species of *Penicillium* and *Diaporthe citri, Phytophthora parasitica* and *P. citrophthora* on citrus fruit (9, 61, 62), and for various organisms parasitizing apples (174).

WOOD-ROTS. Investigations of the development of certain diseases primarily affecting the secondary xylem of trees under natural conditions have demonstrated the activity of saprophytes in preventing or impeding infection. For example, *Phytophthora cactorum,* causing a crown rot of apple trees on artificial inoculation, is isolated readily only from the margin of lesions occurring naturally. Evidence was adduced that the growth of the pathogen was inhibited by the growth of bacteria in all but the marginal regions (182). Similarly, *Stereum purpureum,* which readily infects the surface of newly wounded woody tissues, does so much less readily a month later and is rarely able to establish itself on three-month-old lesions. This was attributed to the colonization of the exposed surface by a variety of micro-organisms which then formed a barrier against invasion by the pathogen (25). Similar effects have been reported for a bacterium against *Ophiostoma ulmi* on elm (73), a species of *Fusarium* against *Deuterophoma tracheiphila* on citrus (156), *Pseudomonas juglandis* against *Dothiorella gregaris* on walnut trees (52) and, at certain times of the year, between *S. purpureum* and *Nectria cinnabarina* or be-

tween the latter and *Botrytis cinerea* on plum trees (128). Studies with the black-knot disease of plums and cherry trees caused by *Dibotryon morbosum* have shown clearly how saprophytes may influence the development of this pathogen. It was observed that *Trichothecium roseum* appeared consistently each year during July and August on conidia-bearing stroma associated with knots on various *Prunus* spp. The fungus was sometimes restricted in its growth but at other times completely covered the fruiting surface. Inoculation of knots with a suspension in water of spores of the antagonist was followed three months later by the disappearance of perithecia and of the perithecial initials which were present at the time of treatment; uninoculated knots showed the normal number of perithecia. Under natural conditions it was noted that perithecia were always more abundant on non-infested than on infested knots and that there was a marked decrease in the number of new knots following heavy infection of old knots by the antagonist. From this and similar evidence it was concluded that *T. roseum* was of some importance in the biological control of *D. morbosum.* Ways in which such control could be enhanced were not reported (98).

LEAF DISEASES. Those caused by facultative parasites or saprophytes will be considered in this section. Comparatively little work has been reported. The rot of *Eichhornia crassipes* caused by *Hypochnus sasakii* was substantially controlled when leaves were simultaneously inoculated with *T. lignorum,* an active parasite of the pathogen in pure culture (117). This antagonist was also found to control the rot of lettuce leaves caused by *Botrytis cinerea;* a species of *Phoma* was also effective. Both antagonists gave better control when added as a suspension in malt or lettuce extract (10). More extensive work along these lines showed that a wide variety of organisms is able to control this disease when added to an inoculum of *B. cinerea* applied as a film over lesions simulating frost damage. Control was particularly effective at higher temperatures and enhanced by pre-inoculation of the saprophytes. Relatively few organisms were effective, however, at the lower temperatures which could be expected in the field and at which progress of the disease was still quite rapid. It was also shown that the pathogen was unable to penetrate areas of leaf tissue already colonized by certain saprophytes. Under conditions

which approximated to those prevailing naturally, a certain amount of control was obtained by spraying seedlings with a suspension of selected antagonists in a one per cent glucose solution (186). In associated work, a considerable amount of evidence was accumulated to support the hypothesis that the activity of saprophytes on dead or moribund leaf tissue substantially reduced disease of seedlings in seedbeds which were over-wintered in the open. Inoculation of seedlings during the winter with organisms isolated from moribund lettuce leaves failed, however, to control the disease. Some control of disease was nevertheless obtained by certain cultural methods which encouraged rapid colonization of dead tissue at ground level by saprophytes normally occurring in the soil (130, 131).

DISCUSSION AND GENERAL CONCLUSIONS

At this stage it will be convenient to deal separately with diseases affecting aerial parts of plants and those which damage roots or other organs below ground. In considering diseases of the shoot system, two general types of infection may be distinguished. In the first the pathogen enters through the intact surface of the host or through one of the natural openings such as stomata or lenticels; in the second entrance is through damaged or moribund tissue. The microbiological problems involved when antagonists are used to control these two types of diseases are likely to be quite different. The early stages of growth of a pathogen on the intact surface of a host are made in a film of moisture containing various substances which have diffused through the cuticle from the tissue beneath. The nature and amounts of such substances have been little investigated; both are likely to vary considerably between plant species and at different times in the life of the individual plant. It seems likely, however, that in its early growth on the host surface, the pathogen relies mainly on reserves within the spore. The antagonist, if it is to act upon the pathogen in the pre-penetration stage, would be subject to the same limiting nutrient conditions, and these would not normally be considered good for antibiotic production. On the other hand, this might be partially offset by the fact that fungi are generally more susceptible to toxins under poor nutrient conditions. Even if an antagonist were able to affect the growth of a pathogen in these circumstances,

