

BIOLOGICAL CONTROL OF SOIL-BORNE FUNGI CAUSING
DAMPING-OFF OF SEEDLINGS.

by

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ABSTRACT

Thirty-four soils were screened in various ways for micro-organisms that were antagonistic to Pythium debaryanum, Corticium praticola and Sclerotium rolfsii and that would protect seedlings of cucumber, lettuce and tomato respectively from damping-off by these fungi. Of 929 bacterial cultures tested, only 73 showed any antagonistic activity on agar media. Seven isolates when applied to seed successfully controlled damping-off of cucumber in sterile soil and three of these did so in non-sterile soil. None of the bacterial isolates, antagonistic to C. praticola on agar, controlled damping-off when applied to lettuce seed but four did so when applied as a drench to infested, non-sterile soil. These, plus a further isolate, were also effective as a root dip to young lettuce before transplanting. Some of these isolates, applied to tomato seed, also controlled damping-off by S. rolfsii in non-sterile soil but generally the spectra of activity were not wide. Successful control, either by treating seed or applying a soil drench, appeared to depend on the establishment of the bacteria in the rhizosphere of seedlings.

Of the fungi tested, only Trichoderma isolates showed any substantial activity but large amounts of wheat-bran cultures of these were required to control Pythium damping-off of cucumber in non-sterile soil and C. praticola in soil was insensitive to them. An isolate of Rhizoctonia solani, avirulent to lettuce controlled damping-off by C. praticola but only when present in large amounts.

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CONTENTS

	Page
TITLE PAGE	1
ABSTRACT	2
ACKNOWLEDGEMENTS	3
CONTENTS	4
ABBREVIATIONS	7
INTRODUCTION	9
LITERATURE REVIEW	10
1. EVALUATION OF PATHOGEN AND HOST COMBINATIONS	22
Materials and Methods	22
Experimental	25
A. Screening test of pathogens on hosts	25
B. Effect of age and concentration of inoculum	25
C. Damping-off in non-sterile soil	27
D. Damping-off of tomato by <u>Sclerotium rolfsii</u> in sterile compost	28
Summary	32
2. ISOLATION OF POTENTIAL ANTAGONISTIC MICRO-ORGANISMS FROM SELECTED SOIL	33
Materials and Methods	33
Experimental	37
A. Suppressive nature of soils	37
B. Tests of antagonism on agar	38
C. Antagonism of selected isolates at different temperatures	38
Summary	47
3. TESTS OF ANTAGONISTS IN STERILE POTTING COMPOST	49
Experimental	49
A. Growth rate of a bacterial isolate in a liquid medium	49
B. Preliminary tests of applying bacteria to seed	49
C. Screening tests for bacterial antagonists against <u>P. debaryanum</u> and <u>C. praticola</u>	51

D. Effects of different suspending fluids on bacterization of lettuce seed against <u>C. praticola</u> damping-off	56
E. Control of damping-off with wheat bran culture of a <u>Trichoderma</u> sp.	58
Summary	61
4. CONTROL OF PYTHIUM DAMPING-OFF IN NON-STERILE SOIL WITH SELECTED ANTAGONISTS	62
Experimental	62
A. Screening test for bacteria against <u>Pythium</u>	62
B. Mechanism of control of <u>Pythium</u> damping-off on cucumber by seed inoculation with bacteria	62
C. Effects of seed inoculation with different inoculum concentrations of a bacterium on control of <u>Pythium</u> damping-off	71
D. Control of <u>Pythium</u> damping-off in non-sterile soil with wheat bran culture of <u>Trichoderma</u>	71
Summary	78
5. SPECTRUM OF ACTIVITY OF BACTERIAL ANTAGONISTS OF <u>PYTHIUM DEBARYANUM</u>	79
Experimental	79
A. Damping-off of tomato by <u>Sclerotium rolfsii</u> in non-sterile soil	79
B. Damping-off of lettuce and tomato by <u>Pythium debaryanum</u> in non-sterile soil	81
C. Control of damping-off of lettuce by <u>Corticium praticola</u>	81
D. Control of damping-off of tomato by <u>Sclerotium rolfsii</u>	85
E. Control of damping-off of lettuce and tomato by <u>Pythium debaryanum</u>	85
Summary	90
6. COMPARISONS OF BACTERIAL AND CHEMICAL SEED TREATMENTS TO CONTROL DAMPING-OFF OF CUCUMBER BY <u>PYTHIUM DEBARYANUM</u>	91
Experimental	91
Summary	94
7. THE LONGEVITY OF BACTERIA ON CUCUMBER SEED	95
Experimental	95
Summary	95

8. CONTROL OF DAMPING-OFF OF LETTUCE BY <u>CORTICIUM PRATICOLA</u> IN NON-STERILE SOIL WITH ANTAGONISTIC MICRO-ORGANISMS	98
Experimental	98
A. Control of damping-off by bacteria on seed in non-sterile soil	98
B. Control of damping-off of lettuce by inoculating soil with selected bacteria	100
C. Control of damping-off of lettuce by dipping seedling roots into suspension of selected bacteria	102
D. Control of damping-off of lettuce by <u>C. praticola</u> with <u>Trichoderma harzianum</u>	105
Summary	109
9. CONTROL OF DAMPING-OFF OF LETTUCE WITH AN AVIRULENT ORGANISM RELATED TO <u>CORTICIUM PRATICOLA</u>	110
Experimental	110
Summary	113
10. SPECTRUM OF ACTIVITY OF BACTERIAL ANTAGONISTS OF <u>CORTICIUM PRATICOLA</u>	114
Experimental	114
A. Control of damping-off of cucumber by <u>Pythium debaryanum</u>	114
B. Control of damping-off of tomato by <u>Sclerotium rolfsii</u>	114
C. Control of damping-off of cucumber and tomato by <u>Corticium rolfsii</u>	116
Summary	121
11. MECHANISM OF CONTROL OF <u>CORTICIUM PRATICOLA</u> DAMPING-OFF OF LETTUCE	122
Experimental	122
Summary	127
12. IDENTIFICATION OF BACTERIAL ANTAGONISTS	128
Experimental	128
DISCUSSION	133
REFERENCES	139
APPENDIX	153

ABBREVIATIONS

a.i.	- active ingredient
ANOVA	- Analysis of variance
<u>c.</u>	- about
C°	- Centigrade degrees
cm	- Centimetres
conc.	- Concentration
Cp	- <u>Corticium praticola</u>
CT	- Controlled Temperature
Cv.	- Cultivar
Df	- Degree of freedom
f.	- forma
F	- variance ratio
Fig.	- Figure
f. sp.	- forma(e) specialis(es)
g	- gram (s)
h	- hour(s)
Inoc.	- Inoculum
kg	- kilogramme(s)
l	- litre(s)
LSR.05	- Least significance range at 0.05 probability level
m	- metre(s)
max	- maximum
mg	- milligramme(s)
ml	- millilitre(s)
min	- minute(s)
MS	- Mean square
No or Nos	- Number(s)
n.s.	- No significant difference

P., pp.	- page, pages
PDA	- Potato dextrose agar
ppm	- parts per million
P.	- Probability level
p.s.i.	- pounds per square inch
r.p.m.	- revolution per minute
Rs ₁ , Rs ₂	- Rhizoctonia solani isolate 1, 2
s	- seconds
s.s.	- Sum of square
SD	- Standard deviation
SE	- Standard error
Sp./Spp.	- Species singular and plural
Sr.	- <u>Sclerotium rolfsii</u>
SV	- Source of variation
trt.	- treatment
wt.	- weight
^w /v	- weight/volume
^w /w	- weight/weight
μg	- microgrammes
μl	- microlitres
μm	- micron
%	- per cent
*	- significant difference, with a probability of 5 per cent ($\underline{P} \leq 0.05$).
**	- significant difference - $\underline{P} \leq 0.01$
***	- significant difference - $\underline{P} \leq 0.001$

1 - B26, 2 - B26, 3 - B26: Isolate number. B, bacterium; prefix 1 from tree nursery soils; prefix 2 from Windsor Park Forest soil and prefix 3 from Imperial College Silwood Park soil.

INTRODUCTION

Seedlings or young plants are often grown in steamed or chemically treated soil to reduce losses from soil-borne fungal pathogens, particularly species of Pythium, Rhizoctonia and Phytophthora. Treating soil with high dosages of fumigants or steaming at 100°C for 30 min or longer tends to produce a biological 'vacuum', and when a pathogen is accidentally re-introduced into treated soil it can spread without microbial competition and produce severe damping-off. One way by which this can be prevented without resorting to fungicidal treatment is to add selected antagonists to soil (Ferguson, 1958). Their establishment in the rhizosphere of the seedling roots may check the development of a subsequently introduced pathogen and may continue the protection when seedlings are transplanted to untreated soil, thus giving effective biological control.

Many attempts have been made to isolate micro-organisms antagonistic to particular plant pathogens and use them to control plant disease. In general, no great difficulty has been experienced in obtaining organisms antagonistic to pathogens in pure culture, and control of disease with these organisms has also been obtained quite often in sterilized soil. There are, however, relatively few instances in which direct application of antagonists has controlled disease under natural conditions in which presumably, the introduced organism has to compete with the normal microflora as well as with the pathogen. The problem of bringing relatively large numbers of an antagonist into intimate association with the pathogen can be considerably simplified if antagonists can be added to seed itself or to drills in which seed is sown. This study describes attempts to control three seedling diseases in this way.

LITERATURE REVIEW

The activity of micro-organisms in controlling plant disease is an aspect of the frequently-observed phenomenon of antagonism. This can be defined as any activity of one organism which in some way adversely affects another organism growing in association with it. The problem of disease control by antagonistic micro-organisms becomes essentially a study of competition between the pathogens concerned and other, non-pathogenic micro-organisms and may be approached in the following ways. First, 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. Second, conditions within the environment may be altered to produce a similar effect by modifying the nature and activity of the micro-flora already present. The first approach requires the isolation and selection of appropriate organisms; it is this aspect that will be reviewed most extensively.

Biological control through introduction of antagonists to the soil apparently was first attempted by Hartley in 1921 against damping-off of a coniferous seedlings, by Millard and Taylor in 1927 against common scab of potato, and by Sanford and Broadfoot in 1931 against take-all disease of wheat (in Baker and Snyder, 1965). In these examples organic manuring was used to increase the activities of soil saprophytic micro-organisms which were implicated in the control achieved. In a similar report Porter (1924) found bacteria to be the most effective antagonists of Fusarium lini and Helminthosporium sativum on agar plates. Some of the bacteria also protected flax and wheat seedlings in soil against these parasites. The control achieved was thought to be due to inhibitors formed by the bacteria during growth. These examples made plant pathologists aware of the possible use of antagonistic micro-organisms to control damping-off diseases and encouraged

further work with antagonists within the bacteria, actinomycetes and fungi.

Bacteria are extremely important in biological control of plant pathogens. They are the most dominant micro-organism in soil and their rapidity of growth and ability to utilize different forms of nutrients under widely different conditions is surpassed by no other group. This feature probably makes them of greater importance than any other group as competitors for nutrients particularly in the rhizosphere and around seed. Some bacteria are anaerobic and, along with few actinomycetes, make biological control possible under anaerobic conditions. Endospores formed by species of Bacillus and Clostridium can survive longer or at higher temperatures than most other microbial propagative units. Some bacteria have flagella and possibly may swim to a substrate or target pathogen if the water-filled soil pores are of sufficient size. Bacteria produce antibiotics, although this apparently is not a strong feature of the class. Certain Bacillus spp., especially Bacillus subtilis, produce antibiotics with activity against all types of soil microflora, including pathogens. Finally, bacteria are small by comparison with other soil micro-organisms and can multiply and form colonies on the surface of hyphae where opportunities for antagonism can be fully realized. Bacteria have limitations, such as their relative inactivity in soils not too dry for growth of fungi and actinomycetes and for disease development.

Actinomycetes are probably second only to bacteria in importance in maintaining satisfactory biological balance in soil, largely because of their widespread ability to produce antibiotics.

Fungi have received most attention as antagonists, possibly because they are easier to handle and identify than bacteria and actinomycetes. However, fungi rank third as potential antagonists in biological control (Baker and Cook, 1974). This does not imply that they are unimportant; on the contrary, some situations clearly suggest fungi as the important antagonists. The

tendency of Trichoderma, Gliocladium and Penicillium species to produce potent broad-spectrum antibiotics is well known. Because of their ability to make rapid growth on organic substrates in dry, coarse-textured and acid soils, and thus reach nutrients available to unicellular micro-organisms only by diffusion (Trinci, 1969), they may outperform bacteria and possibly actinomycetes.

Examples of biological control

An account now follows of the biological control of damping-off fungi by direct use of antagonistic micro-organisms with particular reference to the damping-off fungi used in the present study.

(a) Damping-off by Rhizoctonia

There are numerous reports in the literature of the control of Rhizoctonia seedling diseases by micro-organisms in sterile soil or other soil-like media such as potting compost. Some examples of the micro-organisms used and seedling hosts involved are given in Table 1.

Much work on the biological control of R. solani followed Weindling's discovery of the active parasitism of this pathogen by other fungi. Thus, Weindling and Fawcett (1936) demonstrated control of damping-off of citrus seedlings in peat by the addition of a spore suspension of Trichoderma lignorum. The control of the disease by this antagonist was achieved in non-sterile soil by acidification to pH 4.0. This work was confirmed by Allen and Haenseler (1935) who obtained a significantly increased stand of cucumber and pea seedlings in infected non-sterile soil by incorporating cultures of T. lignorum. Cordon and Haenseler (1939) were able to control damping-off of cucumber and pea by adding Bacillus simplex to a Rhizoctonia-infested non-sterile soil. In comparable work by Wood (1951), almost complete control of lettuce damping-off was obtained when organisms selected for their antagonism to Rhizoctonia on a soil extract agar were added to sterilized sand or soil inoculated with the pathogen. The organisms used included T. viride, Penicillium clavariaeforme

Table 1 Control of Rhizoctonia damping-off of seedlings by isolated antagonists in sterile soil or potting compost

<u>Host</u>	<u>Antagonist used</u>	<u>Medium</u>	<u>Author</u>
Cabbage	<u>Trichoderma lignorum</u>	Soil	Jaarsveld, 1942
	<u>Pyronema confluens</u>		
	<u>Cylindrocarpon didymum</u>		
	<u>Penicillium expansum</u>		
	<u>Absidia spinosa</u>		
Sugar beet	<u>Papulospora stoveri</u>	Soil	Warren, 1948
Sugar beet	<u>Bacillus subtilis</u>	Soil	Dunleavy, 1955
Citrus	<u>Trichoderma lignorum</u>	Soil	Weindling & Emerson, 1936
	<u>Trichoderma viride</u>		
Peas and Radish	<u>Trichoderma viride</u>	Soil	Evans & Gottlieb, 1952
Radish	<u>Bacillus subtilis</u>	Soil	Olsen & Baker, 1968
Pepper	<u>Bacillus</u> spp.	Soil	Broadbent <u>et al.</u> , 1971
Cotton	<u>Trichoderma harzianum</u>	Sand	Curl <u>et al.</u> , 1976

and various species of actinomycetes and spore-forming bacteria. Parallel experiments with non-sterile soil produced quite different results. Although most organisms tested were partially effective in the early stages, the level of control was not maintained although two of the antagonists could be seen growing freely in the soil interstices.

Merriman et al. (1974) obtained significant reductions in symptoms caused by R. solani by inoculating wheat seed with either Streptomyces griseus or Bacillus subtilis. These treatments also increased grain yield and dry weight of wheat in sterile and non-sterile soil. However, when these organisms were applied individually to barley, oat and wheat seed and this was sown at three sites with a known incidence of R. solani, root disease was not effectively controlled, although at one site the seed treatments increased grain yield and total dry weight of the plants. The time of heading was advanced at another site and at two sites there was an increase in tiller number.

Henis et al. (1978) attempted an integrated control of R. solani with chemical and microbial antagonist. They found that wheat bran cultures of Trichoderma harzianum applied to soil at rates of 0.04-0.15g/kg (dry weight basis), protected radish seedlings from damping-off by R. solani and also increased radish germination in non-infested soil. Pentachloronitrobenzene (PCNB) at 4 µg a.i./g soil added with the inoculum of T. harzianum had an additive effect on disease control and a synergistic effect on the decrease in inoculum density of R. solani propagules.

(b) Damping-off by Pythium spp.

There have been similar studies with damping-off diseases caused by Pythium spp., for example that in forest nurseries caused by Pythium debaryanum (Hartley, 1921). Sterilized soil in pots was inoculated with the pathogen and then with cultures of a variety of organisms, including Trichoderma koningi, Trichothecium roseum, species of Chaetomium, Penicillium and Aspergillus,

and some other unidentified fungi and bacteria. In pots inoculated with some of these micro-organisms there was an increased seedling emergence and seedling stand. However, when these particular micro-organisms were applied to seed-beds they were ineffective. Allen and Haenseler (1935) reported that damping-off of cucumber induced by Pythium sp. was appreciably reduced by infesting soil heavily with Trichoderma lignorum. Similarly, Johnson et al. (1952) used species of Spicaria, Penicillium and Aspergillus to control corn root rot caused by Pythium arrhenomanes. Work by Van Luijk (1938) with Pythium spp. parasitizing lucerne showed that weak or non-pathogenic species of Pythium acted as antagonists to virulent strains and led to a reduction of damping-off caused by them. This effect was also observed with Pythium isolated from strawberry plants affected with root rot (Plakidas, 1930).

The difficulty of extending results obtained in pure culture or sterile soil to non-sterile soil was well illustrated in the work by Gregory et al. (1952) on the control of damping-off of alfalfa caused by Pythium debaryanum and Pythium ultimum. In sterile loam both Trichoderma 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.

A survey by Meredith and Semenuik (1946) of the actinomycetes isolated from certain Iowa soils showed that 21% 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 system of corn plants.

The influence of the soil microflora on the development of root rot in sugar cane and corn caused by Pythium arrhenomanes has been extensively studied. A number of actinomycetes from sugar-cane soils proved to be strikingly antagonistic to the pathogen in pure culture. Some of these isolates when applied

to sterilized soil previously infested with P. arrhenomanes reduced root rot of sugar cane (Tims, 1932; Copper et al., 1948 and Johnson et al., 1952). While some forms which were highly antagonistic in pure culture gave control of root rot in the tests, others were quite ineffective. Broadbent et al. (1971) introduced species of Bacillus and Streptomyces into steamed soil and reduced damping-off of pepper caused by Pythium ultimum. They also showed, however, that some micro-organisms inoculated into soil may increase the amount of disease.

Some success in controlling Pythium has been reported by inoculating the seed of susceptible host with antagonists. Wright (1956) was able to protect mustard seedlings from infection by Pythium sp. by dusting the seeds with spores of Trichoderma viride and Penicillium frequentans. Liu and Vaughan (1965) also reported biological control of Pythium ultimum infection of table beet by seed treatment with conidia of the same fungi. This treatment gave better results when the soil was supplemented with KH_2PO_4 or K_2HPO_4 , and with Urea. Attempts to control disease by treating seed in a similar way with bacteria and actinomycetes failed. Mitchell and Hurwitz (1964) also reported that tomato seeds inoculated with rhizosphere bacteria (Arthrobacter sp.) suppressed damping-off caused by P. debaryanum but attempts to control this disease with non-rhizosphere bacteria failed.

A commercial test by A.G. Watson (unpublished in Baker and Cook, 1974) showed that Chrysanthemum cuttings rooted in a steamed propagative medium inoculated with random antagonists were well protected against a high inoculum density of Pythium ultimum when they were transplanted to infested soil. Moist organic matter of near sterility exposed to air-borne contamination for several days provided the inoculum of antagonists that was placed on the propagative beds. Although this method of obtaining antagonists cannot be recommended, the level of success attained shows that empirical methods may give surprising results.

Seedling diseases caused by *Sclerotium rolfsii*

Wells et al. (1972) reported field control of *S. rolfsii* by the addition of an antagonist. Under greenhouse conditions, *Trichoderma harzianum* effectively controlled *S. rolfsii* on blue lupines, tomatoes and peanuts. Under field conditions, one of the three applications of *T. harzianum* inoculum applied to the soil surface was highly effective in reducing *S. rolfsii* damage to tomato transplants. The disadvantage of the method used by these authors was the great bulk of material required to obtain control. In addition, it was difficult to ensure that the same effective dose was delivered on successive dates. This problem was overcome in a similar trial conducted by Backman and Rodriguez-Kabana (1975) by growing *T. harzianum* on diatomaceous earth granules impregnated with 10% molasses. A significant reduction in *S. rolfsii* damage and increase in yield were recorded over the 3 year period. Disease control using 140 kg/hectare of *Trichoderma* granules was equivalent to that achieved using 10% PCNB granules at 112 kg/ha. The biological control system had the advantage of low bulk, no residues and applicability to other pathogen-antagonist systems.

Broadbent et al. (1971) found in Australian soils some *Bacillus* spp. that were antagonistic to *S. rolfsii* in culture, but none was tested in soil against damping-off disease in crops induced by this pathogen.

Applying biological control

It is evident that there are several examples in which biological control of soil-borne parasites can be accomplished by the use of saprophytes known to be antagonistic; either by treating host seeds or infested soil.

Addition of antagonists to infested soil with a pathogen has the best chance of success if the numbers are sufficient to swamp the resident microbial population or when the population of resident micro-organisms has been severely diminished by some other treatment. In either case the population balance is drastically altered to favour antagonists. When antagonists are inoculated

directly to soil infested with a pathogen, it may be necessary to allow the antagonist time to establish before sowing a susceptible host as demonstrated by Liu and Vaughan (1965) on damping-off of table beet by Pythium ultimum. In this case, simultaneous infestation of sterilized soil with P. ultimum and Trichoderma viride or Penicillium frequentans 1 week before seeding significantly reduced beet-seedling damping-off. The antagonists were less effective when mixed with sterilized soil in which P. ultimum had been established or when mixed with non-sterile soil simultaneously with the pathogen and seeding was done immediately after inoculation.

The successful establishment of Rhizobium sp. in legume roots by inoculation of seed before planting and the inoculation of cereal seed with Bacillus subtilis and Streptomyces griseus as a means of increasing growth (Merriman et al, 1974), show the potential of this technique in biological control. Studies in England and Australia have shown that Azotobacter chroococcum can become established in the rhizosphere of several plants when introduced on their seeds.

Because of the rapid sorption and decomposition of antibiotics in soil (Brian, 1957), as well as their cost, it is not feasible to add these materials for disease control. It is considered more promising to use living micro-organisms to produce antibiotics continuously in low concentration in situ. The dominance of the antagonists on seedlings may persist only for a short time, or may be retained for the life of an annual crop. If the susceptibility of the host to the pathogen is brief, even a short protection may be enough to avoid the disease (Chang and Kommendahl, 1968). Addition of a suitable amendment to the soil, or addition of an antagonist-inoculated amendment to the seed or soil, may help the micro-organisms to maintain their dominance.

The importance of different nutrients as a base for bacterization of seed in enhancing antibiotic production, was demonstrated by Sigh et al. (1965).

They obtained appreciable reduction in the incidence of pigeon-pea wilt caused by Fusarium udum under sterile and unsterile soil conditions through seed bacterization with Bacillus subtilis. The maximum reduction was obtained in the treatment where molasses and groundnut cake was used as a base for bacterization, apparently enhancing production of bulbiform (antibiotics) by Bacillus subtilis.

Another method by which biological control can be instituted is to start seedlings and cuttings in propagative beds inoculated with suitable antagonists. Because the antagonists dominate the microflora, the roots and stems become colonized by them. When the seedlings or cuttings are transplanted to pathogen-infested soil, they are thus protected by the antagonistic rhizosphere flora. This dominance may well protect an annual crop through the critical period of susceptibility or primary injury without the antagonistic microflora being perfectly adapted to the crop (the possession principle, see Baker and Cook, 1974). The classical example of this was demonstrated by Olsen and Baker (1978). They started radish seedlings in soil infested with antagonistic Bacillus subtilis and later transplanted to soil infested with Rhizoctonia solani where the seedlings were protected from damping-off. Aldrich and Baker (1970) used Bacillus subtilis to inoculate carnation propagation beds, so that cuttings became infested while rooting; when these rooted cuttings were transplanted to soil infested with Fusarium roseum f. sp. cerealis, some protection was given to the growing plants. Michael and Nelson (1972) also obtained good control of Fusarium roseum f. sp. cerealis by dipping unrooted carnation cuttings in a suspension of bacteria (a pseudomonad isolated from soil). Rooting was not affected and plants grew well for 4 months, although the fungus could be isolated from the symptomless stems. This can also be regarded as an example of biological control whereby the vegetative structure was inoculated with the antagonist before transplanting to a soil infested with the pathogen.

Biological control through the pathogen itself may be possible with avirulent strains. There is increasing evidence that inoculating the host with such strains may prevent subsequent infection by a virulent strain. An example of this was provided by the work of New and Kerr (1972). They reported that when roots of peach seedlings were dipped in a suspension of selected avirulent Agrobacterium radiobacter biotype 2 and wounded before planting in a soil infested by a virulent isolate A. tumefaciens, formation of crown galls was prevented. The avirulent isolate, which was physiologically and serologically indistinguishable from the pathogen, had to be in a population at least equal to that of the pathogen for protection. Langton (1969) placed susceptible tomato cuttings in suspensions of different proportions of microconidia of Fusarium oxysporum f. sp. lycopersici and f. sp. pisi before planting. The greater the ratio of the form pisi, the less severe the wilt symptoms produced.

Some pitfalls encountered in biological control

Cook and Baker (1974) gave a number of explanations for undervaluation and neglect of biological control by plant pathologists. Antagonist populations have often been sought in areas where both the pathogen and disease occur, rather than in soils biologically suppressive to the pathogen. Although individual antagonists may occur in non-suppressive soils, they are unable to express their antagonistic action to a given pathogen either because these soils may lack the necessary substrate for production of effective quantities of antibiotics by the antagonist, or the antibiotic may be sorbed or inactivated by other organisms. Also, the population of resident antagonists in such a soil may be too low to exert any spectacular suppressiveness of the pathogen. Often in searching for individual antagonists too few micro-organisms have commonly been used. Because of the many characteristics required for success it is necessary to work with large numbers of candidates. In glasshouse experiments for screening potential antagonists, the inoculum density of the

pathogen may be raised to unnaturally high levels by addition of cultures when antagonists are tested in soil. This invariably swamps biological control and gives the erroneous conclusion that the antagonists are ineffective. Inoculum density of the pathogen should not exceed that found under severe natural disease conditions. Emphasis has sometimes been placed on unpromising antagonists.

It is evident from the literature presented that much of the work on biological control of soil-borne pathogens has used saprophytes known to be antagonistic, either by treating infested soil or seeds. These antagonists were either encountered accidentally or found associated with the pathogen in a disease complex, it was only in very few instances that antagonists have actually been sought in soil for the specific purpose of using them to control specific disease. When the antagonists are eventually introduced, usually the soil has to be sterilized to establish the antagonist and obtain control. This is in effect a study of antagonism in pure culture using natural substrate. The inability of the introduced antagonist to become established and to multiply in the face of competition with other microflora probably accounts for the fact that there are few reports of disease control under non-sterile soil conditions.

The present investigations were made to find which antagonistic micro-organisms were present in selected soils and to study the possibility of employing the antagonistic micro-organisms for control of damping-off in the glasshouse.

1. EVALUATION OF PATHOGEN AND HOST COMBINATIONS

Several fungi, e.g. Rhizoctonia solani Kühn and Pythium debaryanum Hesse, attack seeds, seedling stems or roots causing respectively seed decay (pre-emergence damping-off), post-emergence damping-off or root rot. The experiments in this section tested the pathogenicity of isolates of Corticium praticola, Pythium debaryanum, Rhizoctonia solani and Sclerotium rolfsii on lettuce, cucumber and tomato to select suitable pathogen/host combinations for further studies. The effects of age and concentration of inoculum on disease incidence were also investigated.

Materials and Methods

Fungi

The following were obtained from the culture collections of the Department of Botany, Imperial College:-

- (i) Two isolates of Rhizoctonia solani Kühn, designated Rs₁ and Rs₂.
- (ii) Pythium debaryanum Hesse was re-isolated from a diseased cucumber seedling. Segments of diseased cucumber shoots, cut to retain 5mm of diseased and 5mm healthy portions, were sterilized for 1 min in 0.1% HgCl₂, rinsed twice in sterile water, dried between sterile filter papers, and plated on 2% MEA. After 4 days at 25°C, a portion of the colony was transferred to plate of PDA. Subcultures to fresh PDA plates were made 4 days later. The growth and morphology of the isolate obtained agreed closely with the C.M.I. description of Pythium debaryanum.
- (iii) Corticium praticola Kotila. The parent culture was acquired from Dr P.H. Williams, Glasshouse Crops Research Station, Rustington, Sussex, and descended from an isolate supplied by the late Professor N.T. Flenje. It was re-isolated from a diseased lettuce seedling by the method outlined in (ii) above.
- (iv) Sclerotium rolfsii Sacc, was re-isolated from a diseased tomato seedling by the method outlined in (ii) above.

Stock cultures of these fungi were maintained on PDA slopes under sterile mineral oil in Universal containers and the cultures used in experiments were renewed from stock cultures at least every 6 months. For experiments, the fungi were grown initially on plates of PDA (oxoid) or V8-juice agar (V8). Other agar used in experiments were: water agar (WA), rose-bengal agar (RBA), Czapek-Dox agar (CzDA) and soil extract agar (SEA). Inoculum for pot experiments was grown on an oatmeal sand medium. The preparation of all media is detailed in Appendix 1.

Host plants

(a) Seed Stocks

Seeds of lettuce (cv. All the year round, Suttons Seeds Ltd), cucumber (cv. Perfection ridge, Elsoms Seeds Ltd) and tomato (cv. Moneymaker, W.W. Johnson and Sons Ltd) were stored in polyethylene bags at 4°C. Germination on moist filter paper was tested every 6 months and, if poor, fresh seed was bought. Germination of seeds used in experiments was: lettuce, 80-95%; cucumber, 80-90% and tomato, 80-96%.

(b) Seedling establishment

Seed was sown either in sterile potting compost (John Innes No 1) or in soil. The soil (except where stated otherwise) was obtained from the Walled Garden of Imperial College at Silwood Park. The top 15cm were collected, air dried, sieved through a 7mm sieve and stored in galvanized metal bins. Any soil which had been more than 4 months in the bins was discarded.

The moisture content of the sieved soil was determined by drying a sample at 100°C to constant weight. The average water content was 15% ^w/w. All weights of soil mentioned in this work are dry weights and all percentages relating to materials added to it, are based on the dry weight of the soil.

Lettuce seeds were lightly watered after sowing and covered with thin polyethylene sheeting until emergence. Sowings of cucumber and tomato seed were not covered. Water was added if the soil appeared dry but no attempt was

made to maintain a constant moisture content of the soil, except in certain experiments which are described later. During the winters of 1977-78, 1978-79 and 1979-80 natural daylight was supplemented by four 400W mercury vapour discharge lamps (Phillips TYP571359) suspended 1m above the glasshouse bench to give a 14h day and a light intensity to the seedling of 500 lumens/cm². The temperature was thermostatically controlled and did not fall below 20°C and rarely exceeded 25°C.

Disease establishment and assessment

Flasks of oatmeal sand were each inoculated with five agar disks (5mm diam) taken from the edge of a mycelial culture and incubated at 25°C for 15 days. The flasks were shaken occasionally during this period to promote even distribution of mycelium throughout the medium and the culture was mixed thoroughly before adding to soil or potting compost. The experimental unit was a 9 x 9cm, 510ml, black plastic pot (Rapidex), filled with potting compost or soil to 3cm below the rim. The inoculum was incorporated into soil or potting compost only in the top layer.

Seedlings were considered to have emerged when their plumules were just above the soil surface. Most emerged 5-8 days after sowing and numbers were recorded on the ninth day. Seed which had not produced seedlings then were considered killed (pre-emergence damping-off). Subsequent death of emerged seedlings (post-emergence damping-off) was derived by subtracting this figure from a final count of seedlings 15 days after sowing. Killed seedlings were not removed at any time. Seedlings of the three hosts became highly resistant to damping-off 2 weeks after sowing and only rarely was there any post-emergence damping-off after 14 days. After recording the final stand, seedlings were removed carefully from the soil and washed free of soil under running tap water to recover as much of the root system as possible. Seedlings from the same pot were dried between blotting paper for c. 5 min and then weighed. In most experiments each treatment was replicated three times and each replicate (pot)

was sown with fifteen tomato or lettuce seeds or ten cucumber seeds. Fresh weights are given as the average per plant, (per replicate).

Most data were subjected to standard analysis of variance (Steel and Torrie, 1969) and means were compared by Duncan's new multiple range test.

Experimental

A. Screening test of pathogens on hosts

The ability of Corticium praticola (Cp), Pythium debaryanum (Pd), and two isolates of Rhizoctonia solani (Rs₁ and Rs₂) to damp-off lettuce, cucumber and tomato was investigated in three experiments each with all fungal isolates but only one host.

Pots were filled with sterile potting compost and the top layer was covered with 100g of a 10% mixture of oatmeal sand culture and potting compost (wet wt of inoculum/dry wt of soil) in which seeds were sown, 5mm deep. Additions of autoclaved oatmeal-sand culture and potting compost served as controls for each fungal isolate and the inclusion of a treatment with potting compost only provided an additional standard for seedling growth. Each treatment was replicated three times and the pots were randomized completely on the glasshouse bench. The results are summarized in Table 1.1 and the full analyses are given in Appendix Table 1.1.

Corticium praticola and Pythium debaryanum caused marked damping-off of all three hosts. P. debaryanum caused only pre-emergence damping-off but C. praticola also killed seedlings that had emerged. The two isolates of Rhizoctonia solani were not pathogenic on lettuce or cucumber but they did reduce significantly the stand of tomato seedlings. Autoclaved inocula of all fungi had no effect on seedling stand. On the basis of these results two parasite-host combinations were chosen for further study: C. praticola - lettuce, P. debaryanum - cucumber.

B. Effects of age and concentration of inoculum

Two experiments investigated the effects of age and concentration of inoculum on the damping-off of cucumber and lettuce by P. debaryanum and

Table 1.1 Damping-off of lettuce, cucumber and tomato by Corticium praticola (Cp), Pythium debaryanum (Pd) and Rhizoctonia solani (Rs₁ and Rs₂) in sterile potting compost

Treatment	Final seedling stand [*]		
	Lettuce	Cucumber	Tomato
Cp	4a	0d	16f
Pd	14b	3d	15f
Rs ₁	45c	24e	27g
Rs ₂	43c	27e	22g
Nil (sterile compost) and all sterile fungus treatments ^{**}	45c	28-30e	37-41h

Values followed by the same letter are not significantly different (comparisons within columns only).

^{*} totals of 3 pots

^{**} for details see Appendix Table 1.1

C. praticola respectively in potting compost. In the first experiment, flasks of oatmeal-sand were inoculated with the two fungi on different days to give 9-day-old and 15-day-old cultures. These were added to pots (each with 300g sterile compost) so that the top layer contained 5%, 10% or 15% fungal inoculum. Sterile compost was added to other pots for controls giving for each fungus eight treatment-combinations which were then replicated three times. The results (Table 1.2 and Appendix Table 1.2) indicated that the amounts of damping-off were not affected by age or amounts of fungal inocula.

In the second experiment, 7-, 9- and 11-day-old inocula of both fungi were prepared and added, as before, to give concentrations of 5%, 10% and 15% in the upper layer of compost in pots. The results (Table 1.3 and Appendix Table 1.3) again showed no significant effect of inoculum age or concentration when used in sterile compost.

C. Damping-off in non-sterile soil

The ability of P. debaryanum and C. praticola to damp-off seedlings of cucumber and lettuce respectively in non-sterile (Walled Garden) soil was investigated next. Different amounts of 7-day-old cultures of these fungi were added to pots, (each with 300g of air-dried, sieved soil) to give an upper layer containing 5%, 10%, 15%, 20%, 25% and 30% fungal inoculum (wet wt inoculum/dry wt soil). Other pots were completely filled with soil (controls). There were four replicate pots for each treatment.

Both fungi effectively damped-off seedlings in this soil (Table 1.4 and Appendix 1.4). C. praticola killed all or most seedlings at each of the inoculum concentrations used but with P. debaryanum there was a more marked relationship between inoculum concentration and seedling death and seedlings which survived tended to have lower fresh weights.

D. Damping-off of tomato by Sclerotium rolfsii in sterile compost

The ability of S. rolfsii to cause damping-off of tomato was investigated in an experiment involving inocula of two different ages (9 and 15 days) added

Table 1.2 Effects of age and concentration of inoculum on damping-off by Pythium debaryanum (Pd) and Corticium praticola (Cp). Expt 1.

Inoculum		Final Seedling Stand*	
Concentration (% W/W)	Age (days)	Cucumber/ Pd	Lettuce/ Cp
Nil	-	27	42
	-	26	42
5	9	6	5
	15	4	3
10	9	4	4
	15	3	2
15	9	3	2
	15	1	1

* totals of 3 pots

Table 1.3 Effects of age and concentration of inoculum on damping-off by Pythium debaryanum (Pd) and Corticium praticola (Cp). Expt 2.

Inoculum		Final Seedling Stand ^x	
Concentration (% w/w)	Age (days)	Cucumber/ Pd	Lettuce/ Cp
Nil	-	26	45
	-	27	45
	-	28	45
5	7	8	7
	9	4	6
	11	3	4
10	7	5	3
	9	3	3
	11	2	3
15	7	5	3
	9	1	2
	11	1	1

^x total of 3 pots

Table 1.4 Damping-off of cucumber by Pythium debaryanum (Pd) and lettuce by Corticium praticola (Cp) in non-sterile soil.

Inoculum concentration (% w/w)	Cucumber/Pd		Lettuce/Cp	
	Seedling stand	Seedling fresh wt (g)	Seedling stand	Seedling fresh wt (g)
Nil	34a	1.2a	53a	0.08a
5	21b	1.2a	3b	0.08a
10	18b	1.1ab	2b	0.05a
15	14c	1.0b	0	-
20	13c	0.9b	0	-
25	10c	0.9b	0	-
30	9c	0.9b	0	-

Values followed by the same letter are not significantly different
(Comparisons within columns only)

Table 1.5 Damping-off of tomato by Sclerotium rolfsii in sterile compost.

Inoculum conc (% ^W / _W)	Seedling stand*			
	Nil	5%	10%	15%
Inoculum age (days): 9	44	31	29	28
15	45	31	27	24

* total of 3 pots

to the upper layer of sterile compost in pots at three different concentrations (5%, 10% and 15% wet inoculum/dry wt soil). The methods were essentially similar to those of the experiment in Section B with P. debaryanum and C. praticola (See Table 1.2).

The results (Table 1.5 and Appendix Table 1.5) showed that S. rolfsii was mildly pathogenic on tomato and that relatively large amounts of inoculum were required to give significant seedling death. The relatively low temperature (20-22°C) throughout the experiment may have partly suppressed the development of this tropical pathogen.

Summary

- 1) From tests in both sterile potting compost and non-sterile soil, two pathogen-host combinations were selected for further studies: Pythium debaryanum/cucumber and Corticium praticola/lettuce.
- 2) The age and concentration of inocula had no effect on the amount of damping-off by these fungi in sterile potting compost but, in non-sterile soil using 7-day-old inocula, larger amounts of P. debaryanum were required to give the severe damping obtained in sterile potting compost. C. praticola was equally effective in damping-off lettuce in sterile potting compost and in non-sterile soil.
- 3) The isolate of S. rolfsii was only slightly pathogenic to tomato in sterile potting compost.

2. ISOLATION OF POTENTIAL ANTAGONISTIC MICRO-ORGANISMS FROM SELECTED SOIL

The aim of the experiments described in this section was to isolate from soil, micro-organisms antagonistic in culture to three fungi previously selected for their ability to damp-off cucumber, lettuce and tomato. An approach suggested by the work of Broadbent et al. (1971) is to search for effective antagonistic micro-organisms in soils which are not conducive to the development of damping-off. However, since such soils were not readily available other approaches were used.

Materials and Methods

A. Selection of soils for isolation of antagonists

The soils selected were of 3 types.

