BIOLOGICAL CONTROL OF SOIL-BORNE PARASITES.

A thesis presented

by

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ABSTRACT

Effects of organic amendments on Corticium praticola appear to depend upon C:N. ratio of material, soil reaction and temperature. In some cases, there was complete control of C. praticola. Fusarium nivale and Fusarium culmorum were checked to lesser degrees by grass meal, under natural conditions.

Materials with high C:N. ratio such as grass meal, bran and cellulose lessened C. praticola, whereas, those with low C:N. ratio such as corn, barley and fish meals increased the disease. Cellulose, containing mainly carbon caused seedling stunting.

High temperatures and acidic soils which reduced disease were correlated with dominance of soil microflora by fungi mainly, and bacteria in part. Low temperatures and alkaline soils which worsened disease were correlated with dominance by actinomycetes. Cultures of fungi isolated from amended soils, lessened disease significantly (P0.01) when simultaneously inoculated into vermiculite with the parasite culture. Bacterial cultures were less effective, but actinomycetes cultures increased disease significantly (P0.01). Without grass meal none of the cultures reduced disease. Vermiculite being very porous, CO_2 from amendment is considered a minor factor in disease control. Actinomycetes antagonized C. praticola on agar, when given growth advantage but increased disease in soil. Antagonism in sterile conditions is considered unsuitable criterion for judging interaction of microorganisms in soil.

Organic amendments produced phytotoxins when freshly added, but later, produced substances, stimulatory to seedling emergence and growth, which perhaps enabled seedlings to escape from the parasite attack, rather physically - non-biological control. Grass meal has prolonged beneficial effects in soils and its efficacy is comparable to that of standard fungicides. The phytotoxins dissipated quickly at high and persisted longer at low temperatures.

How the soil fungi check disease and the actinomycetes increase it, is not fully known, but Penicillium, Trichoderma and Aspergillus species, known to be antagonistic, were often isolated from grass meal treatments.

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INTRODUCTION

Biological control of soil-borne parasites has been much studied in recent time. It is based on the concept of checking soil-borne pathogens by methods other than use of chemical substances - fungicides. Many workers have shown that certain parasites could be reduced by either introducing normal soil saprophytes known to be antagonistic to certain pathogens or adding carbohydrate rich materials, or both together to infested soil. Best results have been reported mainly in sterile soil. Similar achievements in non-sterile soil is scarce. This may not be surprising because of the difficulty in establishing the antagonist in a new habitat which may not favour its growth. Even when the antagonists are successfully introduced into normal soil and the parasite reduced, another problem still remains in applying such methods on a wider scale. With such complicated approach, biological control may fail to compete favourably with use of standard fungicides.

More attempts are recently being made to devise practicable ways of developing methods designed to sustain at a high level of activity of soil microorganisms by the addition of cheap, and readily available organic materials. Indeed, effectiveness of some cultural practices have been interpreted as relating to changes in soil microbial popu-

lation, and biological control could be a means of enhancing and quickening these processes.

Mechanism of disease control by organic amendments is not fully known. Various suggestions normally given are, production of antibiotic substances by soil microorganisms which multiply upon the amendment, direct parasitism of the pathogen by soil saprophytes, struggle for food in which the parasite is at a disadvantage and CO_2 accruing from carbohydrate degradation by soil microflora. Where chitin was applied, it has been suggested that the amendment enhanced multiplication of mycolytic actinomycetes. In spite of much extensive studies, some of these suggestions are still based on indirect evidence, in some cases derived from situations far removed from soil conditions.

Reports presented in this thesis are attempts to find practicable ways of applying cheap and readily available organic materials under natural conditions, and check diseases caused by Corticium praticola, Fusarium culmorum and Fusarium nivale. Sterile soil was used sparingly, in experiments designed to seek for certain explanations, otherwise normal soil was used throughout. The work was tended to be exploratory, but few investigations relate to the mechanism involved in disease control. More attention was given to soil microorganisms in three groups viz, actinomycetes, bacteria and fungi rather than the study of a few isolates.

REVIEW OF LITERATURE

One of the earliest records of toxic substances being produced by soil microorganisms following the addition of organic materials to the soil is that of Greig-Smith (1911, 1915, 1917) who found that dextrose increased the production of bacteriotoxin. Relatively high temperatures, about 20°C, and low moisture content of the soil increased production. So did aeration at first but later caused the toxins to disappear. Many fungi, bacteria and actinomycetes isolated from treated soils were antagonistic to Bacillus vulgatus on agar plates, actinomycetes being the most active in limiting growth of the test bacterium. On the other hand Porter (1924) found bacteria the most effective antagonist of Fusarium lini and Helminthosporium sativum on agar plates. Antagonistic bacteria also protected flax and wheat seedlings in the soil against these parasites. The control achieved was thought to be due to inhibitors formed by the bacteria during growth. These affected H. sativum more than F. lini. But another report by Henry (1931) showed that fungi were considerably more effective in reducing the severity of H. sativum on wheat in a black loam soil, than either bacteria or actinomycetes. Later, Anwar (1949)

investigating the disappearance of H. sativum from soil and the persistence of F. lini in the same soil, found that saprophytic soil fungi and bacteria suppressed the former more than the latter, and that actinomycetes were unimportant in this context.

Most claims for antagonism of soil microorganisms against parasites are based on tests on agar plates. This is not always a good criterion. This was emphasized by Fawcett (1931) who said "Nature does not work with pure cultures alone, but most frequently with associations". Diachun (1939) recorded than when wounded or inoculated corn kennels were pressed into contact with wet soil, injury by Penicillium oxalicum was reduced. He attributed the control to oxygen deficiency in the zone between corn kennels and the soil. There was no antagonism between the soil saprophytes and P. oxalicum in plate cultures. Broadfoot (1933) who reported biological control of Ophiobolus graminis also found that the growth reactions of fungi on culture media was not a reliable indication of their reactions or interactions on a host in the soil.

Potato scab - Streptomyces scabies (Thaxt.) Skinner.

Millard (1923) controlled potato scab caused by Streptomyces scabies (Thaxt.) Skinner by green manuring.

Sanford (1926) reporting similar results suggested that control was due to antibiotic substances produced by soil microorganisms degrading the green manure. Millard and Taylor (1927) carried the investigation further and reported increase of saprophytic organisms in manured soil which tended to "choke" S. scabies. Scab was reduced when sterile soil, amended with grass was simultaneously inoculated with S. scabies and Streptomyces praecox, a normal soil saprophyte.

Waksman (1927) studied the effect of organic matter in the soil and reported increased populations of bacteria and actinomycetes, although the increases of bacteria were ephemeral. However, Sanford (1931) isolated bacteria from amended soil, which completely suppressed growth of S. scabies, but only partially suppressed Rhizoctonia solani in Petri dish experiments. He suggested that the two parasites might require different organic soil amendments for effective control.

Rhizoctonia damping-off diseases

Small (1927) working on "foot rot" of tomatoes caused by Rhizoctonia solani Kühn, applied stable manure to infested soil and reported more disease, but achieved slight control with Ammonium sulphate. Wellman (1932) studying isolates

of Rhizoctonia solani from Crucifers, concluded that some isolates were relatively specific to Crucifers, and that when grain crops or legumes followd Cabbage cropping Rhizoctonia head rot was rarely a serious problem. Weindling (1932, 1934), Weindling and Emerson (1936) and Weindling and Fawcett (1936) found that Trichoderma lignorum and Trichoderma viride, when inoculated separately, into sterile soil gave some control of R. solani on citrus seedlings. T. lignorum parasitised R. solani on agar plates. There was better control in acid than in neutral soil. Later a toxic substance antibiotic to many microorganisms, was isolated from culture filtrate of T. viride Weindling (1937). This toxin was called gliotoxin. Later, Weindling (1947) isolated another toxin from a closely related saprophyte, Gliocladium fimbriatum, which like T. viride attacked R. solani in vivo. Gliotoxin which was thought to be produced by Trichoderma and Gliocladium, was finally found to be a product of Gliocladium only. It persists longer in acid conditions and decomposes quickly in alkaline conditions with loss of sulphur.

Allen et al., (1935) also demonstrated the control of Rhizoctonia damping-off of cucumbers by heavily inoculating sterile infested soil with Trichoderma viride. An active

principle, lethal, was detected in Trichoderma culture filtrates. It was thermolabile and inactivated by oxygen. Blair (1943) continued work on R. solani and reported biological control by adding ground wheat straw to the soil and concluded that this effect depended on (i) negligible cellulose decomposing ability of the parasite (ii) nitrogen starvation of the parasite by rapid utilization of available soil nitrogen by cellulose decomposing microorganisms, which multiplied on the fresh organic material, and (iii) fungistatic effects on R. solani of respiratory carbon dioxide produced by cellulose decomposers. Wood (1951) working on damping-off of Lettuce caused by Rhizoctonia solani found that many organisms which effectively antagonized the parasite in agar cultures, and controlled the disease under sterile conditions, were ineffective in natural soil. Significant results were reported when infested soil was amended with dry grass. The addition of antagonist cultures to a grass amendment, did not give better control significantly. Liming amended soil increased disease. He suggested that the most practicable approach would be to develop practices designed to sustain at a high level of activity of soil microorganisms by the addition of cheap organic amendments.