it would also be necessary to ensure contact between the two unless antibiotics were produced of sufficient potency or in sufficient quantity to act at a considerable distance. In either case it would be necessary to cover the whole of the plant surface liable to infection, with spores of the antagonist, the density depending upon the efficiency with which the organism would act under these rather unsuitable conditions. Even assuming this could be done, another major difficulty would have to be overcome. Environmental conditions for germination and growth of the pathogen and antagonist would be very similar (a wider range for the antagonist would be desirable) so that one might anticipate that growth of the pathogen would be accompanied by growth of the antagonist over the whole area to which it had been applied. Any beneficial effect produced would therefore be non-persistent unless there were a periodic deposition of spores of the antagonist from elsewhere or unless the antagonist produced in the earliest stages of its growth, relatively resistant structures which remained *in situ*. With these considerations in mind one would naturally think of the spore-forming bacteria as a group of organisms most likely to act in these circumstances. The above remarks deal with the problem somewhat theoretically—at this stage this is inevitable in view of the lack of data on the nutrient conditions at the leaf surface and the nature and size of its natural microflora. In practice, at least on a small scale, it would not be difficult to alter both. The spores of many antagonists could easily be produced on a large scale and could readily be applied as a suspension in a nutrient medium. Some control of disease could then be predicted with some confidence and has in fact been obtained in isolated cases. Again, however, one would be faced with the non-persistence of the treatment unless conditions permitted multiplication of the antagonist after its germination and growth. Such conditions are less likely to be obtained in the open than under glass where some control of the environment at the plant surface would be possible. Once the antagonist were established in such a closed environment, it is not difficult to imagine the building-up of a population of antagonists which would have some effect on the growth and establishment of a pathogen. These and other considerations, which will not be dealt with here, would lead to the conclusion that theoretically this type of pathogen could be

controlled, even on a reasonable scale, by spraying plants with a suspension of suitable antagonists in a nutrient medium. Whether such control is likely to be worthwhile except in very special circumstances is very much open to doubt. Its only advantages would be in avoiding toxic sprays and in the rather remote possibility that it might become self-perpetuating. Otherwise it would seem as easy and much cheaper to apply an ordinary fungicide. Here at least one is assured of a certain level of persistence and avoids many of the uncertainties inherent in any biological method of control.

The second group of pathogens do not normally or can not penetrate the intact plant surface, and here considerations apply which are rather different from those outlined above. This type of pathogen normally enters through wounds or dying tissue which, it may be assumed, sooner or later become colonized by a saprophytic flora which does not normally spread to undamaged tissue. The pathogen must either get established before the saprophytes or be able to grow in their presence before spreading to healthy tissue. Evidence has been presented which shows that under natural conditions the microflora on damaged tissue may exclude a particular pathogen; it therefore follows that it might be possible to isolate the organisms responsible and introduce them or others obtained from similar habitats to the surface of the wound. At the same time it would be desirable to alter the nutrient conditions in directions favorable to the antagonist. As before, the problem of non-persistence might arise. Here, however, it might not be so acute, for it might well happen that moribund tissue would normally be produced continuously, thus providing new areas for the antagonist to colonize. This might happen, for example, if the pathogen normally entered through the dead or dying lower leaves of a plant which were closest to the soil surface, since the soil is probably the reservoir of most antagonists. It seems probable that in view of the greater availability of nutrients, antagonistic effects are more likely on the surface of wounded tissue than on intact surfaces of plants. On the other hand, if the normal microflora of the wounded surface is ineffective, greater difficulty might be experienced in introducing a more effective but different flora. Again, it is difficult to see what advantages a biological method of control would have over direct application of fungicides unless

colonization of the dead or dying tissue by an antagonistic flora could be obtained by a simple cultural operation, e.g., building up the soil around the bases of plants. With this, as with the other group of pathogens, direct application of selected antagonists is not likely to become a practical measure as long as alternative methods are available.

In considering diseases affecting underground parts of plants, two other factors assume importance. In the first place many of these diseases are very difficult or expensive to control by direct use of fungicides, and very often alternative methods are not available. There is, therefore, some justification for seeking somewhat unorthodox methods for controlling such diseases. Against this, however, is the fact that the pathogens causing such diseases act in the presence of a diverse microflora which is normally stable and resistant to change. Any radical and persistent alteration in this flora is, therefore, not easily obtained.