- (i) Soils cropped continuously with vegetables without report of incidence of root disease.
- (ii) Soils from undisturbed forest which represent a more balanced ecological habitat.
- (iii) Soils of special interest for various other reasons (e.g. abandoned Forestry Commission nursery).

A total of 34 soils from Imperial College at Silwood Park, Windsor Park, a forest tree nursery at Ascot and a Forestry Commission nursery at Bracknell was examined. The soils included those in which vegetable crops, pine, oak, ornamentals, blackcurrant, hawthorn, beech trees and undisturbed forest were growing. They were collected during the autumn and spring of 1977-78, within the upper 15cm of the profile mainly around the plant roots (rhizosphere). Soils were assayed as soon as possible after collection but some were stored for short periods in polyethylene bags at 25°C.

B. Suppressive nature of soils

The ability of the soils to suppress the three fungi was tested by the method of Broadbent et al. (1971). PDA (3ml) was poured into an aluminium capped tube (25 x 120mm) and autoclaved at 125°C for 15 min. When cooled,

each tube was inoculated with one of the fungi and incubated at 25°C until growth was established. The soil samples were partially-sterilized before they were tested by placing a 100g sample in a 250ml Erlenmeyer flask and immersing this in a water bath at 70-75°C for 30 min. The flask was rotated gently during this period to ensure even heating and the temperature within the soil was determined by placing a thermometer in a sample flask. This indicated a maximum of 60°C, the heat treatment eliminates mucors and other fast growing fungi (Baker, 1962; Bollen, 1969; Baker et al, 1970). It possibly also kills some potential antagonists, but is a compromise between obtaining maximum yield of antagonists and experiencing difficulty with rapid fungal colonizers. Similar soil samples autoclaved at 125°C for 1h were used as controls. A layer (9cm deep) of the soil to be tested was placed over the prepared fungal culture and moistened with sterile distilled water. The tubes were incubated at 25°C for one month, and moistened periodically with sterile distilled water to avoid excessive drying. The growth of the fungus through the soil sample was tested as follows: pieces (5mm long) of hypocotyl taken from 8 to 10-day-old seedlings of dwarf bean (Phaseolus vulgaris) were sterilized in 5% Chlorox (0.5% available Chlorine) for 5 min, rinsed three times with sterile distilled water, and placed on the soil surface. After 1 to 2 days these pieces were transferred to PDA plates which were incubated at 25°C for 4 days and then fungi growing from them were identified. Recovery of the damping-off fungi indicated lack of suppression by the soil. (See Fig. 1).

C. Isolation from soils

(i) Soil sampling

Pooled sampling similar to that recommended by Schmitthenner and Williams (1958) was used. Each soil sample was divided into 4 equal parts, three random soil samples were taken, one from the surface, the second about 2cm and the third about 4cm below the soil surface, and the twelve samples placed together into a sterile, covered jar. The jars were shaken vigorously to mix the samples and kept overnight or for 24h with loose fitting covers. The soil

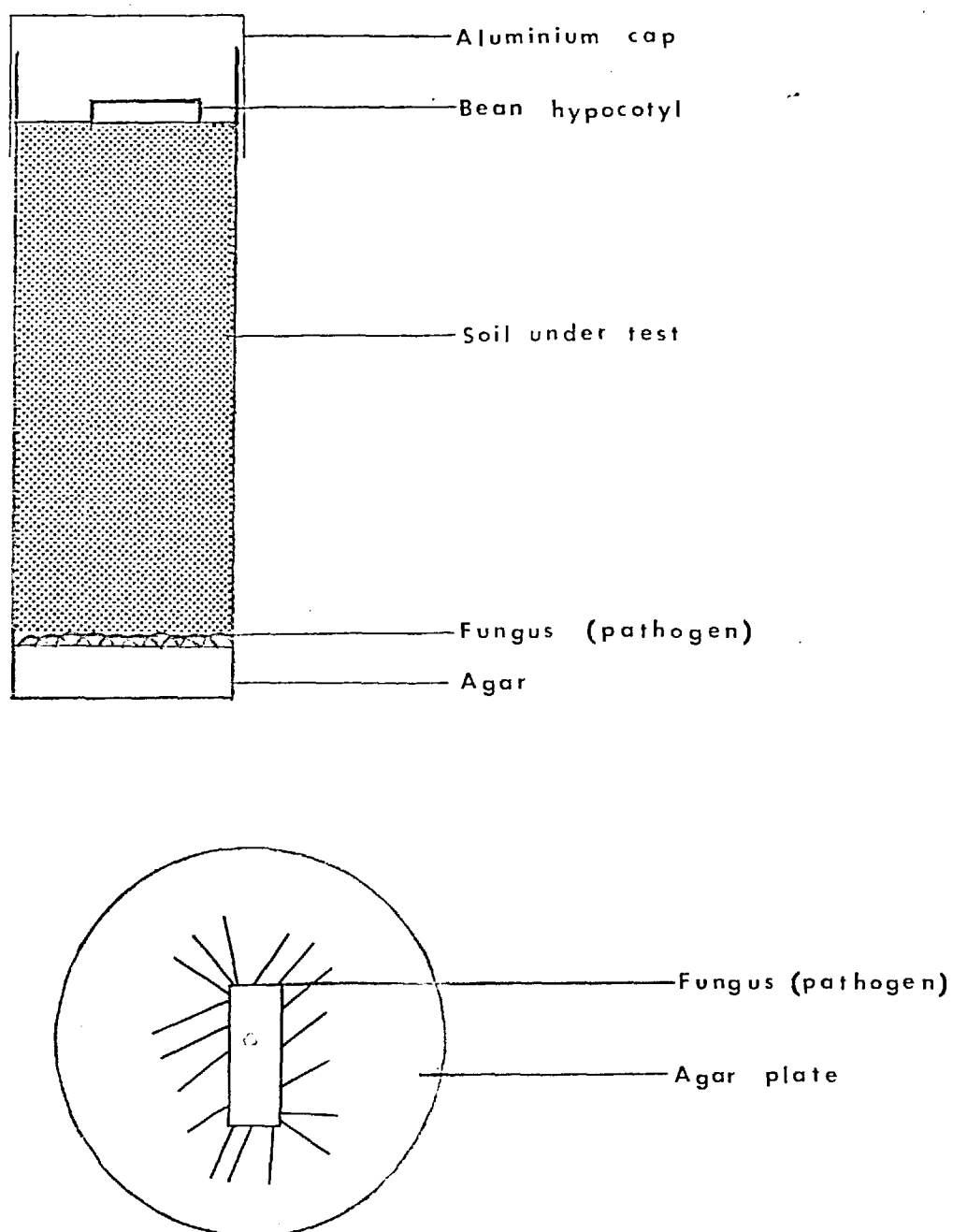


Fig1. Soil-tube method for assaying suppressiveness of a soil.

was spread on sterile blotting paper, and when dry enough it was passed through a 3mm sieve. This sample was used for isolating fungi, bacteria and actinomycetes.

(ii) Dilution plate method

A dilution plate method similar to that described by Timonin (1942) was used. The moisture content of the soil sample was first determined. Then 5g of soil (as dry weight) were placed in 50ml sterile quarter strength Ringer's solution in a 100ml conical flask with a stirrer bar and the soil was dispersed for 30 min by putting the flask on a magnetic stirrer. The resulting suspension was allowed to stand for 30 sec before transferring 5ml to 45ml of sterile ($\frac{1}{4}$ -strength) Ringer's solution with a sterile pipette. Similar transfers, each with fresh pipettes, gave dilutions of 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} and 10^{-6} . One ml from each of the dilutions was spread over the bottom of a sterile petridish, and mixed well with 20ml of an appropriate agar at 45°C .

(iii) Fungal antagonists

Rose bengal agar containing streptomycin (Appendix) was used with a 10^{-4} soil dilution. Four to six plates were prepared for each soil sample and these were incubated at 25°C for 4 to 5 days. All fungal colonies that appeared to be different were subcultured to PDA plates and identified later. Most proved to be species of Aspergillus, Trichoderma, Penicillium and Fusarium.

(iv) Bacteria

A slight modification of the dilution technique was introduced for isolating bacterial antagonists. About 25ml of the 10^{-3} soil suspension was immersed for 10 min in a water bath at $80-85^{\circ}\text{C}$ (to favour isolation of spore-forming bacteria) and a 10^{-5} dilution then prepared from this. Plates incorporating 1ml of this dilution were prepared using one of three media: Yeast-Mannitol agar + congo red (36mg congo red/L), potato dextrose agar (Appendix 1, for Bacillus and Streptomyces spp) and King's medium B (Appendix 1, for isolation of fluorescent pseudomonads). All plates were incubated at 30°C for 5 days. Resulting bacterial colonies that appeared different in

shape, size and colour, were streaked onto dry plates of nutrient agar. At least 25 or more colonies were selected from a given soil sample.

(v) Actinomycetes

Soil samples were first air dried for 4 to 5 days to eliminate vegetative bacterial cells (Williams and Cross, 1974) before preparing a 10^{-6} soil dilution (1 in 6). One ml aliquots of this were mixed with either 10ml of glycerol-casein medium (Appendix 1) or potato dextrose agar (Appendix 1) in petridishes. Plates were incubated at 25°C for 8-10 days and colonies that developed were transferred to potato dextrose agar. Actinomycetes were most prevalent in soils rich in organic matter (e.g. soils from Windsor Park).

D. Test for antagonism on agar

The ability of various bacteria, actinomycetes and fungi isolated to inhibit growth of C. praticola, P. debaryanum and S. rolfsii was tested on potato dextrose, V-8 or soil extract agar. For fungal isolates a 5mm disc, cut from the margin of a 5-day-old colony on PDA, was placed 1cm from the periphery of a V8 plate, diametrically opposite and 7cm from a similar disc of one of the damping-off fungi. Tests with actinomycete isolates were similar but the actinomycete inoculum was a 3mm disc and PDA was the test medium. Bacterial isolates were also tested on PDA, usually four per plate, by streaking them across the agar, each 3.4cm from a central mycelial disc (5mm) of the damping-off fungus. Inoculum of the bacterial isolates was applied on the same day as S. rolfsii but 1-2 days after inoculation with C. praticola or P. debaryanum. All plates were incubated at 25°C for 1 week before inhibition of mycelial growth was determined.

Experimental

A. Suppressive nature of soils

C. praticola, P. debaryanum and S. rolfsii grew through all the soils tested indicating that none was suppressive to the growth of these fungi.

B. Tests of antagonism on agar

The results of tests involving isolates of fungi, bacteria and actinomycetes are summarized in Table 2.1. Relatively few isolates were antagonistic to the growth of C. praticola, P. debaryanum and S. rolfsii as might be expected since none of the soils suppressed the growth of these fungi (see above).

The form of antagonism commonly observed with bacterial and actinomycete isolates was antibiosis. The growth of the test fungus stopped some distance away from the isolate to give a distinct zone free of mycelium (Plate 1, 2 & 3). The hyphae bordering this zone were often discoloured. Some bacterial isolates appeared to cause leakage from mycelia of Pythium giving the colony edge a wet appearance.

The forms of antagonism commonly observed with fungal isolates were competition and parasitism. The most effective fungal antagonists were Trichoderma spp. which grew rapidly over colonies of P. debaryanum and S. rolfsii. All attempts to re-isolate these fungi from zones also occupied by Trichoderma failed. C. praticola was less affected by Trichoderma and was invaded by it on PDA but not on V8 agar. Other fungal isolates, such as species of Penicillium and Rhizoctonia solani stopped the growth of the damping-off fungi but did not penetrate it (Plate 5).

C. Antagonism of selected isolates at different temperatures

The antagonism of selected bacterial isolates at different temperatures was examined to determine the range over which they might so operate in nature.

The procedure resembled that already described and involved dual inoculations of PDA plates with damping-off fungus, applied as a 4mm mycelial disk, and the bacterium, streaked across the plate, 6cm away. Plates were either inoculated simultaneously with both micro-organisms or were inoculated with the bacterium first and then with the fungus under test 2 days later (except with S. rolfsii). Inoculated plates were incubated at 15°, 20°, 25° or 30°. Antagonism was assessed after 7 days by relating growth of the fungus in dual cultures to that in controls with fungus only. There were four replicates for each treatment.

Table 2.1 Antagonism of micro-organisms isolated from soil at three localities to Corticium praticola (Cp), Pythium debaryanum (Pd), and Sclerotium rolfsii (Sr) on agar.

	Total no isolates	No antagonistic to		
		Cp	Pd	Sr
1 Tree nursery soils (14)*				
Fungi	24	2	2	2
Bacteria	350	6	8	6
Actinomycetes	70	30	26	30
2 Windsor Park forest soils (10)*				
Fungi	15	3	3	3
Bacteria	264	14	18	10
Actinomycetes	20	8	6	8
3 Silwood Park soils (10)*				
Fungi	21	1	4	2
Bacteria	315	4	3	4
Actinomycetes	13	1	2	1

* Number of soil samples examined



Plate 1. Inhibition and lysis of Pythium debaryanum by three bacterial isolates.



Plate 2. Reaction of Corticium praticola to four bacterial isolates.
(The colony is inhibited and discoloured by three isolates, but is unaffected by the fourth isolate).



Plate 3. Reaction of Sclerotium rolfsii to four bacterial isolates.
(The colony is inhibited only by one isolate).



Plate 4. Reaction to a Trichoderma sp. of 1) Pythium debaryanum,
2) Sclerotium rolfsii and 3) Corticium praticola. (The
Trichoderma has overrun the colony of Pythium and has stopped the
growth of the other two fungi. It has just begun to penetrate
the colony of C. praticola).



Plate 5. Reaction of Corticium praticola (right) to Rhizoctonia solani (left).
(The growth of C. praticola ceased when the colonies met).

Table 2.2 Effects of three bacterial isolates on growth of Pythium debaryanum on PDA at different temperature (as % reduction in colony diameter relative to control).

		$^{\circ}\text{C}$			
		15°	20°	25°	30°
(a)	Isolate				
	2 - B46	36	44	44	52
	2 - B24	35	41	49	50
	2 - B27	36	42	46	47
(b)	2 - B46	37	55	60	75
	2 - B24	42	56	56	61
	2 - B27	48	52	53	62

(a) Plates inoculated with both micro-organisms simultaneously.

(b) Plates inoculated with bacterium 2 days before fungus.

Table 2.3 Effects of five bacterial isolates on growth of Corticium praticola on PDA at different temperatures (as % reduction in colony diameter relative to control).

		°C			
		15°	20°	25°	30°
(a)	2 - B1	32	32	36	37
	2 - B2	32	37	37	40
	2 - B3	31	35	35	42
	1 - B26	29	34	35	40
	2 - B14	29	33	34	41
(b)	2 - B1	42	46	48	56
	2 - B2	42	43	43	50
	2 - B3	37	41	44	50
	1 - B26	32	43	43	51
	2 - B14	38	41	44	51

(a) Plates inoculated with both micro-organisms simultaneously.

(b) Plates inoculated with bacterium 2 days before fungus.

Table 2.4 Effects of five bacterial isolates on growth of Sclerotium rolfsii on PDA at different temperatures (as % reduction in colony diameter relative to control).

Isolate	$^{\circ}\text{C}$			
	15 $^{\circ}$	20 $^{\circ}$	25 $^{\circ}$	30 $^{\circ}$
2 - B1	25	29	34	41
2 - B2	25	31	36	43
2 - B3	29	34	35	50
1 - B26	20	22	31	40
2 - B14	15	19	29	34

Table 2.5 Competition between inocula of C. praticola (Cp), P. debaryanum (Pd), or S. rolfsii and fungal isolates at different temperatures.

	15°C			20°C			25°C			30°C		
Fungal isolates	Cp	Pd	Sr	Cp	Pd	Sr	Cp	Pd	Sr	Cp	Pd	Sr
<u>Penicillium</u> spp.	+-	+-	+-	+-	+-	+-	+-	+-	+-	+-	+-	+-
<u>Trichoderma</u> spp.	+-	-	-	-	-	-	-	-	-	-	-	-
<u>Aspergillus</u> spp.	+	+	+	+	+	+	+	+	+	+	+	+
<u>Zygorrhynchus</u> spp.	+	+	+	+	+	+	+	+	+	+	+	+
<u>Fusarium</u> spp.	+	+	+	+	+	+	+	+	+	+	+	+
<u>Rhizoctonia solani</u>	+-			+-			+-			+-		

+ = growth of damping-off fungi

- = damping-off fungi growth inhibited

+- = slight check

Fungal isolates were inoculated simultaneously with damping-off fungi on PDA plates as previously described before incubation at various temperatures (see above). When the fungal isolate stopped the growth of the damping-off fungus as both colonies met, overran the latter and sporulated profusely it was recorded as (-), and when the damping-off fungus was little affected by the antagonist as (+). Intermediate effects were occasionally observed (+-), where growth of the damping-off fungus and the fungal isolate stopped when they both met without any further growth of both in culture. The results are summarized in Table 2.2 to 2.5.

Bacterial isolates clearly showed increasing inhibition of damping-off fungi with increase in temperature. The extent to which the total growth of the fungi in culture was inhibited was more, when bacterial isolates were pre-inoculated two days before the fungus. It could be that the bacteria had developed and leaked antifungal substances into the medium before the fungus was inoculated. Temperature appeared not to alter the antagonistic action of fungal isolates against the damping-off fungi except that when C. praticola was overrun at 20°C by Trichoderma, the sporulation of the latter was sparse whereas at 25°C and 30°C sporulation was very dense. Because of the slow growth of S. rolfsii in culture it was more susceptible to Trichoderma attack than either P. debaryanum or C. praticola.

Summary

- 1) None of the selected soils suppressed the growth of damping-off fungi.
- 2) Few isolates of bacteria, actinomycetes and fungi isolated from these soils were antagonistic to the growth of damping-off fungi in culture. Three forms of antagonism were commonly observed; these were antibiosis, competition and parasitism.
- 3) Increase in inhibition of damping-off fungi was observed with increase in temperature and the extent of inhibition was more when bacterial isolates were pre-inoculated 2 days earlier.

- 4) Temperature did not modify the antagonistic action of fungal isolates against the damping-off fungi.

3. TESTS OF ANTAGONISTS IN STERILE POTTING COMPOST

The next series of experiments were designed to screen further the antagonists selected from the agar tests by investigating their ability to control damping-off in sterile potting compost under glasshouse conditions. Bacterial antagonists were applied to seeds and Trichoderma was applied directly to the compost by introducing it on a suitable carrier. The following account includes preliminary experiments which evaluated these techniques.

Experimental

A. Growth rate of a bacterial isolate in a liquid medium

The rate at which one bacterial isolate grew in nutrient broth was studied as a guide for future work involving seed treatments with bacteria.

Inoculum of isolate 1 - B18 was prepared by growing the bacterium in a 2.5% nutrient broth for 24h at 30°. A 250ml Erlenmeyer flask containing 100ml nutrient broth was then inoculated with one ml of this culture and also incubated at 30°. One ml samples were withdrawn daily, diluted serially in sterile phosphate buffer (pH 7.0) and one ml aliquots of selected dilutions were spread over plates of dry nutrient agar. Colonies were counted after 48h at 30°. The results (Fig. 2) show that maximum yield of the viable bacterium was attained in 6-7 days.

B. Preliminary tests of applying bacteria to seed

Isolate 1 - B18, selected for its activity against Pythium on agar, was grown for 7 days at 30° in 100ml nutrient broth. Most cultures were centrifuged at c. 2500g for 10 min and the supernatant passed through a Millipore filter (0.045 μ m) to remove cell fragments. The bacteria were resuspended and washed, by further centrifugation, in three changes of sterile distilled water and then finally made up to 100ml with either sterile water or sterile $\frac{1}{4}$ -strength Ringer's solution. A few cultures were used directly to provide 100ml bacterial suspension. Batches of

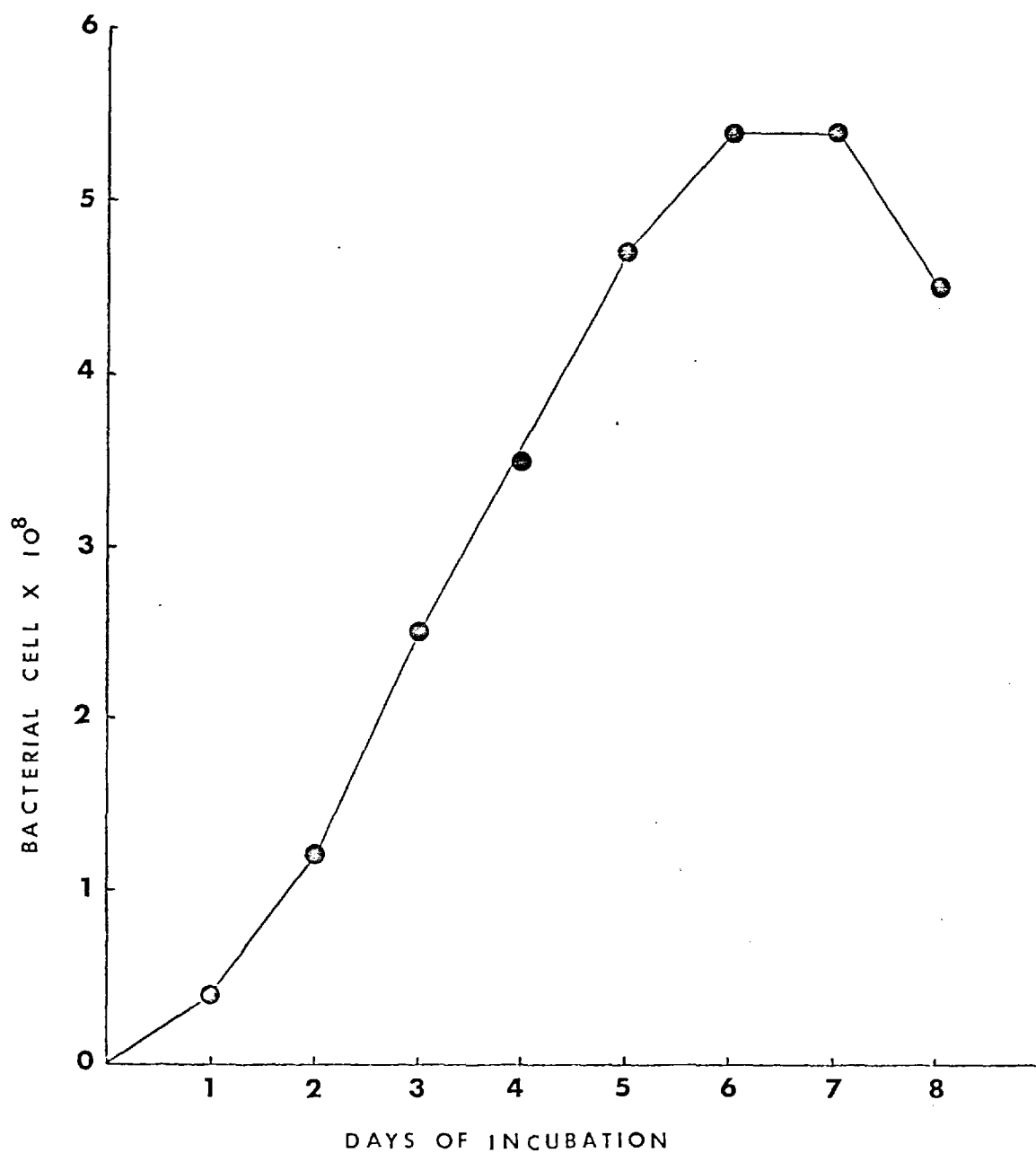


Fig 2. Population curve of bacterium 1-B18 .

cucumber seeds were then soaked for 30 min in the following: a suspension of washed bacteria in (i) sterile water or (ii) sterile $\frac{1}{4}$ -strength Ringer's solution; (iii) a nutrient broth culture of the bacterium; (iv) a cell-free filtrate of the bacterium and (as controls), (v) sterile distilled water and (vi) sterile Ringer's solution.

The seeds were agitated during soaking by placing the flasks on a magnetic stirrer. They were then sown (10 per pot) in sterile potting compost with or without 10% ($^w/w$) of an oatmeal-sand culture of P. debaryanum as described in Section 1B. Each of the 12 treatments (6 seed treatments, + or - Pythium) was replicated four times and the pots were completely randomized on the glasshouse bench. The results are summarized in Table 3.1 and details of the analysis of variance are shown in Appendix Table 3.1.

Soaking seed in a cell-free filtrate of the bacterium or in Ringer's solution had no adverse effect on germination nor did the various treatments involving viable bacteria. In the presence of Pythium seedling stand was poor in all the 'control' treatments and especially so for seed soaked in sterile water or Ringer's solution. Treatments with viable bacteria significantly improved seedling stand over that of the controls but, except for soaking in a nutrient broth culture, did not quite restore it to the levels obtained in sterile potting compost.

C. Screening tests for bacterial antagonists against P. debaryanum and C. praticola

The previous experiments suggested that there was no particular advantage to be gained in washing bacteria and re-suspending them in water, nor in using Ringer's solution. Therefore in the following tests seed was soaked in nutrient broth cultures. In all, 22 bacterial isolates were screened for activity against Pythium damping-off of cucumber in potting compost and 15 isolates similarly for activity against damping-off

Table 3.1 Effect of different seed treatments on the control of Pythium damping-off in sterile potting compost

		Final Seedling Stand	
Seed treatment		+ <u>Pythium</u>	No <u>Pythium</u>
Bacterium 1 - B18 in:-	(i) sterile water	28	35
	(ii) sterile Ringer's soln	26	37
	(iii) nutrient broth culture	30	38
'Controls'	(iv) cell-free filtrate	13	36
	(v) sterile water	8	36
	(vi) sterile Ringer's soln	5	36

of lettuce by C. praticola. The isolates were screened in groups of seven or eight in separate experiments.

Each isolate was grown for 7 days at 30° in 100ml nutrient broth and then either 20g cucumber seed or 10g lettuce seed was soaked in the culture for 30 min. The seeds were separated from the culture by filtration under suction through a double layer of filter paper on a Buchner funnel and then air-dried in a sterile petri dish. Seeds treated in sterile distilled water in a similar manner served as controls.

Pots of sterile potting compost were infested by adding oatmeal-sand cultures of the required fungus at the rate of 10% (^w/_w) for P. debaryanum and 5% (^w/_w) for C. praticola as described in Section 1. These pots and corresponding controls of sterile potting compost were then sown with 10 cucumber seeds or 15 lettuce seeds. Each seed treatment with bacteria was replicated three times and the water control six times. The pots were randomly arranged on the glasshouse bench. Seedling stands and fresh weights were assessed 15 days after sowing. The results (Tables 3.2 and 3.3) are expressed as totals of seedling stand for 3 pots and the mean weight of a single seedling (based on the pot means). The corresponding analyses of variance are given in Appendix Tables 3.2 and 3.3. Several points emerge from the results of tests with P. debaryanum and cucumber:-

(i) None of the bacterial isolates adversely affected seedling stand or seedling fresh weight in sterile compost.

(ii) In Pythium-infested soil all bacterial seed treatments, except that with 3 - B98 and 1 - B32 in Test 1, significantly improved seedling stand over that of the water control. In Test 2, isolates 2 - B77, 1 - B16 and 2 - B27 were particularly effective and so were isolates 2 - B24, 2 - B46 and 1 - B4 in Test 3. Treatments with these isolates gave seedling stands comparable to the corresponding controls (no Pythium) whereas treatments with other isolates did not.

Table 3.2 Effects of treating cucumber seed with selected bacteria on Pythium damping-off in sterile compost.

Bacterium	Final seedling stand		Fresh wt seedling (g)	
	+ <u>Pythium</u>	No <u>Pythium</u>	+ <u>Pythium</u>	No <u>Pythium</u>
<u>Test 1</u>				
3 - B94	15	28	0.48	0.61
2 - B39	15	28	0.53	0.70
2 - B23	13	29	0.52	0.61
1 - B86	12	27	0.47	0.62
2 - B16	11	27	0.44	0.62
1 - B32	8	27	0.51	0.64
3 - B98	6	26	0.43	0.65
Water control	4	28	0.48	0.64
<u>Test 2</u>				
1 - B16	26	28	0.93	0.93
2 - B27	25	29	1.03	1.23
2 - B77	25	27	0.91	1.16
2 - B12	17	27	0.86	1.0
2 - B63	14	28	0.84	0.92
3 - B20	12	28	0.69	0.93
1 - B5	12	28	0.76	0.91
1 - B65	7	26	0.90	0.95
Water control	3	28	0.63	0.96
<u>Test 3</u>				
2 - B46	25	28	1.98	2.21
2 - B24	24	29	2.08	2.14
1 - B4	24	28	1.92	1.98
2 - B31	18	28	1.51	1.71
2 - B33	17	27	1.69	1.86
2 - B60	16	28	1.64	1.77
2 - B28	14	26	1.78	1.94
Water control	4	26	0.84	1.54

Table 3.3 Effects of treating lettuce seed with selected bacteria on damping-off by C. praticola (Cp) in sterile compost.

Bacterium	Final seedling stand		Fresh wt seedling (g)	
	+ Cp	No Cp	+ Cp	No Cp
<u>Test 1</u>				
2 - B76	0	37	-	0.16
2 - B19	0	22	-	0.17
2 - B53	0	15	-	0.15
1 - B26	0	26	-	0.16
2 - B77	0	20	-	0.16
2 - B46	0	8	-	0.16
1 - B7	0	3	-	0.17
Water control	2	42	0.05	0.16
<u>Test 2</u>				
2 - B14	4	8	0.09	0.10
1 - B26	3	37	0.08	0.10
2 - B2	2	23	0.06	0.10
2 - B1	0	20	-	0.10
3 - B63	0	7	-	0.11
1 - B13	0	22	-	0.10
2 - B3	0	36	-	0.10
3 - B19	0	16	-	0.13
Water control	2	42	0.04	0.12

(iii) In sterile potting compost, 2 isolates (2 - B24 and 2 - B46 in Test 3) improved seedling growth significantly over that in the corresponding water control suggesting some stimulatory effect. In Pythium-infested compost, no bacterial isolate in Test 1, only isolate 2 - B27 in Test 2 but all isolates in Test 3 improved seedling fresh weight over that in the corresponding water control. Seedling fresh weight in sterile compost was generally higher than in compost with Pythium but the differences were not significant. The high fresh weight of seedling in Test 3 compared with Tests 1 and 2 was due to different grade of potting compost used.

In contrast to these results, none of the bacterial isolates tested gave any control of damping-off by C. praticola and many adversely affected germination in the absence of the pathogen (Table 3.3). However, the growth of seedlings which did become established in this situation was not affected.

D. Effects of different suspending fluids on bacterization of lettuce seed against C. praticola damping-off

The following experiment was designed to investigate further the adverse effects on seedling establishment noted with some bacteria.

Bacterium 2 - B14 was grown for 7 days at 30° in cultures of 100ml nutrient broth. Suspensions of the washed bacteria were prepared in sterile distilled water and $\frac{1}{4}$ -strength Ringer's solution and a cell-free culture filtrate was prepared as described in Section B. Lettuce seed was soaked in these, in an unfiltered nutrient broth culture and, as controls, in sterile distilled water, Ringer's solution and nutrient broth. The treated seeds were air-dried before planting in pots of compost with or without 5% (w/w) C. praticola. There were four replicates of each treatment and the pots were randomized completely on the glasshouse bench. The experiment was assessed 15 days after sowing.

The results (Table 3.4) showed that damping-off of lettuce by C. praticola could not be controlled by treating seed with bacterium 2 - B14.

Table 3.4 Effects of different suspending fluids on the bacterization of lettuce seed. (Tested against C. praticola (Cp) damping-off in sterile potting compost).

Seed treatment		Final seedling stand		Fresh wt seedling (g)	
		+ Cp	No cp	+ Cp	No Cp
Bacterium 2 - Bl4 in:	(i) water	5	58	0.06	0.13
	(ii) Ringer's soln	4	57	0.05	0.11
	(iii) nutrient broth	4	24	0.05	0.10
'Control'	(iv) cell-free filtrate	0	55	-	0.10
	(v) water	2	51	0.03	0.11
	(vi) Ringer's soln	0	58	-	0.11
	(vii) nutrient broth	0	50	-	0.10

Also, the media in which the bacterium was suspended had little influence on the results.

All the experiments with C. praticola indicated that this fungus grows very rapidly through sterile compost and therefore this tests very severely any attempt to control it biologically. This aspect is considered further in later sections.

E. Control of damping-off with wheat bran culture of a *Trichoderma* spp.

The ability of an isolate of *Trichoderma* to reduce damping-off by *P. debaryanum* and *C. praticola* was tested in sterile potting compost. Preliminary experiments indicated that the *Trichoderma* could be incorporated satisfactorily into the compost on wheat bran, as indicated by the work of Henis et al. (1978). Horticultural vermiculite and sawdust proved to be less satisfactory as carriers.

Erlenmeyer flasks (250ml) containing 10g wheat bran and 20ml tapwater were autoclaved for 1h on 2 successive days and sterile wheat bran was seeded with five, 5mm agar discs of *Trichoderma* obtained from a 5-7 day-old PDA culture. Cultures were incubated first in the dark at 25°C for 3 days, then they were exposed to light for 3 days, shaken vigorously and incubated for 4 more days. This bran culture of *Trichoderma* was then applied to pots of sterile potting compost infested with either 10% (^w/w) *Pythium* or 5% (^w/w) *C. praticola* to give 5, 10, 15, 20, 25 and 30% fresh wt of culture/g dry wt of potting compost. Similar levels of *Trichoderma* were added to pots containing sterile compost only and, as additional controls, sterile wheat bran at rates of 5, 10 or 15% was added to pots with or without the pathogens. Treatments involving *Trichoderma* or sterile wheat bran only were replicated three times; and the controls of potting compost only with or without the pathogens were replicated six times. After preparation of the respective mixtures and sowing them with seeds, all pots were watered lightly and arranged randomly on the glasshouse

Table 3.5 The effect of a Trichoderma sp. on the incidence of damping-off of cucumber by P. debaryanum (Pd) in sterile compost.

% Trichoderma added (^W /w)	No. seedlings				Fresh wt	
	Emerged (9 days)		Survived (28 days)		seedling (g)	
	+ Pd	No Pd	+ Pd	No Pd	+ Pd	No Pd
Nil	4	27	4	27	0.98	1.38
5	9	28	8	28	1.26	1.57
10	16	30	15	30	1.36	1.57
15	17	30	17	30	1.16	1.60
20	24	29	24	29	1.27	1.35
25	25	28	24	28	1.17	1.31
30	26	28	25	28	1.17	1.20
% sterile bran added (^W /w)						
5	0	28	0	28	-	1.52
10	0	27	0	27	-	1.78
15	0	27	0	27	-	2.09

Table 3.6 The effect of a Trichoderma sp. on the incidence of damping-off of lettuce by C. praticola (Cp) in sterile compost.

% Trichoderma added (^w /w)	No. seedlings				Fresh wt	
	Emerged (9 days)		Survived (28 days)		Seedling (g)	
	+ Cp	No Cp	+ Cp	No Cp	+ Cp	No Cp
Nil	3	45	3	45	0.42	0.75
5	14	41	7	41	0.60	0.66
10	17	43	13	43	0.65	0.70
15	35	41	17	41	0.70	0.74
20	33	41	9	41	0.63	0.56
25	28	38	6	38	0.50	0.52
30	26	34	6	34	0.50	0.40
% sterile bran added (^w /w)						
5	0	36	0	36	-	0.71
10	0	27	0	27	-	0.67
15	0	26	0	26	-	0.60

bench. Seedlings were counted 9 and 28 days after sowing to give numbers that emerged and survived respectively. The fresh weight of seedlings that survived was also determined. The results are summarized in Tables 3.5 and 3.6.

The following points emerged from the results:-

- (i) Addition of Trichoderma did not adversely affect seedling stand (shown by results with no Pd, no Cp).
- (ii) Some control of pre-emergence damping-off was obtained by relatively high levels of Trichoderma e.g. 20, 25 and 30% for Pd and 15 to 30% for Cp but whereas seedling survival was also good at these levels of Trichoderma in the experiment with Pd, in compost with Cp few seedlings survived in any treatment involving Cp i.e. Trichoderma was ineffective here in preventing post-emergence damping-off.
- (iii) Wheat bran alone increased severity of damping-off in compost infested with either of the pathogens, showing that the control of damping-off by this preparation was caused specifically by Trichoderma.
- (iv) In Pd or Cp-infested compost, Trichoderma improved seedling fresh weight over that in their corresponding control (i.e. no Trichoderma) but the differences were not significant.

Summary

- (i) Only 7 out of 23 bacterial isolates screened gave a moderate control of Pythium damping-off when applied to cucumber seed.
- (ii) None of the bacterial isolates screened was effective on lettuce seed against C. praticola in sterile compost. This fungus grows rapidly through compost and therefore swamps any attempt to control it biologically.
- (iii) Some control of pre-emergence damping-off of Pythium and C. praticola by Trichoderma was obtained in sterile compost, but post-emergence damping-off of lettuce seedling by Cp could not be prevented.

4. CONTROL OF PYTHIUM DAMPING-OFF IN NON-STERILE SOIL WITH SELECTED ANTAGONISTS

Antagonists that effectively controlled Pythium damping-off of cucumber in sterile compost, were tested further for their ability to control this disease in non-sterile soil.

Experimental

A. Screening test for bacteria against Pythium

The following bacterial isolates were used: 1 - B18, 1 - B4, 2 - B24, 2 - B77, 2 - B27 and 2 - B16. These were grown in nutrient broth as described in Section 3. Cucumber seeds were soaked in these cultures, air-dried in sterile petri-dishes and then planted (10/pot) in Walled Garden soil, with or without 15% (W/W) of oatmeal sand culture of Pythium. Bacterial seed treatments were replicated 3 times and the seed treatment with sterile distilled water was replicated 6 times. Seedling stand and growth were assessed 15 days after sowing.

The results (Table 4.1) showed that all isolates except 1 - B4, gave some control of Pythium damping-off but three isolates (2 - B46, 2 - B24 and 2 - B27) were significantly better in this respect and gave stands comparable with their corresponding controls (without Pythium). Treating seed with the bacteria had no adverse effect on seedling growth and stand and, in the absence of Pythium, isolated 2 - B46 slightly improved seedling growth.

B. Mechanism of control of Pythium damping-off on cucumber by seed inoculation with bacteria.

Successful inoculation of cucumber seeds with bacteria for the specific purpose of biological control of Pythium damping-off was established in previous experiments. However, the numbers of bacteria on seed at inoculation, at sowing and during seedling growth have so far not been investigated. The experiments in this section attempt this, as well as examining further

Table 4.1 Effects of treating cucumber seed with selected bacteria on Pythium damping-off in non-sterile soil

Bacterium	Final seedling stand		Fresh wt seedling (g)	
	+ <u>Pythium</u>	No <u>Pythium</u>	+ <u>Pythium</u>	No <u>Pythium</u>
2 - B46	22	25	2.30	2.48
2 - B24	21	25	2.03	2.32
2 - B27	21	24	2.01	2.13
1 - B16	15	23	1.96	2.12
1 - B18	14	23	1.78	2.01
2 - B77	13	23	1.76	2.02
1 - B4	10	22	1.52	1.98
Water control	8	23	1.51	1.95

the methods of inoculating cucumber seed, with the general aim of determining the possible mode of action of the bacteria against Pythium in non-sterile soil.

The three bacterial isolates, 2 - B24, 2 - B27 and 2 - B46 were each used in separate, similar experiments. They were grown in nutrient broth at 30° for 7 days, and from some of these cultures suspensions of bacteria in sterile distilled water and Ringer's solution were obtained so that cucumber seed could be treated with nutrient broth culture or these suspensions as described in Section 3, Page

The ability of bacteria-treated seed to inhibit Pythium in vitro was checked by plating them on agar inoculated centrally with P. debaryanum. Such checks invariably resulted in a zone of inhibition around the treated seed.

The number of bacteria on seeds (before and after air-drying) was assessed as follows. Three replicate groups of 20 seeds were selected from each treatment. The seeds were shaken on a Whirlimixer in 10ml sterile phosphate buffer (pH 7.0) for 2 min and the resulting bacterial suspension was diluted serially in 10-fold steps in buffer. Then 0.1ml of each dilution was spread over a nutrient agar plate. Colonies were counted after 3 days at 30° and the number of viable bacteria on the seeds estimated from these counts.