Kommedahl and Young (1956) reported significant

decrease in Rhizoctonia solani infection of wheat by adding corn stalks to naturally infested soil, while a contemporary worker Boosalis (1956) found that a high soil temperature of 28°C, stimulated the parasitism of R. solani by Trichoderma and Penicillium vermiculatum in both green manured and unmanured soil. Under more natural conditions and with organic manures of various types, Davey and Papavizas (1959, 1960), Maier (1959) and Papavizas (1960) showed that fresh green amendments with low carbon/nitrogen (C/N) ratio increased disease of beans caused by R. solani, whereas dry amendments hardly ever increased the disease above that in unamended soil. It was suggested that there was a C/N range within which amendments reached maximum effectiveness; above or below this they were less effective.

Supplemental soil nitrogen increased diseases of snap bean (Anderson 1939), bent grass (Couch et al., 1958) and lettuce (Das 1959).

On the direct effect of carbon-dioxide tension in the soil, Durbin (1959) and Papavizas (1960) found that strains attacking hosts at or near ground level were particularly sensitive. Papavizas et al., (1960, 1962) suggested that moisture, temperature, acidity, and nutrient status of the soil influenced the survival and virulence of R. solani. They considered that moisture and temperature affect the

gas content of the soil, and that organic soil amendments which controlled disease in unsterile soil, substantially increase the total soil microflora and that about 60 percent of the total carbon (C) of the organic amendments is evolved as CO_2 because of the general microbial activities associated with decay of plant materials. These views are close to those of Garrett (1937) on the behaviour of Ophiobolus graminis in soil.

The Wheat take-all disease - Ophiobolus graminis

There is much work on biological control of take-all of wheat disease caused by Ophiobolus graminis. Fellows (1929), Sanford and Broadfoot (1933) and Fellows and Ficke (1939) state that this parasite which persists for years in fields successively cropped to wheat, disappears rapidly if either the soil be left fallow or mixed with noninfested soil. Organic manure effectively reduces the disease and the activities of soil saprophytic microorganisms are suggested as responsible for control.

One of the most thorough investigations on a soil-borne parasite and its biological control was made by Garrett (1936,1937) with this disease. He found that the disease spread most rapidly in alkaline, well aerated soils and spread less in acid, poorly aerated soils. He hypothesized that the fungus grows well in aerated alkaline soils

because they are good carbon-dioxide acceptors and because O. graminis is sensitive to CO_2. Garrett (1938) found that addition of nitrogen deficient materials such as glucose, starch, and rye grass meal, to partially sterilized and reinoculated soil, reduced disease. This was attributed to increased respiratory activities of soil microflora. Later, Garrett (1940) suggested that the longevity of O. graminis depends on available nitrogen in the soil, because it grows better and longer in soils amply supplied with nitrogen. However, Fellows (1941) reported reduction of O. graminis in warm, loose soil and its persistence in cool, compact soil. Slagg et al.,(1947) found that certain soil saprophytic fungi, antagonistic to O. graminis on some agar media, produced stimulatory substances on other media.

Cotton root rot disease - Phymatotrichum Omnivorum

One of the main methods of checking this disease is biological, and the results recorded are more/ conclusive than in most other work with soil-borne parasites. King (1926) recorded reduction of disease from 71.7 per cent in 1920 to only 2.2. per cent in 1924 following four years of organic manuring. The efficacy of manural treatment was confirmed by King et al.,(1934) and he suggested among other things that the manure increased the number and activities of

organisms which inhibited the growth and development of the pathogen. Mitchel et al.,(1941 a,b) carried this work further, found good control, after heavy application of organic soil amendment, and correlated control with high temperature and soil moisture, which increased microbial activities. Sclerotia of the parasite buried in amended soil were quickly destroyed whereas those in the untreated soil remained viable much longer.

Fusarial diseases

Nadvodnyuk (1962) reported control of dry rot of potato caused by Fusarium species, by treating infected tubers with dense conidial suspension of Trichoderma koningi. This improved tuber germination in the field by 22.7 percent. There are several reports on chitin in biological control. Mitchell and Alexander (1961, 1962) working on Fusarium solani f. phaseoli and Fusarium oxysporum f. conglutinans reduced disease by treating infested soil with 200 lb. per acre. Khalifa (1965), Buxton et al.,(1965) who worked with Fusarium oxysporum f. pisi on Pea rot, used much more chitin than Mitchel et al.,(1961,1962) and recorded good control only with very large amounts. Both claimed that the amendment increased the numbers of soil micro-organisms mainly mycolytic actinomycetes, basing their claims on antagonism tests

on sterile agar. However, Maurer and Baker (1963) failed to control F. solani f. phaseoli on bean either with chitin or lignin, but when the two were mixed there was significant control of disease. Inclusion of KNO_3 with chitin and lignin gave better control, but this was annulled by the addition of glucose, suggesting that the control without glucose, at least in part, depended on competition for simple carbon substrates.

Beet damping-off - Pythium species.

Liu and Vaughan (1963) reported biological control of Pythium infection of table beet by seed treatment with conidia of Trichoderma viride and Penicillium frequentans, which gave better results when the soil was also supplemented with KH_2PO_4 or K_2HPO_4, and with urea. Corn meal, sucrose, and casein enhanced the virulence of the parasite. Attempts to control the disease with bacterial and actinomycetes seed treatments in a similar way failed.

Presence of antibiotic substances in soil.

Rayner (1934) who worked on the problem of conifer re-establishment in Wareham Heath, claimed to have discovered toxins in the soil, which inhibited growth of the conifers. Nelson-Jones (1941) found that the toxins were of biological

origin and that they operated directly by inhibiting fungal growth. Brian et al.,(1945) studied the same soil and concluded that the toxicity of Wareham Heath soil to mycorrhizal fungi, and its generally low microbial activity were due to accumulation of gliotoxin and other antibiotic substances produced by Penicillium species, which dominated the flora.

Many attempts have been made to extract antibiotic substances from unsterile amended soil. Waksman and Woodruff (1940, 1941) added a bacterial suspension to a known volume of the test soil suspension and thoroughly mixed both with agar. One species of actinomyces was found to produce a specific bacteriostatic and bacteriocidal substance designated actinomycin. Brian (1949, 1951) and Brian et al.,(1951) demonstrated the control of Botrytis cinerea rot of Lettuce seedlings by griseofulvin produced in vivo by Penicillium griseofulvum and by the use of guttation drops from Oat seedlings showed that the antibiotic was absorbed by and translocated in the plant. Grossbard (1948 a, b, 1952) reported production of antibiotic materials in sterile soil by Penicillium patulum and Aspergillus clavatus in organic amended soil. Where no antibiotics could be detected after organic manuring, addition of

glucose restored the ability to produce them. Failure to obtain antibiotics from non-sterile soil supplemented in various ways was ascribed to competition by soil microflora or to the very low concentrations in natural soil.

Gottlieb et al.,(1951) demonstrated production of chloromycitin by Streptomyces venezuelae in sterile but not in natural soil; Gregory et al.,(1952) claimed that antibiotic substances are produced in both sterile and non-sterile soils, but that the soil microflora decomposes them; they found that clavacin was destroyed more quickly in alkaline soil at pH 7.8 than in acid soil at pH 5.3 - 5.6. Jefferys et al.,(1953) compared the effects of antibiotics produced by soil fungi on parasitic and non-parasitic fungi and agreed with Garrett (1950) that plant parasites are on the whole more susceptible to antibiotics than soil saprophytes.

Vasudeva et al.,(1952, 1962, 1963) reported biological control of Pigeon-pea wilt caused by Fusarium udum, by inoculating infested sterile soil with Bacillus subtilis and adding organic manure. An antibiotic substance designated bulbiformin, toxic to F. udum, was extracted from amended soil inoculated with B. subtilis. It was produced in both sterile and non-sterile soils, but much less so in the latter. Production was enhanced by adding glucose,

and the quantity present in amended soil depended on pH and soil temperature. Significant control of the disease was achieved only when the soil was autoclaved, inoculated with B. subtilis and F. udum and amended either with molasses sweet clover or groundnut cake.

Tveit (1953, 1954), Tveit and Moore (1954), Wood and Tveit (1955) and Tveit (1957) reported biological protection of Oat seeds and seedlings against Fusarium nivale, by coating the seeds with perithecia or ascospores of Chaetomium cochliodes or Chaetomium globosum. Attempts to protect Lettuce seedlings against Rhizoctonia solani, in a similar way failed. The control recorded for the disease of Oat equalled that given by a standard organo-mercurial fungicide. Extracts from cultures of these antagonists also protected Oat seedlings. An antibiotic substance called chetomin was demonstrated in guttation drops from Oat plants grown in culture with organic manure.