It will be convenient to deal first with soil-borne pathogens; these are not easily eradicated, once established, primarily because it is difficult to treat the large bulk of soil containing them. This is so even if the area of soil is not uniformly infested because it would not generally be easy to determine accurately the infested areas beforehand. It is apparent, however, that the spread and persistence of soil-borne pathogens may be very much influenced by soil conditions; soil invaders may be more susceptible than soil inhabitants in this respect. For both, the general problem is the same—how to modify the microflora in directions unfavorable to the pathogen. The method of approach to this problem has been largely empirical; this is inevitable, owing to our ignorance of the soil microflora and the lack of techniques for studying it in situ. Relatively little is known of the ways in which the behavior of the pathogen at the root surface differs from its behavior in the soil away from the roots, or of the effect of root exudates in modifying the saprophytic soil flora. There is, of course, good evidence that the composition of the rhizosphere flora is affected by both the type and condition of the plant. Most investigations of soil flora have used dilution plate methods. The types of organisms isolated in this way may well not be those preponderating in the soil as active mycelia. The heavily sporing species are more readily

isolated in this way, and it is these which have been used in most attempts to modify the flora by direct introduction of selected species. These attempts have been largely unsuccessful in the past and are likely to be so in the future unless conditions within the soil are altered at the same time. It is to be noted here that, since pathogens become established, there should be no reason why a particular antagonist should not behave similarly once the right conditions are found. Unfortunately these are not easily determined and the problem of establishing selected saprophytes in unsterile soil on a large scale is likely to be approached empirically for some time. As it happens, soil treatments favoring the growth of an introduced saprophyte may encourage the multiplication of similar types already present. This probably accounts for the fact that, so far, as good control of disease has been obtained by altering soil conditions as by adding antagonists.

There are few detailed studies in microbiological terms of the soil conditions in which pathogens disappear or become inactive. This is likely to be one of the more profitable lines of approach, particularly as it is now well known that the soil flora contains so many organisms antagonistic to particular pathogens in pure culture. In the past, unfortunately, pure culture studies have perhaps over-emphasized antagonism by the production of antibiotics. In spite of much work, the part played by antibiotics in the soil has still to be determined (21, 22, 51, 64, 65, 80, 81, 116). There is, however, some circumstantial evidence that it is significant. Many other factors may be at work, e.g., competition for essential metabolites, local pH changes, stimulation of growth in the rhizosphere. It may be significant that the Mucorales, probably one of the most important groups of soil organisms, are not well known as producers of antibiotics, even in pure culture; other features of their growth may account for their predominance. All these and other facts stress the importance of further detailed studies of the interrelationships between members of the soil flora and of the special relations between them and pathogens. Our knowledge at this level is still fragmentary, even for well known and important diseases, but in view of the tendency to increase the intensity of production and to grow the same crop continuously with the inevitable accumulation of soil-borne pathogens, it becomes

even more necessary to understand the ways in which use can be made of the natural antagonism between soil saprophytes and pathogens.

The problem of controlling soil-borne pathogens is considerably simplified in sterilized or partially sterilized soil. These treatments eliminate the pathogen which may, however, reinfest the soil later. It is generally much easier to establish selected antagonists in soil which has been treated in this way; the aim here would be to colonize the sterilized soil with a flora more antagonistic than the natural one and so prevent reinfestation. Any effect which is produced is likely to be temporary.

Turning finally to diseases caused by seed-borne pathogens, these would seem to be most amenable to control by microbiological methods primarily because the pathogen is confined to a small and known area of plant tissue. While it is unlikely that deep-seated infections will be controlled by applying antagonists externally, there is reasonable hope of success with more superficial infections or surface contaminations where it would not be a difficult matter to apply relatively large populations of antagonists either to the seed or to the soil in which the seed is sown. Various methods of application suggest themselves, such as adding the antagonist as a dust or a suspension to seed drills or to the seed itself, or using a slurry of the antagonist and an inert sticker or a readily available food material. These and similar methods are perfectly feasible and have been used with some success on a small scale (173). Inoculation of the seed with antagonists may not in fact be necessary as the surface is likely to carry a varied microflora which may be stimulated to activity by treatment with various nutrients and incubation under suitable moisture and temperature conditions. While these methods are novel and not impractical, it is not easy to see their advantages over more orthodox treatments such as application of fungicides to the seed or to the drills at the time of sowing. Indeed, while this type of disease is probably the easiest to control by biological methods, it is equally one of the easiest and cheapest to control with fungicides.

In conclusion it may be stated that no great success has attended the direct use of antagonists in control of disease up to the present, and, as far as can be seen, these methods of control are not likely to be able to compete with ordinary fungicidal treatments except

in isolated cases under special conditions. There is, however, a distinct probability that further investigation of the ways in which saprophytes influence the natural establishment and persistence of pathogens will lead to the development of cultural methods for controlling certain diseases, particularly if these are difficult or expensive to control in other ways.

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