Survival of these bacteria in soil with or without Pythium was determined from counts of bacteria in the rhizosphere of seedlings. Samples 4 to 12 seedlings were collected from all replicates of each treatment. The roots were shaken gently to free them of loose soil and were then cut into 3-4cm lengths. The samples were bulked and 2-5 subsamples taken. For each subsample, 1g of roots and closely-adhering soil were shaken for 15 min in 100ml sterile phosphate buffer and then heated at 52° for 20 min to eliminate micro-organisms other than spore-forming bacteria. A dilution

series was prepared from the heat-treated sample and one ml from appropriate dilutions was mixed with nutrient agar cooled to 45°-50°. After incubation at 30° for 3 days, colonies resembling the isolate under test were counted and expressed as numbers per g dry roots. To test whether most of these colonies could inhibit Pythium, 25 colonies were taken from plates of each subsample and the 100 isolates so obtained were plated with P. debaryanum on agar.

Table 4.2 and Fig. 3 summarise the relationship between numbers of bacteria in the suspensions and the numbers retained on seed before sowing. Generally the numbers on seed were directly related to those in the suspensions so that most bacteria were found on seeds dipped in the nutrient broth culture. Within the range of suspensions used the seed surface did not appear to be over-saturated with bacteria since there was no obvious decline in numbers relative to those in suspension. No bacteria were re-isolated from seed treated with a cell-free filtrate of the bacterium, sterile water or Ringer's solution. There was a decline in numbers of bacteria retained on seed after air-drying relative to those on seed before air-drying.

Despite the differences in numbers of bacteria on seed indicated by the above data, the degree of control of Pythium damping-off was similar whether the seed was dipped in a nutrient broth culture or in suspensions made up in sterile water or Ringer's solution (Table 4.3). Comparative lack of control by the cell-free filtrate suggested that control of Pythium damping-off depended on the presence of viable bacteria on the seed. In general, the results otherwise confirmed those previously obtained with these bacteria in sterile potting compost.

The numbers of bacteria resembling the isolates under test that were recovered from the rhizospheres were much lower than those retained by seeds (Table 4.4). Nevertheless, there was a relationship between the two (Fig. 4) and the percentage of bacteria recovered that were antagonistic to Pythium was high in all instances.

Table 4.2 Bacteria on seed (B) in relation to suspension (A)

Bacterium	<u>A</u>	<u>B₁</u> [*]	<u>B₂</u> [✕]	
2 - B46	7.8×10^8	3×10^6	2×10^6	a
	6×10^8	2.5×10^6	1.6×10^6	b
	15×10^8	4.2×10^6	3×10^6	c
2 - B24	4×10^8	1.4×10^6	1×10^6	a
	3.5×10^8	1.2×10^6	0.9×10^6	b
	9×10^8	3.5×10^6	2.3×10^6	c
2 - B27	4.2×10^8	1.6×10^6	1.1×10^6	a
	4.9×10^8	2.1×10^6	1.4×10^6	b
	8.5×10^8	3.9×10^6	2.6×10^6	c

* Before air-drying inoculated seed

✕ After air-drying before sowing inoculated seed

a - water

b - Ringer's solution

c - nutrient broth culture

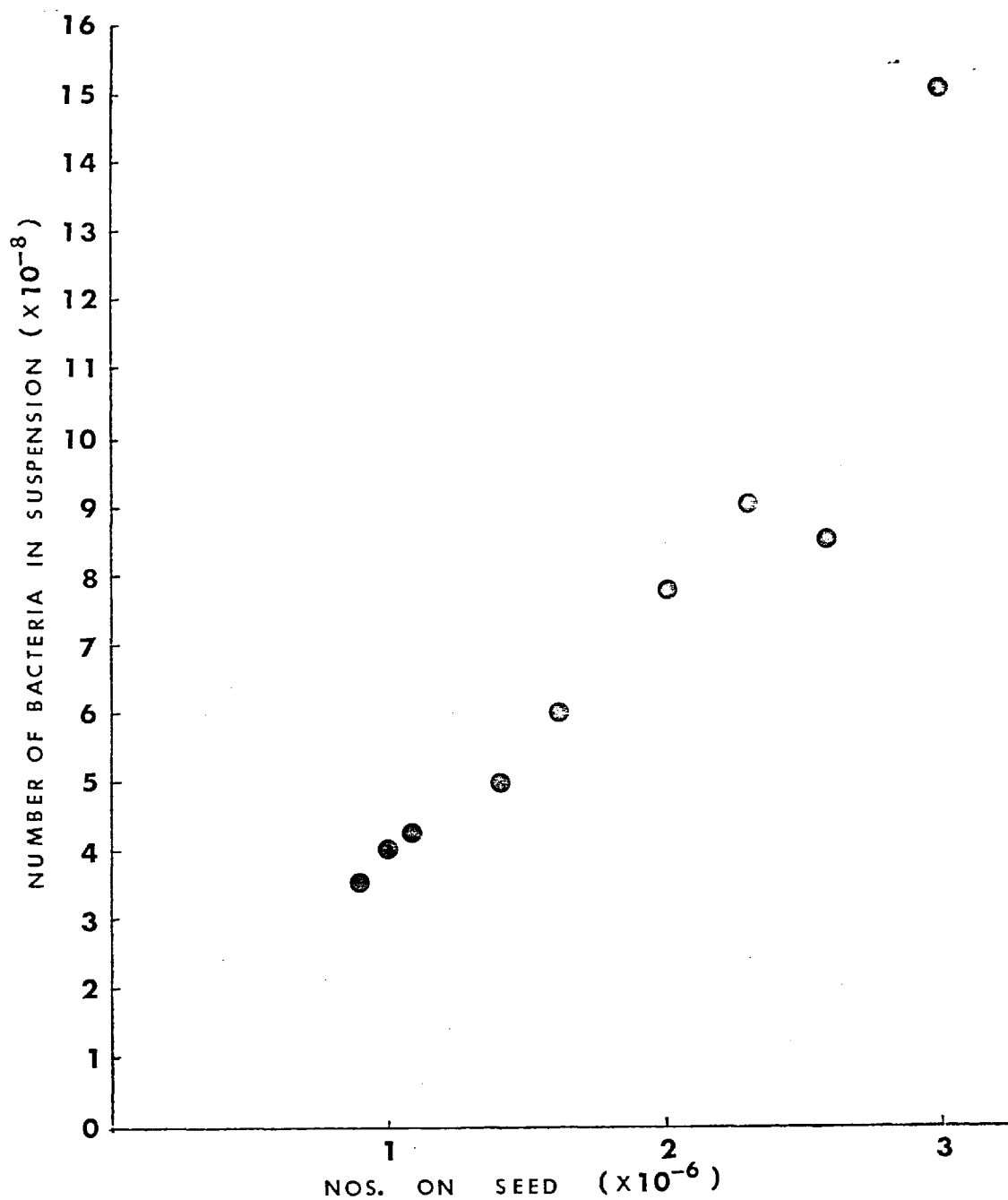


Fig 3. Bacteria on seed in relation to suspension.

Table 4.3 Effect of different methods of inoculating cucumber seed with bacteria on control of Pythium damping-off

(a) Final seedling stand

		<u>+ Pythium</u>			<u>No Pythium</u>		
		A	B	C	A	B	C
Bacterium in:	(i) water	22	21	21	25	24	27
	(ii) Ringer's soln	21	20	21	25	24	25
	(iii) nutrient broth	23	22	22	26	25	26
'Controls':	(iv) cell-free filtrate	10	7	8	24	23	24
	(v) water	5	5	5	24	22	23
	(vi) Ringer's soln	7	4	6	23	22	24

(b) Seedling fresh wt (g)

		<u>+ Pythium</u>			<u>No Pythium</u>		
		A	B	C	A	B	C
Bacterium in:	(i) water	2.08	1.76	1.88	2.14	2.08	2.07
	(ii) Ringer's soln	2.02	1.80	2.08	2.10	1.97	2.11
	(iii) nutrient broth	2.31	1.72	2.05	2.50	2.08	2.25
'Controls':	(iv) cell-free filtrate	1.91	1.63	1.69	1.95	1.89	2.09
	(v) water	1.82	1.21	1.48	1.91	1.87	1.82
	(vi) Ringer's soln	1.65	1.40	1.53	1.88	1.83	1.84

A - Bacterium 2 - B46

B - Bacterium 2 - B24

C - Bacterium 2 - B27

Table 4.4 Bacteria in rhizosphere (R) in relation to seed (S)

Bacterium	<u>S</u>	<u>Rp</u> [*]	<u>Ro</u> [*]	
2 - B46	2×10^6	8.5×10^4	10×10^4	a
	1.6×10^6	7.0×10^4	9.7×10^4	b
	3×10^6	12.0×10^4	11.5×10^4	c
2 - B24	1×10^6	5.0×10^4	6.6×10^4	a
	0.9×10^6	3.6×10^4	5.4×10^4	b
	2.3×10^6	8.2×10^4	8.0×10^4	c
2 - B27	1.1×10^6	5.5×10^4	11.0×10^4	a
	1.4×10^6	5.6×10^4	9.6×10^4	b
	2.6×10^6	7.0×10^4	10.4×10^4	c

^{*} Percentage of colonies tested antagonistic to Pythium in culture ranges from 80-98%.

a - Bacterium in water

b - " " Ringer's solution

c - Nutrient broth culture

Rp - + Pythium

Ro - No Pythium

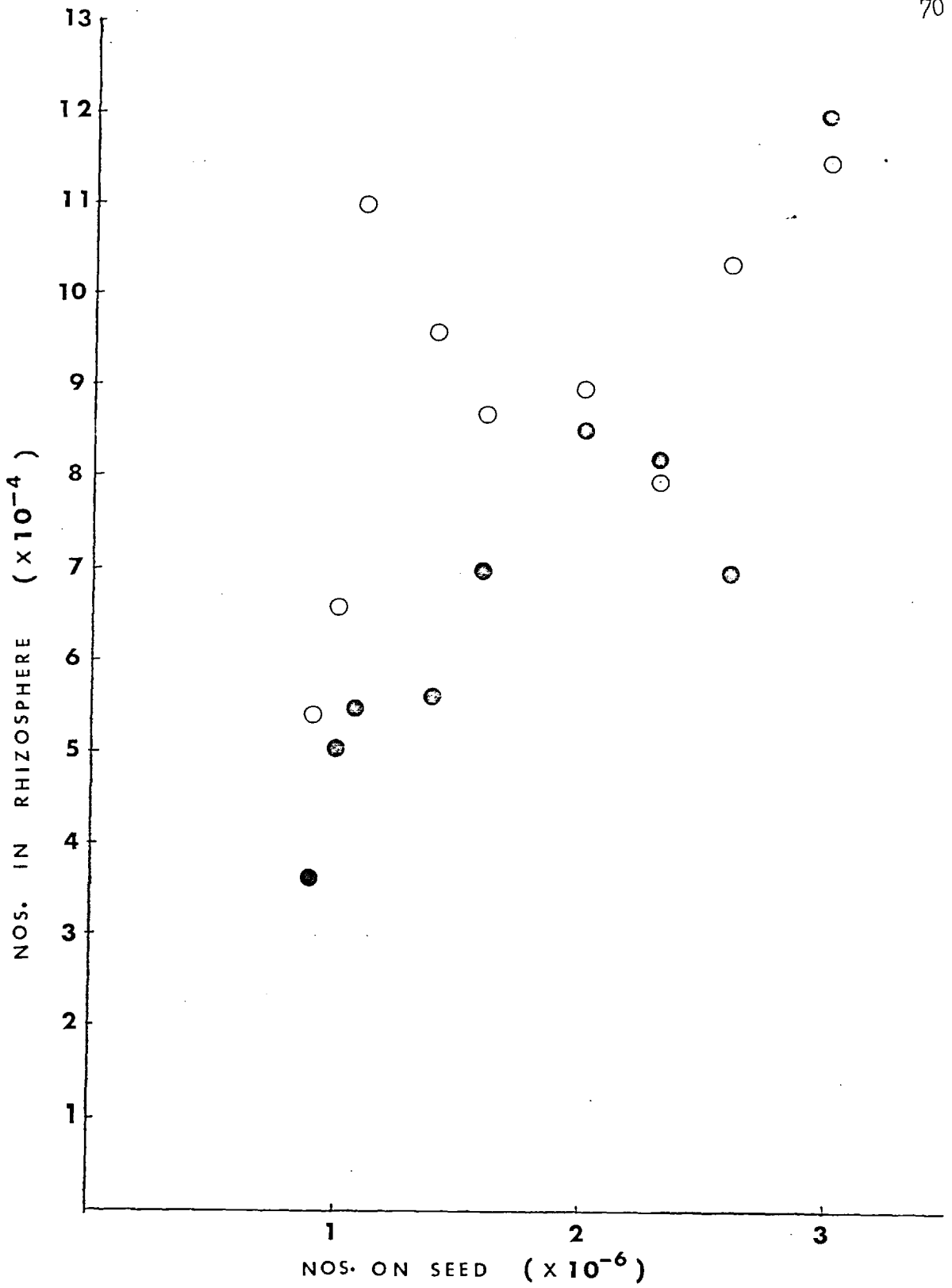


Fig4. Bacteria in rhizosphere(R) in relation to seed(S).

● +Pythium

○ No Pythium

C. Effects of seed inoculation with different inoculum concentrations of a bacterium on control of *Pythium* damping-off

The following experiment examined the effect on *Pythium* damping-off of different concentrations of isolate 2 - B46 on cucumber seed.

The bacterium was grown in nutrient broth at 30° for 7 days and a suspension prepared in sterile distilled water. This was diluted serially to give the seven bacterial suspensions indicated in Table 4.5. Cucumber seeds were soaked in each suspension for 30 min and were then air dried. Some seeds were used to estimate the number of viable bacteria per seed and others were plated on agar inoculated centrally with *P. debaryanum* to check their ability to inhibit fungal growth. The remainder of the seed was planted in Walled Garden soil with or without *Pythium*.

The results are summarised in Tables 4.5 and 4.6. The number of bacteria found on seed was related to the concentration of the bacterial suspension (Fig. 5) but only seed soaked in suspensions containing 6×10^5 or more bacteria per ml inhibited the growth of *P. debaryanum* on agar, (Table 4.6). In *Pythium*-infested soil, seedling stand improved with increasing levels of bacteria but only treatment of seed with suspensions containing 6×10^8 or 6×10^7 bacteria per ml gave seedling stands comparable with the corresponding controls in soil alone.

D. Control of *Pythium* damping-off in non-sterile soil with wheat bran culture of *Trichoderma*

This experiment was identical to that described in Section 3E but used Walled Garden soil instead of sterile compost. Basically a wheat bran culture was incorporated in soil, with or without *Pythium* at 5, 10, 15, 20, 25 or 30% (w/w) and the growth of cucumber seedlings in these mixtures was assessed.

The results (Table 4.7) were also similar to those obtained with sterile compost. Additions of *Trichoderma* in the range 20-30% gave seedling stands and growth comparable to the corresponding controls in soil alone.

Table 4.5 Effect of seed inoculation with different inoculum concentrations of bacterium 2 - B46
on control of Pythium

Bacteria*		Final seedling stand		Fresh wt seedling (g)	
in suspension	per seed	+ <u>Pythium</u>	No <u>Pythium</u>	+ <u>Pythium</u>	No <u>Pythium</u>
6×10^8	16×10^5	22	25	1.99	2.09
6×10^7	4×10^5	20	24	1.76	2.08
6×10^6	1×10^5	18	25	1.67	1.98
6×10^5	7.2×10^4	15	23	1.85	1.98
6×10^4	8×10^3	13	22	1.82	1.94
6×10^3	1×10^2	3	21	0.71	1.68
6×10^2	65	4	21	0.77	1.78

* Cells per ml of suspension

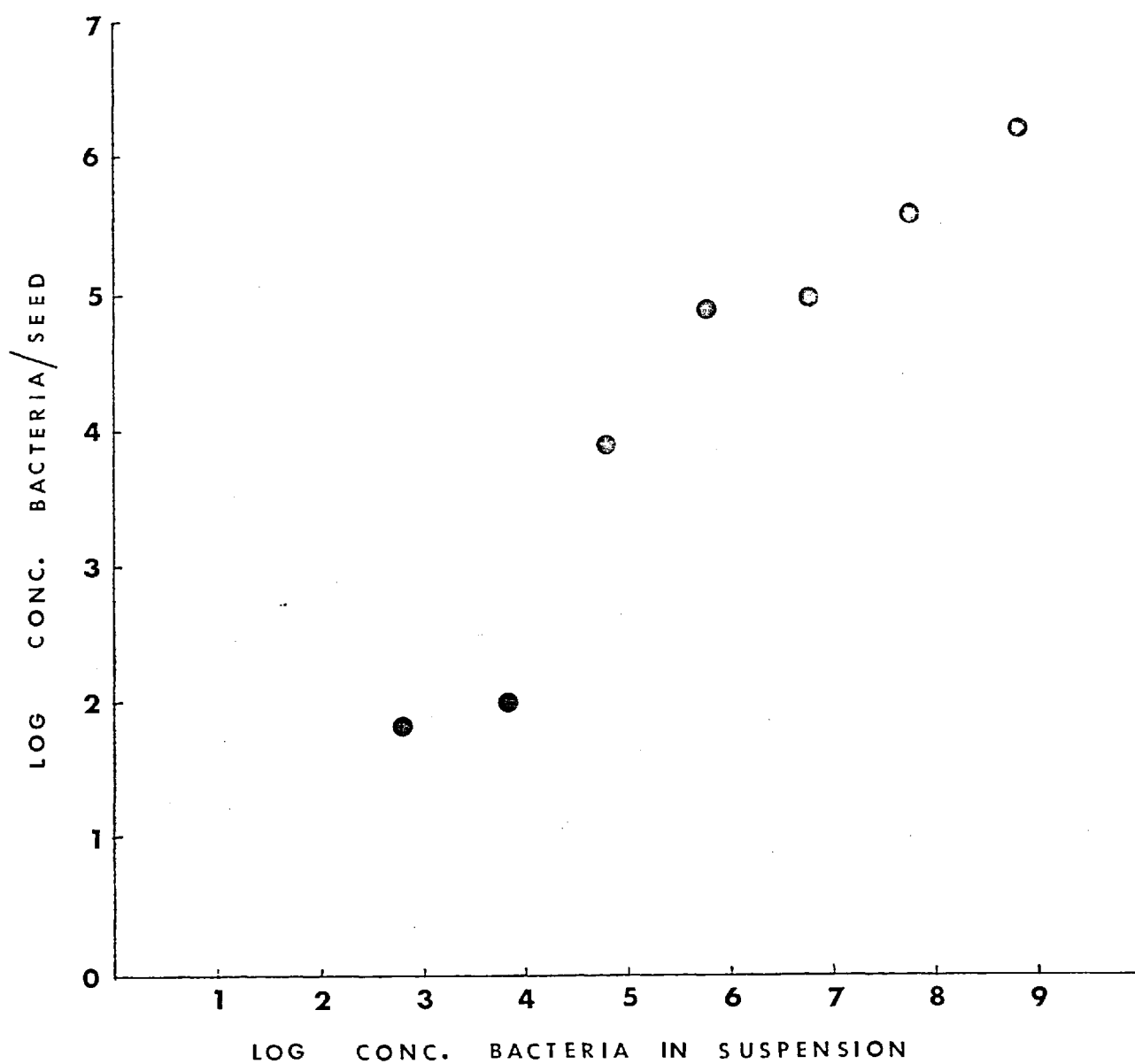


Fig 5. Bacteria on seed in relation to conc. of bacterial suspension.

Table 4.6 Antagonism exhibited by bacterial cells on treated cucumber seed against Pythium on agar

+ = Growth of Pythium - = Pythium growth inhibited +- = slight check

Bacteria		Degree of Antagonism
in suspension	per seed	
6×10^8	16×10^5	-
6×10^7	4×10^5	-
6×10^6	1×10^5	-
6×10^5	7.2×10^4	-
6×10^4	8×10^3	+-
6×10^3	1×10^2	+
6×10^2	65	+

Table 4.7 The effect of Trichoderma on the incidence of damping-off of cucumber by Pythium debaryanum (Pd) in non-sterile soil

% <u>Trichoderma</u> added (^w /w)	No. seedlings				Fresh wt seedling (g)	
	Emerged (9 days)		Survived (28 days)			
	+ Pd	No Pd	+ Pd	No Pd	+ Pd	No Pd
Nil	8	25	8	25	1.46	1.79
5	12	24	12	24	1.55	1.64
10	14	25	14	25	1.98	1.89
15	15	24	15	24	1.73	1.79
20	22	25	21	25	1.62	1.65
25	21	22	21	22	1.25	1.38
30	18	21	15	21	0.96	1.11
% sterile bran added (^w /w)						
5	6	13	6	13	0.80	1.51
10	9	14	9	14	1.01	1.48
15	6	13	6	13	1.10	1.18

Table 4.8 Effect of Trichoderma on Pythium damping-off 7 days after soil inoculation before planting

% <u>Trichoderma</u> added (^w /w)	Final seedling stand		Fresh wt seedling (g)	
	+ <u>Pythium</u>	No <u>Pythium</u>	+ <u>Pythium</u>	No <u>Pythium</u>
Nil	2	24	2.47	2.77
5	16	27	3.00	2.86
10	15	28	2.97	3.14
15	19	27	2.81	3.41
20	25	27	3.03	3.11
25	22	26	3.08	3.34
30	19	28	2.36	3.06
% sterile bran added (^w /w)				
5	20	23	2.58	2.80
10	17	22	2.39	2.66
15	15	21	2.27	2.62



Plate 6. Control of Pythium damping-off of cucumber by bacterial seed treatments.

Untreated seed (A) in soil without Pythium and seed treated with water (B), bacterial isolate 2 - B24 (C), 2 - B27 (D), 2 - B46 (E), 2 - B77 (F), 1 - B18 (G) and 1 - B4 (H) in soil with P. debaryanum.



Plate 7. Control of Pythium damping-off by treating cucumber seed in different cell concentrations of bacterial suspension.

Seed treated in 6×10^8 cells/ml (1), 6×10^7 cells/ml (2), 6×10^6 cells/ml (3), 6×10^5 cells/ml (4), 6×10^4 (5), 6×10^3 cells/ml (6) and 6×10^2 cells/ml (7) in soil infested with P. debaryanum.

No other treatments were satisfactory and additions of bran alone were particularly harmful even in soil with no added Pythium which suggests that this soil contained damping-off fungi that could exploit this added substrate.

In the second experiment, the soil with or without Pythium incorporated with Trichoderma as before, was left in the glasshouse for 7 days after which it was planted with cucumber. The results (Table 4.8) showed an improvement in seedling stands and growth. Addition of bran alone did not severely affect seedling stands and growth as before.

Summary

- (i) Only 3 out of 7 bacterial isolates screened gave a moderate control of Pythium damping-off in non-sterile soil when applied to seed.
- (ii) The control of Pythium damping-off was found to depend upon the presence of viable bacteria on cucumber seed.
- (iii) There was a direct relationship between the concentration of bacteria in suspensions and the number found on cucumber seed after inoculation.
- (iv) The amount of control of Pythium damping-off was found to depend upon the number of viable bacteria on cucumber seed which also determined the population of bacteria that eventually colonized the rhizosphere of seedlings.
- (v) Some control of Pythium damping-off by Trichoderma was obtained in non-sterile soil, and addition of the antagonist to infested soil 7 days before sowing cucumber improved control by Trichoderma.

5. SPECTRUM OF ACTIVITY OF BACTERIA ANTAGONISTS OF *PYTHIUM DEBARYANUM*

Previous experiments showed that three bacterial isolates consistently controlled damping-off by *P. debaryanum* when applied to cucumber seed. This control was achieved under natural conditions in which, presumably, the introduced organisms had to compete with the soil microflora as well as the pathogen. One attribute of an ideal antagonist is that it should have a broad spectrum of activity. It should be effective against different strains of the pathogen and against other pathogens. The next series of experiments were aimed at investigating the effects of these bacteria on other soil-borne fungi and also on *P. debaryanum* of other hosts in non-sterile soil.

Experimental

A. Damping-off of tomato by *Sclerotium rolfsii* in non-sterile soil

An experiment in Section 1D (Page 27) showed that the isolate of *S. rolfsii* was mildly pathogenic on tomato in sterile potting compost. Relatively low temperatures (20-22°C) throughout the experiment were thought to have partly suppressed this tropical pathogen. This experiment investigated damping-off of tomato when a 7-day-old culture of *S. rolfsii* was added to the upper layer of non-sterile soil in pots at six different concentrations (5%, 10%, 15%, 20%, 25% and 30% wet inoculum/dry wt soil). Other pots were completely filled with soil (controls). There were 3 replicate pots for each treatment and the temperature of the glasshouse was maintained at 30°C.

The results (Table 5.1 and Appendix Table 5.1) indicated that the virulence of this fungus on tomato was not altered by keeping the glasshouse at 30°C. However, there was a marked relationship between inoculum concentration and seedling death and seedlings which survived tended to have lower fresh weights, though here differences were not significant.

Table 5.1 Damping-off of tomato by Sclerotium rolfsii

Inoculum concentration % (w/w)	Seedling stand	Seedling fresh wt (g)
Nil	38	0.93
5	29	0.85
10	27	0.78
15	26	0.77
20	22	0.71
25	19	0.69
30	18	0.62

B. Damping-off of lettuce and tomato by *Pythium debaryanum* in non-sterile soil

The methods were essentially similar to those of the previous experiment with *S. rolfsii* except that the glasshouse was maintained at 20-25°C.

The results (Table 5.2 and Appendix Table 5.2) showed that the fungus effectively damped-off lettuce and tomato seedlings in this soil. The fungus killed all or most lettuce seedlings at each of the inoculum concentrations used but with tomato there was a more marked relationship between inoculum concentration and seedling death and the fresh weights of seedlings that survived did not differ within the various treatments.

C. Control of damping-off of lettuce by *Corticium praticola*

The three bacterial isolates that were effective against *P. debaryanum* in non-sterile soil were tested against *C. praticola* in similar soil.

Suitable conditions for damping-off were created by infesting the upper layer of non-sterile soil in pots with 1% ^w/w of oatmeal/sand culture of the pathogen. A suspension (containing $4 - 7.8 \times 10^8$ cells/ml) of each of the bacterial isolates 2 - B24, 2 - B27 and 2 - B46 was prepared in sterile distilled water as described in Section 4. In the first experiment lettuce seed was soaked in bacterial suspensions for 30 min and then air dried. Seed treated with sterile distilled water served as the control. Treated seed were then sown in soil with and without the pathogen. In the second experiment, soil with and without the pathogen was inoculated with 40ml of cell suspension of each bacterium using a sterile atomizer and was kept for 7 days in the glasshouse before planting untreated seeds. There were three replicate pots for each treatment in both experiments. The results are summarized in Tables 5.3 and 5.4 and in Appendix Tables 5.3 and 5.4.

None of the bacteria tested was particularly effective in controlling damping-off by *C. praticola*. Some seedlings survived when either the soil or seed was treated with isolate 2 - B46 but the numbers were significantly less than those in the corresponding controls. This bacterium slightly

Table 5.2 Damping-off of lettuce and tomato by Pythium debaryanum (Pd)
in non-sterile soil

Inoculum concentration % (^w /w)	Lettuce/Pd		Tomato/Pd	
	Seedling stand	Seedling fresh wt (g)	Seedling stand	Seedling fresh wt (g)
Nil	38	0.53	43	0.80
5	7	0.43	22	0.63
10	3	0.38	16	0.61
15	2	0.30	13	0.60
20	1	0.13	7	0.60
25	0	-	6	0.60
30	0	-	3	0.60

Table 5.3 Effects of treating seed with bacterial antagonists of Pythium debaryanum on damping-off of lettuce by Corticium praticola (Cp).

Bacterium	Seedling stand		Seedling fresh wt (g)	
	+ <u>Cp</u>	No <u>Cp</u>	+ <u>Cp</u>	No <u>Cp</u>
2 - B46	11	44	0.14	0.18
2 - B27	8	40	0.12	0.13
2 - B24	0	39	-	0.12
Water (control)	0	40	-	0.17

Table 5.4 Effects of treating soil with bacterial antagonists of Pythium debaryanum on damping-off of lettuce by Corticium praticola (Cp).

Bacterium	Seedling stand		Seedling fresh wt (g)	
	+ <u>Cp</u>	No <u>Cp</u>	+ <u>Cp</u>	No <u>Cp</u>
2 - B46	14	43	0.26	0.34
2 - B27	4	39	0.18	0.22
2 - B24	0	40	-	0.21
Water (controls)	1	40	0.13	0.24

inhibited the growth of C. praticola in culture but the other two isolates did not (Page 55). In uninfested soil there was no evidence that treatments with the bacteria stimulated plant growth.

D. Control of damping-off of tomato by Sclerotium rolfsii

This experiment tested the ability of the bacterial isolates, 2 - B24, 2 - B27 and 2 - B46, to control damping-off by S. rolfsii when applied to tomato seed. The procedure was essentially similar to that used in the previous experiment. Untreated seed and seed treated with suspensions ($4 - 7.8 \times 10^8$ cells/ml) of the bacteria were sown in pots of soil, the upper layer of which was either infested with S. rolfsii (25% oatmeal/sand culture) or not. For this experiment, the glasshouse was maintained at 30°.

The results (Table 5.5) showed that all the bacteria improved seedling emergence in infested soil but only treatment with isolate 2 - B27 gave a stand comparable to the corresponding control. Seedling weights did not differ significantly in any treatments but those from bacteria-treated seed were somewhat higher than from seed treated with water.

E. Control of damping-off of lettuce and tomato by Pythium debaryanum

Two similar experiments investigated whether these same bacterial antagonists applied on lettuce and tomato seeds (as compared with cucumber, see Page 62) could control damping-off by Pythium debaryanum in non-sterile soil.

The methods were the same as used previously. In the first experiment, untreated lettuce seed and seed treated with suspensions ($4 - 7.8 \times 10^8$ cells/ml) of the bacteria were sown in soil with or without 10% (^w/w) P. debaryanum in the upper layer. Tomato seed was used in the second experiment in similar treatments with soil infested with 20% (^w/w)

P. debaryanum. In both experiments, there were three replicates of each treatment. The results are summarised in Table 5.6 and Appendix Table 5.6.

Table 5.5 Effects of treating seed with bacterial antagonists of Pythium debaryanum on damping-off of tomato by Sclerotium rolfsii (Sr).

Bacterium	Seedling stand		Seedling fresh wt (g)	
	+ Sr	No Sr	+ Sr	No Sr
2 - B27	39	45	0.96	1.17
2 - B24	28	43	0.93	1.02
2 - B46	28	44	0.83	0.91
Water control	19	43	0.78	0.88

Table 5.6 Control of damping-off of lettuce and tomato by Pythium debaryanum (Pd) with bacterial antagonists of this fungus

(a) Expt 1. Lettuce/Pd

Bacterium	Seedling stand		Seedling fresh wt (g)	
	+ <u>Pd</u>	No <u>Pd</u>	+ <u>Pd</u>	No <u>Pd</u>
2 - B46	25	43	0.35	0.53
2 - B24	24	39	0.32	0.49
2 - B27	24	40	0.33	0.51
Water control	5	36	0.29	0.39

(b) Expt 2. Tomato/Pd

Bacterium	Seedling stand		Seedling fresh wt (g)	
	+ <u>Pd</u>	No <u>Pd</u>	+ <u>Pd</u>	No <u>Pd</u>
2 - B46	30	42	0.78	0.87
2 - B27	29	43	0.79	0.85
2 - B24	25	40	0.77	0.91
Water control	8	38	0.64	0.79



Plate 8. Activity of three bacteria, antagonistic to P. debaryanum, in preventing damping-off of lettuce by C. pratensis.

Upper row: untreated seed (D), seed treated with bacterial isolate 2 - B27 (A), 2 - B24 (B) and 2 - B46 (C).

Lower row: Untreated soil (D), soil watered with bacterial isolate 2 - B27 (A), 2 - B46 (B) and 2 - B24 (C).



Plate 9. Activity of three bacteria, antagonistic to P. debaryanum, in preventing damping-off of tomato by S. rolfsii.

Upper row: infested soil, and lower row, uninfested soil, planted with untreated seed (D), or seed treated with bacterial isolate 2 - B46 (A), 2 - B27 (B) or 2 - B24 (C).

All bacterial isolates significantly improved seedling stand of lettuce and tomato in infested soil compared with the water control where damping-off was severe. However, none gave sufficient control of damping-off to restore seedling stands to the levels obtained in non-infested soil. This is contrary to the observation made in a previous similar experiment with cucumber (Page 62) and may be related to the numbers of bacteria retained on seed of different size.

There were no significant differences between the bacterial isolates in controlling damping-off nor did seedling weight differ in the treatments involving infested soil. However, seedling weight in soil infested with P. debaryanum was significantly less than in the corresponding control, indicating that the surviving seedlings were not free of infection. Indeed, in these instances obvious lesions could be seen on the roots. With tomato, seedling weights were similar in infested and non-infested soil.

Summary

- (i) Bacterial antagonists of Pythium debaryanum could not effectively control damping-off of lettuce by Corticium praticola in non-sterile soil.
- (ii) These bacteria on tomato seed reduced damping-off by Sclerotium rolfsii in non-sterile soil, but only isolate 2 - B27 gave a stand comparable with the corresponding control in soil without S. rolfsii.
- (iii) These bacteria gave a moderate control of damping-off by P. debaryanum on lettuce and tomato. But they were not as effective on seed of these hosts against the pathogen as they were on cucumber seed.

6. COMPARISONS OF BACTERIAL AND CHEMICAL SEED TREATMENTS TO CONTROL DAMPING-OFF OF CUCUMBER BY PYTHIUM DEBARYANUM

Control of damping-off of cucumber by P. debaryanum in non-sterile soil was achieved by treating seed with some bacterial antagonists. This control was compared with that achieved by treating seed with fungicides recommended for use on vegetable seed against soil-borne fungi.

Bacterial isolates 2 - B24, 2 - B27 and 2 - B46 were used in this study and the following three fungicides.

(i) Mil-Col'30 an aqueous suspension containing 300g/l of draxozolon (4-[2-Chlorophenylhydrazono]-3-methyl-5-isoxazolone) supplied by Plant Protection Ltd, (Jealott's Hill Research Station, Bracknell, UK) and recommended for Pythium, Fusarium and certain other rots.

(ii) Orthocide WP, a wettable powder containing 50% Captan (3a, 4, 7, 7a-tetrahydro-N-(trichloro methane phenyl) phthalimide); supplied by Murphy Chemical Co Ltd, Wheathampstead, Herts.

(iii) Fernasan A, a wettable powder containing 80% thiram (tetramethylthiram disulphide); supplied by Plant Protection Ltd, Yalding, Kent and recommended for pre-emergence damping-off and seed decay in peas.

Seeds were treated:-

(i) with Mil-Col'30 by dipping them in a mixture of 2 parts fungicide: 1 part water for 3-4 min. (The relative quantities of seed and fungicide were based on the recommendation of 250ml fungicide to 100kg seed).

(ii) with Orthocide or Fernasan A by shaking them in a bottle with 0.02 a.i. of the fungicide per 10g seed.

(iii) with bacteria, by immersing them in a suspension containing $4-7.8 \times 10^8$ cells/ml for 30 min and then air-drying.

Untreated cucumber seed and seed treated with one of the bacterium or chemicals were then sown in pots of soil and in pots in which the upper layer was contaminated with 15% (^w/w) P. debaryanum. There were three replicates of each treatment.

Table 6.1 Control of Pythium damping-off by bacterial and chemical seed treatments

Treatment	Seedling stand	Seedling fresh wt (g)
Chemical - Drazoxolon	25	2.05
- Captan	23	1.90
- Thiram	22	1.81
Bacterium: 2 - B27	22	1.97
2 - B ⁴⁶	22	1.82
2 - B24	21	1.82
Nil - No <u>Pythium</u>	22	1.73
- + <u>Pythium</u>	4	1.00



Plate 10. Control of Pythium damping-off by bacterial and chemical seed treatments.

Untreated seed (A) and seed treated with bacterial isolate 2 - B24 (B), 2 - B27 (C) and 2 - B46 (D) and with fungicide Drazoxolon (E), Captan (F) and Thiram (G) in soil with P. debaryanum. Untreated seed in non-infested soil (H).

The results (Table 6.1) showed that all bacteria and chemicals were equally effective in controlling damping-off and there were no significant differences in seedling stand or seedling fresh weight between the bacterial and chemical treatments.

Summary

The control of Pythium damping-off by bacteria seed treatments was comparable with treating cucumber seed with fungicides recommended for use on vegetable seed against soil-borne fungi.

7. THE LONGEVITY OF BACTERIA ON CUCUMBER SEED

This experiment investigated the effects on the subsequent control of Pythium damping-off of storing seed treated with bacteria.

Cucumber seeds were soaked in suspensions of isolates 2 - B24 (4×10^8 cells/ml), 2 - B27 (4×10^8 cells/ml), 2 - B46 (8×10^8 cells/ml) or water, and then air-dried. They were then sown immediately, or after storage in sterile petri-dishes for 1, 2 or 4 wk, in pots of soil or soil with the upper layer infested with 15% (w/w) P. debaryanum. The numbers of bacteria on the seed before sowing were assessed by plating diluted washings of the seed as described on Page 64. There were three replicates of each treatment. The results are summarised in Table 7.1 and the detailed results are given in Appendix Table 7.1.

The numbers of viable bacteria per seed decreased with storage (Table 7.1a) and so did the viability of the seed, whether treated with bacteria or not, as judged by seedling stands in non-infested soil (Table 7.1b). These two factors probably accounted for the decline in the control of damping-off by bacteria on seed with storage which was apparent both in the figures for seedling stand (Table 7.1c) and those for seedling fresh weight (Table 7.1d). It was also observed that fresh seed germinated more rapidly than stored seed. This may be another factor contributing to the lower seedling stands with stored seed since this would aggravate any damping-off by fungi, other than Pythium, that are present in this soil.

Summary

When seeds treated with bacteria were stored at 4°C, the number of viable bacteria on the seed decreased and so also did the viability of the seed. These two factors could have been responsible for the decline in control of Pythium damping-off when stored seed was planted in infested soil.

Table 7.1 Effects of storing cucumber seed treated with bacteria

(a) Viability of bacteria

Storage Period (wk)	<u>Viable bacteria/seed</u>		
	2 - B24	2 - B27	2 - B46
0	9.7×10^5	10.7×10^5	18.2×10^5
1	3.3×10^5	2×10^5	5×10^5
2	9×10^4	8.2×10^4	7.2×10^4
4	8.3×10^4	7.7×10^4	7×10^4

(b) Viability of seed

Storage period (wk)	<u>Seedling stand in non-infested soil (max = 30) of seed treated with</u>			
	2 - B24	2 - B27	2 - B46	Water
0	24	23	24	22
1	22	22	23	21
2	21	21	20	19
4	18	17	18	16

(c) Control of Pythium damping-off

Storage period (wk)	<u>Seedling stand in soil infested with <u>Pythium</u> of seed treated with</u>			
	2 - B24	2 - B27	2 - B46	Water
0	21	21	22	5
1	19	17	21	5
2	15	13	12	4
4	12	10	9	3

Storage period (wk)	<u>% control* with</u>		
	2 - B24	2 - B27	2 - B46
0	88	91	92
1	86	77	91
2	71	62	60
4	67	59	50

* seedling stand in table above as % of corresponding controls in (b).

Table 7.1 (Cont)

(d) Fresh weight (g) of emerged seedlings

Storage period (wk)	Mean seedling wt in non-infested soil, seed treated with			
	2 - B24	2 - B27	2 - B46	Water
0	2.1	2.1	2.3	1.9
1	1.6	1.7	1.7	1.4
2	1.6	1.6	1.4	1.3
4	1.4	1.4	1.4	1.3

Storage period (wk)	Mean seedling wt in soil infested with <u>Pythium</u> , seed treated with			
	2 - B24	2 - B27	2 - B46	Water
0	2.0	2.0	2.1	1.1
1	1.5	1.6	1.6	0.9
2	1.3	1.2	1.1	0.8
4	1.1	1.2	1.1	0.7

8. CONTROL OF DAMPING-OFF OF LETTUCE BY CORTICIUM PRATICOLA IN NON-STERILE SOIL WITH ANTAGONISTIC MICRO-ORGANISMS

Previous experiments (pp. 56 - 57) showed that none of the bacterial isolates, found to be antagonistic to C. praticola in culture, was effective when applied to lettuce seed in controlling damping-off by this fungus in sterile potting compost. In this situation, C. praticola grew freely throughout the compost, uninhibited by other soil micro-organisms. The possibility that it might be more readily controlled if it were present at lower inoculum levels in non-sterile soil was investigated next and also other methods of applying the antagonistic bacteria.