Evans and Gottlieb (1952) reporting production of gliotoxin in amended sterile soil inoculated with Trichoderma viride and the control of Rhizoctonia solani damping-off disease of pea and radish in sterile and non-sterile soils by soil inoculation with T. viride, detected no toxin in unsterile soil. This report however contradicts

Weindling (1941) who found that gliotoxin was a product of Gliocladium fimbriatum and not T. viride. Wright (1952) showed convincingly that gliotoxin is produced in unsterile Wareham Heath soil which is acidic. Gliotoxin is produced after soil amendment with 5 percent dried clover. Wright (1956) also extracted gliotoxin and frequentin from Oat seed coats, coated with thoroughly washed T. viride and Penicillium frequentans. She failed to demonstrate antibiotics produced under similar conditions by three actinomycetes.

Some pit-falls encountered in biological control.

Certain Lettuce diseases originally attributed to bacteria (Brown, 1918; Thomas, 1922; Hannon, 1953; Marlatt, 1955), and the unknown disease of Lettuce (Mix, 1945), have been ascribed by Grogan et al.,(1956) to fertilizers containing ammonia. These symptoms resemble those induced by organic soil amendments which would liberate ammonia on decomposition. Nitrites, also damaging to plants may be produced in this way. In the presence of carbohydrates and potassium, ammonia is detoxified by plants and converted to amines.

Patrick et al.,(1962), Toussoun et al.,(1962) and Toussoun and Patrick (1963) showed that toxic substances

from amended soil delayed or inhibited seed germination, and induced discoloration and necrosis of roots in the absence of parasites, lettuce, beans, broccoli and tobacco were affected in this way. However, the toxicity declined 25 days after amendment and 35 days after treatment soil extracts stimulated germination and growth of seedlings. The toxins increased the pathogenicity of Rhizoctonia solani f. phaseoli. They suggested that the toxins increase cell permeability and allow exudation of amino-acids which stimulate growth of the parasite.

It is evident from the literature presented that much of the work on biological control of soil-borne parasites has used saprophytes known to be antagonistic; either by treating infested soil or the seeds. Usually the soil had to be sterilized to establish the antagonist and obtain control. Such methods are laborious, complicated, somewhat unreliable and unsuitable for practical use.

To operate biological control of a soil-borne parasite, effectively, economically and on a large scale, the organic material must be cheap and easily obtainable, because of the large quantities required for control. Consequently, the use of materials not conforming to these needs may not in the foreseable future, play much part. Secondly there

was no direct evidence that the control recorded with chitin soil amendment was due to mycolytic actinomycetes, derived as it were, from sterile agar experiments. Many workers had earlier in this literature review warned against deductions based entirely on such premise. Only a few papers relate to the use of cheap readily available organic materials, that give good control in normal soil.

MATERIALS AND METHODS

A. The Parasites

The following fungi were used:-

1. Corticium praticola Kolita, obtained from stock culture collections of the Department of Botany, Imperial College. The parent culture was acquired from Dr. P.H. Williams, Glasshouse Crops Research Station, Rustington, Sussex, and descended from an isolate supplied by Professor N.T. Flentje.

2. Fusarium culmorum (W.G. Smith) Sacc. reisolated from a diseased wheat seedling , and identified by a method similar to that described by Colhoun and Park (1964). Segments of diseased wheat shoots, cut to retain 7 mm. of diseased and 7 mm. healthy portion, were sterilized for 1 min. in 0.1 per cent $HgCl_2$ solution, rinsed twice in sterile water, dried between sterile filter papers, placed on malt extract agar p. 26 , and incubated at 25°C. Hyphal tips from the edge of a colony were transferred to plates of potato sucrose agar. This was repeated four days later. The growth, morphological and conidial characteristics of the pure culture obtained in this way conformed closely with the description of Fusarium culmorum by Colhoun and Park (1964).

3. Fusarium nivale (Fr.) Ces. was isolated from infected Oat seeds by a method based on those of Noble and Montgomerie

(1956), and Colhoun and Park (1964)

Oat seeds were immersed for 10 seconds in calcium hypochlorite solution, supplying 1 per cent available chlorine, and placed on potato sucrose agar, without rinsing, 10 seeds per plate and incubated at about 15°C. in daylight. After 7 days the pink sterile, stranded mycelium of F. nivale had grown away from other seed borne fungi. Hyphal tips of it were taken with a sterile needle and transferred to fresh plates. Pure cultures obtained in this way were identified by methods recommended by Gordon (1952), Colhoun and Park (1964), i.e. from cultures grown on p.s.a at pH 6.5, in the dark at 25°C. Four days later, 5 mm. disks containing hyphal tips were transferred to fresh p.s.a plates and incubated at 23°C. under florescent light at 100 ft. for 12 hr. per day. Concentric rings of sterile and conidia-bearing hyphae developed. The conidia were uniform in size, small, evenly curved and mainly 3-septate, conforming with description of Fusarium nivale by Colhoun and Park (1964).

B. Cultural methods.

Five mm. disks of agar, containing hyphal tips, were used to inoculate agar plates and hyphal tips taken with sterile needle for test tube cultures. For Petri-dish cul-

tures Corticium praticola was grown on glucose-nitrate-agar, and Fusariun culmorum on Potato dextrose agar. Stock cultures of C. praticola and F. culmorum were maintained on V-8 juice agar, at 22°C. - 25°C. and sub-cultured every four months. Both parasites remained viable and pathogenic to Lettuce and Wheat respectively throughout the experimental period.

C. Nutrient media used for growing fungi

All chemicals used, except where otherwise stated, were A.R. grade.

Agar media

All the agar media were steamed for 30 minutes and autoclaved for 15 minutes at 15 p.s.i unless otherwise stated.

(i) Glucose-nitrate agar

Glucose	10.0 g.
$NaNO_3$	2.0 g.
KH_2PO_4	1.0 g.
$MgSO_4 \cdot 7H_2O$	0.5 g.
Agar	20.0 g.
Water	1000 ml.

(ii) Potato dextrose agar

Dextrose (glucose)	10 g.
Agar	15 g.

Potato Extract (peeled & sliced)	200g.
Water	1000 ml.

pH 6.5

(iii) Potato sucrose agar

Sucrose	20 g.
Agar	20 g.
Potato Extract (peeled & sliced)	200 g.
Water	1000 ml.

pH 6.5

(iv) Malt extract agar

Dextrose	20 g.
Peptone	1 g.
Malt extract (oxoid)	20 g.
Agar	25 g.
Water	1000 ml.

(v) V-8 juice agar

V-8 juice	100 ml.
Agar	20 g.
$CaCO_3$	1.5 g.

(vi) Modified Thornton's Medium (Thornton 1922)

Agar	15.0 g.
K_2HPO_4	1.0 g.
$MgSO_4 \cdot 7H_2O$	0.2 g.

$CaCl_2$	0.1 g.
NaCl	0.1 g.
$FeCl_3$	Trace
KNO_3	0.5 g.
Asparagine	0.5 g.
Mannitol	1.0 g.
Water	1000 ml.

Modified by Johnson (1959) by addition of 0.25 g. yeast extract to 1000 ml. of medium. The medium + yeast extract was used.

(vii) Bean extract glucose agar

Glucose	40 g.
Dwarf French bean flour	40 g.
$CaCO_3$	3 g.
Agar	17 g.
Water	1l.

40 g. Dwarf French Bean flour was simmered with 500 ml. water for 35 minutes, and filtered through muslin. 40 g. glucose, 3 g. $CaCO_3$ and 17 g. agar were added to the filtrate which was made up to 1000 ml. with water. The pH of the medium was 5.5. and 5.9 and this was adjusted with 2.5. ml. IN.NaOH to pH 8.

(viii) Glycerol asparaginate agar (Conn 1921)

Glycerol	10.0 ml.

K_2HPO_4	1.0 g.
Sodium asparaginate	1.0 g.
Agar	15.0 g.
Water	1000 ml.

3 g. $CaCO_3$ was added per 1000 ml. of medium to adjust pH to pH 7.0.

(ix) Soil Extract agar.

Glucose	1.0 g.
K_2HPO_4	0.5 g.
Agar	15.0 g.
Soil extract	
Water	900 ml.

pH 6.8-7.0

Preparation of soil extract

1000 g. of air dried sieved garden soil, with 1000 ml. of water was autoclaved at 15 p.s.i. pressure for 30 minutes. A small amount of $CaCO_3$ was added before vacuum filtering through double filter papers in a Buchnor funnel. This was repeated until the filtrate became clear. It was then bottled and sterilized in 100 ml. lots.

(x) Water agar

Agar	20.0 g.
Water	1000 ml.

(xi) Peptone dextrose agar plus rose bengal and Streptomycin. (Martin, 1950; Johnson, 1957)

Agar	20.0 g.
KH_2PO_4	1.0 g.
$MgSO_4 \cdot 7H_2O$	0.5 g.
Peptone	5.0 g.
Dextrose	10.0 g.
Water	1000 ml.

Rose bengal 1:30,000 added before autoclaving;

Streptomycin 1: 33,000 autoclaved separately from rest of materials.

Sand media

1. Cornmeal/ sand medium

Corn-meal	4.0 g.
Washed white sand	196.0 g.
Water	20.0 ml.

Corn-meal and white sand were thoroughly mixed in 500 ml. Erlenmeyer flasks before adding 20 ml. of water. The medium was autoclaved for 2hrs at 15 p.s.i. pressure and shaken after autoclaving to break up large aggregates.

2. Oat-meal/ sand medium

Oat-meal	4.0 g.
Washed white sand	196.0 g.
Tap water	20.0 ml.

D. The Hosts.

The host plants were as follows:-

1. Lettuce (Lactuca sativa L.) variety "All the year round" supplied by Sutton and Sons Ltd.

2. Wheat (Triticum vulgare L.) variety "Svenno" supplied by E. Dixon and Sons. (Ware) Ltd.

3. Oat (Avena sativa L.)

Single lots of seeds were stored in polythene bags at 4°C., and were sampled every six months for germination tests on moist filter papers. Average percentage germination of the seeds throughout the experimental period was as follows:-

Lettuce	99.6%
Wheat	88.2%
Oat	89.1%

Infection tests with Oat seed.

The Oat seed was treated as for F. nivale isolation from seeds (p 23), with ten seeds on each of four agar plates. There was an average of 3 per cent infection throughout the experimental period.

E. The soil.

The soil used, except where otherwise stated was obtained from the "Walled Garden" of Imperial College Field

Station, Silwood Park. The top six inches was collected, and air dried, and sieved through a ¼ inch sieve and stored in metal dust bins with loosely fitting lids. Any soil which had been more than four months in the bins was discarded.

Moisture content of the soil.

The moisture content of the sieved soil was determined by placing a known weight in an oven at 100°C. and weighing to constant weight. The average water content of sieved soil was 15 per cent (i.e. 100 g. sieved, air dried soil contained 15 g. water). All weights of soil mentioned in this work are dry weights and all percentages relating to materials added to it, are based on dry weight of the soil.

Watering of host plants.

Lettuce seeds were lightly watered after sowing and covered with thin polythene until emergence. Wheat and Oat pottings were not covered. More water was added if the soil appeared dry. No attempt was made to maintain a constant moisture content of the soil, except where otherwise stated.

E_2. Peat/sand mixture

Equal volumes of washed, air dried, fine sand and peat were thoroughly mixed with a fertilizer of the following composition:-

Fertilizer materials	Quantities per cu.ft. of Peat/sand mixture
Magnesium limestone	126.0 g.
$CaCO_3$	42.0 g.
Hoof and horn meal	42.0 g.
KNO_3	4.2 g.
K_2SO_4	4.2 g.

The mixture was lightly watered after seed sowing.

F. Greenhouse Illumination and Temperature

During the 1963-1964 and 1964-1965 winter months, natural daylight was supplemented with artificial light, for 14 hr. a day in the green house at Silwood Park by four fluorescent tubes kept about 6 ft. apart and suspended 3ft. 6 inch. above the green house potting tables. The temperature, during the winter months was thermostatically controlled and did not fall below 16°C. and rarely exceeded 17°C. Light intensity at the tops of pots was 500 lumens per sq. ft.

During the 1965-66 winter months the work was done in South Kensington. The temperature here, kept close to 28°C. and daylight, was supplemented with five batteries of fluorescent tubes placed $4\frac{1}{2}$ inch. apart and 2ft. 6 inc. above the potting table, for 14 hr. each day. The light inten-

sity at the tops of pots was 900 lumens per sq. ft.

G. Soil inoculation techniques

Agar inoculum

Six to ten day old agar cultures were homogenized by passing through 1/8th inch sieve and thoroughly mixed. Amount of inoculum is given as wet weights of the agar culture, per unit dry weight of the soil. Corticium praticola was grown on Glucose nitrate agar, Fusarium culmorum on Potato dextrose agar, 20 ml. to a 9 cm. Petri dish at 22°C. - 25°C.

Cornmeal/sand and Oatmeal/sand cultures.

Corticium praticola was grown in Cornmeal/sand and Fusarium culmorum in Oatmeal/sand. These were inoculated with 8,5 mm. agar plugs taken from the edge of cultures on agar plates and incubated at 25°C. for 2 - 3 weeks, with occasional shaking to promote regular distribution of sclerotia and mycelia in the cultures. They were homogenized by passing through a 1/8 inch sieve and well mixed before adding to soil. The amount of inoculum is given as wet weights of the culture per unit dry weight of dry soil.

H. Materials used for soil amendment.

The following organic materials were obtained from W.A. Lidstone Ltd. The oils, Albuminoids and Fibre per-

centage composition of some of the materials supplied by the dealers is given below:

Material	% Oils	% Albuminoide	% Fibre
1. Middlings (Calcot)			7.5
2. Bran			12.5
3. Linseed cake	7.5	28	
4. Fish meal	4	66	
5. Barley meal			
6. Middlings			11
7. Maize meal	2.5	8.5	
8. Molassine meal			
9. Grass meal			

Other materials

1. Peptone
2. Starch (Potato)
3. Wood cellulose (Solka Floc)
4. Soluble cellulose (cellulose gum)
5. Sodium polypectate
6. Casein
7. Casein hydrolysate
8. Glucose (dextrose)
9. Sodium Nitrate
10. Ammonium sulphate
11. Fungal mycelium (powder)

I. Disease symptoms and assessment.

(i) Corticium praticola and Lettuce

Corticium praticola causes pre-emergence and post-emergence killing of Lettuce seedlings. Seedlings which failed to emerge were regarded as having been killed by the parasite - pre-emergence damping-off. Any seedling showing the plumule just above the soil was regarded as having emerged. Normally emergence was complete in 6 to 8 days after sowing. The number of emerged seedlings was recorded on the 9th day, and any further killings were regarded as post-emergence damping-off. It was easy to determine whether a seedling had been killed by C.praticola by microscopic examination for infection cushions. Killed seedlings were not removed. The final stand of seedlings was obtained 28 days after seed sowing, because it was known that 3 week old seedlings were highly resistant to the parasite although with obvious lesions just above soil level. Only rarely was there post-emergence damping-off after 21 days.

When recording final stand of seedlings, pots were shaken to reveal seedlings which had been attacked by the parasite below the soil level, but which appeared healthy above ground.

Recording and Statistical analysis

In most experiments each treatment was replicated four times. Three inch plastic pots were each sown with 20 equally spaced Lettuce seeds. The results are presented in two ways.

(a) Numbers of emerged seedlings, numbers of seedlings damped-off and post-emergence, and final stand. In graphs and histograms, disease Indices are the total percentage kill (pre-emergence and post-emergence)

(b) For statistical analyses, the final stand of seedlings was expressed as percentage seeds sown and analysis of variance applied after sine transformation of the original datta $p = \sin^2 \theta$

Where p = percentage final stand and θ = transformed value for p.

Generally, the tables show transformed values of p. Least significant differences at .05, .01 and .001 levels of probability are given and significant differences between the controls and various treatments are indicated as * = P.05 ** = P.01 and *** = P.001.

(ii) Fusarium culmorum and Wheat

Fusarium culmorum causes pre-, and post-emergence, killing and root and foot rot of wheat seedlings. It affects

shoot length and weights of seedlings. Experiments were done as four replicates in five inch clay pots. Amount of inoculum is expressed as wet weight of the Oatmeal/sand culture per unit dry weight of the soil. Twenty seedswere sown per pot, normally 24 hr. after soil inoculation. Watering was done as necessary, but no attempt was made to keep the moisture content between narrow limits.

Pre-emergence killing of seedlings was recorded fourteen days after seed sowing and all seedlings which failed to emerge were recorded as pre-emergence killing. This also applied to the controls.

Disease was assessed 3 to 4 weeks after seed sowing. Seedlings were carefully removed from the soil and washed under running tap water, to recover as much of the root system free from soil as possible. Washed seedlings were placed on blotting papers for measurements of roots, shoots, and assessment of disease. Finally, all seedlings from the same pot were dried between blotting papers for about five minutes and then weighed to give fresh weights.

(iii) Fusarium nivale and Oat

Fusarium nivale causes little or no pre-emergence killing of seedlings. It affects mainly the root and shoot lengths of seedlings and their weights. The procedures here

were similar to those used for F. culmorum except that because F. nivale is seed-borne, soil was not inoculated before seed sowing.

Disease assessment with F. culmorum and F. nivale.

There were five disease Ratings and each was given a numerical value:

Disease	Disease Rating	Numerical value.
Healthy	0	0
Very mild rot	+	25
Mild Rot	++	50
Severe rot	+++	75
Dead or very severe rot	++++	100

Disease Index = $\frac{\Sigma d\, n}{N}$

where d n = Numerical value of disease rating

N = Number of seeds sown

J. Technique for assaying survival of C. praticola in soil.

Baiting with Lettuce seeds was devised because this fungus was not readily isolated from soil by dilution plates, even when the soil was plated immediately after inoculation with C. praticola.

Any seedlings remaining in soil in pots were removed with minimum disturbance of the soil. Twenty or forty fresh

Lettuce seeds were pressed into the soil, to a depth of 5 mm. and the soil watered as required. Ten seeds were later sampled at random at one time from each treatment, and placed individually into sterile tubes with covers. They were washed separately in the tubes in 15 ml. tap water by mechanically shaking for five minutes. After three water changes, sterile distilled water was used for three final washings. The seeds were finally dried between two sterile filter papers, without touching one another, and ten placed on plates of peptone/dextrose agar with rose bengal and streptomycin. Later, it was easy to determine which seed had Corticium praticola growing from it; colonies normally turned brown, with characteristic amorphous microsderotia, and hyphae with peculiar constrictions at the points of branching. Usually, the parasite emerged either from the micropylar or chalazal end, or both, and spread in a fan like way, from the seed. With the food base advantage, C. praticola normally grew faster than most of the other fungi present.