Experimental

A. Control of damping-off by bacteria on seed in non-sterile soil

The first experiment (Appendix Table 8.1a) showed that five selected bacterial isolates were not effective on lettuce seed against damping-off by C. praticola when soil was infested with this pathogen at inoculum concentrations of 1%, 3% or 5% (^w/_w).

A second experiment tested the viability and numbers of the bacteria on treated seed and their ability to control damping-off in soil infested with 1% or 0.5% (^w/_w) C. praticola. Suspensions of the bacteria were prepared in sterile distilled water from 7-day-old nutrient broth cultures grown at 30°. Lettuce seeds were soaked in these suspensions or water for 30 min and then air-dried for 1h. The numbers of bacteria on the seeds were estimated by plating out diluted washings of a seed sample and the ability of bacteria-treated seed to inhibit C. praticola was examined by placing some seed on agar plates inoculated centrally with this fungus. The remainder of the seed was sown in pots of soil only or in soil infested with C. praticola at 0.5% or 1% (^w/_w). The results (Table 8.1b and Appendix Table 8.1b) showed that while many viable bacteria remained on seed after air-drying and these were capable of

Table 8.1b Effects of treating lettuce seed with selected bacteria on damping-off of lettuce by C. praticola in non-sterile soil

Bacterium	No. bacteria per		Inhibition of <u>Cp</u> in culture	Seedling stand		
	ml suspension (x 10 ⁸)	seed (x 10 ⁶)		Control No <u>Cp</u>	0.5% <u>Cp</u>	1.0% <u>Cp</u>
2 - B1	1.2	1.0	+	44	2	1
2 - B2	2.4	2.6	+	43	4	3
2 - B3	1.4	1.8	+	45	3	2
1 - B26	2.7	2.5	+	45	4	3
2 - B14	2.2	1.1	+	43	5	3
Water	-	-		41	3	1

inhibiting C. praticola in culture, little control of damping-off was obtained in infested soil. It was thus clear that seed inoculated with bacterial antagonists was not a successful method of biological control of this pathogen and other methods of introducing these antagonists were tried.

B. Control of damping-off of lettuce by inoculating soil with selected bacteria

The aim of this experiment was to investigate the possibility of controlling damping-off of lettuce by inoculating soil infested with C. praticola with selected bacteria.

The five bacterial isolates used in the previous experiment (see Section 8A) were grown for 7 days in nutrient broth at 30°. The washed cell concentrate from 600ml of culture was suspended in 300ml sterile distilled water and the concentration of bacterial cells per ml of each suspension was adjusted to the levels used in Section 8A. Pots were filled with non-sterile soil and the upper layer was inoculated with 1% w/w of oatmeal sand culture of the pathogen. Other pots were completely filled with soil (controls). For treatments involving bacteria the top of the soil was sprayed with 40ml of the bacterial suspension using a sterile atomizer. Control pots were treated with sterile distilled water. The pots were then randomly arranged on the glasshouse bench and incubated for 7 days before lettuce seeds were sown. There were 3 replicate pots for each treatment.

The results (Table 8.2) showed that four isolates, 2 - B2, 2 - B3, 2 - B14, and 1 - B26 gave a significant control of damping-off. Seedling stands with these isolates in infested soil were similar to that obtained using untreated soil in non-infested soil. However, in this soil three of the bacteria (2 - B3, 2 - B14 and 1 - B26) gave seedling stands that were significantly better than the water control suggesting that there were other damping-off fungi in the soil and that these were controlled

Table 8.2 Control of damping-off of lettuce by inoculating soil infested with Corticium praticola with selected bacteria

Bacterium	No. of bacteria per ml suspension	<u>Seedling stand</u>		<u>Seedling fresh wt (g)</u>	
		+ <u>Cp</u>	No <u>Cp</u>	+ <u>Cp</u>	No <u>Cp</u>
2 - B14	2.2×10^8	28	39	0.35	0.48
1 - B26	2.7×10^8	27	34	0.31	0.40
2 - B3	1.4×10^8	26	37	0.30	0.48
2 - B2	2.4×10^8	23	28	0.33	0.39
2 - B1	1.2×10^8	12	25	0.27	0.36
Water	-	5	22	0.19	0.29

by the bacteria. The net result was that the bacteria-treated soil did not always give comparable seedling stands in infested and non-infested soil, eg. 2 - B14.

These features of the seedling stands were reflected in the figures for seedling fresh weight. Particularly, in non-infested soil treated with 2 - B2, 2 - B3, 2 - B14 and 1 - B26 bigger seedlings were produced than in untreated soil, either because pathogens other than C. praticola were controlled and/or the bacteria stimulated plant growth.

C. Control of damping-off of lettuce by dipping seedling roots into suspension of selected bacteria

The following experiments investigated the use of bacterial suspensions as root dips to control attack by C. praticola on transplanted lettuce seedlings.

The pathogenicity to transplants was first established by growing lettuce for 7 days in sterile potting compost and then transplanting them to pots (six per pot) of sterile compost or non-sterile Walled Garden soil either without C. praticola or with 0.5%, 1%, 3% or 5% (^w/w) of the fungus in the upper layer. There were three pots per treatment. All or most of the seedlings were killed after 15 days where C. praticola was present (Table 8.3).

In the second part of the experiment, lettuce seedlings were similarly grown in sterile compost for 7 days. They were then removed carefully, the roots were washed free of most compost by dipping in sterile distilled water and the seedlings were placed for 30 min in a beaker containing 100ml bacterial suspension so that their roots and stem bases were covered. Other seedlings were dipped only in sterile distilled water (controls). The seedlings were then planted in non-sterile soil only or in soil infested with 1% (^w/w) C. praticola. All the bacterial isolates controlled damping-off when used in this way (Table 8.4), even isolate 2 - B1 which was

Table 8.3 Damping-off of lettuce by Corticium praticola in sterile potting compost and non-sterile soil

Inoculum concentration % ^w /w	Seedling stand	
	Compost	Non-sterile soil
Nil	18	18
0.5	3	5
1	1	2
3	0	0
5	0	0

Table 8.4 Control of Corticium praticola damping-off in lettuce by dipping seedlings into suspension of selected bacteria

Bacterium	No. of cells per ml suspension	Seedling stand		Seedling fresh wt (g)	
		+ <u>Cp</u>	No <u>Cp</u>	+ <u>Cp</u>	No <u>Cp</u>
2 - B1	1.2×10^8	16	18	2.07	2.28
2 - B2	2.4×10^8	16	18	2.45	2.65
2 - B14	2.2×10^8	16	18	2.61	2.88
1 - B26	2.7×10^8	15	18	2.25	2.84
2 - B3	1.4×10^8	14	18	2.29	2.67
Water	-	0	18	-	2.27

relatively ineffective when applied to the soil surface (Table 8.2).

Seedling fresh weights were similar in all treatments giving no indication of stimulation of plant growth by these bacteria.

A further experiment tested the effect of varying the concentration of bacteria in the suspension used as a root dip. Isolate 2 - B1 was chosen for this study. A suspension in sterile distilled water, containing 1.2×10^8 cells/ml, was prepared from a 7-day-old nutrient broth culture and 10-fold dilutions were derived from this as indicated in Table 8.5. Lettuce seedlings grown for 7 days in sterile compost were dipped in the various bacterial suspensions or in sterile water (as described above) and were then planted in soil with 1% (^w/w) C. praticola or in soil without this fungus.

The result (Table 8.5 and Appendix Table 8.5) showed that there was a marked relationship between inoculum concentration of the bacterium and seedling deaths. The best control was obtained using a suspension of 1.2×10^8 bacteria per ml. The degree of control was progressively less with more dilute suspensions and no control was obtained using a suspension of 1.2×10^3 bacteria/ml.

D. Control of damping-off of lettuce by Corticium praticola with Trichoderma harzianum

A previous experiment (See Section 3) showed that biological control of damping-off of lettuce by C. praticola in sterile compost could not be achieved with wheat bran culture of Trichoderma sp. isolated from some selected soils. Certain species of Trichoderma, especially, T. harzianum have been reported (Henis et al., 1977) to be effective on Rhizoctonia solani, a fungus related to C. praticola. The effect of T. harzianum on damping-off of lettuce by C. praticola was therefore examined in non-sterile soil.

The methods were essentially the same as those of experiment in (p. 58). Pots were filled with non-sterile soil and the upper layer was

Table 8.5 Control of Corticium praticola damping-off in lettuce by dipping seedlings into suspension of bacterium 2 - B1 at different cell concentrations

Concentrations of cell/ml of suspension	<u>Seedling stand</u>		<u>Seedling fresh wt (g)</u>	
	+ <u>Cp</u>	No <u>Cp</u>	+ <u>Cp</u>	No <u>Cp</u>
1.2×10^8	15	18	2.53	2.90
1.2×10^7	14	18	2.65	2.68
1.2×10^6	12	17	2.38	2.60
1.2×10^5	10	18	2.29	2.44
1.2×10^4	8	18	2.17	2.52
1.2×10^3	5	18	2.14	2.38
1.2×10^2	3	18	2.03	2.47

Table 8.6 Control of damping-off of lettuce by Corticium praticola with wheat bran culture of Trichoderma harzianum

Trichoderma preparation (% w/w)	Seedling stand		Seedling fresh wt (g)	
	+ <u>Cp</u>	No <u>Cp</u>	+ <u>Cp</u>	No <u>Cp</u>
Nil	2	33	0.20	0.29
5	5	28	0.20	0.27
10	0	43	-	0.24
15	0	37	-	0.29
20	0	40	-	0.28
25	0	43	-	0.28
30	0	33	-	0.28
Sterile wheat bran				
5	0	41	-	0.31
10	0	38	-	0.22
15	0	37	-	0.28



Plate 11. Control of damping-off of lettuce by C. praticola, by inoculating soil with bacterial cell-suspensions.

Upper row, infested soil and lower row, non-infested soil treated with water (A), or bacterial suspensions of isolates 2 - B1 (B), 2 - B2 (C), 2 - B3 (D), 2 - B14 (E) and 1 - B26 (F).



Plate 12. Differential effectiveness of five bacterial isolates (B-F) in controlling damping-off of lettuce by dipping roots of seedlings in bacterial suspensions before transplanting into soil infested with C. praticola.

Upper row and lower row, infested soil treated with water (A), or bacterial suspensions of isolates 2 - B1 (B), 2 - B2 (C), 2 - B3 (D), 2 - B14 (E), 1 - B26 (F).

inoculated simultaneously with 1% ^w/w of oatmeal sand culture of C. praticola and different amounts of wheat bran culture to give an upper layer containing 5%, 10%, 15%, 20%, 25% and 30% T. harzianum inoculum (wet wt inoculum/dry wt soil). Other pots were completely filled with soil and the upper layer inoculated with similar concentrations of T. harzianum inoculum as described above (controls). The pots were kept in the glasshouse for 7 days before sowing with lettuce seed. Disease was assessed 28 days later.

The result (Table 8.6) showed that this isolate of T. harzianum could not control damping-off of lettuce by C. praticola. Indeed, when added in large amounts, the antagonist seemed to increase the amount of pre-emergence damping-off resulting in total death of the seedlings. Sterile wheat bran also increased damping-off in a similar manner. It can thus be concluded that C. praticola unlike R. solani, was generally resistant to the antagonistic action of Trichoderma species in soil.

Summary

- (i) Control of damping-off of lettuce by C. praticola could not be achieved by treating seed with bacterial antagonists.
- (ii) Four isolates (2 - B2, 2 - B3, 2 - B14 and 1 - B26) gave a moderate control of damping-off of lettuce when applied to soil infested with C. praticola, 7 days before planting lettuce.
- (iii) These isolates plus a further isolate (2 - B1) controlled damping-off of lettuce when applied as a root dip to young lettuce before transplanting to infested soil with C. praticola. The amount of control depended on the concentration of the bacterial suspension.
- (iv) The isolate of Trichoderma harzianum could not control damping-off of lettuce by C. praticola in non-sterile soil.

9. CONTROL OF DAMPING-OFF OF LETTUCE WITH AN AVIRULENT ORGANISM RELATED TO CORTICIUM PRATICOLA

An isolate of Rhizoctonia solani not pathogenic to lettuce was found to stop the growth of C. praticola in culture (see Section 2). Several workers (Curren, 1967; Langton, 1969; Meyer and Maraite, 1971 and New and Kerr, 1972) have reported biological control using a micro-organism related to the pathogen but avirulent to the given host. The next experiments investigated the protection of lettuce from damping-off by inoculating soil infested with C. praticola with this avirulent isolate of R. solani.

Each fungus was grown for 7 days on oatmeal-sand medium (as described in Section 1). Six different inoculum mixtures were then prepared in non-sterile (Walled Garden) soil. Each contained 1% (^W/_W) C. praticola plus one of the following levels of R. solani: 5%, 10%, 15%, 20%, 25% and 30% (^W/_W). These mixtures were added to pots containing 300g soil to provide an infested upper layer. There were three pots of each mixture plus appropriate controls.

In the first experiment (Test 1) each pot was planted with lettuce immediately; in the second (Test 2) pots were kept in the glasshouse for 7 days before planting with lettuce. In both instances, damping-off was assessed 15 days after planting.

The results (Table 9.1) showed that additions of R. solani significantly reduced damping-off by C. praticola. Where pots were planted immediately after the preparation of fungal mixtures, control of damping-off was related to the amount of R. solani added but even at 30% R. solani seedling stands were not comparable to those of the corresponding controls. Allowing the mixtures to stand for 7 days in the glasshouse before sowing lettuce improved control by R. solani and seedling stands at 25% and 30% R. solani were not significantly different from the controls.

Table 9.1 Control of Corticium praticola damping-off of lettuce by inoculating infested soil with non-pathogenic isolate of Rhizoctonia solani

	% <u>Rhizoctonia</u> added (^W /w)	Final seedling stand		Seedling fresh wt (g)	
		+ <u>Cp</u>	No <u>Cp</u>	+ <u>Cp</u>	No <u>Cp</u>
Test 1 [*]					
	Nil	3	33	0.25	0.30
	5	12	34	0.25	0.30
	10	14	34	0.27	0.33
	15	16	34	0.30	0.31
	20	16	37	0.34	0.34
	25	19	35	0.33	0.37
	30	22	35	0.33	0.33
Test 2 ^{**}					
	Nil	2	34	0.20	0.30
	5	16	38	0.30	0.32
	10	24	39	0.30	0.32
	15	25	37	0.33	0.33
	20	26	37	0.36	0.33
	25	29	37	0.33	0.37
	30	30	39	0.33	0.35

* Seeding was done immediately after soil inoculation

** Seeding was done 7 days after soil inoculation



Plate 13. Effect of adding nil (A), 5% (B), 10% (C), 15% (D), 20% (E), 25% (F) and 30% (G) R. solani on the damping-off of lettuce by C. praticola

Summary

(i) Some control of damping-off of lettuce by C. praticola was achieved by mixed inoculation with an avirulent isolate of R. solani.

(ii) Allowing the mixture to stand for 7 days before sowing lettuce improved control by R. solani.

(iii) The greater the population of R. solani, the less severe was the damping-off by C. praticola.

10. SPECTRUM OF ACTIVITY OF BACTERIAL ANTAGONISTS OF CORTICIUM PRATICOLA

The experiments in this section investigated the ability of bacterial antagonists of Corticium praticola to control damping-off by other soil-borne fungi and damping-off by C. praticola of other hosts.

ExperimentalA. Control of damping-off of cucumber by Pythium debaryanum

The following bacterial isolates were used: 2 - B1, 2 - B2, 2 - B3, 1 - B26, and 2 - B14. They were grown in nutrient broth at 30° for 7 days and from these cultures suspensions of bacteria in sterile distilled water were obtained as described in Section 8. The concentration of bacteria in each suspension was adjusted to the levels used in Section 8 and cucumber seeds were treated in these suspensions. Seeds treated in sterile distilled water served as the control. Treated seeds were planted (10/pot) in Walled Garden soil, with or without 15% (w/w) of oatmeal-sand culture of Pythium. There were three replicates of each treatment and pots were randomly arranged on the glasshouse bench.

The results (Table 10.1) showed that all bacterial isolates gave some control, but none gave stands comparable with their corresponding controls. These isolates were thus not as effective on cucumber seed against damping-off by P. debaryanum as the 3 isolates used previously with this fungus (Table 4.3). Treating seed with bacteria had no adverse effect on seedling growth and stand in the absence of Pythium.

B. Control of damping-off of tomato by Sclerotium rolfsii

The experimental procedure was essentially similar to that of Section A. Seeds with the bacterial suspensions were planted (15/pot) in Walled Garden soil, with or without 25% (w/w) of oatmeal sand culture of S. rolfsii. Each treatment was replicated three times and pots were randomly arranged on the glasshouse bench. The temperature of the glasshouse was kept at 30°C throughout the experiment. Seedlings were counted 9 and 15 days after

Table 10.1 Control of Pythium damping-off of cucumber by treating seed with selected bacterial antagonists of Corticium praticola

Bacterium	No. of bacteria per ml suspension	Final seedling stand		Fresh wt (g) seedling	
		+ <u>Pythium</u>	No <u>Pythium</u>	+ <u>Pythium</u>	No <u>Pythium</u>
2 - B3	1.4×10^8	16	25	1.83	2.16
2 - B1	1.2×10^8	15	27	1.94	2.12
2 - B2	2.4×10^8	11	23	1.45	2.00
1 - B26	2.7×10^8	10	24	1.46	1.92
2 - B14	2.2×10^8	9	24	1.34	2.02
Water	-	4	22	1.32	1.74

sowing to give numbers that emerged and survived respectively. The fresh weight of seedlings that survived was also determined. The results are summarized in Table 10.2 and Appendix Table 10.2.

Treating tomato seed with any of the isolates significantly controlled pre-emergence damping-off by S. rolfsii. The number of seedlings that emerged from bacteria-treated seed in soil plus S. rolfsii was generally lower than in the corresponding controls in soil alone but not significantly so, except for isolate 2 - B1. However, there was some post-emergence damping-off of older seedlings in soil with S. rolfsii and this was most severe in pots where seeds were treated with water or isolate 1 - B26. Only seed treatments with isolates 2 - B2 and 2 - B3 eventually gave stands comparable with their corresponding controls. The bacteria had no adverse effect on seedling growth.

C. Control of damping-off of cucumber and tomato by Corticium praticola

The study investigated the ability of five bacterial antagonists of C. praticola to control damping-off of this fungus on cucumber and tomato. Applications of these bacteria to seed or directly to soil were also compared.

The suspensions were prepared and used at the same concentrations as in previous experiments, i.e. 2 - B1 (1.2×10^8 cells/ml), 2 - B2 (2.4×10^8 cells/ml), 2 - B3 (1.4×10^8 cells/ml), 2 - B14 (2.2×10^8 cells/ml) and 1 - B26 (2.7×10^8 cells/ml). In the first series of experiments, each suspension was applied to Walled Garden soil with and without 1% (w/w) of oatmeal sand culture of the fungus at the rate of 40ml per pot using the method described in Section 8B. Soil treated with sterile distilled water served as the control. The pots were kept in the glasshouse for 7 days and then sown with either cucumber or tomato seeds.

In the second series of experiments, the suspensions were applied to either tomato or cucumber seeds and the treated seeds were planted in soil

Table 10.2 Control of S. rolfsii (Sr) damping-off of tomato by treating seed with selected bacterial antagonists of Corticium praticola

Bacterium	No. seedlings					
	Emerged (9 days)		Survived (15 days)		Fresh wt seedling (g)	
	+ <u>Sr</u>	No <u>Sr</u>	+ <u>Sr</u>	No <u>Sr</u>	+ <u>Sr</u>	No <u>Sr</u>
2 - B3	38	44	37	44	1.32	1.42
2 - B2	36	43	35	43	1.21	1.32
2 - B14	35	40	29	40	1.32	1.40
2 - B1	34	42	30	42	1.29	1.38
1 - B26	33	40	25	40	1.27	1.42
Water	19	38	10	38	0.92	1.30

with and without 1% (^w/w) C. *praticola*. All treatments were replicated 3 times and disease was assessed by counting seedling stands 9 and 15 days to give numbers that emerged and survived respectively. The fresh weight of seedlings that survived was also determined. The results are summarized in Table 10.3 to 10.6.

C. *praticola* caused severe pre-emergence damping-off of cucumber seed and treating seed with the bacterial isolates improved seedling stand only slightly. The fresh weight of seedlings that survived was also generally lower (in some instances significantly lower) than in the corresponding controls. Treating soil with bacterial suspensions 7 days before planting considerably improved seedling stand in infested soil but not to the level of the corresponding controls. However, seedling growth, as determined by fresh weight was not significantly different from that of the corresponding controls, indicating some protection of seedlings that became established. In many respects these results were similar to those of a previous experiment with lettuce as host (See Table 8.1) though these soil treatments with bacteria were generally more effective.

By comparison, all bacterial isolates gave some control of pre-emergence damping-off of tomato and two isolates in particular, 2 - B3 and 1 - B26, partially controlled post-emergence damping-off to give the highest stands in infested soil. However, these were still not comparable to the seedling stands in the corresponding controls without C. *praticola*. The seedlings which survived in these two treatments were comparable in fresh weight to those in the controls but in the other treatments they were not. As with the previous experiment with cucumber, control of damping-off was much improved by adding the bacteria to soil and here isolate 1 - B26 gave stands comparable with its corresponding control in soil alone. Also, seedling fresh weights were not significantly different from those of the controls.

Table 10.3 Effects of treating cucumber seed with selected bacteria on damping-off by C. praticola in non-sterile soil

Bacterium	Final seedling stand		Fresh wt Seedling (g)	
	+ <u>Cp</u>	No <u>Cp</u>	+ <u>Cp</u>	No <u>Cp</u>
2 - B3	8	25	1.22	1.78
1 - B26	7	24	1.23	1.83
2 - B1	5	24	0.82	1.79
2 - B14	5	23	1.35	1.58
2 - B2	4	23	0.97	1.89
Water	0	22	-	1.72

Table 10.4 Effects of treating soil with selected bacteria on damping-off of cucumber by C. praticola

Bacterium	Final seedling stand		Fresh wt Seedling (g)	
	+ <u>Cp</u>	No <u>Cp</u>	+ <u>Cp</u>	No <u>Cp</u>
2 - B3	16	27	1.67	1.91
1 - B26	15	25	1.60	1.70
2 - B14	13	26	1.51	1.82
2 - B2	13	25	1.60	1.72
2 - B1	11	25	1.41	1.71
Water	0	24	-	1.66

Table 10.5 Effects of treating tomato seed with selected bacteria on damping-off by C. praticola in non-sterile soil

Bacterium	No. seedling				Fresh wt seedling (g)	
	Emerged (9 days)		Survived (15 days)			
	+ <u>Cp</u>	No <u>Cp</u>	+ <u>Cp</u>	No <u>Cp</u>	+ <u>Cp</u>	No <u>Cp</u>
1 - B26	33	38	25	38	1.40	1.75
2 - B3	29	36	22	36	1.52	1.83
2 - B2	24	33	16	33	1.32	1.73
2 - B14	24	33	15	33	1.18	1.60
2 - B1	22	34	12	34	1.23	1.60
Water	19	32	8	32	1.10	1.48

Table 10.6 Effects of treating soil with selected bacteria on damping-off of tomato by C. praticola

Bacterium	Final seedling stand		Fresh wt seedling (g)	
	+ <u>Cp</u>	No <u>Cp</u>	+ <u>Cp</u>	No <u>Cp</u>
1 - B26	33	39	1.90	1.89
2 - B3	27	36	1.56	1.84
2 - B2	27	35	1.46	1.87
2 - B14	22	40	1.19	1.71
2 - B1	21	35	1.33	1.78
Water	10	33	1.10	1.50

This series of experiments thus illustrated that bacterial antagonists of C. praticola were more effective when applied to soil than to seed. It could be that applying them to soil 7 days before sowing allowed them to compete successfully with the population of C. praticola. This was investigated in the next experiment.

Summary

- (i) The bacterial antagonists of Corticium praticola on cucumber seed gave some control of Pythium damping-off in non-sterile soil, but the control obtained was not comparable with that of the bacterial antagonists specifically selected against Pythium.
- (ii) The bacterial antagonists of C. praticola also gave moderate control of pre-emergence damping-off of tomato by S. rolfsii, but only isolate 2 - B2 and 2 - B3 gave good protection to seedlings from post-emergence damping-off.
- (iii) These bacteria were more effective against damping-off of cucumber and tomato by C. praticola when applied to soil than to seed.

11. MECHANISM OF CONTROL OF CORTICIUM PRATICOLA DAMPING-OFF OF LETTUCE

This experiment investigated the mechanism of control of damping-off of lettuce by inoculating soil infested with C. praticola with selected bacteria.

Suspensions of isolates 2 - B1 (1.2×10^8 cells/ml), 2 - B2 (2.4×10^8 cells/ml), 2 - B3 (1.4×10^8 cells/ml), 2 - B14 (2.2×10^8 cells/ml) and 1 - B26 (2.7×10^8 cells/ml) were prepared as described in Section 8A. Pots were filled with non-sterile soil and the upper layer was either infested with 1% ^w/w of oatmeal sand culture of the pathogen or not. For treatments involving bacteria the top of the soil was sprayed with 40ml of the bacterial suspension whereas control pots were treated with sterile distilled water. The pots were randomly arranged on the glasshouse bench and incubated for 7 days before lettuce seeds were planted. Each treatment was replicated three times.

Soil samples for the quantitative estimation of C. praticola were collected from the pots 7 and 28 days after inoculation. Five cores of soil (5cm long) were taken from each replicate within a treatment and these were bulked and mixed in a wide-neck, polypropylene bottle by agitating in a Whirlimixer before taking subsamples for the preparation of soil suspensions.

C. praticola was estimated in these samples by a method essentially similar to that of Ko and Hora (1971) for determining Rhizoctonia except that in the medium Metazoxolon [4 - (3 - Chlorophenylhydrazone) - 3 methyl - 5 isoxazolono] ICI was used instead of Dexon because this could not be obtained in the UK. Poured plates were kept in the dark for 12h before use.

Soil plates were prepared as follows. A subsample of 0.5g soil was moistened with 2 drops ($2 \times 10 \mu\text{l}$) of sterile distilled water, compacted with a spatula and then divided into ten soil aggregates on a plate of Ko and Hora's medium. Ten plates per treatment were used, i.e. 100 soil

aggregates per treatment. Each soil aggregate was examined microscopically after 48h at 25° and C. praticola was identified by its morphological characters (Butler and Bracker, 1970). The number of soil aggregates per plate with C. praticola growing from them was thus a measure of the propagules per 0.05g (fresh weight) soil. The results are given as an average for 10 replicates and as propagules per g of soil on dry weight basis.

The number of antibiotic-producing bacteria in the rhizosphere of lettuce seedlings was estimated by the method described by Neal (1971). Essentially the method is a bilayer technique. For the first layer, 15ml portions of potato dextrose agar containing 50ppm Acti-dione was poured into petridishes and allowed to dry overnight at 30°C. Eight, approximately equidistant, circular areas were marked lightly on the agar with a sterile cork borer (1cm diameter). The rhizosphere soil was prepared as described in Section 4 and a 10-fold dilution-series of the soil was made. A 0.1ml pipette (graduated in hundredths) was used to deliver eight replicate 0.01ml samples from each dilution to the centre of the prescribed circles on the medium. After 2h the plates were inverted and incubated at 30°C. Plates showing growth after 96h were then covered with a second agar layer, consisting of 10ml PDA containing 100ppm streptomycin. After the second layer hardened, the plates were inverted, and the growth inhibiting substances were allowed to diffuse into the layer overnight at 30°C before placing a 5mm disk of C. praticola centrally on the agar surface. The plates were then inverted and incubated at 30°C for 96h. The number of zones in which C. praticola was inhibited was then counted and from these counts the number of antibiotic-producing bacteria were estimated by referring to the plate dilution-frequency response table given in Harris and Sommers (1968). Numbers were adjusted to a dry soil weight basis.

The results (Fig. 6) showed that, except for soil with isolate 1 - B26, the population of C. praticola was lower but not significantly in soils

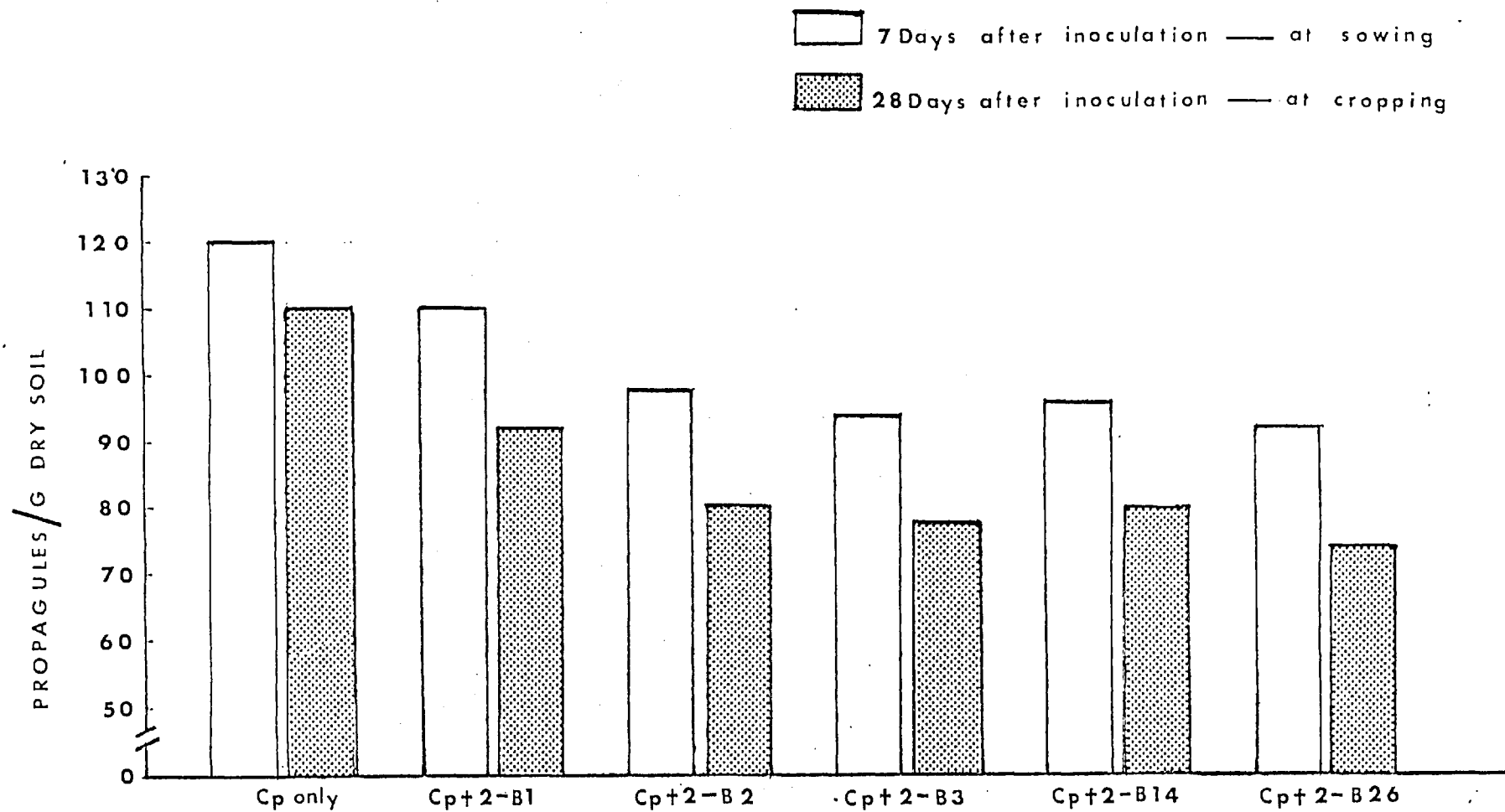


Fig 6. Population of C. praticola (Cp) in soil inoculated with Cp + bacteria.

Table 11.1 Control of damping-off of lettuce by inoculating soil infested with C. praticola (Cp) with selected bacteria

Bacterium	Final seedling stand		Fresh wt seedling (g)	
	+ <u>Cp</u>	No <u>Cp</u>	+ <u>Cp</u>	No <u>Cp</u>
1 - B26	28	39	0.30	0.32
2 - B3	27	41	0.30	0.31
2 - B14	26	38	0.26	0.30
2 - B2	24	39	0.27	0.30
2 - B1	14	37	0.20	0.29
Water Control	4	36	0.19	0.29

Table 11.2 Number of antibiotic producing bacteria in the rhizosphere of lettuce seedlings

Bacterium	Nos. of bacteria/g dry soil ($\times 10^4$)*	
	+ <u>C. praticola</u>	No <u>C. praticola</u>
2 - B1	1.02	4.22
2 - B2	2.28	5.81
2 - B3	4.22	10.20
2 - B14	3.06	5.81
1 - B26	4.22	10.20
Water	0	0

* For details of method, see text P.

treated with bacteria than the controls with C. praticola alone when lettuce seed was planted. This possibly accounts for the control of damping-off obtained (Table 11.1). Significantly, the seedling stand in infested soil treated with isolate 2 - B1 was lower than in the other bacterial treatments and this could be related to the highest population of C. praticola recorded from this soil in comparison to other bacterial treatments.

After 28 days the population of C. praticola declined still further (Fig. 6) but in soil treated with isolate 2 - B1 it remained higher than in soil with the other bacteria and was still significantly lower in soil treated with isolate 1 - B26 than in the controls with C. praticola alone. No C. praticola was detected in pots with soil only by the method used.

Estimates of antibiotic-producing bacteria (Table 11.2) suggested that the rhizosphere of lettuce seedlings was colonized by the bacterial isolates added to soil. Isolate 2 - B1 appeared least effective in this respect and this could have contributed to its relatively poor control of damping-off. Generally, the development of these bacteria was more profuse in the rhizosphere of seedlings in soil without C. praticola.

Summary

The population of C. praticola was decreased when bacterial antagonists were applied as soil drenches to infested soil. This factor together with the population build up of bacteria in the rhizosphere of lettuce seedlings possibly account for the control of damping-off obtained.

12. IDENTIFICATION OF BACTERIAL ANTAGONISTS

Attempts were made to identify the bacterial isolates that controlled the damping-off fungi in non-sterile soil.

Pure cultures of each of the eight bacterial isolates were transferred to and incubated on nutrient agar slants for 48h. Suspensions were prepared from these cultures and these were streaked onto nutrient agar plates. Inoculated plates were incubated for 2-3 days at 30°C and the colour, shape, size of colonies, their outline, elevation and transparency were observed with aid of the binocular dissecting microscope and a hand lens. The motility of the bacteria was also determined in hanging drops. Preston and Morrell's (1962) modification of Gram's method was used (Cruickshank, 1972), to determine the staining reactions of the bacteria. Gram-staining revealed the presence of colourless areas in the vegetative organisms, giving an indication of possession of endospores by the organisms. To confirm this bacterial films were stained by the method described by Dowson (1957) for spores.

Tests were performed, as detailed below, to determine the physiological characteristics and biochemical activities of the bacteria. All tests were carried out in five replicates per isolate and controls without bacteria were also included in each test. The procedures (with slight modification) used were as given by Hugh and Leifson (1953), Dowson (1957), Bergey (1948), Harrigan and McCane (1976) and the laboratory schedules on bacteriology for the M.Sc. course in Plant Pathology of Department of Botany, Imperial College which are based on Hayward et al. (1961).

(i) Oxidation vs Fermentation of carbohydrates

The medium of Hugh and Leifson (1953) was modified for use as follows: peptone (oxoid), 1.0g; NaCl, 2.5g; distilled water, 450ml. Ninety ml of this basic medium was added to 0.3g agar in a 250ml conical flask and sterilized in an autoclave. A 10% glucose solution was prepared, sterilized

by autoclaving and 10ml of this was added to 90ml basic medium. Bromothymol blue was dissolved in sterile distilled water to give a 1% solution and 0.3ml of this was added to each 100ml glucose-peptone medium and mixed thoroughly. Five ml of the medium + indicator were then dispensed with a sterile pipette into sterile 10ml test tubes and allowed to set. Duplicate tubes were stab-inoculated with each bacterial isolate. The agar in one tube of each pair was covered with a layer of liquid paraffin (BP grade) to a depth 6 to 12mm. Inoculated tubes were incubated at 25° and examined over a period of 14 days for colour changes.

(ii) Hydrolysis of soluble starch, gelatin and casein

The following agar medium was dispensed in 90ml quantities into 250ml flask and sterilized by autoclaving: peptone (oxoid), 5g; yeast extract (Difco), 3g; agar, 20g; distilled water, one litre; adjusted to pH 7.2. Ten ml quantities of sterile 4% (w/v) gelatin; 4% (w/v) casein; or 2% (w/v) soluble starch were added to the molten medium from which plates were poured. The plates were inoculated with the bacterial isolates by streaking. After incubation for 6 days at 25° starch plates were flooded with Gram's iodine; gelatin and casein plates with acid mercuric chloride solution. Zones of hydrolysis were recorded.

(iii) Voges-Proskauer reaction (acetoin production)

The following medium was dispensed in 5ml quantities in 10ml tubes and autoclaved: NaCl, 5g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2g; $\text{NH}_4\text{H}_2\text{PO}_4$, 1g; K_2HPO_4 , 1g; sodium citrate, 2.77g; glucose, 5g; sodium fumarate, 10g; distilled water, one litre. The tubes were stab-inoculated with the bacterial isolates. After incubation for 2 days at 25°C a pinch of creatine was added to each culture followed by one ml 40% KOH. The cultures were shaken vigorously and allowed to stand for 15min. A pink colour indicates dia-acetyl derived from acetoin by oxidation.

(iv) Nitrite formation from nitrate and nitrite destruction

The following medium was used: peptone (oxoid), 10g; K_2HPO_4 , 5g; yeast extract, 1g; KNO_3 , 1g or $NaNO_2$, 0.1g; agar, 3.0g; distilled water, one litre; adjusted to pH 7.0. Ten ml of this medium were dispensed into 20ml McCartney bottles. At 5 days after inoculation with the bacterial isolates, nitrite formation or nitrite destruction was tested by adding 0.5ml each of sulphanilic acid and Dimethyl- α -naphthylamine to each culture. Colour reactions were noted and recorded.

(v) Gas production

Procedures in Bergey's manual of determinative bacteriology (6th Edition) were followed.

(vi) Egg-yolk reaction (Hydrolysis of lecithin)

The procedure in the Harrigan and McCance (1976) was followed.

(vii) Oxidase test

Kovac's method (1956) was used.

(viii) Catalase

Loopfuls of agar growth were emulsified in 10 vol - H_2O_2 on a slide and examined microscopically for evolution of oxygen.

(ix) H_2S production and Ammonia production

The recognised standard methods as described by Dowson (1957) were used.

(x) Indole production

The test solution consisted to 1% tryptophane in a broth of 1% peptone and 0.5% NaCl and this was sterilized in 10ml portions in test-tubes. The tubes were inoculated with an homogenous suspension of the bacteria. Dry strips of filter paper impregnated with oxalic acid were inserted into each tube, and secured in position by cotton wool plugs. The tubes were incubated for five days at $25^\circ C$ after which colour reactions on the paper strips were observed and recorded.

(xi) Action on litmus milk

The medium consisted of 100g litmus milk (oxoid) added to one litre distilled water. This was strained through muslin, and distributed in 10ml portions into tubes and steam sterilized for 5 min at 121°C . When sufficiently cooled, one ml of bacterial suspension was introduced into each tube and incubated at 25°C for 5 days. Colour reactions were observed and recorded.