The survival of the parasite is expressed as the percentage of seed giving colonies of the parasite.

K. Techniques for estimating microbial population of soils.

(i) Soil sampling

Pooled sampling similar to that recommended by

Schmitthenner and Williams (1958) was used. From each of four replicate pots of soil, three random soil samples were taken, one from the surface, the second about one inch and the third about two inches below the soil surface, and the twelve samples placed together into a sterile, covered jar. The jars were vigorously shaken to mix the samples and kept overnight or for 24 hr. with loose fitting covers. They were then spread on a sterile blotting paper, and when dry enough passed through 3 mm. sieve. The same sample pool was used for isolating fungi, bacteria and actinomycetes.

(ii) Dilution Plate Method

A dilution plate method similar to that described by Timonin (1940) was used. It is not an entirely adequate technique but is about the only one that could be used in the time available for this work.

Details are as follows:-

The moisture content of the soil sample was first determined. Five g. of soil (as dry weight) was placed in 50 ml. sterile water in a medicine bottle, and shaken mechanically for ten minutes, allowed to stand for 30 seconds; 5 ml. were then transferred with a sterile pipette into 45 ml. sterile water. This was shaken before transferring 5 ml. to another 45 ml. of sterile water. The transfers continued until

dilutions of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} were obtained. At each transfer the pipette was vigorously shaken to avoid sedimentation at the inner side of the pipette, while the soil suspension was being dispensed; a fresh sterile pipette was used for each transfer. One ml. from the dilutions was spread over the bottom of a sterile Petri dish, and 20 ml. of appropriate agar at 45°C. was poured into the dish shaken so that the soil suspension became thoroughly mixed with it. This was done inside a sterile chamber.

(iii) Fungal population

Peptone dextrose agar plus rose bengal and streptomycin was used with soil dilution of 10^{-4}. This medium prevented growth of most bacteria and actinomycetes and impeded spread of the fungal colonies. Four to six plates were used for each estimate of population. They were incubated at 25°C. and colonies were counted after 4 - 5 days.

(iv) Bacterial population

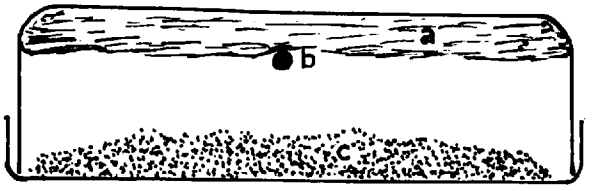
Modified Thornton's (Thornton, 1922; Johnson, 1959) was used with dilution at 10^{-6}. Plates were incubated at 25°C. and colonies counted after four days.

Actinomycetes also appear in this medium, but mostly only after 6 days so they did not interfere with bacterial counting.

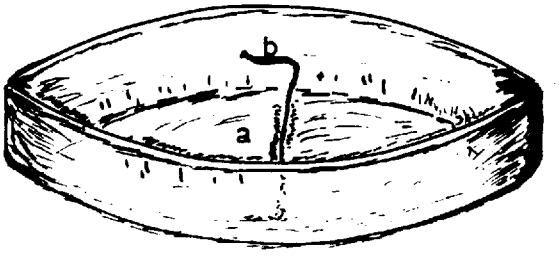
(v) Actinomycetes population

Two types of agar media were used. The method recommended by Tsoa et al., (1960) was used with both. Soil was pulverized in a clean mortar and thinly spread in enamel trays at room temperature for 8 days. This eliminated most fungal hyphae and non-spore forming bacteria. After 8 days the soil was stored in a fresh sterile glass jar with a close lid. Ten g. was mixed with 1g. of powdered $CaCO_3$ and kept in a closed inverted plastic Petri dish at 25°C. for another 8 days. High relative humidity was maintained within the dish by saturating with sterile water 10 filter papers positioned with wire in the Petri dish bottom, acting as the top. (See fig. I). The filter papers were kept moist for the eight days. A soil dilution of 10^{-6} was used. The few bacterial colonies that appeared presented no problem, because they appeared by the fourth day whereas the actinomycetes colonies appeared only after six days and were not counted until after the eight day. Where many actinomycetes colonies appeared close near one another, there were indications that some were suppressed, in circular zones of inhibition that appeared free from colonies. If one carefully transferred a bit of the agar at the middle of such a zone to another plate an actinomycete colony often developed. So zones clearly defined in this way were counted as colonies.

Fig.1. SOIL INCUBATION FOR ACTINOMYCETES ISOLATION.



Petri dish bottom



a. Moist sterile filter papers.

b. Bent wire.

c. Mixed soil and $CaCO_3$.

The media used were:

(a) Glycerol asparaginate agar (Conn 1921) was found suitable and convenient when aim was to count the number of actinomycetes present. But it did not distinguish well between different types of colonies. The next medium did this

(b) Soybean-meal glucose agar (Tsao et al., 1960) showed various colours from red to violet and clearly showed how many of each group was present.

EXPERIMENTAL

1. Factors affecting the virulence of Corticium praticola Kotila.

The virulence of Corticium praticola on lettuce in normal and steam sterilized soil and the effects of inoculum concentration were investigated.

Air dried and sieved garden soil (p. 30), was steam sterilized for 2 hr. and thinly spread on a polythene sheet for four days with occasional mixing and turning over. The experiment was done as series I. in ordinary soil and series II. in steam sterilized soil. Each series consisted of six treatments of various inoculum concentrations with four replicates. 270 g. soil was put into 3 inch pots. Treatments B. to F. of each series were inoculated with Corticium praticola grown on glucose nitrate agar (p. 25), at 1 to 5 percent (wet wt. inoculum/dry wt. soil) levels. Twenty lettuce seeds were sown 5 mm. deep in each pot with the aid of a metal disk perforated with twenty equidistant holes, 24 hr. after inoculation. The pots were lightly watered, covered with thin polythene sheets and were not watered again until the seedlings emerged, after which they were watered uniformly when necessary.

The experiment was done in January, in the Silwood

Table 1. Effects of soil sterilization and inoculum concentration on the virulence of Corticium praticola.

TREATMENT	Percentage kill (in angles)	
Series I		
NORMAL SOIL		
A_1, Control soil only	3.2	
B_1, 1% Corticium praticola	48.0	*** A_1/B_1
C_1, 2% " "	65.9	
D_1, 3% " "	68.2	** B_1/D_1
E_1, 4% " "	81.1	*** B_1/E_1
F_1, 5% " "	90.0	*** B_1/F_1
Series II		
STERILE SOIL		
A_2, control soil only	4.6	
B_2, 1% Corticium praticola	67.4	** B_1/B_2
C_2, 2% " "	74.0	
D_2, 3% " "	83.6	* D_1/D_2
E_2, 4% " "	90.0	
F_2, 5% " "	90.0	

* Treatment means which are compared for significant differences are indicated with letters

L.S.D., P = 0.05 = 12.57 angles *

" " " P = 0.01 = 16.93 " **

" " " P = 0.001 = 22.31 " ***

Figures are means of 4 replicates.

Park green house with additional lighting for 14 hr. each day (p.32).

The final stand of seedlings was taken 28 days after sowing and the pre-, and post-emergence killing was obtained as percentage of seed sown. The data was analysed statistically after angular transformation of the percentage kill figures. The results are shown in Table 1. and Table 1a. (Appendix)

These results show significant differences (P = 0.001) between treatments. Table 1b (Appendix). There were significant differences in virulence between series I and II, at 1 percent level of inoculum (P=0.01) Table 1. Corticium praticola was more pathogenic in steam sterilized soil than in normal soil. As the amount of inoculum increased these differences in virulence became less and at 5 percent had disappeared because all seedlings were killed in both types of soil.

Within series.

In each, the virulence of C. praticola increased with increase in inoculum. Thus in the normal soil series there were significant differences between 1 percent and 3 percent levels (P = 0.01) but between 1 and 4 percent levels, the difference in virulence became more significant (P = 0.001).

In summary Corticium praticola was more virulent in sterile than in normal soil and virulence increased with amount of inoculum. At high levels of inoculum the parasite killed almost all seedlings in both soils. These results were confirmed in a second experiment in which nutrient composition of the culture of C. praticola was investigated.

2. Effects of soil, nutrient status and concentration of inoculum on virulence of Corticium praticola.

Three types of soil, normal and sterilized soil and sterile peat/sand (p. 32) were used. The peat/sand and soil were separately steamed for 2 hr. and spread on polythene sheets for four days with occasional turning over and mixing. After the fourth day 270 g. (dry weight) of the soils were placed in 3 inch plastic pots. Corticium praticola was grown on glucose nitrate agar (G.N.A.) and on agar containing 1/10 of the nutrients listed on p.25 (G.N.A/10). The pots were inoculated with 1,2,3. 4 and 5 percent (wet weight inoculum/dry wt. soil) of C. praticola (p.33).

Each treatment was replicated four times and there were thirty treatment combinations in the experiment, two nutrient levels, three soil types and five inoculum levels (2 x 3 x 5). The experiment was done in Silwood Park green house during January.

The final stand of lettuce seedlings was counted 28 days after sowing and from these figures, the percentage kill was obtained. Tables 2a and 2b (Appendix) give details of the statistical treatment on angular transformed values of percentage kill. Table 2. summarizes the results.

Cultures of Corticium praticola on G.N.A. medium were significantly ($P = 0.01$) more virulent than were those on G.N.A/10 medium (Table 2a. Appendix). The mean percentage kills (transformed to angles) being 56 and 30 respectively. On plates of G.N.A/10 the parasite grew very scantily but grew very luxuriantly in G.N.A. Also the parasite was more virulent in sterile than in normal soil with percentage kills of 48 and 34 respectively. It was about as pathogenic in peat/sand as in sterile soil.

Disease failed to develop in normal soil inoculated with G.N.A/10 culture, but did so in sterile soil and peat/sand. Disease was severe in all types of soil inoculated with G.N.A. culture, and also at all levels of inoculum. The low level of disease in G.N.A/10 inoculated soil, may have been due to poor growth of the parasite because of inadequate nutrients and its inability to survive antagonism, by soil microorganisms. When these were partly eliminated by soil sterilization the parasite was able to grow through the soil.

Table 2. Effects of soil, inoculum concentration and nutrient status on the virulence of Corticium praticola.

TREATMENT	Percentage kill (in angles)									
	G.N.A./10					G.N.A.				
	A Control Soil only	B 1% C.p.	C 2% C.p.	D 3% C.p.	E 5% C.p.	A Control Soil only	B 1% C.p.	C 2% C.p.	D 3% C.p.	E 5% C.p.
NORMAL SOIL	13.8	15.7	9.2	15.7	24.0 *	6.5	40.6 ***	58.7 ***	74.0 ***	79.0 ***
STERILE SOIL	26.4	33.0	39.3 **	37.7 *	49.4 ***	0.0	68.0 ***	65.3 ***	76.5 ***	83.5 ***
STERILE HEAT/SAND	9.2	26.5 ***	45.0 ***	47.9 ***	49.4 ***	11.1	55.6 ***	57.0 ***	78.8 ***	77.8 ***

* Treatment means compared with the control for significant differences. L.S.D., P = 0.05 = 9.5 angles *
P = 0.01 = 12.6 " **
P = 0.001 = 16.2 " ***

Figures are means of 4 replicates. G.N.A. = glucose nitrate agar.

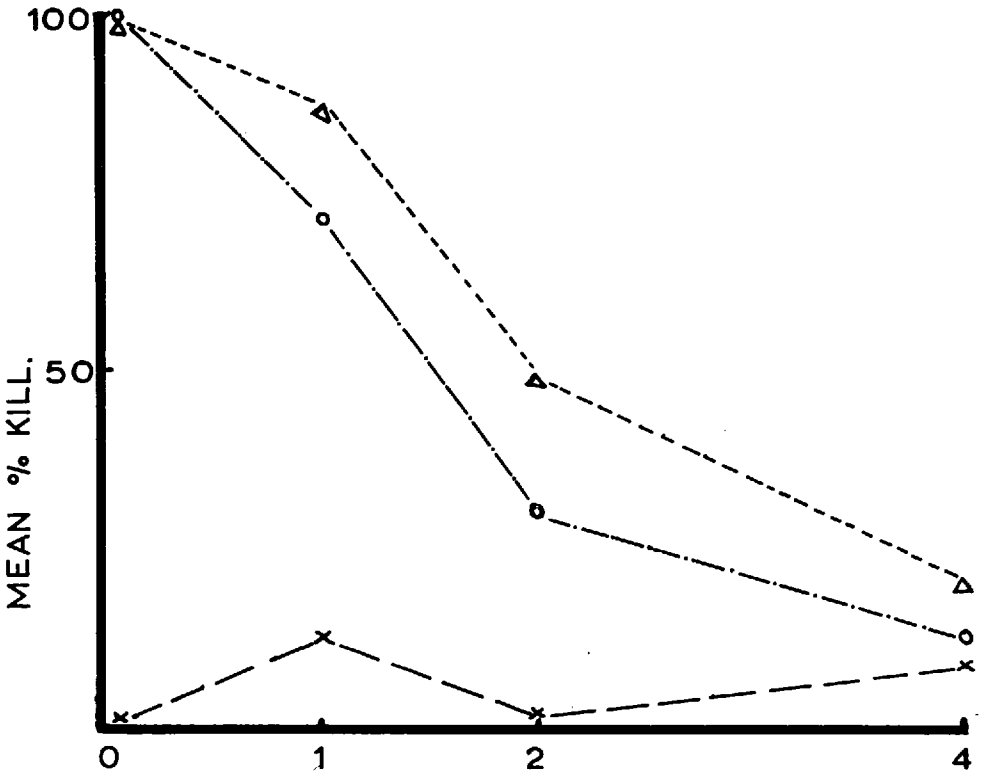
This showed well in the G.N.A. treatment where disease was severe irrespective of previous soil treatments, thus suggesting the importance of energy source for the spread of the parasite.

3. The effect of extra nutrients on the persistance of Corticium praticola in soil.

It has been noticed that whereas Corticium praticola grown on glucose nitrate agar (G.N.A.) was very virulent to lettuce, if seeds were sown 24 hr. after soil inoculation; virulence diminished very quickly with time. Also when G. N.A/10 was used, there was less disease than when G.N.A was used, even when seeds were sown 24 hr. after soil inoculation. So it seemed possible that adding extra nutrient materials to the inoculum, just before soil inoculation would make the parasite persist longer in soil. An experiment was designed to test this.

C. praticola was grown on 20 ml. G.N.A. in Petri dishes at 25°C. for 7 days and transferred to a cold room at 4°C for 6 hr. They were brought out of the cold room and the agar inverted on to the lids. 20 ml. fresh G.N.A. or plain agar at 45°C. were poured on to the C. praticola cultures. The lot with plain agar was designated G.N.A. while the lot with extra 20 ml. of glucose nitrate agar was designated 2 G.N.A. Both were kept for 24 hr. before passing each, separately through $\frac{1}{4}$ inch sieve so that the old and new agar remained together. The inoculum was then mixed with the soil. Seeds were sown 24 hrs (0 week) and 1,2 and 4 weeks after soil inoculation. This experiment was done in the

Fig. 2. THE EFFECT OF EXTRA NUTRIENTS ON THE PERSISTENCE OF CORTICIUM PRATICOLA IN THE SOIL.



green house at Imperial College, South Kensington, with lighting and heat control of the green house as described in p. 32. The final stand of seedlings were counted 28 days after seed sowing.

Table 3. Appendix shows the percentage kill of seedlings and the data are summarized in Figure 2. It is evident that C. praticola killed off all seedlings from the G.N.A. and 2 G.N.A. inocula when seed were sown 24 hr. after soil inoculation i.e. 0 week series. But a week later, disease declined rapidly in both treatments and did so more rapidly, with the inoculum supplied with extra nutrients, a somewhat unexpected result. After four weeks the effect of inoculation had almost disappeared.

The extra glucose nitrate agar which had not been colonized by C. praticola before soil inoculation, may have provided a good base for soil microorganisms, which multiplied on it at the high temperature ($28^{\circ}C.$) of this experiment and may have antagonized the parasite to produce the result obtained.

4. Screening tests for organic materials (seed treatment).

The following organic materials were screened for control of Corticium praticola by seed treatment.

1. Grass meal.
2. Cellulose (Solka Floc)
3. Starch (potato)
4. Sodium polypectate
5. Peptone
6. Casein
7. Casein hydrolysate
8. Soluble cellulose (cellulose gum)
9. Glucose (dextrose)
10. Sodium nitrate
11. Ammonium nitrate.

Each material was used in a separate experiment (Table 4.). The soil was inoculated with 3 percent C. praticola sand/corn meal culture (p. 33). Some seeds were treated with Thiram (0.01g. of Thiram to 2.5g. seed). Other lettuce seeds were soaked in water for 10 min., dried between blotting papers and placed into specimen tubes containing the organic materials. For (Sodium polypectate, Casein hydrolysate and soluble cellulose) unsoaked seeds were used, as prior soaking caused seeds and organic materials to stick together. Excess material was shaken off before the seeds

Table 4. Screening tests for organic materials (seed treatment).

Final stand as % seed sown. Standard error of means are given.