The results of the tests are summarized in Table 12.1. There were differences between cultural characteristics of the 8 bacterial isolates. In general, after 48h on nutrient agar plates, small round colonies about 0.5-1mm diameter appeared. These colonies grew to a size that varied from 2 to 6mm in diameter after 4-5 days at 30°C . The colour of the colonies on agar varied from slight yellow to cream coloured, becoming brown in some isolates. These colonies were either round or irregular, with a dull or glossy surface; becoming thick and opaque; wrinkled in some isolates and smooth in others.

All the isolates were Gram-positive and the cells were rod-shaped arranged in clusters or grouped in chains. All possessed endospores which were either located centrally or at the terminal end of the cells. They were all motile and capable of fermenting and oxidizing glucose. They are catalase positive. From the results of the Gram-stain, possession of spores and various biochemical tests, it can be concluded that all the bacterial isolates are Bacillus species.

Table 12.1 Characters of bacterial isolates

Characteristics	2 - B1	2 - B2	2 - B3	2 - B14	2 - B24	2 - B27	2 - B46	1 - B26
Shape	Rods in chains	Rods in clusters	Rods in clusters	Rods in clusters	Rods in clusters	Rods in chains	Rods in chains	Rods in clusters
Endospore formation	T	C	T	T	C	C	T	C
Gram-reaction	+	+	+	+	+	+	+	+
Motility	+	+	+	+	+	+	+	+
Metabolism of glucose								
(i) oxidative	+	+	+	+	+	+	+	+
(ii) fermentative	+	+	+	+	+	+	+	+
Acid from mannitol	+	+	+	+	+	+	+	+
Gas production	-	+	+	-	-	-	-	-
Starch hydrolysis	+	+	+	-/+	+	+	+	+
Gelatin hydrolysis	+	+	+	+	-/+	+	-/+	-/+
Casein hydrolysis	+	+	-	+	-	+	+	-
Lecithin hydrolysis (egg yolk reaction)	+	+	+	+	-	+	-	-
Ammonia production	-	-	-	-	-	-	-	-
H ₂ S production	+	+	+	+	+	+	+	+
Acetoin reaction (Voges-Proskauer)	+	+	+	-	+	-	+	+
Oxidase test	+	+	+	-/+	-	-	-	+
Catalase test	+	+	+	+	+	+	+	+
Action on litmus milk	+	+	+	+	+	+	+	+
Action on tryptophane	-	-	-	-	-	-	-	-

KEY

T = Endospore located at terminal end of cell
 C = Endospore located centrally

+ = Positive reaction
 - = No reaction
 -/+ = Variable reaction

DISCUSSION

Although, in this study of biological control, three pathogen-host combinations were used, most work was done with Pythium debaryanum on cucumber and Corticium praticola on lettuce, so the discussion will be concerned largely with these two systems. The preliminary experiments showed some marked differences between them. Although both fungal pathogens readily damped-off seedlings of their respective hosts in sterile compost, larger amounts of P. debaryanum inoculum were required to produce comparable seedling death in non-sterile, Walled Garden soil which itself suggests that this fungus competed less well against indigenous soil micro-flora. In contrast, C. praticola appeared to grow well in this soil and cause damping-off of lettuce at very low concentrations of inoculum.

Despite an extensive search for soils that were suppressive to the selected fungal pathogens, none was found and it was thus not surprising that when these soils were screened in detail for micro-organisms antagonistic to these fungi, relatively few were found. Thus of all the fungal isolates tested on agar for antagonism, only isolates of Trichoderma showed any substantial activity. Similarly, of 929 bacterial cultures obtained from the 34 soils examined, only 73 showed any antagonism on agar media to C. praticola, P. debaryanum or S. rolfsii (Table 2.1). Most of these effective bacteria were isolated from soils collected throughout Windsor Park Forest which represents a more balanced ecological habitat than the other sites. Similar results were obtained by Merriman et al. (1974). They obtained more bacteria and fungi that were antagonistic to Rhizoctonia solani from soils in undisturbed pasture than from soils continuously planted with wheat.

There were further reductions in the selection of bacterial cultures obtained in the agar plate tests when these were examined in sterile compost and subsequently in (non-sterile) soil for the control of damping-off. Thus

only 7 cultures of the 23 that were antagonistic to P. debaryanum on agar, successfully controlled damping-off of cucumber in sterile compost (Table 3.2) and only 3 similarly controlled damping-off in Walled Garden soil (Table 4.1).

The main problems in these situation appear to be establishing contact between the antagonist and the fungal pathogen and to maintain an effective population of the introduced antagonist. Since Pythium becomes particularly active around germinating seeds, treatment of the seed with the antagonist seemed the obvious approach. Experiments using this technique suggested that the control of Pythium damping-off of cucumber in soil depended on the presence of viable bacteria on seed. Treating seed with cell-free filtrate gave poor control either because antifungal materials were removed during filtration or because they were rapidly adsorbed or decomposed in soil.

The numbers of bacteria that can be accommodated on each seed is thus critical. For example, tests with isolate 2 - B46 indicated that cucumber seed soaked in suspensions containing 6×10^8 or 6×10^7 cells per ml gave seedling stands in soil infested with Pythium comparable with the corresponding controls in soil alone; seed soaked in more dilute suspensions did not. Within the range of bacterial suspensions used, the numbers of bacteria on seed were directly related to those in suspension (Fig. 3 and Fig. 5). It might have been possible, therefore, to increase further the population of bacteria on seed. It seems doubtful that this would have increased the control obtained with isolate 2 - B46 (Table 4.5) but it might have improved the control obtained with other isolates.

There is good circumstantial evidence that the three bacterial isolates (2 - B46, 2 - B24, 2 - B27) which effectively controlled damping-off by Pythium in soil were able to colonize the rhizosphere of cucumber seedlings. Thus, there was a direct relationship between the numbers of these bacteria on seed and the numbers of bacteria isolated from the rhizosphere that were

antagonistic to Pythium on agar. The relationship was more marked for seedling roots obtained from Pythium-infested soil (Fig. 4). Confirmation that these rhizosphere bacteria were those derived from treated seed would require a more specific test such as that afforded by the fluorescent antibody technique (Heimer, 1967). However, in their study of biological control of P. debaryanum, Mitchell and Hurwitz (1965) also found coating tomato seed with lytic bacteria (Arthrobacter sp.) isolated from the rhizosphere allowed it to proliferate on the young seedling roots and protected them from attack by this pathogen. Ridge (1970) also suggested that to be effective seed inoculation must result in development of the antagonist in the rhizosphere and that the numbers there would be dependent to some extent on the initial seed inoculum.

While some success was obtained in the control of Pythium damping-off by 3 bacterial isolates the practical value of this method needs to be judged in relation to other factors. One such factor is the degree of control compared with that of a standard fungicide. When tested all 3 bacterial isolates gave seedling stands comparable to seed treated with captan, thiram or drazoxolon (Table 6.1). However, an ideal antagonist should provide protection against other soil-borne fungal pathogens as would a broad-spectrum fungicide such as thiram or captan. In this respect, the three bacteria were less successful. Only one isolate, 2 - B27, when applied to tomato seed resulted in a seedling stand in soil with S. rolfsii comparable to that in soil without the pathogen. None, applied to seeds, controlled C. praticola. All 3 isolates did, however, give some control of Pythium damping-off when used on lettuce and tomato seed but less than with cucumber, probably because fewer bacteria were retained on the smaller seed. Broadbent et al. (1971) also found that antagonists from soil varied in their spectra of activity, inhibiting only one and others all 9 pathogens examined.

A further factor which must be considered in the practical application of seed bacterization is the longevity of the bacteria on treated seed. When treated cucumber seeds were stored at 4°C the viability of the bacteria decreased and so, to a limited extent did the viability of the seed, whether treated or not. The net result was a fall in the percentage control of damping-off from an initial value of 88-92% to 50-67% after 4 weeks' storage (Table 7.1). This plus the problem of drying treated seeds suggest that the method would only be practical if it could be used prior to planting.

In contrast to the results with P. debaryanum and cucumber, no bacterial isolate that was selected for its antagonism on agar to C. praticola controlled damping-off by this fungus in either sterile compost or non-sterile soil when applied to lettuce seed. However, four isolates (2 - B2, 2 - B3, 2 - B14 and 1 - B26) were effective when applied to infested soil, 7 days before planting lettuce (Table 8.2) and these, plus a further isolate (2 - B1) controlled the pathogen when applied as a root dip to young lettuce before transplanting to infested soil (Table 8.4). The success of this latter method depended very largely on the concentration of the bacterial suspension.

There was some indication from direct plating of C. praticola from treated soil that the population of this pathogen was decreased by soil drenches of bacteria (Fig. 6). However, with some bacterial treatments the decline in C. praticola was relatively small and populations remained not significantly different from the untreated control. This suggested that a reduction in the population of C. praticola did not itself account for the control obtained but that a build-up of bacteria in the rhizosphere was also involved. Some evidence was obtained that bacteria antagonistic to C. praticola did develop in the rhizosphere of lettuce seedlings which survived and this, plus the success of the root dip method, suggest that early establishment of the bacteria in the rhizosphere is essential for the

control of C. praticola. Possibly, when applied to lettuce seed, the bacteria fail to colonize the root more rapidly than does C. praticola itself, which apparently is an aggressive pathogen that can rapidly develop even when introduced into non-sterile soils in small quantities.

Tests of the same bacteria with C. praticola and cucumber gave similar results (Tables 10.3 and 10.4); only soil applications gave any control but this was less satisfactory than with lettuce. Tests with tomato gave slightly different results. Here five bacterial isolates showing some activity against C. praticola controlled this pathogen to similar extents when applied to tomato seed or watered on infested soil (Tables 10.5 and 10.6). The key factor in this variability seems to be the virulence of C. praticola to the various hosts. Thus some 30-50% of tomato seed produced seedlings in soil infested with C. praticola whereas few or no seedlings of cucumber developed in this soil without treatments of bacteria (Tables 10.4, 10.5 and 10.6). Control of Pythium damping-off of cucumber by applying the bacteria to seed was relatively poor but initially gave good control of S. rolfsii on tomato. This latter result was not entirely unexpected since these isolates were able to inhibit the growth of S. rolfsii in culture. However, damping-off of older tomato seedlings occurred in infested soil and only seed treatments with isolates 2 - B2 or 2 - B3 eventually gave seedling stands comparable with the corresponding controls without S. rolfsii.

In contrast to the experiments with bacteria, those that attempted biological control with fungal antagonists were relatively disappointing. Although isolates of Trichoderma were strongly antagonistic to P. debaryanum in culture, high levels (20%, 25% and 30%) of Trichoderma in wheat bran were required to give any control of damping-off in infested soil, and incorporation of this into soil without Pythium indicated some adverse effects on seedling growth. There were similar adverse effects when

Trichoderma and C. praticola were combined in experiments on the control of lettuce damping-off. Indeed, Trichoderma in large amounts seemed to increase the amount of damping-off. Experiments to control C. praticola on lettuce with an isolate of Rhizoctonia solani, avirulent on this host, were also not encouraging since large amounts of R. solani were required to give effective control and this fungus also proved to be pathogenic to tomato.

This study with three common damping-off fungi has illustrated some of the pitfalls of seeking a biological control agent. While some of the bacteria initially showed great promise as antagonists, few finally succeeded in controlling the pathogens in non-sterile soil, their spectra of activity were limited and applying them to seed was successful for one disease and not for another. Of the methods examined, the control of P. debaryanum by bacterization of seed appears most promising. It requires further studies on the application of the bacteria to seed and methods for improving viability during storage. Such studies, if pursued, would need to be linked with toxicological studies on the bacteria to ensure that they do not constitute a health hazard to those handling them.

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Appendix 1 Nutrient media

All chemicals used were A.R. grade and all agar media were steamed for 30 min and autoclaved for 15 min at 125°C unless otherwise stated.

(i) Czapek (Dox) agar (CzDA)

Czapek Dox agar (oxoid)	45.4g
Water	1 litre

(ii) Glycerol-casein medium

Glycerol	10g
Casein (vitamin free)	0.3g
KNO ₃	2g
NaCl	2g
K ₂ HPO ₄	2g
MgSO ₄ ·7H ₂ O	0.05g
CaCO ₃	0.02g
FeSO ₄ ·7H ₂ O	0.01g
Agar	20g
Distilled water	1 litre
*Cycloheximide	50mg
*Nystatin	20mg

* Williams and Cross (1974); added after autoclaving the rest medium.

Glycerol was substituted for soluble starch in starch-casein medium used by Küster and Williams (1974).

(iii) Ko and Hora's Medium

K ₂ HPO ₄	1g
MgSO ₄ ·7H ₂ O	0.5g
KCl	0.5g
FeSO ₄ ·7H ₂ O	0.01g
NaNO ₂	0.2g
*Gallic acid	0.4g
Agar	20g
Distilled water	1 litre
*Metazoxolon (ICI)	100 ppm
*Chloramphenicol	50mg
*Streptomycin sulphate	50mg

* Added after sterilizing and before pouring plates.

(iv) King's Medium B

Proteose (Difco)	20g
Peptone (Oxoid)	20g
Ionagar (Oxoid)	12g
Glycerol	8ml
K ₂ SO ₄	1.5g
MgSO ₄ .7H ₂ O	1.5g
Distilled water	940ml
* Penicillin G	75,000 units
* Novobiocin	45mg
* Cycloheximide	75mg

* Were mixed together in 3ml of 95% ethanol, then diluted with 50ml of sterile distilled water and added after autoclaving the rest medium. pH of basal medium was adjusted to 7.2 before sterilizing.

(v) Malt extract agar (MEA)

Dextrose (Oxoid)	20g
Peptone	1g
Malt extract (Oxoid)	20g
Agar	25g
Water	1 litre

(vi) Oatmeal/sand medium

Scott's Porage Oats	49
Washed white sand	196g
Tap water	25ml

The oats and sand were thoroughly mixed c. 25ml water in 500ml Erlenmeyer flasks and autoclaved for 1h at 125°. After autoclaving the flasks were shaken to break up large aggregates.

(vii) Potato dextrose agar (PDA)

Dextrose (Oxoid)	20g
Agar	20g
Potato (peeled and sliced)	200g
Water	1 litre

Prepared as described in the 'Plant Pathologists Pocketbook' and adjusted to pH 6.5

(viii) Potato dextrose agar (Oxoid)

PDA (Oxoid)	39g
Water	1 litre

(ix) Rose bengal agar (RBA)

Dextrose (Oxoid)	10g
Yeast extract	0.5g
K_2HPO_4	0.5g
$MgSO_4 \cdot 7H_2O$	0.5g
Peptone	0.5g
*Rose bengal	0.05g
*Streptomycin sulphate	0.03g
Water	1 litre

* Added after sterilizing and before pouring plates.

(x) Soil extract agar (SEA)

Glucose	1g
K_2HPO_4	0.5g
Agar	15g
Soil extract	900ml
Adjust to pH 6.8-7.0	

One kilogramme of air-dried, sieved garden soil was added to 1 litre of tap water and autoclaved at 125° for 30 min. $CaCO_3$ (c. 2g) was added before vacuum filtering the soil suspension through double filter papers in a Buchner funnel. The filtration was repeated until soil extract became clear. It was then bottled and re-sterilized in 100ml lots.

(xi) V8 juice agar

V8 juice*	100ml
Agar	20g
$CaCO_3$	1.5g
Water	1 litre

* Manufactured by Campell's Soups Ltd, King's Lynn, Norfolk, England.

(xii) Water agar (WA)

Agar	20g
Water	1 litre

(xiii) Yeast mannitol agar

Yeast extract (oxoid)	10g
Mannitol	10g
K_2HPO_4	0.5g
$MgSO_4 \cdot 7H_2O$	0.2g
NaCl	0.1g
$CaCO_3$	3g
Agar	15g
Distilled water	1 litre
Congo red	36mg

Sterilized at (120°C) 15 psi for 20 min. The composition was as given by Fred and Waksman, (1928).

Appendix Table 1.1 Damping-off of lettuce, cucumber and tomato by Cp, Pd and Rs in sterile potting compost.

(a) Lettuce

<u>Treatment</u>	<u>Seedling stand</u>	<u>Total</u>	<u>Mean</u>
Cp	1, 1, 2	4	1.3
Sterile Cp	15, 15, 15	45	15.0
Pd	5, 5, 4	14	4.7
Sterile Pd	15, 15, 15	45	15.0
Rs ₁	15, 15, 15	45	15.0
Sterile Rs ₁	15, 15, 15	45	15.0
Rs ₂	15, 13, 15	43	14.3
Sterile Rs ₂	15, 15, 15	45	15.0
Sterile compost	15, 15, 15	45	15.0

Analysis of variance

Source of variation	df	SS	MS	F
Between groups	8	679.19	84.90	108.85 ^{***}
Within groups	18	14	0.78	
TOTAL	26			

$$SE = 0.51$$

Ranked Mean: 1.3, 4.7, 14.3, 15.0, 15.0, 15.0, 15.0, 15.0, 15.0

Means not underscored by the same line are significantly different ($P \leq 0.01$).

(b) Cucumber

<u>Treatment</u>	<u>Seedling stand</u>	<u>Total</u>	<u>Mean</u>
Cp	0, 0, 0	0	0
Sterile Cp	9, 9, 10	28	9.3
Pd	1, 1, 1	3	1.0
Sterile Pd	10, 9, 9	28	9.3
Rs ₁	9, 8, 7	24	8.0
Sterile Rs ₁	9, 10, 10	29	9.7
Rs ₂	10, 7, 10	27	9.0
Sterile Rs ₂	10, 10, 10	30	10.0
Sterile compost	9, 9, 10	28	9.3

Analysis of variance

Source of variation	df	SS	MS	F
Between groups	8	364.96	45.62	77.32 ^{***}
Within groups	18	10.67	0.59	
TOTAL	26			

$$SE = 0.44$$

Ranked Means: 0, 1.0, 8.0, 9.0, 9.3, 9.3, 9.3, 9.7, 10.0

Means not underscored by the same line are significantly different ($P \leq 0.01$).

(c) Tomato

<u>Treatment</u>	<u>Seedling stand</u>	<u>Total</u>	<u>Mean</u>
Cp	6, 5, 5	16	5.3
Sterile Cp	14, 12, 14	40	13.3
Pd	6, 5, 4	15	5.0
Sterile Pd	11, 14, 12	37	12.3
Rs ₁	9, 8, 10	27	9.0
Sterile Rs ₁	14, 12, 14	40	13.3
Rs ₂	8, 8, 6	22	7.3
Sterile Rs ₂	12, 13, 12	37	12.3
Sterile compost	13, 14, 14	41	13.7

Analysis of variance

Source of variation	df	SS	MS	F
Between groups	8	303.4	37.93	36.47 ^{***}
Within groups	18	18.67	1.04	
TOTAL	26			

$$SE = 0.59$$

Ranked Means: 5.0, 5.3, 7.3, 9.0, 12.3, 12.3, 13.3, 13.3, 13.7

Means not underscored by the same line are significantly different ($P \leq 0.01$).

Appendix Table 1.2 Effects of age and concentration of inoculum on damping-off
by Pd and Cp. Expt 1.

(a) Cucumber/Pd

Inoculum				
Concentration (% W/W)	Age (days)	Seedling stand	Total	Mean
Nil	-	10, 8, 9	27	9.0
	-	8, 10, 8	26	8.7
5	9	3, 2, 1	6	2.0
	15	1, 2, 1	4	1.3
10	9	2, 1, 1	4	1.3
	15	2, 1, 0	3	1.0
15	9	1, 2, 0	3	1.0
	15	1, 0, 0	1	0.3

Analysis of variance

Source of variation	df	SS	MS	F
Treatment	7	269.16	38.45	
Inoculum conc.	3	267.50	89.17	56.44 ^{***}
Inoculum age	1	1.5	1.5	0.95 ns
Age x conc.	4	0.16	0.04	0.03 ns
Residual	8	12.67	1.58	
TOTAL	23			

SE = 0.73

Ranked Means: 0.3, 1.0, 1.0, 1.3, 1.3, 2.0, 8.7, 9.0

Means not underscored by the same line are significantly different ($P \leq 0.01$).

(b) Lettuce/Cp

<u>Inoculum</u>				
Concentration (% ^w /w)	Age (days)	Seedling stand	Total	Mean
Nil	-	15, 15, 12	42	14.0
	-	15, 14, 13	42	14.0
5	9	2, 2, 1	5	1.7
	15	2, 1, 0	3	1.0
10	9	2, 1, 1	4	1.3
	15	1, 1, 0	2	0.7
15	9	1, 0, 1	2	0.7
	15	0, 0, 1	1	0.3

Analysis of variance

Source of variation	df	SS	MS	F
Treatment	7	770.63		
Inoculum conc.	3	769.13	256.38	153.52 ^{***}
Inoculum age	1	1.04	1.04	0.62 ns
Age x conc.	4	0.46	0.12	0.07 ns
Residual	8	13.33	1.67	
TOTAL	23			

$$SE = 0.75$$

Ranked Means: 0.3, 0.7, 0.7, 1.0, 1.3, 1.7, 14.0, 14.0

Means not underscored by the same line are significantly different ($P \leq 0.01$)

Appendix Table 1.3 Effects of age and concentration of inoculum on damping-off of Pd and Cp. Expt 2.

(a) Cucumber/Pd

Inoculum				
Concentration (% ^W /W)	Age (days)	Seedling stand	Total	Mean
Nil	-	8, 10, 8	26	8.7
	-	8, 10, 9	27	9.0
	-	10, 10, 8	28	9.3
5	7	3, 4, 1	8	2.7
	9	2, 1, 1	4	1.3
	11	1, 1, 1	3	1.0
10	7	2, 2, 1	5	1.7
	9	1, 1, 1	3	0.7
	11	1, 0, 1	2	0.7
15	7	1, 2, 2	5	1.7
	9	0, 1, 0	1	0.3
	11	0, 0, 1	1	0.3

Analysis of variance

Source of variation	df	SS	MS	F
Treatment	11	426.31		
Inoculum conc	3	415.86	138.62	206.90 ^{***}
Inoculum age	2	5.06	2.53	3.78 n.s.
Age x conc	6	5.39	0.93	1.34 n.s.
Residual	24	16	0.67	
TOTAL	35			

$$SE = 0.47$$

Ranked Means: 0.3, 0.3, 0.7, 1.0, 1.0, 1.3, 1.7, 1.7, 2.7, 8.7, 9.0, 9.3

Means not underscored by the same line are significantly different ($P \leq 0.01$)

(b) Lettuce/Cp

Inoculum				
Concentration	Age	Seedling		
(% ^w /w)	(days)	stand	Total	Mean
Nil	-	15, 15, 15	45	15.0
	-	15, 15, 15	45	15.0
	-	15, 15, 15	45	15.0
5	7	2, 3, 2	7	2.3
	9	2, 2, 2	6	2.0
	11	1, 3, 0	4	1.3
10	7	1, 2, 0	3	1.0
	9	1, 2, 0	3	1.0
	11	2, 1, 0	3	1.0
15	7	1, 1, 1	3	1.0
	9	0, 1, 1	2	0.7
	11	0, 0, 1	1	0.3

Analysis of variance

Source of Variation	df	SS	MS	F
Treatment	11	1297.64	117.97	
Inoculum conc.	3	1295.42	431.81	814.73 ^{***}
Inoculum age	2	1.06	0.53	1 ns
Age x conc.	6	1.16	0.19	0.35 ns
Residual	24	12.67	0.53	
TOTAL	35			

SE = 0.42

Ranked Means: 0.3, 0.7, 1.0, 1.0, 1.0, 1.0, 1.3, 2.0, 2.3, 15.0, 15.0, 15.0Means not underscored by the same line are significantly different ($P \leq 0.01$).

Appendix Table 1.4 Damping-off of cucumber by Pythium debaryanum (Pd) and lettuce by Corticium praticola (Cp) in non-sterile soil.

(a) Cucumber/Pd

(i) Seedling stand

Inoc. conc. (% ^W/w)

Replicate	Nil	5	10	15	20	25	30
1	9	6	5	3	3	3	3
2	9	5	5	4	3	3	2
3	8	6	4	4	3	2	2
4	8	4	4	3	4	2	2
Total	34	21	18	14	13	10	9
Mean	8.5	5.3	4.5	3.5	3.3	2.5	2.3

ANOVA

SV	df	SS	MS	F
Between groups	6	111	18.5	47.44 ^{***}
Within groups	21	8.25	0.39	
Total	27			

SE = 0.31

Ranked Means: 2.3, 2.5, 3.3, 3.5, 4.5, 5.3, 8.5

Means not underscored by the same line are significantly different ($P \leq 0.01$)

(ii) Seedling fresh wt (g)

Inoc. conc. (% ^W/w)

Replicate	Nil	5	10	15	20	25	30
1	1.0	0.9	1.1	1.2	0.9	0.9	1.0
2	1.2	1.1	1.1	0.9	0.8	0.8	0.9
3	1.1	1.4	1.1	1.1	0.9	1.2	0.8
4	1.3	1.2	1.1	0.9	0.9	0.7	0.9
Total	4.6	4.6	4.4	4.1	3.5	3.6	3.6
Mean	1.2	1.2	1.1	1.0	0.9	0.9	0.9

ANOVA

SV	df	SS	MS	F
Between groups	6	0.36	0.06	3 [*]
Within groups	21	0.41	0.02	
Total	27			

$$SE = 0.07$$

Ranked Means: 0.9, 0.9, 0.9, 1.0, 1.1, 1.2, 1.2

Means not underscored by the same line are significantly different ($P \leq 0.01$).

(b) Lettuce/Cp

(i) Seedling stand

Inoc. conc. (% ^W/w)

Replicate	Nil	5	10	15	20	25	30
1	13	1	1	0	0	0	0
2	14	1	0	0	0	0	0
3	12	0	1	0	0	0	0
4	14	1	0	0	0	0	0
Total	53	3	2	0	0	0	0
Mean	13.3	0.8	0.5	0	0	0	0

ANOVA

SV	df	SS	MS	F
Between groups	6	585.36	97.56	464.57 ^{***}
Within groups	21	4.5	0.21	
Total	27			

$$SE = 0.23$$

Ranked Means: 0, 0, 0, 0, 0.5, 0.8, 13.3

Means not underscored by the same line are significantly different ($P \leq 0.01$).

(ii) Seedling fresh wt (g)

Inoc. conc. (% ^W/w)

Replicate	Nil	5	10	15	20	25	30
1	0.075	0.1	0.1	-	-	-	-
2	0.079	0.1	0.1	-	-	-	-
3	0.092	0.1	0	-	-	-	-
4	0.080	0	0	-	-	-	-
Total	0.326	0.3	0.2	-	-	-	-
Mean	0.082	0.075	0.05	-	-	-	-

ANOVA

SV	DF	SS	MS	F
Between groups	6	0.035	0.0058	6.4
Within groups	21	0.018	0.0009	
Total	27			

$$SE = 0.02$$

Ranked Means: 0.082, 0.075, 0.05

The means are not significantly different.

Appendix Table 1.5 Damping-off of tomato by Sclerotium rolfsii in sterile potting compost.

Inoculum				
Concentration	Age	Seedling	Total	Mean
% (^w / _w)	(days)	Stand		
Nil	-	15, 14, 15	44	14.7
	-	15, 15, 15	45	15.0
5	9	12, 11, 8	31	10.3
	15	12, 9, 10	31	10.3
10	9	11, 9, 9	29	9.7
	15	10, 9, 8	27	9.0
15	9	8, 11, 9	28	9.3
	15	8, 9, 7	24	8.0

Analysis of variance

Source of variation	df	SS	MS	F
Treatment	7	142.63	20.38	6.42
Inoculum concentration	3	139.13	46.38	14.63 ^{***}
Inoculum age	1	1.04	1.04	9.33 ns
Age x concentration	4	2.46	0.62	0.22 ns
Residual	8	25.33	3.17	
Total	23			

SE = 1.03

Ranked Means: 8.0, 9.0, 9.3, 9.7, 10.3, 10.3, 14.7, 15.0

Means not underscored by the same line are significantly different ($P \leq 0.05$).

Appendix Table 2.2 Effects of three bacterial isolates on growth of Pd on PDA at different temperatures (as % reduction in colony diameter relative to control).

(a) 2 - B46

Replicate	Bacterial pre-inoculated in days							
	15°C		20°C		25°C		30°C	
	0	2	0	2	0	2	0	2
1	36	37.5	46.25	55	43.75	58.75	52.05	75
2	37.5	37.5	43.75	56.18	43.75	58.75	48.75	75
3	33.75	37.5	42.5	53.69	43.75	61.25	53.75	75
4	36.25	37.5	43.75	55	43.75	62.5	53.75	75
Total	143.5	150	176.25	218.87	175	241.25	208.75	300
Mean	35.87	37.5	44.06	54.97	43.75	60.31	52.19	75

(b) 2 - B24

1	35	42.5	41.25	56.25	47.5	55	50	61.25
2	33.75	42.5	41.25	56.25	48.75	57.5	50	61.25
3	35	42.5	41.25	56.25	48.75	56.25	50	61.25
4	36.25	42.5	41.25	56.25	50	57.5	50	61.25
Total	140	170	165	225	195	226.25	200	245
Mean	35	42.5	41.25	56.25	48.75	56.56	50	61.25

(c) 2 - B27

1	36.2	48.4	42.5	52.2	45	54	46.6	61.9
2	36.2	48.4	42.5	52.2	43	52	46.6	61.9
3	36.2	48.4	42.5	52.5	46	53.5	46.6	61.9
4	36.2	48.4	42.5	52.2	48.76	54.1	46.6	61.9
Total	144.8	193.6	170	208.8	182.76	213.6	186.4	247.6
Mean	36.2	48.4	42.5	52.2	45.69	53.4	46.6	61.9

Appendix Table 2.3 Effects of five bacterial isolates on growth of Cp on PDA at different temperatures (as % reduction in colony diameter relative to control).

(a) 2 - B1

Bacterial pre-inoculated in days

Replicate	15°C		20°C		25°C		30°C	
	0	2	0	2	0	2	0	2
1	32	42.5	32.5	46.25	36.25	48.75	37.5	55
2	32	42.5	32.5	46.25	36.25	47.5	37.5	56.25
3	32	42.5	32.5	46.25	36.25	47.5	37.5	55
4	32	42.5	32.5	46.25	36.25	48.75	37.5	56.25
Total	128	170	130	185	145	192.5	150	222.5
Mean	32	42.5	32.5	46.25	36.25	48.13	37.5	55.63

(b) 2 - B2

1	32.5	42	37.5	42.5	37.5	42.5	41.25	50
2	32.5	42	37.5	42.5	37.5	42.5	40	50
3	32.5	42	37.5	43.75	37.5	43.75	40	50
4	32.5	42	37.5	43.75	37.5	43.75	40	50
Total	130	168	150	172.5	130	172.5	161.25	200
Mean	32.5	42	37.5	43.12	37.5	43.12	40.31	50

(c) 2 - B3

1	31.25	37.5	35	40	35	43.75	42.5	50
2	31.25	37.5	33.75	41.25	33.75	43.75	42.5	50
3	31.25	37.5	35	41.25	35	43.75	42.5	50
4	31.25	37.5	35	40	35	43.75	42.5	50
Total	125	150	138.75	162.5	138.75	175	170	200
Mean	31.25	37.5	34.69	40.62	34.69	43.75	42.5	50

(d) 1 - B26

1	28.75	32.5	33.75	43.75	33.75	43.75	40	52.5
2	28.75	32.5	33.75	41.25	35	43.75	38.75	50
3	28.75	32.5	33.75	43.75	35	41.25	40	50
4	28.75	32.5	33.75	43.75	35	43.75	40	52.5
Total	115	130	135	172.5	138.75	172.5	158.75	205
Mean	28.75	32.5	33.75	43.12	34.69	43.12	39.69	51.25

(e) 2 - B14

1	26.25	33.75	33.75	41.25	33.75	43.75	41.25	51.25
2	28.75	33.75	32.5	41.25	35	43.75	42.5	51.25
3	30	33.75	33.75	41.25	34	43.75	42.5	51.25
4	30	33.75	32.5	41.25	33.75	43.75	42.5	51.25
Total	115	135	132.5	165	136.5	175	168.75	205
Mean	28.75	33.75	33.12	41.25	34.13	43.75	42.19	51.25

Appendix Table 2.4 Effects of five bacterial isolates on growth of Sr on PDA at different temperatures (as % reduction in colony diameter relative to control).

(a) <u>2 - B1</u>	0°C			
	15°C	20°C	25°C	30°C
Replicate				
1	25	28.75	34.37	40.75
2	25	28.75	35.62	41.5
3	25	28.75	35	41
4	25	28.75	32.49	41.75
Total	100	115	137.42	165
Mean	25	28.75	34.37	41.25
(b) <u>2 - B2</u>				
1	25	30.75	35.62	42.81
2	26	31.50	35.62	42.81
3	24	32.50	35.62	42.81
4	25	30.25	35.62	42.81
Total	100	125	142.48	171.24
Mean	25	31.25	35.62	42.81
(c) <u>2 - B3</u>				
1	28.75	32.5	34	50
2	28.75	32.5	36	50
3	28.75	32.5	35	50
4	28.75	32.5	35	50
Total	115	130	140	200
Mean	28.75	32.5	35	50
(d) <u>1 - B26</u>				
1	20	22.5	31.25	40
2	20	22.5	31.25	40
3	20	22.5	31.25	40
4	20	22.5	31.25	40
Total	80	90	125	160
Mean	20	22.5	31.25	40
(e) <u>2 - B14</u>				
1	14	19.5	28.75	35
2	15	18.76	28.75	34
3	15	17.5	28.75	35
4	15	19	28.75	33.48
Total	59	74.76	115	137.48
Mean	14.75	18.69	28.75	34.37

Appendix to Fig. 2 Population count (number of bacterial cells per ml $\times 10^7$)
of bacterium 1 - B18 grown in nutrient broth.

Replicates	Days of incubation							
	1	2	3	4	5	6	7	8
1	1	10	23	32	49	54	52	45
2	2.7	15	20	36	50	56	54	48
3	4	13	26	35	52	55	55	46
4	5	14	25	35	47	54	55	39
5	4	13	25	35	45	54	54	44
6	4.3	12	28	37	45	55	54	47
7	3.5	14	27	36	46	57	54	46
8	5	11	25	38	46	58	53	46
9	3	11	25	31	46	54	54	45
10	4.5	9	26	35	44	43	55	39
Total	42	122	250	350	470	540	540	445
Mean	4.2	12.2	25	35	47	54	54	44.5

Appendix Table 3.1 Effect of different seed treatments on the control of Pythium damping-off in sterile potting compost.

	Bacterium 1 - Bl8 in			'Controls'		
(a) + <u>Pythium</u>	Sterile water	Sterile Ringer's soln	Nutrient broth culture	Cell-free filtrate	Sterile water	Sterile Ringer's soln
Final seedling	7, 8	7, 7, 6, 6	7, 7, 8, 8	3, 3, 4	2, 2	1, 1, 2, 1
stand	7, 6			3	3, 1	
Total	28	26	30	13	8	5
Mean	7.0	6.5	7.5	3.3	2.0	1.3
(b) No <u>Pythium</u>						
Final seedling	9, 9	8, 10, 10, 9	10, 10, 9	9, 10, 9	9, 9	10, 7, 10
stand	9, 8		9	8	10, 8	9
Total	35	37	38	36	36	36
Mean	8.8	9.3	9.5	9.0	9.0	9.0

ANOVA

SV	df	SS	MS	F
Trt	11	394.67	35.88	
Soil trt	1	243	243	276.44 ~~HEH~~
Seed trt	5	81.17	16.23	18.44 ~~HEH~~
Soil trt x seed trt (inter)	5	70.50	14.10	16.02 ~~HEH~~
Residual	25	22	0.88	
Total	47			

SE = 0.47

Ranked means: 1.3, 2.0, 3.3, 6.5, 7.0, 7.5, 8.8, 9.0, 9.0, 9.0, 9.0, 9.3, 9.5

Means underscored are not significantly different ($P \leq 0.05$).

Appendix Table 3.2 Effects of treating cucumber seed with selected bacteria on Pythium damping-off in sterile compost.

(i) Seedling stand

Test 1

	Bacterium							
(a) + <u>Pythium</u>	3 - B94	2 - B39	2 - B23	1 - B86	2 - B16	1 - B32	3 - B98	Water control
Final seedling	5, 5	5, 5	4, 4	3, 5	3, 3	2, 3	3, 2	1, 1, 2, 1, 2
stand	5	5	5	4	5	3	1	2
Total	15	15	13	12	11	8	6	9
Mean	5.0	5.0	4.3	4.0	3.7	2.7	2.0	1.5

(b) No Pythium

Final seedling	9, 9	10, 9	9, 10	9, 9	9, 10	9, 9	9, 9	10, 9, 9, 9, 9
stand	10	9	10	9	8	9	8	10
Total	28	28	29	27	27	27	26	56
Mean	9.3	9.3	9.7	9.0	9.0	9.0	8.7	9.3

ANOVA

SV	df	SS	MS	F	
Between groups	15	518.37	34.56	84.29 ^{****}	SE = 0.64
Within groups	38	15.5	0.41		
Total	53	533.87			

Ranked Means: 1.5, 2.0, 2.7, 3.7, 4.0, 4.3, 5.0, 5.0, 8.7, 9.0, 9.0, 9.0, 9.3, 9.3, 9.3, 9.7

Means not underscored by the same line are significantly different ($P \leq 0.01$).

Test 2Bacterium(a) + Pythium

	1 - B16	2 - B27	2 - B77	2 - B12	2 - B63	3 - B20	1 - B5	1 - B65	Water control
Final seedling	9, 8	9, 8, 8	9, 9, 7	5, 6	4, 4	4, 4	4, 5	2, 2	1, 1, 2, 1, 1, 1
stand	9			6	6	4	3	3	
Total	26	25	25	17	14	12	12	7	7
Mean	8.7	8.3	8.3	5.7	4.7	4.0	4.0	2.3	1.2

(b) No Pythium

Final seedling	9, 10	10, 10	9, 9, 9	9, 8	9, 9	9, 9	9, 9	8, 9	10, 9, 9
stand	9	9		10	10	10	10	9	10, 8, 10
Total	28	29	27	27	28	28	28	26	56
Mean	9.3	9.7	9.0	9.0	9.3	9.3	9.3	8.7	9.3

ANOVA

SV	df	SS	MS	F	
Between groups	17	515.76	30.34	63.21 ^{***}	SE = 0.48
Within groups	42	20.17	0.48		
Total	59	535.93			

Ranked Means: 1.2, 2.3, 4.0, 4.0, 4.7, 5.7, 8.3, 8.3, 8.7, 8.7, 9.0, 9.0, 9.3, 9.3, 9.3, 9.3, 9.7

Means not underscored by the same line are significantly different ($P \leq 0.01$).

Test 3Bacterium

(a) + <u>Pythium</u>	2 - B46	2 - B24	1 - B4	2 - B31	2 - B33	2 - B60	2 - B28	Water Control
Final seedling	8, 8	8, 8	8, 8	6, 6	5, 6	6, 5	5, 4	2, 2, 1, 1, 1, 2
stand	9	8	8	6	6	5	5	
Total	25	24	24	18	17	16	14	9
Mean	8.3	8.0	8.0	6.0	5.7	5.3	4.7	1.5

(b) No Pythium

Final seedling	9, 9	10, 9	9, 9	9, 9	9, 9	8, 10	8, 9	9, 8, 9, 9, 9
stand	10	10	10	10	9	10	9	8
Total	28	29	28	28	27	28	26	52
Mean	9.3	9.7	9.3	9.3	9.0	9.3	8.7	8.7

ANOVA

SV	df	SS	MS	F	
Between groups	15	345.33	23.02	76.73 ^{REKRE}	SE = 0.55
Within groups	38	11.5	0.30		
Total	53	356.83			

Ranked Means: 1.5, 4.7, 5.3, 5.7, 6.0, 8.0, 8.0, 8.3, 8.7, 8.7, 9.0, 9.3, 9.3, 9.3, 9.3, 9.7

Means not underscored by the same line are significantly different ($P \leq 0.01$).

(ii) Seedling fresh wt (g)

Test 1

Bacterium

(a) + <u>Pythium</u>	3 - B94	2 - B39	2 - B23	1 - B86	2 - B16	1 - B32	3 - B98	Water control
Seedling fresh wt (g)	0.52 0.42 0.50	0.56 0.52 0.50	0.90 0.30 0.37	0.40 0.50 0.50	0.40 0.57 0.34	0.54 0.50 0.50	0.33 0.30 0.65	0.50, 0.50, 0.40 0.50, 0.50, 0.50
Total	1.44	1.58	1.57	1.40	1.31	1.54	1.28	2.90
Mean	0.48	0.53	0.52	0.47	0.44	0.51	0.43	0.48

(b) No Pythium

Seedling fresh wt (g)	0.63 0.51 0.70	0.62 0.74 0.75	0.60 0.60 0.62	0.60 0.57 0.68	0.71 0.53 0.63	0.60 0.64 0.68	0.61 0.59 0.75	0.60, 0.60, 0.69 0.65, 0.72, 0.57
Total	1.84	2.11	1.82	1.85	1.87	1.92	1.95	3.83
Mean	0.61	0.70	0.61	0.62	0.62	0.64	0.65	0.64

ANOVA

SV	df	SS	MS	F
Between groups	15	0.38	0.025	2.27*
Within groups	38	0.42	0.011	
Total	53	0.80		

SE = 0.10

Ranked Means: 0.43, 0.44, 0.47, 0.48, 0.48, 0.51, 0.52, 0.53, 0.61, 0.61, 0.62, 0.62, 0.64, 0.64, 0.65, 0.70

Means not underscored by the same line are significantly different ($P \leq 0.01$).

Test 2Bacterium(a) + Pythium

	1 - B16	2 - B27	2 - B77	2 - B12	2 - B63	3 - B20	1 - B5	1 - B65	Water control
Seedling fresh	0.90	1.02	0.75	0.82	0.95	0.95	0.80	0.82	0.65, 0.60, 0.65, 0.70
wt (g)	0.90	1.08	0.90	0.90	0.55	0.65	0.73	0.91	0.55, 0.65
	0.98	0.98	1.08	0.86	1.03	0.47	0.76	0.98	
Total	2.78	3.08	2.73	2.56	2.53	2.07	2.29	2.71	3.80
Mean	0.93	1.03	0.91	0.86	0.84	0.69	0.76	0.90	0.63

(b) No Pythium

Seedling fresh	0.93	0.95	1.04	1.08	0.90	0.91	0.82	1.04	0.98, 0.96, 1.08
wt (g)	0.85	1.08	0.50	0.90	0.90	0.91	0.84	0.93	0.90, 0.93, 0.90
	1.02	1.65	0.95	1.02	0.95	0.98	1.08	0.89	
Total	2.80	3.68	3.49	3	2.75	2.80	2.74	2.86	5.75
Mean	0.93	1.23	1.16	1.0	0.92	0.93	0.91	0.91	0.96

ANOVA

SV	df	SS	MS	F	
Between groups	17	1.29	0.076	3.62 ^{***}	SE = 0.14
Within groups	42	0.89	0.021		
Total	59	2.18			

Ranked Means: 0.63, 0.69, 0.76, 0.84, 0.86, 0.90, 0.91, 0.91, 0.92, 0.93, 0.93, 0.95, 0.96, 1.0, 1.03, 1.16, 1.23

Means not underscored by the same line are significantly different ($P \leq 0.01$).

Test 3

Bacterium

(a) + <u>Pythium</u>	2 - B46	2 - 24	1 - B4	2 - 31	2 - 33	2 - 60	2 - 28	Water control
Seedling fresh	1.8	2.24	2.1	1.42	1.6	1.5	2	0.66, 1.05, 0.75
wt (g)	1.96	2.03	1.82	1.4	1.47	1.46	1.71	1.0, 0.8, 0.78
	2.18	1.96	1.93	1.71	1.99	1.95	1.62	
Total	5.94	6.23	5.75	4.53	5.06	4.91	5.33	5.04
Mean	1.98	2.08	1.92	1.51	1.69	1.64	1.78	0.84

(b) No Pythium

Seedling fresh	2.33	2	2	1.71	1.8	1.9	2.06	1.78, 1.64, 1.34
wt (g)	2.11	2.35	1.95	1.68	2.02	1.72	1.69	1.46, 1.5, 1.5
	2.2	2.06	1.99	1.74	1.75	1.7	2.06	
Total	6.64	6.41	5.94	5.13	5.57	5.32	5.81	9.22
Mean	2.21	2.14	1.98	1.71	1.86	1.77	1.94	1.54

ANOVA

SV	df	SS	MS	F
Between groups	15	7.37	0.495	17.68 ^{***}
Within groups	38	1.06	0.028	
Total	53	8.43		

SE = 0.17

Ranked Means: 0.84, 1.51, 1.54, 1.64, 1.69, 1.71, 1.77, 1.78, 1.86, 1.92, 1.94, 1.98, 1.98, 2.08, 2.14, 2.21

Means not underscored by the same line are significantly different ($P \leq 0.01$).

Appendix Table 3.3 Effects of treating lettuce seed with selected bacteria on damping-off of C. praticola (Cp) in sterile
(i) Seedling stand compost.

Test 1

	Bacterium							
(a) + Cp	2 - B76	2 - B19	2 - B53	1 - B26	2 - B77	2 - B46	1 - B7	Water control
Final seedling stand	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	1, 0, 1, 0, 1, 1
Total	0	0	0	0	0	0	0	4
Mean	0	0	0	0	0	0	0	0.7
(b) No Cp								
Final seedling stand	12, 13 12	8, 6 8	6, 3, 6	9, 8 9	7, 7 6	3, 2, 3	1, 1, 1	14, 15, 15, 13 13, 14
Total	37	22	15	26	20	8	3	84
Mean	12.3	7.3	5.0	8.7	6.7	2.7	1.0	14.0

ANOVA

SV	df	SS	MS	F	
Between groups	15	1373.17	91.54	208.05 ^{XXXX}	SE = 0.66
Within groups	38	16.66	0.44		
Total	53	1389.83			

Ranked Means: 0, 0, 0, 0, 0, 0, 0, 0.7, 1.0, 2.7, 5.0, 6.7, 7.3, 8.7, 12.3, 14.0

Means not underscored by the same line are significantly different ($P \leq 0.01$).

Test 2

	Bacterium								
(a) + Cp	2 - B14	1 - B26	2 - B2	2 - B1	3 - B63	1 - B13	2 - B3	3 - B19	Water control
Final seedling	1, 2, 1	1, 1, 1	1, 1, 0	0, 0	0, 0	0, 0	0, 0	0, 0	1, 0, 0, 1, 2, 0
stand				0	0	0	0	0	
Total	4	3	2	0	0	0	0	0	4
Mean	1.3	1.0	0.7	0	0	0	0	0	0.7
(b) No Cp									
Final seedling	2, 3	12, 13	9, 7	6, 7	3, 3	6, 8	14, 10	6, 5	15, 15
stand	3	12	7	7	1	8	12	5	12, 13, 15, 14
Total	8	37	23	20	7	22	36	16	84
Mean	2.7	12.3	7.7	6.7	2.3	7.3	12.0	5.3	14.0

ANOVA

SV	df	SS	MS	F	
Between groups	17	1491.4	87.73	113.94 ^{***}	SE = 0.88
Within groups	42	32.33	0.77		
Total	59	1523.73			

Ranked Means: 0, 0, 0, 0, 0, 0.7, 0.7, 1.0, 1.3, 2.3, 2.7, 5.3, 6.7, 7.3, 7.7, 12.0, 12.3, 14.0

Means not underscored by the same line are significantly different ($P \leq 0.01$).

(ii) Seedling fresh wt (g)

Test 1

Bacterium

(a) + Cp	2 - B76	2 - B19	2 - B53	1 - B26	2 - B77	2 - B46	1 - B7	Water control
Seedling fresh	-, -	-, -	-, -	-, -	-, -	-, -	-, -	0.07, 0.07, 0.07, -, -
wt (g)	-	-	-	-	-	-	-	0.08
Total	-	-	-	-	-	-	-	0.29
Mean	-	-	-	-	-	-	-	0.05
(b) No Cp								
Seedling fresh	0.16	0.18	0.15	0.16	0.16	0.16	0.17	0.16, 0.16, 0.16,
	0.16	0.16	0.14	0.16	0.16	0.16	0.17	0.16, 0.16, 0.16
wt (g)	0.17	0.16	0.15	0.16	0.16	0.16	0.17	
Total	0.49	0.50	0.44	0.48	0.48	0.48	0.51	0.96
Mean	0.16	0.17	0.15	0.16	0.16	0.16	0.17	0.16

ANOVA

SV	df	SS	MS	F	
Between groups	15	0.31	0.02	66.67 ^{***}	SE = 0.017
Within groups	38	0.01	0.0003		
Total	53	0.42			

Ranked Means: 0.05, 0.15, 0.16, 0.16, 0.16, 0.16, 0.16, 0.17

Means not underscored by the same line are significantly different ($P \leq 0.01$).

Test 2

Bacterium

(a) + Cp

	2 - B14	1 - B26	2 - B2	2 - B1	3 - B63	1 - B13	2 - B3	3 - B19	Water control
Seedling fresh	0.10	0.08	0.08	-, -	-, -	-, -	-, -	-, -	0.08, 0.06, -
	0.10	0.01							
wt (g)	0.07	0.06	0.01	-	-	-	-	-	-, -, 0.07
Total	0.27	0.24	0.18	-	-	-	-	-	0.21
Mean	0.09	0.08	0.06	-	-	-	-	-	0.04

(b) No Cp

Seedling fresh	0.1	0.1	0.1	0.1	0.12	0.1	0.1	0.15	0.1, 0.1
	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.12	0.1, 0.1, 1.15, 0.16
wt (g)	0.1	0.1	0.1	0.1	0.11	0.09	0.1	0.12	
Total	0.3	0.3	0.3	0.3	0.33	0.29	0.3	0.39	0.71
Mean	0.1	0.1	0.1	0.1	0.11	0.10	0.1	0.13	0.12

ANOVA

SV	df	SS	MS	F	
Between groups	17	0.127	0.0075	15.63 ^{***}	SE = 0.022
Within groups	42	0.02	0.00048		
Total	59	0.147			

Ranked Means: 0.04, 0.06, 0.08, 0.09, 0.1, 0.1, 0.1, 0.1, 0.1, 0.1, 0.11, 0.12, 0.13

Means not underscored by the same line are significantly different ($P \leq 0.01$).

Appendix Table 3.4 Effects of different suspending fluids on the bacterization of lettuce seed. (Tested against C. praticola (Cp) damping-off in sterile potting compost).

(i) Seedling stand

(a) + Cp	Bacterium 2 - Bl4 in			'Controls'			
	Water	Ringer's soln	Nutrient broth	Cell-free filtrate	Water	Ringer's soln	Nutrient broth
Final seedling	1, 2, 1	1, 1, 1	1, 1, 1	0, 0, 0	0, 1, 1	0, 0, 0	0, 0, 0
stand	1	1	1	0	0	0	0
Total	5	4	4	0	2	0	0
Mean	1.3	1.0	1.0	0	0.5	0	0

(b) No Cp

Final seedling	15, 15	15, 15	7, 6, 6	15, 14	14, 11	15, 14	12, 12
stand	14, 14	14, 13	5	14, 12	13, 13	15, 14	14, 12
Total	58	57	24	55	51	58	50
Mean	14.5	14.3	6.0	13.8	12.8	14.5	12.5

ANOVA

SV	df	SS	MS	F
Between groups	13	2266.71	174.36	348.72 ^{***}
Within groups	42	21	0.5	
Total	55	2287.71		

SE = 0.35

Ranked Means: 0, 0, 0, 0.5, 1.0, 1.0, 1.3, 6.0, 12.5, 12.8, 13.8, 14.3, 14.5, 14.5

Means not underscored by the same line are significantly different ($P \leq 0.01$).

(ii) Seedling fresh wt (g)

(a) + Cp	Bacterium 2 - B14 in			'Controls'			
	Water	Ringer's soln	Nutrient broth	Cell-free filtrate	Water	Ringer's soln	Nutrient broth
Seedling fresh	0.05, 0.06	0.05, 0.05	0.05, 0.05	-, -	-, 0.05	-, -	-, -
wt (g)	0.05, 0.06	0.05, 0.05	0.05, 0.05	-, -	-, 0.05	-, -	-, -
Total	0.22	0.20	0.20	-	0.10	-	-
Mean	0.06	0.05	0.05	-	0.03	-	-
(b) No Cp							
Seedling fresh	0.1, 0.15	0.12, 0.12	0.1, 0.1	0.1, 0.1	0.12, 0.12	0.12, 0.10	0.1, 0.1
wt (g)	0.12, 0.16	0.1, 0.1	0.1, 0.1	0.1, 0.1	0.10, 0.10	0.11, 0.11	0.1, 0.1
Total	0.53	0.44	0.40	0.40	0.44	0.44	0.40
Mean	0.13	0.11	0.10	0.10	0.11	0.11	0.10

ANOVA

SV	df	SS	MS	F	
Between groups	13	0.116	0.0089	63.57 ^{***}	SE = 0.0059
Within groups	42	0.006	0.00014		
Total	55	0.122			

Ranked Means: 0.03, 0.05, 0.05, 0.06, 0.10, 0.10, 0.10, 0.11, 0.11, 0.11, 0.13

Means not underscored by the same line are significantly different ($P \leq 0.01$).

Appendix Table 3.5 The effect of a Trichoderma sp. on the incidence of damping-off of cucumber by P. debaryanum (Pd) in sterile compost.

(i) Seedling stand

	% Trichoderma added (W/W)							% sterile bran added (W/W)		
(a) + Pd	Nil	5	10	15	20	25	30	5	10	15
Emerged seedling	2, 2, 2	4, 3	5, 5	5, 7	9, 9	9, 7	7, 10	0, 0	0, 0	0, 0
stand	1, 1, 1	2	6	5	6	9	9	0	0	0
Total	9	9	16	17	24	25	26	0	0	0
Mean	1.5	3.0	5.3	5.7	8.0	8.3	8.7	0	0	0

(b) No Pd

Emerged seedling	9, 9, 9	9, 9	10, 10	10, 10	9, 10	10, 10	10, 10	10, 9	9, 9	9, 9
stand	9, 8, 10	10	10	10	10	8	8	9	9	9
Total	54	28	30	30	29	28	28	28	27	27
Mean	9.0	9.3	10.0	10.0	9.7	9.3	9.3	9.3	9.0	9.0

ANOVA

SV	df	SS	MS	F	
Between groups	19	878.45	46.23	72.23 ^{***}	SE = 0.8
Within groups	46	29.5	0.64		
Total	65	907.95			

Ranked Means: 0, 0, 0, 1.5, 3.0, 5.3, 5.7, 8.0, 8.3, 8.7, 9.0, 0.9, 9.0, 9.3, 9.3, 9.3, 9.3, 9.7, 10.0, 10.0

Means not underscored by the same line are significantly different ($P \leq 0.01$).

Appendix Table 3.5 (Cont)

	% Trichoderma added (^w /w)							% sterile bran added (^w /w)		
(a) + Pd	Nil	5	10	15	20	25	30	5	10	15
Survived seedling stand	2, 2, 2 1, 1, 1	4, 2 2	5, 5 5	5, 7 5	9, 6 9	8, 7 9	7, 9 9	0, 0 0	0, 0 0	0, 0 0
Total	9	8	15	17	24	24	25	0	0	0
Mean	1.5	2.7	5.0	5.7	8.0	8.0	8.3	0	0	0
(b) No Pd										
Survived seedling stand	9, 10, 8 9, 9, 9	9, 9 10	10, 10 10	10, 10 10	9, 10 10	10, 10 8	10, 10 8	10, 9 9	9, 9 9	9, 9 9
Total	54	28	30	30	29	28	28	28	27	27
Mean	9.0	9.3	10.0	10.0	9.7	9.3	9.3	9.3	9.0	9.0
<u>ANOVA</u>										
SV	df	SS	MS	F						
Between groups	19	881.61	46.40	80.00 ^{***}	SE = 0.76					
Within groups	46	26.83	0.58							
Total	65	908.44								
Ranked Means: 0, 0, 0, 1.5, 2.7, 5.0, 5.7, 8.0, 8.0, 8.3, 9.0, 9.0, 9.0, 9.3, 9.3, 9.3, 9.3, 9.7, 10.0, 10.0										

Means not underscored by the same line are significantly different ($P \leq 0.01$).

Appendix Table 3.5 (Cont) (ii) Seedling fresh wt (g)

	% Trichoderma added (W/w)							% sterile bran added (W/w)		
	Nil	5	10	15	20	25	30	5	10	15
(a) + Pd										
Seedling fresh wt (g)	0.85	1.5	1.4	1.18	1.35	1.14	1.03	-	-	-
	0.8	1.0	1.33	1.1	1.19	1.3	1.16	-	-	-
	1.0	1.25	1.35	1.2	1.28	1.06	0.32	-	-	-
	1.10									
	1.10									
	1.0									
Total	5.85	3.78	4.08	3.48	3.82	3.50	3.51	-	-	-
Mean	0.98	1.26	1.36	1.16	1.27	1.17	1.17	-	-	-
(b) No Pd										
Seedling fresh wt (g)	1.61	1.5	1.68	1.55	1.34	1.25	1.19	1.71	1.69	2.07
	1.22	1.5	1.51	1.79	1.28	1.27	1.30	1.4	1.88	2.24
	1.79	1.7	1.51	1.45	1.42	1.42	1.12	1.46	1.78	1.96
	1.22									
	1.21									
	1.24									
Total	8.29	4.79	4.70	4.79	4.04	3.94	3.61	4.57	5.35	6.27
Mean	1.38	1.57	1.57	1.60	1.35	1.31	1.20	1.52	1.78	2.09

ANOVA

SV	df	SS	MS	F
Between groups	19	18.66	0.982	51.68***
Within groups	46	0.88	0.019	
Total	65	19.54		

SE = 0.14

Ranked Means: 0.98, 1.16, 1.17, 1.17, 1.20, 1.26, 1.27, 1.31, 1.35, 1.36, 1.38, 1.52, 1.56, 1.56, 1.60, 1.78, 2.09Means not underscored by the same line are significantly different ($P \leq 0.01$).

Appendix Table 3.6 The effect of a Trichoderma sp. on the incidence of damping-off of lettuce by C. praticola (Cp) in sterile compost.

(i) Seedling stand

	% Trichoderma added (W/w)							% Sterile bran		
	Nil	5	10	15	20	25	30	5	10	15
(a) + Cp										
Emerged seedling	1, 2, 1, 0	6, 3	5, 4	10, 13	10, 10	7, 11	7, 8	0, 0	0, 0	0, 0
stand	2, 0	5	8	12	13	10	11	0	0	0
Total	6	14	17	35	33	28	26	0	0	0
Mean	1.0	4.7	5.7	11.7	11.0	9.3	8.7	0	0	0

(b) No Cp

Emerged seedling	15, 15, 15	15, 14	14, 14	14, 13	15, 12	13, 13	12, 12	11, 11	10, 9	7, 8
stand	15, 15, 15	12	15	14	14	12	10	14	8	11
Total	90	41	43	41	41	38	34	36	27	26
Mean	15.0	13.7	14.3	13.7	13.7	12.7	11.3	12.0	9.0	8.7

ANOVA

SV	df	SS	MS	F
Between groups	19	1813.09	95.43	57.84 ^{****}
Within groups	46	76	1.65	
Total	65	1889.09		

SE = 1.28

Ranked Means: 0, 0, 0, 1.0, 4.7, 5.7, 8.7, 8.7, 9.0, 9.3, 11.0, 11.3, 11.7, 12.0, 12.7, 13.7, 13.7, 13.7, 14.3, 15.0

Means not underscored by the same line are significantly different ($P \leq 0.01$).

Appendix Table 3.6 (Cont)

	% Trichoderma added (^w / _w)							% sterile bran added (^w / _w)		
(a) + Cp	Nil	5	10	15	20	25	30	5	10	15
Survived seedling	1, 2, 1, 0	3, 2	3, 5	5, 5	4, 3	1, 3	1, 1	0, 0	0, 0	0, 0
stand	2, 0	2	5	7	2	2	4	0	0	0
Total	6	7	13	17	9	6	6	0	0	0
Mean	1.0	2.3	4.3	5.7	3.0	2.0	2.0	0	0	0
(b) No Cp										
Survived seedling	15, 15, 15	15, 14	14, 14	14, 13	15, 12	13, 13	12, 12	11, 11	10, 9	7, 8
stand	15, 15, 15	12	15	14	14	12	10	14	8	11
Total	90	41	43	41	41	38	34	36	27	26
Mean	15.0	13.7	14.3	13.7	13.7	12.7	11.3	12.0	9.0	8.7

ANOVA

SV	df	SS	MS	F
Between groups	19	2134.86	112.36	112.36 ^{***}
Within groups	46	50.67	1.10	
Total	65	2185.53		

SE = 1.05

Ranked Means: 0, 0, 0, 1.0, 2.0, 2.0, 2.3, 3.0, 4.3, 5.7, 8.7, 9.0, 11.3, 12.0, 12.7, 13.7, 13.7, 14.3, 15.0

Means not underscored by the same line are significantly different ($P \leq 0.01$).

Appendix Table 3.6

	(ii) Seedling fresh wt (g)							% sterile bran added (^w /w)		
	% Trichoderma added (^w /w)									
(a) + Cp	Nil	5	10	15	20	25	30	5	10	15
Seedling fresh wt (g)	0.5	0.4	0.7	0.65	0.6	0.5	0.5	-	-	-
	0.68	0.8	0.6	0.75	0.5	0.5	0.4	-	-	-
	-	0.6	0.64	0.70	0.8	0.5	0.6	-	-	-
	0.74									
	-									
	0.58									
Total	2.5	1.8	1.94	2.10	1.90	1.50	1.5	-	-	-
Mean	0.42	0.6	0.65	0.70	0.63	0.5	0.5	-	-	-
(b) No Cp										
Seedling fresh wt (g)	0.8	0.52	0.56	0.75	0.5	0.5	0.4	0.67	0.65	0.6
	0.7	0.7	0.75	0.8	0.55	0.05	0.46	0.75	0.07	0.6
	0.75	0.75	0.80	0.67	0.64	0.55	0.35	0.7	0.65	0.6
	0.8									
	0.7									
	0.75									
Total	4.50	1.97	2.11	2.22	1.69	1.55	1.21	2.12	2.0	0.18
Mean	0.75	0.66	0.70	0.74	0.56	0.52	0.40	0.71	0.67	0.6

ANOVA

SV	df	SS	MS	F
Between groups	19	3.56	0.19	9.5 ^{***}
Within groups	46	0.81	0.02	
Total	65	4.37		

SE = 0.14

Ranked Means: 0.40, 0.42, 0.50, 0.50, 0.52, 0.56, 0.60, 0.60, 0.63, 0.65, 0.66, 0.67, 0.70, 0.70, 0.71, 0.74, 0.75Means not underscored by the same line are significantly different ($P \leq 0.01$).

Appendix Table 4.1 Effects of treating cucumber seed with selected bacteria on Pythium damping-off in non-sterile soil.

(i) Seedling stand

	Bacterium							
(a) + <u>Pythium</u>	2 - B46	2 - B24	2 - B27	1 - B16	1 - B18	2 - B77	1 - B4	Water control
Final seedling stand	8, 7, 7	8, 6, 7	7, 7, 7	5, 4 6	4, 6 5	4, 4 5	4, 3 3	3, 3, 3, 2 2, 3
Total	22	21	21	15	14	13	10	16
Mean	7.3	7.0	7.0	5.0	4.7	4.3	3.3	2.7
(b) No <u>Pythium</u>								
Final seedling stand	9, 8, 8	9, 8, 8	8, 8, 8	7, 8 8	7, 8 8	8, 8 7	7, 7 8	7, 8, 8 8, 7, 9
Total	25	25	24	23	23	23	22	47
Mean	8.3	8.3	8.0	7.7	7.7	7.7	7.3	7.8

ANOVA

SV	df	SS	MS	F	
Between groups	15	203.75	13.58	30.86 ^{***}	SE = 0.66
Within groups	38	16.84	0.44		
Total	53	220.59			

Ranked Means: 2.7, 3.3, 4.3, 4.7, 5.0, 7.0, 7.0, 7.3, 7.3, 7.7, 7.7, 7.7, 7.8, 8.0, 8.3, 8.3

Means not underscored by the same line are significantly different ($P \leq 0.01$).

(ii) Seedling fresh wt (g)

Bacterium

(a) + <u>Pythium</u>	2 - B46	2 - B24	2 - B27	1 - B16	1 - B18	2 - B77	1 - B4	Water control
Seedling fresh wt (g)	2.33	1.9	1.84	1.83	1.6	2.05	1.33	1.33, 1.37
	2.33	2.2	2.0	2.3	2	1.4	1.67	1.5, 1.77
	2.25	2.0	2.2	1.76	1.76	1.84	1.55	1.5, 1.6
Total	6.91	6.10	6.04	5.89	5.36	5.29	4.55	9.07
Mean	2.30	2.03	2.01	1.96	1.78	1.76	1.52	1.51
(b) No <u>Pythium</u>								
Seedling fresh wt (g)	2.49	2.5	2.17	2.15	1.94	1.93	1.83	1.97, 1.88
	2.63	2.3	2.13	2.1	2.1	2.07	1.99	1.87, 1.86
	2.32	2.16	2.1	2.1	2.0	2.06	2.13	2.08, 1.81
Total	7.44	6.96	6.40	6.35	6.04	6.06	5.93	11.67
Mean	2.48	2.32	2.13	2.12	2.01	2.02	1.98	1.95

ANOVA

SV	df	SS	MS	F	
Between groups	15	3.77	0.251	9.30 ^{***}	SE = 0.16
Within groups	38	1.06	0.027		
Total	53	4.83			

Ranked Means: 1.51, 1.52, 1.76, 1.78, 1.95, 1.96, 1.98, 2.01, 2.01, 2.02, 2.03, 2.12, 2.13, 2.30, 2.32, 2.48

Means not underscored by the same line are significantly different ($P \leq 0.01$).

Appendix Table 4.2 Bacteria on seed (B) in relation to suspension (A)

(i) Number of bacteria per ml of suspension (A)

	Bacterium in		
	Water	Ringer's soln	Nutrient broth
(a) <u>2 - B46</u>			
Replicate	80, 73, 78, 80, 80 75, 79, 75, 78, 82	60, 62, 58, 65, 55 57, 60, 60, 63, 60	200, 100, 190, 110, 220 80, 180, 120, 160, 140
Total ($\times 10^7$)	780	600	1500
Mean ($\times 10^8$)	7.8	6.0	15
(b) <u>2 - B24</u>			
Replicate	43, 40, 39, 45, 40 30, 36, 46, 40, 41	29, 36, 38, 40, 31 32, 39, 35, 36, 34	84, 97, 98, 86, 98 97, 90, 85, 81, 84
Total ($\times 10^7$)	400	350	900
Mean ($\times 10^8$)	4	3.5	9.0
(c) <u>2 - B27</u>			
Replicate	44, 36, 50, 49, 37 46, 34, 40, 48, 36	38, 54, 32, 62, 49 52, 57, 59, 40, 47	86, 70, 74, 90, 90 85, 80, 96, 84, 95
Total ($\times 10^7$)	420	490	850
Mean ($\times 10^8$)	4.2	4.9	8.5

(ii) Number of bacteria ($\times 10^6$) on seed before air-drying (B_1)

Seed treated with bacterium in			
(a) <u>2 - B46</u>	Water	Ringer's soln	Nutrient broth
Replicate	3.0, 2.0, 2.6, 2.4, 4.1 2.3, 3.9, 3.6, 3.0, 3.0	2.0, 1.6, 2.3, 3.3, 2.6 1.8, 3.8, 3.6, 2.0, 2.0	6.0, 4.5, 3.5, 3.0, 4.0 5.6, 4.7, 3.0, 3.6, 4.3
Total	30	25	42
Mean	3.0	2.5	4.2
(b) <u>2 - B24</u>			
Replicate	0.9, 0.7, 1.1, 2.6, 1.7 1.8, 1.4, 1, 1.2, 1.6	1.0, 0.8, 0.8, 1.2, 1.6 2.0, 1.7, 1.4, 0.7, 0.8	3.0, 3.1, .30, 3.0, 3.5 3.6, 4.0, 3.8, 4.2, 3.8
Total	14	12	35
Mean	1.4	1.2	3.5
(c) <u>2 - B27</u>			
Replicate	0.8, 0.9, 1.2, 1.8, 2.6 2, 1.4, 1.9, 2.1, 1.3	1.6, 1.6, 2.0, 2.4, 2.0 2.6, 2.3, 2.9, 2.2, 1.4	3.6, 3.6, 4.0, 4.2, 3.8 4.5, 4.2, 3.6, 3.6, 3.9
Total	16	21	39
Mean	1.6	2.1	3.9

(iii) Number of bacteria ($\times 10^6$) on seed after air-drying (B_2)

	Seed treated with bacterium in		
(a) <u>2 - B46</u>	Water	Ringer's soln	Nutrient broth
Replicate	2.0, 2.4, 1.4, 1.6, 1.2 3.0, 1.8, 1.8, 2.4, 2.4	1.1, 1.2, 1.8, 2.0, 1.3 1.6, 1.2, 2.2, 1.6, 2.0	3.2, 2.7, 2.9, 2.3, 2.3 4.2, 3.5, 3.9, 3.0, 2.0
Total	20.0	16.0	30.0
Mean	2.0	1.6	3.0
(b) <u>2 - B24</u>			
Replicate	1.0, 0.8, 0.6, 1.2, 1.0 1.0, 1.4, 1.2, 0.8, 1.0	0.9, 0.8, 1.2, 0.9, 1.1 0.9, 1.0, 0.8, 0.6, 0.8	2.0, 2.3, 2.1, 1.8, 2.7 3.0, 2.6, 3.2, 3.3, 1.0
Total	10	9.0	23.0
Mean	1.0	0.9	2.3
(c) <u>2 - B27</u>			
Replicate	1.1, 0.9, 0.8, 1.2, 1.3 0.6, 1.2, 1.5, 1.4, 1.0	1.7, 1.8, 0.9, 1.2, 1.3 1.6, 1.2, 1.5, 1.4, 1.4	2.5, 2.1, 2.9, 3.0, 2.6 2.5, 2.8, 3.2, 2.4, 2.0
Total	11.0	14.0	26.0
Mean	1.1	1.4	2.6

Appendix Table 4.3 Effect of different methods of inoculating cucumber seed with bacteria on control of Pythium damping-off

(A) Seedling stand (i) 2 - B46

	Bacterium in			'Controls'		
(a) + <u>Pythium</u>	Sterile water	Sterile Ringer's soln	Nutrient broth	Cell-free filtrate	Sterile water	Sterile Ringer's soln
Final seedling stand	8, 7, 7	7, 7, 7	8, 8, 7	3, 3, 4	2, 2, 1	3, 2, 2
Total	22	21	23	10	5	7
Mean	7.3	7.0	7.7	3.3	1.7	2.3
(b) No <u>Pythium</u>						
Final seedling stand	9, 8, 8	9, 9, 7	9, 9, 8	9, 7, 8	8, 8, 8	8, 8, 7
Total	25	25	26	24	24	23
Mean	8.3	8.3	8.7	8.0	8.0	7.7

ANOVA

SV	df	SS	MS	F	
Between groups	11	210.97	19.18	45.67 ^{***}	SE = 0.37
Within groups	24	10	0.42		
Total	35	220.97			

Ranked Means: 1.7, 2.3, 3.3, 7.0, 7.3, 7.7, 7.7, 8.0, 8.0, 8.3, 8.3, 8.7

Means not underscored by the same line are significantly different ($P \leq 0.01$).

(ii) 2 - B24

(a) + <u>Pythium</u>	Bacterium in			'Controls'		
	Sterile water	Sterile Ringer's soln	Nutrient broth	Cell-free filtrate	Sterile water	Sterile Ringer's soln
Final seedling stand	7, 7, 7	7, 7, 6	8, 8, 6	2, 3, 2	1, 2, 2	1, 1, 2
Total	21	20	22	7	5	4
Mean	7.0	6.7	7.3	2.3	1.7	1.3
(b) No <u>Pythium</u>						
Final seedling stand	7, 8, 9	8, 8, 8	8, 9, 8	7, 8, 8	7, 7, 8	7, 7, 8
Total	24	24	25	23	22	22
Mean	8.0	8.0	8.3	7.7	7.3	7.3

ANOVA

SV	df	SS	MS	F	
Between groups	11	230.75	20.98	49.95 ^{***}	SE = 0.37
Within groups	24	10	0.42		
Total	35	240.75			

Ranked Means: 1.3, 1.7, 2.3, 6.7, 7.0, 7.3, 7.3, 7.3, 7.7, 8.0, 8.0, 8.3

Means not underscored by the same line are significantly different ($P \leq 0.01$).

(iii) 2 - B27

	Bacterium in			'Controls'		
	Sterile water	Sterile Ringer's soln	Nutrient broth	Cell-free filtrate	Sterile water	Sterile Ringer's soln
(a) + <u>Pythium</u>						
Final seedling stand	7, 7, 7	7, 7, 7	7, 8, 7	4, 2, 2	2, 2, 1	2, 1, 3
Total	21	21	22	8	5	6
Mean	7.0	7.0	7.3	2.7	1.7	2.0
(b) No <u>Pythium</u>						
Final seedling stand	8, 10, 9	9, 7, 9	8, 8, 10	7, 9, 8	8, 8, 7	8, 8, 8
Total	27	25	26	24	23	24
Mean	9.0	8.3	8.7	8.0	7.7	8.0

ANOVA

SV	df	SS	MS	F	
Between groups	11	238.89	21.72	32.42 ^{XXXX}	SE = 0.47
Within groups	24	16	0.67		
Total	35	254.89			

Ranked Means: 1.7, 2.0, 2.7, 7.0, 7.0, 7.3, 7.7, 8.0, 8.0, 8.3, 8.7, 9.0

Means not underscored by the same line are significantly different ($P \leq 0.01$).

(B) Seedling fresh wt (g) (i) 2 - B46

(a) + <u>Pythium</u>	Bacterium in			'Controls'		
	Sterile water	Sterile Ringer's soln	Nutrient broth	Cell-free filtrate	Sterile water	Sterile Ringer's soln
Seedling fresh wt (g)	1.62	1.80	1.96	1.7	1.88	1.6
	2.49	2.25	2.96	2.06	2.25	1.45
	2.12	2.0	2.0	1.98	1.34	1.9
Total	6.23	6.05	6.92	5.74	5.47	4.98
Mean	2.08	2.02	2.31	1.91	1.82	1.65

(b) No Pythium

Seedling fresh wt (g)	2.10	2.20	2.58	2.0	2.0	1.67
	2.40	2.0	2.20	2.0	2.0	1.97
	1.93	2.0	2.72	1.85	1.72	2.0
Total	6.43	6.30	7.50	5.85	5.72	5.64
Mean	2.14	2.10	2.50	1.95	1.91	1.88

ANOVA

SV	df	SS	MS	F	
Between groups	11	1.68	0.153	1.72 n.s.	SE = 0.17
Within groups	24	2.13	0.089		
Total	35	3.81			

Ranked Means: 1.65, 1.82, 1.88, 1.91, 1.91, 1.95, 2.02, 2.08, 2.10, 2.14, 2.31, 2.50

Means not underscored by the same line are significantly different ($P \leq 0.01$).

(ii) 2 - B24

(a) + <u>Pythium</u>	Bacterium in			'Controls'		
	Sterile water	Sterile Ringer's soln	Nutrient broth	Cell-free filtrate	Sterile water	Sterile Ringer's soln
Seedling fresh wt (g)	2.01 1.46 1.8	1.7 1.4 2.3	1.84 1.7 1.62	1.42 1.62 1.85	1.18 1.2 1.25	1.5 1.4 1.3
Total	5.27	5.40	5.16	4.89	3.63	4.20
Mean	1.76	1.80	1.72	1.63	1.21	1.40
(b) No <u>Pythium</u>						
Seedling fresh wt (g)	2.2 2.08 1.95	1.98 1.93 2	2.14 2.2 1.9	1.78 1.93 1.98	2 1.46 2.14	1.74 2.1 1.65
Total	6.23	5.91	6.24	5.69	5.60	5.49
Mean	2.08	1.97	2.08	1.89	1.87	1.83

ANOVA

SV	df	SS	MS	F
Between groups	11	2.2	0.20	4 ^{***}
Within groups	24	1.19	0.05	
Total	35	3.39		

SE = 0.13

Ranked Means: 1.21, 1.40, 1.63, 1.72, 1.76, 1.80, 1.83, 1.87, 1.89, 1.97, 2.08, 2.08

Means not underscored by the same line are significantly different ($P \leq 0.01$).

(iii) 2 - B27

	Bacterium in			'Controls'		
(a) + <u>Pythium</u>	Sterile water	Sterile Ringer's soln	Nutrient broth	Cell-free filtrate	Sterile water	Sterile Ringer's soln
Seedling fresh wt (g)	1.9 1.89 1.86	2 1.92 2.33	2.17 2.08 1.9	1.21 1.67 2.19	1.23 1.75 1.46	1.49 1.44 1.65
Total	5.65	6.25	6.15	5.07	4.44	4.58
Mean	1.88	2.08	2.05	1.69	1.48	1.53
(b) No <u>Pythium</u>						
Seedling fresh wt (g)	2.07 1.93 2.2	1.97 1.87 2.5	2.08 2.25 2.43	2.28 1.75 2.25	1.9 1.79 1.76	1.59 2.25 1.68
Total	6.20	6.34	6.76	6.28	5.45	5.52
Mean	2.07	2.11	2.25	2.09	1.82	1.84

ANOVA

SV	df	SS	MS	F
Between groups	11	1.99	0.18	3*
Within groups	24	1.54	0.06	
Total	35	3.53		

SE = 0.14

Ranked Means: 1.48, 1.53, 1.69, 1.82, 1.84, 1.88, 2.05, 2.07, 2.08, 2.09, 2.11, 2.25

Means not underscored by the same line are significantly different ($P \leq 0.01$).

Appendix Table 4.4 Bacteria in rhizosphere (R) in relation to seed (S).

2 - B46

Seed inoculated with bacteria in

	Water	Ringer's soln	Nutrient broth
(a) + <u>Pythium</u> (Rp)			
Nos of bacteria in	4, 10, 8	5, 3, 7	14, 10, 15
rhizosphere ($\times 10^4$)	7, 9, 7, 8 10, 12, 10	6, 3, 9, 2 12, 14, 9	15, 8, 12, 14 12, 7, 13
Total	85	70	120
Mean	8.5	7.0	12.0
(b) No <u>Pythium</u> (Ro)			
Nos of bacteria in	10, 10, 12	7, 8, 9, 10	13, 12, 15
rhizosphere ($\times 10^4$)	13, 8, 10, 14, 8, 7, 8	10, 12, 11 7, 12, 11	12, 11, 10, 13 8, 9, 12
Total	100	97	115
Mean	10.0	9.7	11.5

2 - B24

(a) + <u>Pythium</u> (Rp)			
Nos bacteria in	8, 6, 9, 7	1, 1, 4, 6	9, 9, 6, 11
rhizosphere ($\times 10^4$)	1, 6, 2, 1 3, 7	4, 2, 2, 4 7, 5	8, 10, 5, 7 7, 10
Total	50	36	82
Mean	5.0	3.6	8.2

(b) No <u>Pythium</u> (Ro)			
Nos of bacteria in	3, 5, 7, 5	2, 5, 7, 6	8, 8, 9, 10
rhizosphere ($\times 10^4$)	9, 8, 5, 6 10, 8	6, 4, 5, 4 9, 6	8, 7, 6, 7 8, 9
Total	66	54	80
Mean	6.6	5.4	8.0

2 - B27

(a) + <u>Pythium</u> (Rp)			
Nos of bacteria in	6, 3, 6, 7	4, 4, 7, 8	9, 7, 7, 5
rhizosphere ($\times 10^4$)	8, 5, 5, 5 6, 4	7, 9, 3, 2 6, 6	10, 6, 8, 9 4, 5
Total	55	56	70
Mean	5.5	5.6	7.0

(b) No <u>Pythium</u> (Ro)			
Nos of bacteria in	11, 12, 14	8, 9, 7, 8	12, 13, 10, 11
rhizosphere ($\times 10^4$)	15, 10, 9, 8 7, 11, 13	9, 10, 13 10, 10, 12	9, 9, 12, 9 10, 9
Total	110	96	104
Mean	11.0	9.6	10.4

Appendix Table 4.5 Effect of seed inoculation with different inoculum concentrations of bacterium 2 - B46 on control of Pythium damping-off on cucumber.