Seed Treatment	A Soil only	B C.p. only	C C.p. + Thiram	D C.p. + Treatment	E Treatment only
1. Grass meal	93.8±3.8	1.3±1.5	56.3±2.1	5.00±2.0	100.0±0.0
2. Cellulose (Solka Floc)	87.5±3.2	7.5±3.2	75.0±6.4	31.3 ±7.8	78.8±8.7
3. Starch(potato)	95.0±2.0	6.3±4.7	82.5±7.2	2.5 ±1.0	97.5±2.5
4. Sodium polypectate	97.5±1.4	13.8±4.7	78.8±2.3	20.0 ±2.0	73.8±12.8
5. Peptone	96.3±2.2	2.5±1.4	48.8±8.5	2.5 ±2.5	76.3±8.2
6. Casein	95.0±2.0	2.5±2.5	75.0±5.4	5.0 ±3.5	61.3±13.7
7. Casein hydrolysate	90.0±3.5	2.5±2.5	66.3±7.7	0.0 ±0.0	51.2±8.7
8. Soluble cellulose	82.5±10.1	2.5±2.5	77.5±2.5	8.8 ±5.1	88.8±4.3
9. Glucose (dextrose)	93.8±4.7	3.8±2.4	67.5±7.2	2.5 ±2.5	82.5±3.2
10. Sodium nitrate	95.0±3.5	3.8±2.4	73.8±3.1	10.0 ±4.5	81.3±4.7
11. Ammonium sulphate	88.8±2.1	2.5±2.5	70.0±6.1	20.0 ±2.8	78.8±5.5

A = Control soil only

B = 3% C. praticola only

C = 3% C. praticola + Thiram (seed treated)

D = 3% C. praticola + organic material (seed treated)

E = organic material (seed treated only).

Figures are means of 4 replicates.

sown. The experiment was done at Silwood Park in July.

The final stand of surviving seedlings were recorded 28 days after sowing and the results are summarized in Table 4. Of the eleven materials tested, cellulose lessened the disease by about 24 per cent, ammonium sulphate by 18 percent, grass meal, sodium polypectate, casein, soluble cellulose and sodium nitrate by about 6 percent. Peptone and glucose made no difference whereas starch, casein hydrolysate made the disease worse by about 5 percent.

Thiram gave good protection in each of the eleven tests and only casein and casein hydrolysate diminished seed germination in the controls without C. praticola.

5. Screening tests for organic materials by soil amendment.

The following organic materials were screened as soil amendments for control of C. praticola.

1. Grass meal.
2. Maize meal
3. Barley meal
4. Molassine meal
5. Linseed cake
6. Bran

Each material was separately used at 0.5, 1 and 2 per cent of final weight of soil per pot. The soil was inoculated with 3 percent C. praticola, sand/corn meal culture,

Table 5. Screening tests for organic materials (soil amendment).

Final stand of seedlings as % of seed sown. Standard errors of means are given

Soil Treatment	A Soil only	B C.p. only	C C.p.+0.5% material	D C.p. + 1% material	E C.p.+ 2% material
1. Grass meal	91.3±2.5	46.3±6.8	78.8±2.4	68.8±7.2	26.3±10.9
2. Maize meal	97.5±1.4	61.3±2.4	27.5±11.0	2.5±1.4	0.0± 0.0
3. Barley meal	92.5±4.3	52.5±9.6	18.8±7.5	32.5±3.2	13.8± 5.8
4. Molassine meal	98.8±1.2	47.5±5.9	48.8±5.5	42.5±14.8	52.5± 6.5
5. Linseed cake	80.0±7.3	53.8±2.4	51.3±12.9	41.3±8.2	0.0± 0.0
6. Bran	93.8±3.1	42.5±10.9	55.0±5.4	57.5±6.6	62.5± 1.4

A = Control soil only
B = 3% C. praticola only
C = 3% C. praticola + 0.5% amendment
D = " " " + 1% "
E = " " " + 2% "
Figures are means of four replicates.

amended with the various organic materials and kept watered for two weeks before sowing 20 lettuce seeds per pot. The six experiments were done at the same time in Silwood Park green house in September.

Each treatment was done in four replicates. At the end of two weeks, top inch of each soil was loosened before seed sowing. Surviving seedlings were counted 28 days later and their percentage based on number of seeds sown, is recorded in Table 5.

Grass meal and bran gave good control of the disease and maize meal and barley meal made the disease worse. Molassine meal made little or no difference whereas linseed cake increased disease with increase in amounts of amendment. Grass meal improved the final stand by about 33 percent at 0.5 percent amendment. This improvement fell to 23 percent at 1 percent and disease became worse than in the control by 20 percent at the 2 percent level. Bran gave about 13 percent improvement at 0.5 percent level, 15 percent at 1 percent and 20 percent at the 2 percent level. In both maize meal and linseed cake treatments, seeds did not emerge at the 2 percent levels.

Disease was less in the control treatments (B, 3% C. praticola only) than in similar treatments in Exp. 4 where seeds were sown 24 hr. after soil inoculation.

6. The Effect of time on soil amendment.

The screening tests had revealed that materials with high nitrogen content worsened the disease and those high in

carbohydrate either made no difference or lessened the disease. Materials representing the three categories were used. It was also decided to leave amended soil for longer periods before sowing seeds. In previous experiments it was noted that C. praticola failed to persist for long in the soil, so in this experiment amended soil was inoculated just 24 hr. before seed sowing. The following materials were used:

1. Fish meal
2. Peptone
3. Starch (potato)
4. Wood cellulose

Each material was used separately as 0.5, 1 and 2 percent of the final weight of soil per pot. The experiment was done in two series: 1. amended soil was kept for 2 weeks before use and series II in which inoculation was done 4 weeks after amendment.

In each series treatments were quadruplicated and the final stand of seedlings was counted 28 days after sowing. The results are shown in Table 6. Fish meal and peptone gave slight but insignificant control in the 2 week series, but worsened the disease in the fourth week. Starch increased disease in the second week but reduced disease in the fourth week. There was better control of disease at 0.5 percent than at either 1 or 2 percent level. Wood cellulose

Table 6. The effect of time on soil amendment.

Final stand of seedlings as % of seed sown.
Standard errors of means are given.

	A	B	C	D	E
	Soil only	C.p only	C.p.+0.5% material	C.p.+1% material	C.p. +2% material
A. Two week series					
1. Fish meal	93.8±2.4	26.3±6.5	25.0±5.4	35.0±3.5	0.0±0.0
2. Peptone	87.3±3.5	11.3±3.1	22.5±3.2	28.8±8.3	13.8±4.7
3. Starch (potato)	90.0±3.5	41.3±7.2	36.3±4.7	32.5±3.2	32.5±7.8
4. Wood cellulose	90.0±5.7	38.8±1.3	51.3±6.9	46.3±6.2	62.5±5.2
B. Four week series					
1. Fish meal	95.0±2.0	20.0±4.1	3.8±1.8	7.5±5.1	1.3±1.3
2. Peptone	77.5±5.2	12.5±6.6	0.0±0.0	0.0±0.0	0.0±0.0
3. Starch (potato)	82.5±4.8	16.3±3.1	38.8±3.7	25.0±2.0	27.5±7.2
4. Wood cellulose	92.5±3.1	12.5±5.2	46.3±8.5	53.8±4.3	62.5±3.2

A = Control soil only

B = 3% C. praticola only

C = 3% " " + 0.5% amendment

D = 3% " " + 1% "

E = 3% " " + 2% "

Figures are means of four replicates.

lessened the disease in both the two and four weeks series and at all levels. Generally its effectiveness increased with dose, particularly in the fourth week treatment when disease was diminished by about 35, 41 and 50 percent at 0.5, 1 and 2 percent amendments.

The surviving seedlings in both starch and wood cellulose treatments were stunted and unhealthy. Their sizes are recorded in the next experiment. Stunting of seedlings occured in both 2 wk. and 4 wk. series.

7. Seedling stunting by starch and cellulose amendments.

Four-week old lettuce seedlings from Exp. 6 were sampled at random, ten from each treatment. Soil particles were washed away in 1/8th inch sieve under tap water and bits of roots were collected. Primary roots were measured from tip of hypocotyl and the shoot from hypocotyl to the tip of the longest leaf. Leaf areas were determined with EEL UNICALVO leaf area measuring machine. Roots, shoots and severed leaves were dried separately at 80°C. for three days to constant weights.

The results as means of ten seedlings are shown in Table 7. It is clear that both starch and cellulose amendments reduce growth greatly, leaves being most affected. For both amendments the ratio of leaf area in normal soil

Table 7. Seedling stunting by starch and cellulose amendments. Seedling sizes (mean of ten).

Treatment	Mean short length (cm.)	Mean Root length (cm.)	Mean Leaf Area (Cm^2.)	Mean dry wt. (seedling) (mg.)
1. Starch potato				
A. Control, soil only	10.15	7.97	12.08	16.88
B. 3% C. praticola only	8.72	5.71	8.44	10.84
C. 3% C.p. + 0.5% starch	2.85	4.62	0.80	2.54
D. 3% C.p. + 1% starch	2.64	6.12	0.76	2.34
E. 3% C.p. + 2% starch	2.54	6.74	1.28	2.67
2. Wood Cellulose				
A. Control, soil only	10.75	7.56	9.44	14.11
B. 3% C. praticola only	9.71	6.57	8.96	13.01
C. 3% C.p. + 0.5% cellulose	4.87	5.99	1.36	3.31
D. 3% C.p. + 1% cellulose	3.39	5.01	1.16	2.72
E. 3% C.p. + 2% cellulose	3.07	5.10	0.44	2.35

to that of amended soil was about 10:1, those of dry weights about 7:1 and shoot lengths 4:1. Effects on root

lengths were not so outstanding. Seedlings in amended soil were chlorotic and those left in the pots grew slowly if at all for some time and then became still more chlorotic and died.