(A) Seedling stand

	Bacteria in suspension						
	6×10^8	6×10^7	6×10^6	6×10^5	6×10^4	6×10^3	6×10^2
(a) + <u>Pythium</u>							
Final seedling stand	7, 8, 7	7, 6, 7	7, 5, 6	4, 6, 5	5, 4, 4	1, 1, 1	1, 1, 2
Total	22	20	18	15	13	3	4
Mean	7.3	6.7	6.0	5.0	4.3	1.0	1.3
(b) No <u>Pythium</u>							
Final seedling stand	9, 8, 8	8, 8, 8	9, 8, 8	9, 7, 7	6, 8, 8	7, 7, 7	8, 6, 7
Total	25	24	25	23	22	21	21
Mean	8.3	8.0	8.3	7.7	7.3	7.0	7.0

ANOVA

SV	df	SS	MS	F	
Between groups	13	222.29	17.10	65.77 ^{JEJEJE}	SE = 0.29
Within groups	28	7.33	0.26		
Total	41	229.62			

Ranked Means: 1.0, 1.3, 4.3, 5.0, 6.0, 6.7, 7.0, 7.0, 7.3, 7.3, 7.7, 8.0, 8.3, 8.3

Means not underscored by the same line are significantly different ($P \leq 0.01$)

(B) Seedling fresh wt (g)

		Bacteria in suspension						
(a) + <u>Pythium</u>		6×10^8	6×10^7	6×10^6	6×10^5	6×10^4	6×10^3	6×10^2
Seedling fresh wt (g)		1.8	1.74	1.65	1.83	1.82	0.92	0.7
		1.92	1.7	1.64	1.91	1.86	0.5	0.6
		2.25	1.83	1.73	1.8	1.77	0.7	1.0
Total		5.97	5.27	5.02	5.54	5.45	2.12	2.30
Mean		1.99	1.76	1.67	1.85	1.82	0.71	0.77
(b) No <u>Pythium</u>								
Seedling fresh wt (g)		2.2	1.72	1.95	2.07	1.83	1.81	1.78
		1.83	2.39	1.9	1.78	1.95	1.58	1.9
		2.25	2.13	2.09	2.08	2.04	1.66	1.66
Total		6.25	6.24	5.94	5.93	5.82	5.05	5.34
Mean		2.09	2.08	1.98	1.98	1.94	1.68	1.78

ANOVA

SV	df	SS	MS	F
Between groups	13	7.46	0.57	17.27 ^{***}
Within groups	28	0.92	0.033	
Total	41	8.38		

SE = 0.10

Ranked Means: 0.71, 0.77, 1.67, 1.68, 1.76, 1.78, 1.82, 1.85, 1.94, 1.98, 1.98, 1.99, 2.08, 2.09

Means not underscored by the same line are significantly different ($P \leq 0.01$).

Appendix Table 4.5 (Cont)

Number of bacteria per seed

Replicate	Nos of bacteria in suspension						
	6×10^8	6×10^7	6×10^6	6×10^5	6×10^4	6×10^3	6×10^2
1	27×10^5	4×10^5	1×10^5	8×10^4	8×10^3	2×10^2	70
2	20×10^5	4×10^5	1×10^5	5×10^4	6×10^3	4×10^2	60
3	13×10^5	6×10^5	0.9×10^5	3×10^4	7×10^3	0	0
4	16×10^5	8×10^5	1.1×10^5	7×10^4	7×10^3	0	0
5	10×10^5	1×10^5	1×10^5	10×10^4	10×10^3	0	0
6	10×10^5	1×10^5	1×10^5	10×10^4	10×10^3	0	0
Total	96×10^5	24×10^5	6×10^5	43×10^4	48×10^3	6×10^2	135
Mean	16×10^5	4×10^5	1×10^5	7.2×10^4	8×10^3	1×10^2	65

Appendix Table 4.7 The effect of Trichoderma on the incidence of damping-off of cucumber by P. debaryanum (Pd) in non-sterile soil

(A) <u>Seedling stand</u>	% Trichoderma added (^w /w)							% sterile bran added (^w /w)		
(a) + <u>Pd</u>	Nil	5	10	15	20	25	30	5	10	15
Emerged seedling stand	2	3	5	4	8	6	6	3	3	1
	3	4	5	6	8	8	5	1	2	3
	3	5	4	5	6	7	7	2	4	2
	3									
	2									
	3									
Total	16	12	14	15	22	21	18	6	9	6
Mean	2.7	4.0	4.7	5.0	7.3	7.0	6.0	2.0	3.0	2.0
(b) No <u>Pd</u>										
Survived seedling stand	9	8	9	8	9	7	7	3	4	4
	9	8	8	8	7	7	6	4	5	4
	9	8	8	8	9	8	8	6	5	5
	9									
	6									
	8									
Total	50	24	25	24	25	22	21	13	14	13
Mean	8.3	8.0	8.3	8.0	8.3	7.3	7.0	4.3	4.7	4.3

ANOVA

SV	df	SS	MS	F
Between groups	19	327.76	17.25	20.78 ^{****}
Within groups	46	38	0.83	
Total	65	365.76		

SE = 0.91

Ranked Means: 2, 2, 2.7, 3, 4, 4.3, 4.3, 4.7, 4.7, 5.0, 6.0, 7, 7, 7.3, 7.3, 8, 8, 8.3, 8.3, 8.3

Means not underscored by the same line are significantly different ($P \leq 0.01$).

Appendix Table 4.7 (cont)

	% Trichoderma added (W/w)							% sterile bran added (W/w)		
	Nil	5	10	15	20	25	30	5	10	15
(a) + <u>Pd</u>										
Survived seedling stand	2	3	5	4	8	6	3	3	3	1
	3	4	5	6	6	8	5	2	2	3
	3	5	4	5	7		7	1	4	2
	3									
	2									
	3									
Total	16	12	14	15	21	21	15	6	9	6
Mean	2.7	4.0	4.7	5.0	7.0	7.0	5.0	2.0	3.0	2.0
(b) No <u>Pd</u>										
Survived seedling stand	9	8	9	8	9	7	7	3	4	4
	9	8	8	8	7	7	6	4	5	4
	9	8	8	8	9	8	8	5	5	5
	9									
	6									
	8									
Total	50	24	25	24	25	22	21	13	14	13
Mean	8.3	8.0	8.3	8.0	8.3	7.3	7.0	4.3	4.7	4.3

ANOVA

SV	df	SS	MS	F
Between groups	19	325.02	17.11	18.20 ^{***}
Within groups	46	43.34	0.94	
Total	65	368.36		

SE = 0.97

Ranked Means: 2, 2, 2.7, 3, 4, 4.3, 4.3, 4.7, 4.7, 5, 5, 7, 7, 7, 7.3, 8, 8, 8.3, 8.3, 8.3Means not underscored by the same line are significantly different ($P \leq 0.01$).

Appendix Table 4.7 (Cont) (B) Seedling fresh wt (g)

		% Trichoderma added (^w /w)						% sterile bran added (^w /w)										
		Nil	5	10	15	20	25	30	5	10	15							
(a) + <u>Pd</u>	Seedling fresh wt (g)	1.41	1.33	2.03	1.7	1.6	1.12	0.9	0.9	0.9	1.3							
		1.63	1.63	2.1	2	1.67	1.26	1.1	0.6	1.1	1.2							
		1.2	1.68	1.81	1.5	1.58	1.36	0.89	0.9	1.02	0.8							
		1.78																
		1.4																
		1.35																
	Total	8.76	1.55	1.98	1.73	1.62	1.25	0.96	0.80	1.01	1.10							
	Mean	1.46	1.55	1.98	1.73	1.62	1.25	0.96	0.80	1.01	1.10							
(b) No <u>Pd</u>	Seedling fresh wt (g)	1.48	1.75	1.9	1.92	1.51	1.28	1.2	1.37	1.5	0.8							
		1.79	1.6	2	1.6	1.53	1.28	1.07	2	1.5	1.4							
		2.09	1.57	1.79	1.85	1.93	1.58	1.06	1.15	1.43	1.35							
		1.5																
		1.87																
		2.01																
	Total	10.74	4.92	5.69	5.37	4.96	4.14	3.33	4.52	4.43	3.55							
	Mean	1.79	1.64	1.89	1.79	1.65	1.38	1.11	1.51	1.48	1.19							
<u>ANOVA</u>																		
SV	df	SS	MS	F														
Between groups	19	6.70	0.35	8.13 ^{***}	SE = 0.21													
Within groups	46	1.98	0.043															
Total	65	8.68																

Ranked Means:

0.8, 0.96, 1.01, 1.10, 1.11, 1.18, 1.25, 1.38, 1.46, 1.48, 1.51, 1.55, 1.62, 1.64, 1.65, 1.73, 1.78, 1.79, 1.89, 1.98

Means not underscored by the same line are significantly different ($P \leq 0.01$).

Appendix Table 4.8 Effect of Trichoderma on Pythium damping-off 7 days after soil inoculation before planting.

(A) Seedling stand

	% Trichoderma added (W/w)							% sterile bran added (W/w)		
(a) + <u>Pythium</u>	Nil	5	10	15	20	25	30	5	10	15
Final seedling stand	3	7	5	6	8	9	7	7	6	5
	2	5	5	6	9	6	6	6	6	5
	3	4	5	7	8	7	6	7	5	5
Total	8	16	15	19	25	22	19	20	17	15
Mean	2.7	5.3	5.0	6.3	8.3	7.3	6.3	6.7	5.7	5.0

(b) No Pythium

Final seedling stand	8	10	9	10	9	8	9	8	8	7
	8	9	10	9	9	9	9	8	7	7
	8	8	9	8	9	9	10	7	7	7
Total	24	27	28	27	27	26	28	23	22	21
Mean	8.0	9.0	9.3	9.0	9.0	8.7	9.3	7.7	7.3	7.0

ANOVA

SV	df	SS	MS	F
Between groups	19	182.98	9.63	18.52 ^{***}
Within groups	40	20.67	0.53	
Total	59	203.65		

SE = 0.42

Ranked Means: 2.7, 5, 5, 5.3, 5.7, 6.3, 6.3, 6.7, 7.0, 7.3, 7.3, 7.7, 8.0, 8.3, 8.7, 9.0, 9.0, 9.0, 9.3, 9.3

Means not underscored by the same line are significantly different ($P \leq 0.01$).

(B) Seedling fresh wt (g)

	% Trichoderma added (^W / _W)							% sterile bran added (^W / _W)		
(a) + <u>Pythium</u>	Nil	5	10	15	20	25	30	5	10	15
Seedling fresh wt (g)	1.75	2.7	3	3.08	3.2	3.24	2.29	3.1	2.17	1.64
	2.77	3	3	2.5	3	3	2.15	2	2.1	2.4
	2.88	3.30	2.82	2.86	2.9	3	2.64	2.65	2.91	2.76
Total	7.4	9	8.92	8.44	9.10	9.24	7.08	7.75	7.18	6.80
Mean	2.47	3.0	2.97	2.81	3.03	3.08	2.36	2.58	2.39	2.27
(b) No <u>Pythium</u>										
Seedling fresh wt (g)	2.63	2.76	3.69	3.4	2.87	3.56	2.78	3.15	2.56	2.87
	2.74	3.19	2.57	3.58	3.29	3.34	3.09	2.6	2.98	2.26
	2.95	2.64	3.17	3.25	3.18	3.11	3.32	2.65	2.44	2.74
Total	8.32	8.59	9.43	10.23	9.34	10.01	9.19	8.40	7.98	7.87
Mean	2.77	2.86	3.14	3.41	3.11	3.34	3.06	2.80	2.66	2.62

ANOVA

SV	df	SS	MS	F
Between groups	19	5.93	0.31	2.58
Within groups	40	4.83	0.12	
Total	59	10.76		

SE = 0.2

Ranked Means:

2.27, 2.36, 2.39, 2.47, 2.58, 2.62, 2.66, 2.77, 2.80, 2.81, 2.86, 2.97, 3, 3.03, 3.06, 3.08, 3.11, 3.14, 3.34, 3.41

Means not underscored by the same line are significantly different ($P \leq 0.01$).

Appendix Table 5.1 Damping-off of tomato by Sclerotium rolfsii in non-sterile soil

(i) Seedling stand

Replicate	Inoc. conc. (% ^w /w)						
	Nil	5	10	15	20	25	30
1	14	10	9	9	7	7	6
2	12	9	9	8	7	6	6
3	12	10	9	9	8	6	6
Total	38	29	27	26	22	19	18
Mean	12.7	9.7	9.0	8.7	7.3	6.3	6.0

ANOVA

SV	df	SS	MS	F	
Between groups	6	93.91	15.65	41.18 ^{***}	SE = 0.36
Within groups	14	5.33	0.38		
Total	20	99.24			

Ranked Means: 6.0, 6.3, 7.3, 8.7, 9.0, 9.7, 12.7

Means underscored by the same line are not significantly different.

(ii) Seedling fresh wt (g)

Replicate	Inoc. conc. (% ^w /w)						
	Nil	5	10	15	20	25	30
1	0.80	0.80	0.95	0.78	0.70	0.57	0.45
2	0.95	0.71	0.68	0.69	0.78	0.81	0.78
3	1.03	1.05	0.70	0.85	0.65	0.70	0.62
Total	2.78	2.56	2.33	2.32	2.13	2.08	1.85
Mean	0.93	0.85	0.78	0.77	0.71	0.69	0.62

ANOVA

SV	df	SS	MS	F	
Between groups	6	0.19	0.032	1.88 n.s.	SE = 0.075
Within groups	14	0.24	0.017		
Total	20				

Ranked Means: 0.62, 0.69, 0.71, 0.77, 0.78, 0.85, 0.93

The means are not significantly different.

Appendix Table 5.2 Damping-off of lettuce and tomato by Pythium debaryanum (Pd) in non-sterile soil

(a) Lettuce/Pd

(i) Seedling stand

Replicate	Inoc. conc. (% ^w /w)						
	Nil	5	10	15	20	25	30
1	11	2	0	0	0	0	0
2	14	3	1	1	1	0	0
3	13	2	2	1	0	0	0
Total	38	7	3	2	1	0	0
Mean	12.7	2.3	1.0	0.7	0.3	0	0

ANOVA

SV	df	SS	MS	F	
Between groups	6	378.47	63.08	101.74 ^{***}	SE = 0.45
Within groups	14	8.67	0.62		
Total	20	387.14			

Ranked Means: 0, 0, 0.3, 0.7, 1.0, 2.3, 12.7

Means underscored by the same line are not significantly different.

(ii) Seedling fresh wt (g)

Replicate	Inoc. conc. (% ^w /w)						
	Nil	5	10	15	20	25	30
1	0.60	0.40	0.50	0.50	0.40	-	-
2	0.50	0.50	0.65	0.40	0	-	-
3	0.50	0.40	0	0	0	-	-
Total	1.60	1.30	1.15	0.90	0.40	-	-
Mean	0.53	0.43	0.38	0.30	0.13	-	-

ANOVA

SV	df	SS	MS	F	
Between groups	4	0.27	0.067	1.36 n.s.	SE = 0.13
Within groups	10	0.49	0.049		
Total	14	0.76			

Ranked Means: 0.13, 0.30, 0.38, 0.43, 0.53

The means are not significantly different.

(Appendix Table 5.2 Cont).

(b) Tomato/Pd(i) Seedling stand

Replicate	Inoc. conc. (% W/W)						
	Nil	5	10	15	20	25	30
1	14	7	6	4	3	2	1
2	14	7	5	4	2	2	1
3	15	8	5	5	2	2	1
Total	43	22	16	13	7	6	3
Mean	14.3	7.3	5.3	4.3	2.3	2.0	1.0

ANOVA

SV	df	SS	MS	F	
Between groups	6	374.48	62.41	260.04 ^{***}	SE = 0.28
Within groups	14	3.33	0.24		
Total	20	377.81			

Ranked Means: 1.0, 2.0, 2.3, 4.3, 5.3, 7.3, 14.3

Means underscored by the same line are not significantly different.

(ii) Seedling fresh wt (g)

Replicate	Inoc. conc. (% W/W)						
	Nil	5	10	15	20	25	30
1	0.80	0.65	0.60	0.60	0.70	0.60	0.60
2	0.69	0.60	0.62	0.60	0.50	0.60	0.60
3	0.92	0.64	0.60	0.60	0.60	0.60	0.60
Total	2.41	1.89	1.82	1.80	1.80	1.80	1.80
Mean	0.80	0.63	0.61	0.60	0.60	0.60	0.60

ANOVA

SV	df	SS	MS	F	
Between groups	6	0.10	0.0167	4.64 ^{***}	SE = 0.035
Within groups	14	0.05	0.0036		
Total	20	0.15			

Ranked Means: 0.60, 0.60, 0.60, 0.60, 0.61, 0.63, 0.80

Means underscored by the same line are not significantly different.

Appendix Table 5.3 Effects of treating seed with bacterial antagonists of Pythium debaryanum on damping-off of lettuce by Corticium praticola (Cp)

(A) Seedling stand

	Bacterium			Water control
	2 - B46	2 - B27	2 - B24	
(a) + Cp	2 - B46	2 - B27	2 - B24	Water control
Seedling stand	3, 5, 3	4, 2, 2	0, 0, 0	0, 0, 0
Total	11	8	0	0
Mean	3.7	2.7	0	0
(b) No Cp				
Seedling stand	15, 14, 15	13, 13, 14	13, 14, 12	13, 12, 15
Total	44	40	39	40
Mean	14.7	13.3	13.0	13.3

ANOVA

SV	df	SS	MS	F	
Between groups	7	900.5	128.64	154.99 ^{XXXX}	SE = 0.53
Within groups	16	13.33	0.83		
Total	23	913.83			

Ranked Means: 0, 0, 2.7, 3.7, 13.0, 13.3, 13.3, 14.7

Means underscored by the same line are not significantly different ($P \leq 0.01$).

(B) Seedling fresh wt (g)

	Bacterium			Water control
	2 - B46	2 - B27	2 - B24	
(a) + Cp	2 - B46	2 - B27	2 - B24	Water control
Seedling fresh wt (g)	0.16, 0.17 0.10	0.10, 1.15 0.10	-, - -	-, - -
Total	0.43	0.35	-	-
Mean	0.14	0.12	-	-
(b) No Cp	0.13, 0.20 0.20	0.17, 0.13 0.10	0.13, 0.13 0.11	0.20, 0.12 0.19
Total	0.53	0.40	0.37	0.51
Mean	0.18	0.13	0.12	0.17

ANOVA

SV	df	SS	MS	F	
Between groups	5	0.012	0.0024	1.4 n.s.	SE = 0.023
Within groups	12	0.014	0.0017		
Total	17	0.026			

Ranked Means: 0.12, 0.12, 0.13, 0.14, 0.17, 0.18

The Means are not significantly different ($P \leq 0.01$).

Appendix Table 5.4 Effects of treating soil with bacterial antagonists of Pythium debaryanum on damping-off of lettuce by Corticium praticola (Cp)

(A) Seedling stand

	Bacterium			Water control
	2 - B46	2 - B27	2 - B24	
(a) + Cp				
Seedling stand	7, 4, 3	1, 2, 1	0, 0, 0	1, 0, 0
Total	14	4	0	1
Mean	4.7	1.3	0	0.3
(b) No Cp				
Seedling stand	15, 14, 14	13, 13, 13	13, 13, 14	12, 14, 14
Total	43	39	40	40
Mean	14.3	13.0	13.3	13.3

ANOVA

SV	df	SS	MS	F	
Between groups	7	395.96	127.99	145.44 ^{***}	SE = 0.54
Within groups	16	14	0.88		
Total	23	409.96			

Ranked Means: 0, 0.3, 1.3, 4.7, 13.0, 13.3, 13.3, 14.3

Means underscored by the same line are not significantly different ($P \leq 0.01$).

(B) Seedling fresh wt (g)

	Bacterium			Water control
	2 - B46	2 - B27	2 - B24	
(a) + Cp				
Seedling fresh wt (g)	0.30, 0.22 0.25	0.20, 0.15 0.20	-, - -	0.13, - -
Total	0.77	0.55	-	0.13
Mean	0.26	0.18	-	0.13
(b) No Cp				
Seedling fresh wt (g)	0.35, 0.46 0.20	0.20, 0.30 0.17	0.20, 0.20 0.23	0.20, 0.22 0.31
Total	1.01	0.67	0.63	0.73
Mean	0.34	0.22	0.21	0.24

ANOVA

SV	df	SS	MS	F	
Between groups	6	0.14	0.023	4.6 ^{***}	SE = 0.041
Within groups	14	0.07	0.005		
Total	20	0.21			

Ranked Means: 0.13, 0.18, 0.21, 0.22, 0.24, 0.26, 0.34

Means underscored by the same line are not significantly different ($P \leq 0.01$).

Appendix Table 5.5 Effects of treating seed with bacterial antagonists of Pythium debaryanum on damping-off by Sclerotium rolfsii (Sr)

(A) Seedling stand

	Bacterium			
	2 - B27	2 - B24	2 - B46	Water control
(a) + Sr				
Seedling stand	14, 15, 10	11, 7, 10	9, 8, 11	7, 6, 6
Total	39	28	28	19
Mean	13.0	9.3	9.3	6.3
(b) No Sr				
Seedling stand	15, 15, 15	14, 14, 15	14, 15, 15	13, 15, 15
Total	45	43	44	43
Mean	15.0	14.3	14.7	14.3

ANOVA

SV	df	SS	MS	F	
Between groups	7	222.96	31.85	15.93 ^{***}	SE = 0.82
Within groups	16	32	2		
Total	23	254.96			

Ranked Means: 6.3, 9.3, 9.3, 13.0, 14.3, 14.3, 14.7, 15.0

Means underscored by the same line are not significantly different ($P \leq 0.05$).

(B) Seedling fresh wt (g)

	Bacterium			
	2 - B27	2 - B24	2 - B46	Water control
(a) + Sr				
Seedling fresh wt (g)	1.06, 0.85 0.96	1.16, 0.90 0.74	1.25, 0.72 0.52	0.64, 0.80 0.90
Total	2.87	2.80	2.49	2.34
Mean	0.96	0.93	0.83	0.78
(b) No Sr				
Seedling fresh wt (g)	1.11, 1.32 1.09	0.76, 1.11 1.19	0.96, 0.93 0.84	0.95, 0.77 0.91
Total	3.52	3.06	2.73	2.63
Mean	1.17	1.02	0.91	0.88

ANOVA

SV	df	SS	MS	F	
Between groups	7	0.35	0.05	1.32 n.s.	SE = 0.11
Within groups	16	0.60	0.038		
Total	23	0.95			

Ranked Means: 0.78, 0.83, 0.88, 0.91, 0.93, 0.96, 1.02, 1.17

The means are not significantly different ($P \leq 0.01$).

Appendix Table 5.6 Control of damping-off of lettuce and tomato by Pythium debaryanum (Pd) with bacterial antagonists of this fungus

(A) Expt 1: Lettuce/Pd

(i) Seedling stand

	Bacterium			
	2 - B46	2 - B24	2 - B27	Water control
(a) + Pd				
Seedling stand	8, 9, 8	8, 8, 8	8, 9, 7	1, 2, 2
Total	25	24	24	5
Mean	8.3	8.0	8.0	1.7
(b) No Pd				
Seedling stand	15, 14, 14	15, 11, 13	13, 14, 13	12, 12, 12
Total	43	39	40	36
Mean	14.3	13.0	13.3	12.0

ANOVA

SV	df	SS	MS	F	
Between groups	7	368.66	52.67	66.67 ^{***}	SE = 0.51
Within groups	16	12.67	0.79		
Total	23	381.33			

Ranked Means: 1.7, 8.0, 8.0, 8.3, 12.0, 13.0, 13.3, 14.3

Means underscored by the same line are not significantly different ($P \leq 0.05$).

(ii) Seedling fresh wt (g)

	Bacterium			
	2 - B46	2 - B24	2 - B27	Water control
(a) + Pd				
Seedling fresh wt (g)	0.39, 1.30 0.35	0.30, 0.30 0.37	0.32, 0.35 0.33	0.25, 0.30 0.30
Total	1.04	0.97	1.00	0.85
Mean	0.35	0.32	0.33	0.29
(b) No Pd				
Seedling fresh wt (g)	0.54, 0.57 0.49	0.45, 0.58 0.44	0.58, 0.48 0.48	0.33, 0.40 0.45
Total	1.60	1.47	1.54	1.18
Mean	0.53	0.49	0.51	0.39

ANOVA

SV	df	SS	MS	F	
Between groups	7	0.19	0.027	10.8 ^{***}	SE = 0.029
Within groups	16	0.04	0.0025		
Total	23	0.23			

Ranked Means: 0.29, 0.32, 0.33, 0.35, 0.39, 0.49, 0.51, 0.53

Means underscored by the same line are not significantly different ($P \leq 0.05$).

(Appendix Table 5.6 Cont)

(B) Expt 2: Tomato/Pd

(i) Seedling stand

	Bacterium			
	2 - B46	2 - B27	2 - B24	Water control
(a) + Pd				
Seedling stand	10, 0, 11	11, 9, 9	8, 9, 8	3, 3, 2
Total	30	29	25	8
Mean	10.0	9.7	8.3	2.7
(b) No Pd				
Seedling stand	15, 13, 14	14, 15, 14	14, 12, 14	11, 15, 12
Total	42	43	40	38
Mean	14.0	14.3	13.3	12.7

ANOVA

SV	df	SS	MS	F	
Between groups	7	319.62	45.66	36.53***	SE = 0.65
Within groups	16	20	1.25		
Total	23	339.62			

Ranked Means: 2.7, 8.3, 9.7, 10.0, 12.7, 13.3, 14.0, 14.3Means underscored by the same line are not significantly different ($P \leq 0.05$).(ii) Seedling fresh wt (g)

	Bacterium			
	2 - B46	2 - B27	2 - B24	Water control
(a) + Pd				
Seedling fresh wt (g)	0.93, 0.80 0.60	0.77, 0.98 0.61	0.70, 0.85 0.77	0.65, 0.65 0.62
Total	2.33	2.36	2.32	1.92
Mean	0.78	0.79	0.77	0.64
(b) No Pd				
Seedling fresh wt (g)	0.90, 0.72 0.98	0.81, 0.84 0.90	0.89, 0.89 0.95	0.69, 0.77 0.92
Total	2.60	2.55	2.73	2.38
Mean	0.87	0.85	0.91	0.79

ANOVA

SV	df	SS	MS	F	
Between groups	7	0.14	0.02	2 n.s.	SE = 0.058
Within groups	16	0.20	0.01		
Total	23	0.34			

Ranked Means: 0.64, 0.77, 0.78, 0.79, 0.79, 0.85, 0.87, 0.91The means are not significantly different ($P \leq 0.01$).

Appendix Table 6.1 Control of Pythium damping-off by bacterial and chemical seed treatments

(A) Seedling stand

<u>Treatment</u>	<u>Seedling stand</u>	<u>Total</u>	<u>Mean</u>
Chemical - Drazoxolon	8, 8, 9	25	8.3
Captan	8, 7, 8	23	7.7
Thiram	7, 7, 8	22	7.3
Bacterium: 2 - B27	8, 7, 7	22	7.3
2 - B46	8, 7, 7	22	7.3
2 - B24	8, 7, 6	21	7.0
Nil - No <u>Pythium</u>	7, 8, 7	22	7.3
+ <u>Pythium</u>	1, 2, 1	4	1.3

ANOVA

SV	df	SS	MS	F	
Between groups	7	102.29	14.61	34.79 ^{***}	SE = 0.37
Within groups	16	6.67	0.42		
Total	23	108.96			

Ranked Means: 1.3, 7.0, 7.3, 7.3, 7.3, 7.3, 7.7, 8.3

Means underscored by the same line are not significantly different ($P \leq 0.05$).

(B) Seedling fresh wt (g)

<u>Treatment</u>	<u>Seedling fresh wt (g)</u>	<u>Total</u>	<u>Mean</u>
Chemical - Drazoxolon	1.89, 1.85, 2.38	6.14	2.05
Captan	2.04, 1.93, 1.93	5.70	1.90
Thiram	1.77, 1.97, 1.70	5.44	1.81
Bacterium: 2 - B27	1.79, 2.13, 2.00	5.92	1.97
2 - B46	1.50, 2.00, 1.95	5.42	1.82
2 - B24	1.95, 1.80, 1.70	5.45	1.82
Nil - No <u>Pythium</u>	1.80, 1.67, 1.71	5.18	1.73
+ <u>Pythium</u>	1.0, 1.0, 1.0	3.0	1.0

ANOVA

SV	df	SS	MS	F	
Between groups	7	2.20	0.31	3.88	SE = 0.16
Within groups	16	1.27	0.08		
Total	23	3.47			

Ranked Means: 1.0, 1.73, 1.81, 1.82, 1.82, 1.90, 1.97, 2.05

Means underscored by the same line are not significantly different ($P \leq 0.05$).

Appendix Table 7.1 Effects of storing cucumber seed treated with bacteria.

(a) Viability of bacteria

<u>2 - B24</u>	Storage period (wk)	Viable bacteria/seed	Total	Mean
	0	$2 \times 10^5, 3 \times 10^5, 19 \times 10^5, 14 \times 10^5, 10 \times 10^5, 10 \times 10^5$	58×10^5	9.7×10^5
	1	$1.9 \times 10^5, 2.5 \times 10^5, 4.6 \times 10^5, 4 \times 10^5, 3 \times 10^5, 3.8 \times 10^5$	19.8×10^5	3.3×10^5
	2	$7 \times 10^4, 9 \times 10^4, 10 \times 10^4, 8 \times 10^4, 10 \times 10^4, 10 \times 10^4$	54×10^4	9×10^4
	4	$8 \times 10^4, 8 \times 10^4, 7 \times 10^4, 7 \times 10^4, 10 \times 10^4, 10 \times 10^4$	50×10^4	8.3×10^4
<u>2 - B27</u>				
	0	$3 \times 10^5, 3 \times 10^5, 16 \times 10^5, 22 \times 10^5, 10 \times 10^5, 10 \times 10^5$	64×10^5	10.7×10^5
	1	$1.5 \times 10^5, 2 \times 10^5, 2 \times 10^5, 2.5 \times 10^5, 2 \times 10^5, 2 \times 10^5$	12×10^5	2×10^5
	2	$9 \times 10^4, 10 \times 10^4, 5 \times 10^4, 5 \times 10^4, 10 \times 10^4, 10 \times 10^4$	49×10^4	8.2×10^4
	4	$8 \times 10^4, 8 \times 10^4, 4 \times 10^4, 6 \times 10^4, 10 \times 10^4, 10 \times 10^4$	46×10^4	7.7×10^4
<u>2 - B46</u>				
	0	$3 \times 10^5, 3 \times 10^5, 29 \times 10^5, 24 \times 10^5, 30 \times 10^5, 20 \times 10^5$	109×10^5	18.2×10^5
	1	$2 \times 10^5, 2.4 \times 10^5, 7.6 \times 10^5, 9 \times 10^5, 5 \times 10^5, 4 \times 10^5$	30×10^5	5×10^5
	2	$7 \times 10^4, 8 \times 10^4, 6 \times 10^4, 2 \times 10^4, 10 \times 10^4, 10 \times 10^4$	43×10^4	7.2×10^4
	4	$7 \times 10^4, 5 \times 10^4, 6 \times 10^4, 4 \times 10^4, 10 \times 10^4, 10 \times 10^4$	42×10^4	7×10^4

(b) Viability of seed

Seedling stand in non-infested soil of seed treated with

	Water				2 - B24				2 - B27				2 - B46			
Storage period (wk)	0	1	2	4	0	1	2	4	0	1	2	4	0	1	2	4
Replicate																
1	8	7	6	5	8	7	7	7	8	7	8	5	8	7	7	6
2	7	7	6	6	8	8	6	6	8	8	6	6	9	9	7	6
3	7	7	7	5	8	7	8	5	7	7	7	6	7	7	6	6
Total	22	21	19	16	24	22	21	18	23	22	21	17	24	23	20	18
Mean	7.3	7.0	6.3	5.3	8.0	7.3	7.0	6.0	7.7	7.3	7.0	5.7	8.0	7.7	6.7	6.0

ANOVA

SV	df	SS	MS	F
Between groups	15	30.47	2.03	4.06
Within groups	32	16.01	0.50	
Total	47	46.48		

SE = 0.41

Ranked Means: 5.3, 5.7, 6.0, 6.0, 6.3, 6.7, 7.0, 7.0, 7.0, 7.3, 7.3, 7.3, 7.7, 7.7, 8.0, 8.0

Means underscored by the same line are not significantly different ($P \leq 0.05$).

(c) Control of Pythium damping-off

Seedling stand in soil infested with Pythium of seed treated with

	<u>2 - B24</u>				<u>2 - B27</u>				<u>2 - B46</u>				<u>Water</u>			
Storage period (wk)	0	1	2	4	0	1	2	4	0	1	2	4	0	1	2	4
Replicate																
1	7	7	5	5	7	5	4	3	7	7	4	3	1	1	1	1
2	7	6	5	3	6	6	5	4	7	7	4	4	2	2	1	1
3	7	6	5	4	8	6	4	3	8	7	4	2	2	2	2	1
Total	21	19	15	12	21	17	13	10	22	21	12	9	5	5	4	3
Mean	7.0	6.3	5.0	4.0	7.0	5.7	4.3	3.3	7.3	7.0	4.0	3.0	1.7	1.7	1.3	1.0

ANOVA

SV	df	SS	MS	F
Between groups	15	221.65	14.78	42.33 ^{***}
Within groups	32	11.33	0.35	
Total	47	232.98		

SE = 0.34

Ranked Means: 1.0, 1.3, 1.7, 1.7, 3.0, 3.3, 4.0, 4.0, 4.3, 5.0, 5.7, 6.3, 7.0, 7.0, 7.0, 7.3

Means underscored by the same line are not significantly different ($P \leq 0.05$).

(d) Fresh wt (g) of emerged seedling

		Mean seedling wt in non-infested soil of seed treated with															
		2 - B24				2 - B27				2 - B46				Water			
Storage period (wk)		0	1	2	4	0	1	2	4	0	1	2	4	0	1	2	4
Replicate																	
1		2.2	1.75	1.92	1.4	2.1	1.7	1.86	1.5	2.3	1.7	1.2	1.24	1.96	1.5	1.0	1.05
2		2	1.5	1.33	1.3	2.2	1.57	1.6	1.4	2.2	1.65	1.7	1.64	2.13	1.4	1.7	1.4
3		2	1.67	1.4	1.3	2.1	1.7	1.2	1.4	2.53	1.8	1.38	1.2	1.63	1.38	1.3	1.3
Total		6.2	4.92	4.65	4.1	6.4	4.97	4.66	4.3	7.03	5.15	4.28	4.08	5.72	4.28	4	3.75
Mean		2.1	1.6	1.6	1.4	2.1	1.7	1.6	1.4	2.3	1.7	1.4	1.4	1.9	1.4	1.3	1.3

ANOVA

SV	df	SS	MS	F
Between groups	15	4.63	0.31	9.7 ^{***}
Within groups	32	1.02	0.032	
Total	47	5.65		

SE = 0.1

Ranked Means: 1.3, 1.3, 1.4, 1.4, 1.4, 1.4, 1.4, 1.6, 1.6, 1.6, 1.7, 1.7, 1.9, 2.1, 2.1, 2.3

Means underscored by the same line are not significantly different ($P \leq 0.05$).

Mean seedling wt in soil infested with Pythium, seed treated with

	2 - B24				2 - B27				2 - B46				Water			
Storage period (wk)	0	1	2	4	0	1	2	4	0	1	2	4	0	1	2	4
1	2.17	1.65	1.1	1	1.95	1.66	1.25	1.15	1.96	1.4	1.6	1.23	1	0.97	1	0.7
2	1.80	1.39	1.35	1.4	1.77	1.6	1.55	1.5	2.1	1.72	1	0.93	1	0.68	0.85	0.7
3	2.0	1.50	1.4	0.9	2.20	1.45	0.9	1	2.2	1.53	0.8	1.1	1.3	1.1	0.5	0.6
Total	5.97	4.54	3.85	3.30	5.92	4.71	3.7	3.65	6.26	4.65	3.4	3.26	3.3	2.75	2.35	2
Mean	2	1.5	1.3	1.1	2	1.6	1.2	1.2	2.1	1.6	1.1	1.1	1.1	0.9	0.8	0.7

ANOVA

SV	df	SS	MS	F
Between groups	15	7.39	0.49	7.10 ^{***}
Within groups	32	2.22	0.069	
Total	47	9.61		

SE = 0.15

Ranked Means: 0.7, 0.8, 0.9, 1.1, 1.1, 1.1, 1.1, 1.2, 1.2, 1.3, 1.5, 1.6, 1.6, 2, 2, 2.1

Means underscored by the same line are not significantly different ($P \leq 0.05$).

Appendix Table 8.1a Effects of treating lettuce seed with selected bacteria on damping-off of lettuce by C. praticola in non-sterile soil

Bacterium	Seedling stand			
	Control	1%	3%	5%
	No <u>Cp</u>	<u>Cp</u>	<u>Cp</u>	<u>Cp</u>
2 - B1	43	2	0	0
2 - B2	42	3	0	0
2 - B3	45	2	0	0
1 - B26	45	3	1	0
2 - B14	41	4	0	0
Water	42	2	0	0

Appendix Table 8.1b Effects of treating lettuce seed with selected bacteria on damping-off of lettuce by C. praticola in non-sterile soil

(A) No. of bacteria per ml suspension

Bacterium	No. of bacteria/ml suspension ($\times 10^7$) (Replicate)	Total ($\times 10^7$)	Mean ($\times 10^8$)
2 - B1	12, 13, 10, 11, 10, 12, 14, 11, 14, 13	120	1.2
2 - B2	20, 20, 28, 30, 24, 25, 24, 23, 21, 25	240	2.4
2 - B3	17, 10, 10, 18, 20, 15, 15, 17, 10, 8	140	1.4
1 - B26	24, 29, 30, 33, 25, 26, 30, 30, 23, 20	270	2.7
2 - B14	20, 30, 20, 23, 18, 14, 27, 29, 19, 20	220	2.2
Water	-	-	-

(B) No. bacteria per seed

Bacterium	No. of bacteria per seed ($\times 10^5$) (Replicate)	Total ($\times 10^5$)	Mean ($\times 10^5$)
2 - B1	2, 1, 1, 0, 0, 1, 2, 1, 1, 1	10	1.0
2 - B2	3, 3, 4, 2, 1, 1, 3, 2, 4, 3	26	2.6
2 - B3	2, 2, 2, 2, 3, 1, 1, 2, 2, 1	18	1.8
1 - B26	2, 3, 4, 4, 3, 3, 2, 2, 1, 1	25	2.5
2 - B14	1, 1, 1, 0, 2, 3, 2, 1, 0, 0	11	1.1

Appendix Table 8.1b (Cont)

(C) Seedling stand

Bacteria

	2 - B1	2 - B2	2 - B3	1 - B26	2 - B14	Water
(a) + 0.5% <u>Cp</u>						
Seedling stand	0, 1, 1	2, 1, 1	1, 1, 1	2, 2, 0	2, 2, 1	1, 1, 1
Total	2	4	3	4	5	3
Mean	0.7	1.3	1.0	1.3	1.3	1.0
(b) + 1% <u>Cp</u>						
Seedling stand	1, 0, 0	1, 1, 1	0, 1, 1	1, 2, 0	1, 1, 1	1, 0, 0
Total	1	3	2	3	3	1
Mean	0.3	1.0	0.7	1.0	1.0	0.3
(c) No <u>Cp</u>						
Seedling stand	15, 14, 15	14, 14, 15	15, 15, 15	15, 15, 15	14, 14, 15	14, 14, 13
Total	44	43	45	45	43	41
Mean	14.7	14.3	15.0	15.0	14.3	13.7

ANOVA

SV	df	SS	MS	F	
Between groups	17	2214.1	130.24	419.35 ^{***}	SE = 0.32
Within groups	36	11.33	0.31		
Total	53	2225.43			

Ranked Means: 0.3, 0.3, 0.7, 0.7, 1.0, 1.0, 1.0, 1.0, 1.0, 1.3, 1.3, 1.7, 13.7, 14.3, 14.3, 14.7, 15.0, 15.0

Means underscored by the same line are not significantly different ($P \leq 0.01$).

Appendix Table 8.2 Control of damping-off of lettuce by inoculating soil infested with Corticium praticola with selected bacteria

(A) Seedling stand	Bacterium					
(a) + <u>Cp</u>	2 - B14	1 - B26	2 - B3	2 - B2	2 - B1	Water
Seedling stand	11, 8, 9	10, 8, 9	9, 9, 8	6, 9, 8	4, 3, 5	1, 2, 2
Total	28	27	26	23	12	5
Mean	9.3	9.0	8.7	7.7	4.0	1.7
(b) No <u>Cp</u>						
Seedling stand	14, 15, 10	11, 12, 11	12, 14, 11	10, 8, 10	7, 9, 9	7, 8, 7
Total	39	34	37	28	25	22
Mean	13.0	11.3	12.3	9.3	8.3	7.3

ANOVA

SV	df	SS	MS	F
Between groups	11	369.56	33.57	19.52 ^{***}
Within groups	24	41.33	1.72	
Total	35	410.89		

SE = 0.75

Ranked Means: 1.7, 4.0, 7.3, 7.7, 8.3, 8.7, 9.0, 9.3, 9.3, 11.3, 12.3, 13.0.

Means not underscored by the same line are significantly different ($P \leq 0.01$).

Appendix Table 8.2 (Cont)

(B) Seedling fresh wt (g)

(a) + <u>Cp</u>	Bacterium					
	2 - B14	1 - B26	2 - B3	2 - B2	2 - B1	Water
Seedling fresh wt (g)	0.4	0.34	0.30	0.33	0.25	0.2
	0.3	0.3	0.32	0.35	0.2	0.2
	0.35	0.3	0.29	0.32	0.35	0.17
Total	1.05	0.94	0.91	1.0	0.80	0.57
Mean	0.35	0.31	0.30	0.33	0.27	0.19
(b) No <u>Cp</u>						
Seedling fresh wt (g)	0.4	0.4	0.5	0.4	0.32	0.32
	0.55	0.4	0.5	0.4	0.4	0.26
	0.5	0.4	0.45	0.36	0.35	0.28
Total	1.45	1.2	1.45	1.16	1.07	0.86
Mean	0.48	0.40	0.48	0.39	0.36	0.29

ANOVA

SV	df	SS	MS	F
Between groups	11	0.24	0.022	13.75 ¹⁰⁰⁰
Within groups	24	0.04	0.0016	
Total	35	0.28		

SE = 0.023

Ranked Means:

0.19, 0.27, 0.29, 0.30, 0.31, 0.33, 0.35, 0.36, 0.39, 0.40, 0.48, 0.48

Means underscored by the same line are not significantly different ($P \leq 0.01$).

Appendix Table 8.3 Damping-off of lettuce seedling by Corticium praticola in sterile potting compost and non-sterile soil

	Inoculum concentration % ^w /w				
	Nil	0.5%	1%	3%	5%
(a) Compost					
Seedling stand	6, 6, 6	1, 0, 2	0, 1, 0	0, 0, 0	0, 0, 0
Total	18	3	1	0	0
Mean	6.0	1.0	0.3	0	0
(b) Non-sterile soil					
Seedling stand	6, 6, 6	2, 1, 2	1, 0, 1	0, 0, 0	0, 0, 0
Total	18	5	2	0	0
Mean	6.0	1.3	0.7	0	0

ANOVA

SV	df	SS	MS	F
Between groups	11	155.37	14.12	83.06 ^{XXXX}
Within groups	24	4	0.17	
Total	35	159.37		

SE = 0.24

Ranked Means: 0, 0, 0, 0.3, 0.7, 1.0, 1.3, 6, 6

Means underscored by the same line are not significantly different ($P \leq 0.05$).

Appendix Table 8.4 Control of C. praticola damping-off in lettuce by dipping seedlings into suspension of selected bacteria.

(A) Seedling stand

(a) + <u>Cp</u>	Bacterium					
	2 - B1	2 - B2	2 - B14	1 - B26	2 - B3	Water
Seedling stand	4	6	5	4	4	0
	6	5	6	5	6	0
	6	5	5	6	4	0
Total	16	16	16	15	14	0
Mean	5.3	5.3	5.3	5.0	4.7	0

(b) No Cp

Seedling stand	6	6	6	6	6	6
	6	6	6	6	6	6
	6	6	6	6	6	6
Total	18	18	18	18	18	18
Mean	6.0	6.0	6.0	6.0	6.0	6.0

ANOVA

SV	df	SS	MS	F
Between groups	11	93.64	8.51	23.64 ^{***}
Within groups	24	8.67	0.36	
Total	35	102.31		

SE = 0.35

Ranked Means: 0, 4.7, 5.0, 5.3, 5.3, 5.3, 6.0, 6.0, 6.0, 6.0, 6.0, 6.0

Means underscored by the same line are not significantly different ($\underline{P} \leq 0.05$).

Appendix Table 8.4 (Cont)

(B) Seedling fresh wt (g)

(a) + <u>Cp</u>	Bacterium					Water
	2 - B1	2 - B2	2 - B14	1 - B26	2 - B3	
Seedling fresh wt (g)	2.83	3.57	2.75	1.8	2.05	-
	1.88	2.0	1.83	2.92	2.28	-
	1.50	1.78	3.25	2.04	2.53	-
Total	6.21	7.35	7.83	6.76	6.86	-
Mean	2.07	2.45	2.61	2.25	2.29	-
(b) No <u>Cp</u>						
Seedling fresh wt (g)	2.92	2.12	3.08	3.05	2.38	1.5
	2.07	3.08	2.38	2.78	2.78	2.25
	1.87	2.75	3.17	2.69	2.85	3.07
Total	6.84	7.95	8.63	8.52	8.01	6.82
Mean	2.28	2.65	2.88	2.84	2.67	2.27

ANOVA

SV	df	SS	MS	F
Between groups	10	2.12	0.21	0.6 n.s.
Within groups	22	7.69	0.35	
Total	32			

SE = 0.34

Ranked Means:

2.07, 2.25, 2.27, 2.28, 2.29, 2.45, 2.71, 2.65, 2.67, 2.84, 2.88The means are not significantly different ($P \leq 0.05$).

Appendix Table 8.5 Control of Corticium praticola damping-off in lettuce by dipping seedlings into suspension of bacterium 2 - B1 at different cell concentrations

(A) Seedling stand

	Concentration of cell/ml of suspension						
(a) + <u>Cp</u>	1.2×10^8	1.2×10^7	1.2×10^6	1.2×10^5	1.2×10^4	1.2×10^3	1.2×10^2
Seedling stand	6, 4, 5	5, 4, 5	4, 4, 4	4, 3, 3	3, 2, 3	2, 2, 1	1, 1, 1
Total	15	14	12	10	8	5	3
Mean	5.0	4.7	4.0	3.3	2.7	1.7	1.0

(b) No Cp

Seedling stand	6, 6, 6	6, 6, 6	6, 6, 5	6, 6, 6	6, 6, 6	6, 6, 6	6, 6, 6
Total	18	18	17	18	18	18	18
Mean	6.0	6.0	5.7	6.0	6.0	6.0	6.0

ANOVA

SV	df	SS	MS	F	
Between groups	13	120.96	9.30	48.95 ^{XXXX}	SE = 0.25
Within groups	28	5.33	0.19		
Total	41	126.29			

Ranked Means: 1.0, 1.7, 2.7, 3.3, 4.0, 4.7, 5.0, 5.7, 6.0, 6.0, 6.0, 6.0, 6.0, 6.0

Means underscored by the same line are not significantly different ($\underline{P} \leq 0.01$).

Appendix Table 8.5 (Cont)

(B) Seedling fresh wt (g)

(a) + <u>Cp</u>	Concentrations of cell/ml of suspension						
	1.2×10^8	1.2×10^7	1.2×10^6	1.2×10^5	1.2×10^4	1.2×10^3	1.2×10^2
Seedling fresh wt (g)	3.06, 2.1 2.43	2.65, 2.2 3.09	2.16, 1.91 3.08	1.86, 2.92 2.08	2, 2.56 1.94	2.43, 1.76 2.24	1.89, 2.2 2
Total	7.59	7.94	7.15	6.86	6.50	6.43	6.09
Mean	2.53	2.65	2.38	2.29	2.17	2.14	2.03
(b) No <u>Cp</u>							
Seedling fresh wt (g)	3.5, 2.85 2.35	2.22, 2.98 2.83	2.75, 1.86 3.19	3.35, 2 1.98	3.02, 2 2.55	3.12, 3 2.03	2.95, 2.43 2.02
Total	8.70	8.03	7.80	7.33	7.57	7.15	7.40
Mean	2.90	2.68	2.60	2.44	2.52	2.38	2.47

ANOVA

SV	df	SS	MS	F	
Between groups	13	2.14	0.16	0.59 n.s.	SE = 0.3
Within groups	28	7.45	0.27		
Total	41	9.59			

Ranked Means: 2.03, 2.14, 2.17, 2.29, 2.38, 2.44, 2.47, 2.52, 2.53, 2.60, 2.65, 2.68, 2.90

The means are not significantly different ($P \leq 0.05$).

Appendix Table 8.6 Control of damping-off of lettuce by Corticium praticola with wheat bran culture of Trichoderma harzianum

(A) Seedling stand

	Trichoderma preparation (% ^w /w)							Sterile wheat bran		
(a) + <u>Cp</u>	Nil	5	10	15	20	25	30	5	10	15
Seedling stand	1	2	0	0	0	0	0	0	0	0
	1	2	0	0	0	0	0	0	0	0
	0	1	0	0	0	0	0	0	0	0
Total	2	5	0	0	0	0	0	0	0	0
Mean	0.7	1.7	0	0	0	0	0	0	0	0

(b) No Cp

Seedling stand	11	11	14	11	11	14	11	15	12	12
	10	13	15	11	14	15	10	13	11	12
	12	14	14	15	15	14	12	13	15	13
Total	33	38	43	37	40	43	33	41	38	37
Mean	11.0	12.7	14.3	12.3	13.3	14.3	11.0	13.7	12.7	12.3

ANOVA

SV	df	SS	MS	F	
Between groups	19	2402.33	126.44	118.17 ^{***}	SE = 0.6
Within groups	40	42.67	1.07		
Total	59	2445.00			

Ranked Means: 0, 0, 0, 0, 0, 0, 0, 0, 0, 0.7, 1.7, 11.0, 11.0, 12.3, 12.3, 12.7, 13.3, 13.7, 14.3, 14.3

Means underscored by the same line are not significantly different ($P \leq 0.05$).

(B) Seedling fresh wt (g)

	Trichoderma preparation (% w/w)							Sterile wheat bran		
	Nil	5	10	15	20	25	30	5	10	15
(a) + <u>Cp</u>										
Seedling fresh wt (g)	0.2	0.2	-	-	-	-	-	-	-	-
	0.2	0.2	-	-	-	-	-	-	-	-
	-	0.2	-	-	-	-	-	-	-	-
Total	0.4	0.6	-	-	-	-	-	-	-	-
Mean	0.20	0.2	-	-	-	-	-	-	-	-
(b) No <u>Cp</u>										
Seedling fresh wt (g)	0.23	0.27	0.23	0.32	0.26	0.26	0.3	0.30	0.23	0.3
	0.27	0.3	0.24	0.3	0.3	0.3	0.3	0.32	0.23	0.3
	0.38	0.25	0.25	0.25	0.28	0.29	0.25	0.32	0.21	0.23
Total	0.88	0.82	0.72	0.87	0.84	0.85	0.84	0.94	0.67	0.83
Mean	0.29	0.27	0.24	0.29	0.28	0.28	0.28	0.31	0.22	0.28

ANOVA

SV	df	SS	MS	F	
Between groups	11	0.09	0.008	4 ^{***}	SE = 0.08
Within groups	24	0.05	0.002		
Total	35	0.14			

Ranked Means: 0.2, 0.2, 0.22, 0.24, 0.27, 0.28, 0.28, 0.28, 0.28, 0.29, 0.29, 0.31

The means are not significantly different ($P \leq 0.05$).

Appendix Table 9.1 Control of Corticium praticola damping-off of lettuce by inoculating infested soil with non-pathogenic isolate of Rhizoctonia solani

(A) Test 1

(i) Seedling stand

	% <u>Rhizoctonia</u> added (^w /w)						
(a) + <u>Cp</u>	Nil	5	10	15	20	25	30
Seedling stand	2	5	5	6	6	7	8
	0	3	4	5	6	7	6
	1	4	5	5	4	5	8
Total	3	12	14	16	16	19	22
Mean	1.0	4.0	4.7	5.3	5.3	6.3	7.3
(b) No <u>Cp</u>							
Seedling stand	10	11	12	11	12	11	11
	12	11	10	11	13	12	13
	11	12	12	12	12	12	11
Total	33	34	34	34	37	35	35
Mean	11.0	11.3	11.3	11.3	12.3	11.7	11.7

ANOVA

SV	df	SS	MS	F
Between groups	13	543.13	41.78	50.34 ^{***}
Within groups	28	23.33	0.83	
Total	41	566.46		

SE = 0.53

Ranked Means:

1.0, 4.0, 4.7, 5.3, 5.3, 6.3, 7.3, 11.0, 11.3, 11.3, 11.3, 11.7, 11.7, 12.3

Means underscored by the same line are not significantly different ($P \leq 0.01$).

Appendix Table 9.1A (Cont)

(ii) Seedling fresh wt (g)

	% Rhizoctonia added (^w /w)						
	Nil	5	10	15	20	25	30
(a) + <u>Cp</u>							
Seedling fresh wt (g)	0.3	0.25	0.3	0.3	0.4	0.3	0.3
	-	0.2	0.3	0.3	0.3	0.35	0.34
	0.2	0.3	0.2	0.3	0.33	0.35	0.34
Total	0.50	0.75	0.80	0.90	1.03	1.00	0.98
(b) No <u>Cp</u>							
Seedling fresh wt (g)	0.3	0.2	0.33	0.3	0.3	0.31	0.32
	0.3	0.45	0.3	0.3	0.32	0.34	0.3
	0.3	0.25	0.35	0.33	0.39	0.45	0.36
Total	0.90	0.90	0.98	0.93	1.01	1.10	0.98
Mean	0.30	0.30	0.33	0.31	0.34	0.37	0.33

ANOVA

SV	df	SS	MS	F
Between groups	13	0.09	0.0069	1.44 n.s.
Within groups	28	0.12	0.0043	
Total	41	0.21		

SE = 0.038

Ranked Means:

0.25, 0.25, 0.27, 0.30, 0.30, 0.30, 0.31, 0.33, 0.33, 0.33, 0.33, 0.34, 0.34, 0.3

Means are not significantly different ($\underline{P} \leq 0.05$).

Appendix Table 9.1 (Cont)

(B) Test 2

(i) Seedling stand

	% Rhizoctonia added (^w / _w)						
	Nil	5	10	15	20	25	30
(a) + <u>Cp</u>							
Seedling stand	0	5	9	8	8	9	10
	2	4	8	8	7	8	9
	0	7	7	9	11	12	11
Total	2	16	24	25	26	29	30
Mean	0.7	5.3	8.0	8.3	8.7	9.7	10.0
(b) No <u>Cp</u>							
Seedling stand	13	12	14	14	12	12	12
	10	12	13	12	12	14	14
	11	14	12	11	13	11	13
Total	34	38	39	37	37	37	39
Mean	11.3	12.7	13.0	12.3	12.3	12.3	13.0

ANOVA

SV	df	SS	MS	F
Between groups	13	481.16	37.01	20.45 ^{***}
Within groups	28	50.67	1.81	
Total	41	531.83		

SE = 0.78

Ranked Means:

0.7, 5.3, 8.0, 8.3, 8.7, 9.7, 10.0, 11.3, 12.3, 12.3, 12.3, 12.7, 13.0, 13.0

Means not underscored by the same line are significantly different ($P \leq 0.01$).

Appendix Table 9.1B

(ii) Seedling fresh wt (g)

	% Rhizoctonia added (^w / _w)						
	Nil	5	10	15	20	25	30
(a) + <u>Cp</u>							
Seedling fresh wt (g)	-	0.3	0.3	0.33	0.45	0.35	0.36
	0.2	0.3	0.3	0.3	0.3	0.3	0.3
	-	0.3	0.3	0.35	0.32	0.35	0.34
Total	0.2	0.9	0.9	0.98	1.07	1.00	1.00
Mean	0.2	0.3	0.3	0.33	0.36	0.33	0.33
(b) No <u>Cp</u>							
Seedling fresh wt (g)	0.3	0.32	0.3	0.4	0.39	0.42	0.35
	0.3	0.3	0.3	0.3	0.3	0.4	0.35
	0.3	0.34	0.36	0.3	0.3	0.3	0.35
Total	0.9	0.96	0.96	1.00	0.99	1.12	1.05
Mean	0.3	0.32	0.32	0.33	0.33	0.37	0.35

ANOVA

SV	df	SS	MS	F
Between groups	13	0.21	0.016	6.4 ~~xxx~~
Within groups	28	0.07	0.0025	
Total	41	0.28		

SE = 0.029

Ranked Means:

0.2, 0.3, 0.3, 0.32, 0.32, 0.33, 0.33, 0.33, 0.33, 0.33, 0.35, 0.36, 0.37Means not underscored by the same line are significantly different ($\underline{P} \leq 0.05$).

Appendix Table 10.1 Control of Pythium damping-off of cucumber by treating seed with selected bacterial antagonists of Corticium praticola

(A) Seedling stand

	Bacterium					
(a) + <u>Pythium</u>	2 - B3	2 - B1	2 - B2	1 - B26	2 - B14	Water
Seedling stand	6	6	4	3	3	1
	5	4	4	4	3	1
	5	5	3	3	3	2
Total	16	15	11	10	9	4
Mean	5.3	5.0	3.7	3.3	3.0	1.3
(b) No <u>Pythium</u>						
Seedling stand	9	10	8	8	8	7
	8	9	7	8	7	8
	8	8	8	8	9	7
Total	25	27	23	24	24	22
Mean	8.3	9.0	7.7	8.0	8.0	7.3

ANOVA

SV	df	SS	MS	F
Between groups	11	214.33	19.48	44.27 ^{***}
Within groups	24	10.67	0.44	
Total	35	225.00		

SE = 0.38

Ranked Means:

1.3, 3.0, 3.3, 3.7, 5.0, 5.3, 7.3, 7.7, 8.0, 8.0, 8.3, 9.0

Means not underscored by the same line are significantly different ($P \leq 0.01$).

Appendix Table 10.1 (Cont)

(B) Seedling fresh wt (g)

	Bacterium					Water
	2 - B3	2 - B1	2 - B2	1 - B26	2 - B14	
(a) + <u>Pythium</u>						
Seedling fresh wt (g)	2.5	2.1	1.55	1.35	1.2	1.35
	1.8	2.33	1.3	1.5	1.3	1.3
	1.2	1.48	1.5	1.53	1.52	1.3
Total	5.50	5.81	4.35	4.38	4.02	3.95
Mean	1.83	1.94	1.45	1.46	1.34	1.32
(b) No <u>Pythium</u>						
Seedling fresh wt (g)	1.75	2.5	2.05	2.2	1.69	1.53
	2.1	2	1.72	1.8	2.04	2.15
	2.63	1.87	2.23	1.76	2.32	1.68
Total	6.48	6.37	6.0	5.76	6.05	5.36
Mean	2.16	2.12	2.0	1.92	2.02	1.79

ANOVA

SV	df	SS	MS	F
Between groups	11	3.10	0.28	2.25 n.s.
Within groups	24	2.56	0.11	
Total	35	5.66		

SE = 0.19

Ranked Means:

1.32, 1.34, 1.45, 1.46, 1.79, 1.83, 1.92, 1.94, 2.00, 2.02, 2.12, 2.16

The means are not significantly different.

Appendix Table 10.2 Control of S. rolfsii (Sr) damping-off of tomato by treating seed with selected bacterial antagonists of Corticium praticola

(A) Seedling stand

	Bacterium					
	2 - B3	2 - B2	2 - B14	2 - B1	1 - B26	Water
(a) + <u>Sr</u>						
Emerged seedling stand	10	10	11	12	11	7
	14	13	14	12	11	6
	14	13	10	10	11	6
Total	38	36	35	34	33	19
Mean	12.7	12.0	11.7	11.3	11.0	6.3
(b) No <u>Sr</u>						
Emerged seedling stand	15	15	14	15	14	14
	15	14	14	13	13	11
	14	14	12	14	13	13
Total	44	43	40	42	40	38
Mean	14.7	14.3	13.3	14.0	13.3	12.7

ANOVA

SV	df	SS	MS	F
Between groups	11	161.22	14.66	8.79 ^{***}
Within groups	24	40	1.67	
Total	35	201.22		

SE = 0.75

Ranked Means:

6.3, 11.0, 11.3, 11.7, 12.0, 12.7, 12.7, 13.3, 13.3, 14.0, 14.3, 14.7

Means not underscored by the same line are significantly different ($P \leq 0.05$).

Appendix Table 10.2 (Cont)

(a) + <u>Sr</u>	Bacterium					Water
	2 - B3	2 - B2	2 - B14	2 - B1	1 - B26	
Survived seedling stand	13	10	9	9	10	3
	10	12	10	10	8	4
	14	13	10	11	7	3
Total	37	35	29	30	25	10
Mean	12.3	11.7	9.7	10.0	8.3	3.3
(b) No <u>Sr</u>						
Survived seedling stand	15	15	12	14	14	14
	15	14	14	15	13	11
	14	14	14	13	13	13
Total	44	43	40	42	40	38
Mean	14.7	14.3	13.3	14.0	13.3	12.7

ANOVA

SV	df	SS	MS	F
Between groups	11	346.3	31.48	23.15 ^{***}
Within groups	24	32.67	1.36	
Total	35	378.97		

SE = 0.67

Ranked Means:

3.3, 8.3, 9.7, 10.0, 11.7, 12.3, 12.7, 13.3, 13.3, 14.0, 14.3, 14.7

Means not underscored by the same line are significantly different ($P \leq 0.01$).

Appendix Table 10.2 (Cont)

(B) Seedling fresh wt (g)

(a) + <u>Sr</u>	Bacterium					Water
	2 - B3	2 - B2	2 - B14	2 - B1	1 - B26	
Seedling fresh wt (g)	1.38	1.5	1.4	1.4	1.15	1.2
	1.25	1.29	1.1	1.1	1.17	0.75
	1.33	1.17	1.12	1.36	1.50	0.80
Total	3.96	3.96	3.62	3.86	3.82	2.75
Mean	1.32	1.32	1.21	1.29	1.27	0.92
(b) No <u>Sr</u>						
Seedling fresh wt (g)	1.5	1.36	1.5	1.4	1.62	1.36
	1.45	1.3	1.5	1.2	1.3	1.4
	1.3	1.3	1.2	1.55	1.35	1.15
Total	4.25	3.96	4.20	4.15	4.27	3.90
Mean	1.42	1.32	1.40	1.38	1.42	1.30

ANOVA

SV	df	SS	MS	F	
Between groups	11	0.61	0.055	2.04 n.s.	SE = 0.095
Within groups	24	0.64	0.027		
Total	35	1.25			

Ranked Means:

0.92, 1.21, 1.27, 1.29, 1.30, 1.32, 1.32, 1.32, 1.38, 1.40, 1.40, 1.42

Means not underscored by the same line are significantly different ($\underline{P} \leq 0.05$).

Appendix Table 10.3 Effects of treating cucumber seed with selected bacteria on damping-off by C. praticola in non-sterile soil

(A) Seedling stand

	Bacterium					
(a) + <u>Cp</u>	2 - B3	1 - B26	2 - B1	2 - B14	2 - B2	Water
Seedling stand	2	3	2	1	2	0
	3	3	1	2	2	0
	3	1	2	2	0	0
Total	8	7	5	5	4	0
Mean	2.7	2.3	1.7	1.7	1.3	0
(b) No <u>Cp</u>						
Seedling stand	9	8	9	8	8	7
	8	8	7	8	7	8
	8	8	8	7	8	7
Total	25	24	24	23	23	22
Mean	8.3	8.0	8.0	7.7	7.7	7.3

ANOVA

SV	df	SS	MS	F
Between groups	11	363.22	33.02	66.04 ^{***}
Within groups	24	12	0.5	
Total	35	375.22		

SE = 0.41

Ranked Means:

0, 1.3, 1.7, 1.7, 2.3, 2.7, 7.3, 7.7, 7.7, 8.0, 8.0, 8.3

Means not underscored by the same line are significantly different ($P \leq 0.05$).

Appendix Table 10.3 (Cont)

(B) Seedling fresh wt (g)

	Bacterium					
	2 - B3	1 - B26	2 - B1	2 - B14	2 - B2	Water
(a) + <u>Cp</u>						
Seedling fresh wt (g)	1.35	0.9	0.9	1.5	1.4	-
	0.97	1.6	0.8	1.3	1.5	-
	1.33	1.2	0.75	1.25	-	-
Total	3.65	3.70	2.45	4.05	2.90	-
Mean	1.22	1.23	0.82	1.35	0.97	-
(b) No <u>Cp</u>						
Seedling fresh wt (g)	1.88	1.65	1.95	1.6	1.83	1.7
	1.9	1.93	1.8	1.75	2.1	1.8
	1.55	1.9	1.63	1.38	1.75	1.65
Total	5.33	5.48	5.38	4.73	5.68	5.15
Mean	1.78	1.83	1.79	1.58	1.89	1.72

ANOVA

SV	df	SS	MS	F
Between groups	10	4.18	0.42	4.38 ^{***}
Within groups	22	2.11	0.096	
Total	32	6.29		

SE = 0.18

Ranked Means:

0.82, 0.97, 1.22, 1.23, 1.35, 1.58, 1.72, 1.78, 1.79, 1.83, 1.89
Means not underscored by the same line are significantly different ($P \leq 0.05$).

Appendix Table 10.4 Effects of treating soil with selected bacteria on damping-off of cucumber by C. *praticola*

(A) Seedling stand

	Bacterium					
(a) + <u>Cp</u>	2 - B3	1 - B26	2 - B14	2 - B2	2 - B1	Water
Seedling stand	6	6	3	4	4	0
	5	4	5	5	3	0
	5	5	5	5	4	0
Total	16	15	13	13	11	0
Mean	5.3	5.0	4.3	4.3	3.7	0
(b) No <u>Cp</u>						
Seedling stand	10	9	9	9	8	8
	9	9	8	9	9	8
	8	7	9	7	8	8
Total	27	25	26	25	25	24
Mean	9.0	8.3	8.7	8.3	8.3	8.0

ANOVA

SV	df	SS	MS	F
Between groups	11	258.56	23.51	15.99 ^{***}
Within groups	24	35.33	1.47	
Total	35	293.89		

SE = 0.7

Ranked Means:

0, 3.7, 4.3, 4.3, 5.0, 5.3, 8.0, 8.3, 8.3, 8.3, 8.7, 9.0

Means not underscored by the same line are significantly different ($P \leq 0.05$).

Appendix Table 10.4 (Cont)

(B) Seedling fresh wt (g)

	Bacterium					
	2 - B3	1 - B26	2 - B14	2 - B2	2 - B1	Water
(a) + <u>Cp</u>						
Seedling fresh wt (g)	1.55	1.8	1.43	1.73	1.2	-
	1.95	1.49	1.3	1.5	1.4	-
	1.5	1.52	1.8	1.3	1.62	-
Total	5	4.81	4.53	4.53	4.22	-
Mean	1.67	1.60	1.51	1.51	1.41	-
(b) No <u>Cp</u>						
Seedling fresh wt (g)	2.3	1.75	2.1	2.0	1.95	1.85
	1.93	1.6	1.75	1.83	1.63	1.43
	1.5	1.85	1.6	1.45	1.55	1.7
Total	5.73	5.20	5.45	5.28	5.13	4.98
Mean	1.91	1.73	1.82	1.76	1.71	1.66

ANOVA

SV	df	SS	MS	F
Between groups	10	0.65	0.065	1.08 n.s.
Within groups	22	1.32	0.06	
Total	32	1.97		

SE = 0.14

Ranked Means:

1.41, 1.51, 1.51, 1.60, 1.66, 1.67, 1.71, 1.73, 1.76, 1.82, 1.93The means are not significantly different ($P \leq 0.01$).

Appendix Table 10.5 Effects of treating tomato seed with selected bacteria on damping-off by C. praticola in non-sterile soil

(A) Seedling stand

	Bacterium					
	1 - B26	2 - B3	2 - B2	2 - B1 ⁴	2 - B1	Water
(a) + <u>Cp</u>						
Emerged seedling stand	13	9	8	6	7	6
	11	9	8	8	8	7
	9	11	8	10	7	6
Total	33	29	24	24	22	19
Mean	11.0	9.7	8.0	8.0	7.3	6.3
(b) No <u>Cp</u>						
Emerged seedling stand	13	11	10	11	10	10
	13	13	12	11	12	11
	12	13	11	11	12	11
Total	38	36	33	33	34	32
Mean	12.7	12.0	11.0	11.0	11.3	10.7

ANOVA

SV	df	SS	MS	F
Between groups	11	134.78	12.25	10.47 ^{***}
Within groups	24	28	1.17	
Total	35	162.78		

SE = 0.62

Ranked Means:

6.3, 7.3, 8.0, 8.0, 9.7, 10.7, 11.0, 11.0, 11.3, 12.0, 12.7

Means not underscored by the same line are significantly different ($P \leq 0.05$).

Appendix Table 10.5 (Cont)

	Bacterium					Water
	1 - B26	2 - B3	2 - B2	2 - B14	2 - B1	
(a) + <u>Cp</u>						
Survived seedling stand	10	8	6	3	3	2
	9	6	5	5	4	3
	7	8	5	7	5	3
Total	25	22	16	15	12	8
Mean	8.3	7.3	5.3	5.0	4.0	2.7
(b) No <u>Cp</u>						
Survived seedling stand	13	11	10	11	12	10
	13	12	12	11	10	11
	12	13	11	11	12	11
Total	38	36	33	33	34	32
Mean	12.7	12.0	11.0	11.0	11.3	10.7

ANOVA

SV	df	SS	MS	F
Between groups	11	398.22	36.20	19.89***
Within groups	24	43.67	1.82	
Total	35	441.89		

SE = 0.78

Ranked Means:

2.7, 4.0, 5.0, 5.3, 7.3, 8.3, 10.7, 11.0, 11.0, 11.3, 12.0, 12.7

Means not underscored by the same line are significantly different ($P \leq 0.05$).

Appendix Table 10.5 (Cont)

(B) Seedling fresh wt (g)

	Bacterium					
	1 - B26	2 - B3	2 - B2	2 - B14	2 - B1	Water
(a) + <u>Cp</u>						
Seedling fresh wt (g)	1.47	1.65	1.15	1.2	1.4	1.1
	1.38	1.5	1.26	1.35	1.1	1.2
	1.35	1.4	1.55	1.0	1.2	1.0
Total	4.20	4.55	3.96	3.55	3.70	3.30
Mean	1.40	1.52	1.32	1.18	1.23	1.10
(b) No <u>Cp</u>						
Seedling fresh wt (g)	1.97	1.7	1.6	1.8	1.5	1.4
	1.58	2.0	1.6	1.8	1.8	1.4
	1.7	1.8	2.0	1.2	1.5	1.65
Total	5.25	5.50	5.20	4.80	4.80	4.45
Mean	1.75	1.83	1.73	1.60	1.60	1.48

ANOVA

SV	df	SS	MS	F
Between groups	11	1.85	0.17	4.86 ^{***}
Within groups	24	0.83	0.035	
Total	35	2.68		

SE = 0.11

Ranked Means:

1.10, 1.18, 1.23, 1.32, 1.40, 1.48, 1.52, 1.60, 1.60, 1.73, 1.75, 1.83

Means not underscored by the same line are significantly different ($P \leq 0.05$).

Appendix Table 10.6 Effects of treating soil with selected bacteria on damping-off of tomato by C. praticola

(A) Seedling stand

	Bacterium					
(a) + <u>Cp</u>	1 - B26	2 - B3	2 - B2	2 - B14	2 - B1	Water
Seedling stand	10	9	11	7	8	3
	14	8	8	8	6	4
	9	10	8	7	7	3
Total	33	27	27	22	21	10
Mean	11.0	9.0	9.0	7.3	7.0	3.3
(b) No <u>Cp</u>						
Seedling stand	14	14	12	13	11	11
	12	10	10	14	13	11
	13	12	13	13	11	11
Total	39	36	35	40	35	33
Mean	13.0	12.0	11.7	13.3	11.7	11.0

ANOVA

SV	df	SS	MS	F
Between groups	11	282.56	25.69	14.19 ³⁰²⁸
Within groups	24	43.33	1.81	
Total	35	325.89		

SE = 0.78

Ranked Means:

3.3, 7.0, 7.3, 9.0, 9.0, 11.0, 11.0, 11.7, 11.7, 12.0, 13.0, 13.3

Means not underscored by the same line are significantly different ($P \leq 0.05$).

Appendix Table 10.6 (Cont)

(B) Seedling fresh wt (g)

	Bacterium					
	1 - B26	2 - B3	2 - B2	2 - B14	2 - B1	Water
(a) + <u>Cp</u>						
Seedling fresh wt (g)	2	1	2	1.33	1.75	1
	1.71	1.67	1.37	1	1	1.3
	2	2	1	1.23	1.25	1
Total	5.71	4.67	4.37	3.56	4.0	3.30
Mean	1.90	1.56	1.46	1.19	1.33	1.10
(b) No <u>Cp</u>						
Seedling fresh wt (g)	2	2	1.8	1.63	1.33	1.33
	1.67	1.58	1.8	1.5	2	1.8
	2	1.95	2	2	2	1.36
Total	5.67	5.53	5.60	5.12	5.33	4.49
Mean	1.89	1.84	1.87	1.71	1.78	1.50

ANOVA

SV	df	SS	MS	F
Between groups	11	2.63	0.24	2.53*
Within groups	24	2.27	0.095	
Total	35	4.90		

SE = 0.18

Ranked Means:

1.10, 1.19, 1.33, 1.46, 1.50, 1.56, 1.71, 1.78, 1.84, 1.87, 1.89, 1.90Means not underscored by the same line are significantly different ($P \leq 0.01$).

Appendix to Fig. 6 Population of Corticium praticola (Cp) in soil inoculated with Cp+ bacteria

No Corticium sp. was detected in controls.

7 days after inoculation (at sowing) - Propagules/g dry soil

Cp only	- 100, 120, 100, 100, 120, 120, 140, 140, 120, 140	Mean = 120
Cp + 2 - B1	- 140, 100, 80, 80, 100, 80, 120, 140, 120, 140	Mean = 110
Cp + 2 - B2	- 80, 80, 100, 120, 80, 100, 80, 120, 100, 120	Mean = 98
Cp + 2 - B3	- 80, 80, 100, 80, 120, 80, 100, 100, 100, 100	Mean = 94
Cp + 2 - B14	- 100, 100, 100, 80, 100, 80, 80, 100, 100, 120	Mean = 96
Cp + 1 - B26	- 100, 80, 100, 80, 100, 100, 100, 80, 80, 100	Mean = 92

28 days after inoculation (after cropping) - Propagules/g dry soil

Cp only	- 140, 100, 100, 100, 80, 120, 120, 120, 120	Mean = 110
Cp + 2 - B1	- 100, 100, 100, 80, 80, 80, 80, 100, 100, 100	Mean = 92
Cp + 2 - B2	- 60, 80, 80, 80, 80, 80, 80, 100, 80, 80	Mean = 80
Cp + 2 - B3	- 80, 80, 60, 60, 80, 80, 100, 80, 80, 80	Mean = 78
Cp + 2 - B14	- 80, 80, 80, 100, 80, 60, 60, 80, 80, 100	Mean = 80
Cp + 1 - B26	- 80, 80, 80, 80, 60, 60, 80, 80, 80, 60	Mean = 74

Soil treatment	<u>% soil aggregates with <u>C. praticola</u></u>	
	7 days	28 days after sowing
Cp only	60	55
Cp + 2 - B1	55	46
Cp + 2 - B2	49	40
Cp + 2 - B3	47	39
Cp + 2 - B14	48	40
Cp + 1 - B26	46	37

Comparisons of % soil aggregates with Cp based on test given in Bailey (1959) pp 38-39.

Only Cp vs Cp + 1 - B26 for 7 and 28 days are significant.

d = 1.98 and 2.55 respectively.

Appendix Table 11.1 Control of damping-off of lettuce by inoculating soil infested with C. praticola (Cp) with selected bacteria

(A) Seedling stand

	Bacterium					Water Control
	1 - B26	2 - B3	2 - B14	2 - B2	2 - B1	
(a) + <u>Cp</u>						
Final seedling stand	9	10	8	7	4	2
	10	8	10	9	4	1
	9	9	8	8	6	1
Total	28	27	26	24	14	4
Mean	9.3	9.0	8.7	8.0	4.7	1.3
(b) No <u>Cp</u>						
Final seedling stand	13	14	12	13	13	12
	13	15	12	14	12	11
	13	12	14	12	12	13
Total	39	41	38	39	37	36
Mean	13.0	13.7	12.7	13.0	12.3	12.0

ANOVA

SV	df	SS	MS	F
Between groups	11	474.97	43.18	45.94 ^{xxx}
Within groups	24	22.67	0.94	
Total	35	497.64		

SE = 0.56

Ranked Means:

1.3, 4.7, 8.0, 8.7, 9.0, 9.3, 12.0, 12.3, 12.7, 13.0, 13.0, 13.7

Means not underscored by the same line are significantly different ($P \leq 0.05$).

Appendix Table 11.1 (Cont)

(B) Seedling fresh wt (g)

	Bacterium					Water Control
	1 - B26	2 - B3	2 - B14	2 - B2	2 - B1	
(a) + <u>Cp</u>						
Seedling fresh wt (g)	0.34	0.3	0.2	0.25	0.2	0.17
	0.3	0.3	0.3	0.2	0.2	0.2
	0.26	0.29	0.27	0.35	0.2	0.2
Total	0.90	0.89	0.77	0.80	0.60	0.57
Mean	0.30	0.30	0.26	0.27	0.20	0.19
(b) No <u>Cp</u>						
Seedling fresh wt (g)	0.3	0.33	0.3	0.3	0.23	0.33
	0.33	0.3	0.29	0.3	0.33	0.26
	0.32	0.3	0.3	0.3	0.30	0.28
Total	0.95	0.93	0.89	0.90	0.86	0.87
Mean	0.32	0.31	0.30	0.30	0.29	0.29

ANOVA

SV	df	SS	MS	F
Between groups	11	0.06	0.0055	4.2
Within groups	24	0.03	0.0013	
Total	35	0.09		

SE = 0.021

Ranked Means:

0.19, 0.20, 0.26, 0.27, 0.29, 0.29, 0.30, 0.30, 0.30, 0.30, 0.31, 0.32
Means not underscored by the same line are significantly different ($P \leq 0.05$).

Appendix Table 11.2 Number of antibiotic producing bacteria in the rhizosphere of lettuce seedling

(a) + C. praticola

Bacterium	Nos at each dilution level						Total	Nos/ml of suspension	Nos/g dry root x 10 ⁴
	10 ¹	10 ²	10 ³	10 ⁴	10 ⁵	10 ⁶			
2 - B1	3	3	0	0	0	0	6	102	1.02
2 - B2	6	3	0	0	0	0	9	228	2.28
2 - B3	8	3	0	0	0	0	11	422	4.22
2 - B14	8	2	0	0	0	0	10	306	3.06
1 - B26	8	3	0	0	0	0	11	422	4.22

(b) No C. praticola

2 - B1	8	3	0	0	0	0	11	422	4.22
2 - B2	6	6	0	0	0	0	12	581	5.81
2 - B3	8	6	0	0	0	0	14	1020	10.20
2 - B14	7	5	0	0	0	0	12	581	5.81
1 - B26	8	6	0	0	0	0	14	1020	10.20