The stunting probably resultedfrom the activities of soil microorganisms which in multiplying upon the carbohydrate supplied in the amendment, utilized the readily available soil nitrogen, and caused the lettuce seedlings to suffer from nitrogen starvation. The symptoms on the stunted seedlings resembled those normally associated with nitrogen defficiency.

8. Effects of dried grass and cornmeal in the control of Corticium praticola.

In the screening tests, it was found that grass meal gave the best control and cornmeal was the worst. Further work on the effectiveness of the two materials was done as follows.

The grass material was cropped straight from the field, when it had aged, turning yellow and most of it was already dead. It was air-dried and cut into bits before adding to the soil at 1, 2 and 3 percent of the final weight of soil per pot. The pots were kept aside, well watered for 2 weeks before some were inoculated with 2

Table 8. Effects of dried grass and cornmeal in the control of Corticium praticola.

Final stand of seedlings as % of seed sown.
Standard errors of means are given.

	A	B	C	D	E
	2%C.p. only	Control Soil only	2% C.p.+1% material	2% C.p.+2% material	2%C.p.+3% material
1. Dried grass	35.0±8.8	86.3±3.7	78.8±4.3	55.0±18.1	27.5± 9.2
2. Corn meal	25.0±9.4	90.0±3.5	0.0±0.0	1.3±1.3	6.3±3.2

Figures are means of four replicates.

percent C. praticola (wet wt. inoculum/dry wt. soil), grown on glucose nitrate agar (p.33). Seeds were sown 24 hr. later. The experiment was done in April at Silwood Park. Each treatment consisted of four replicates.

Surviving seedlings were counted 28 days after sowing. The results are recorded in Table 8. as final stand of seedlings as percent seed sown. Standard errors of the means are given.

Dried grass improved seedling stand by 44 percent at 1 percent level, and 20 percent at 2 percent level. These

Plate 1. Effects of dried grass and corn-meal in the control of Corticium praticola.



C.p. - Corticium praticola G.m. - grass-manure.



C.m. - Corn-meal

confirm earlier findings on the efficacy of grass meal in disease control.

Cornmeal treatment increased disease at all levels, again confirming earlier findings on disease increase. At 1 percent level, no seedlings emerged in the cornmeal series (Plate 1).

In the dried grass treatments, surviving seedlings were stunted and the stunting increased with increase in amendment dose (Plate 1.). Thus seedlings in 2 percent dried grass treatment were more stunted than those in the 1 percent treatment. These stunting effects were similar to those caused by wood cellulose in previous experiments. However, the adverse effects in the higher levels of amendment could be due to more micro-organims which utilized the available soil nitrogen, more than at lower levels of amendment. The low final stand of seedlings in treatments with heavy dried grass amendment could be due to phytotoxins normally associated with decaying vegetable matters.

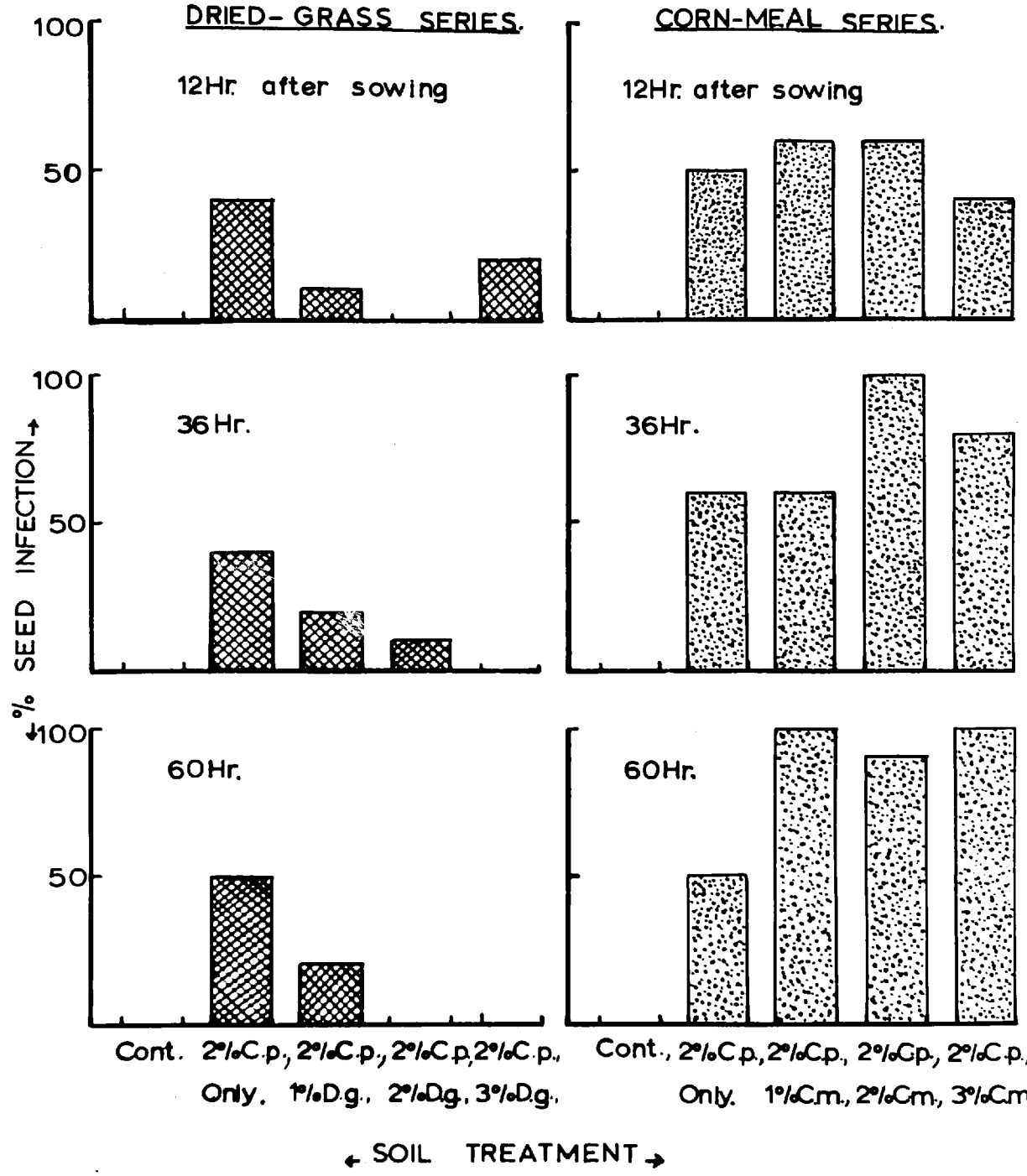
All pots of soil in this experiment were preserved after the seedlings were removed and kept well watered for further work on the persistence of C. praticola in soil which is described in the next experiment.

9. Persistence of C. praticola in soil 90 days after inoculation.

In the previous experiments it was observed that cornmeal increased damping-off of lettuce, whereas grass meal or dried grass lessened it. It was not clear whether the worsening of disease by cornmeal was because of toxins produced during its degradation, or because the parasite multiplied in such soil. In most cases disease was more severe in cornmeal treated soil than in controls which were inoculated with same quantity of C. praticola.

Attempts made to study the survival of the parasite, by dilution plates failed (p.38). However, baiting with lettuce seeds was devised for this study. Pots originally used for Exp. 8 were kept for 90 days from the time of inoculation, before use. The top 1 inch of soil was loosened and 40 lettuce seeds were sown (about 5mm. deep) per pot. Two of the four pots of each treatment were used and two kept back. The pots were lightly watered after sowing and covered with thin polythene sheets. Ten seeds were taken at random, 12, 36 and 60 hr. after sowing. Each seed was treated as described (p.38) and 10 seeds from the same treatment were plated in the same Petri dish on peptone/dextrose agar with rose bengal and streptomycin. All seeds infected by C. praticola always showed the para-

Fig. 3. PERSISTENCE OF C. PRATICOLA IN THE SOIL, 90 DAYS AFTER INOCULATION.



site from the second day after planting, because it normally grew faster than other contaminants (p.39).

The survival of the parasite is expressed as the percentage of seed giving colonies of the parasite. The results are summarized in Figure 3. It is clear that C. praticola persisted more in cornmeal than dried grass amended soil. The grass meal series showed decline of the parasite with increase in amendment dose, though the 12 hr. series gave inconsistent result. Thus there was more survival at 3 percent than at either 1 or 2 percent level. As the seeds stayed longer in the soil, more of them would be expected to be attacked by the parasite, but 3 percent dried grass series showed 20 percent survival after 12 hr. and none after 36 and 60 hr.

In the cornmeal series, there was increase in survival percentage with increase in amendment, except the 12 hr. series which showed less survived at 3 percent amendment than at either 1 or 2 percent (Figure 3).

These results suggest that the decline in disease control with increase in dried grass amendment (Exp. 8) was not due to increase in C. praticola propagule in the soil. They support rather the suggestion that toxins might have been responsible for reducing the final stand.

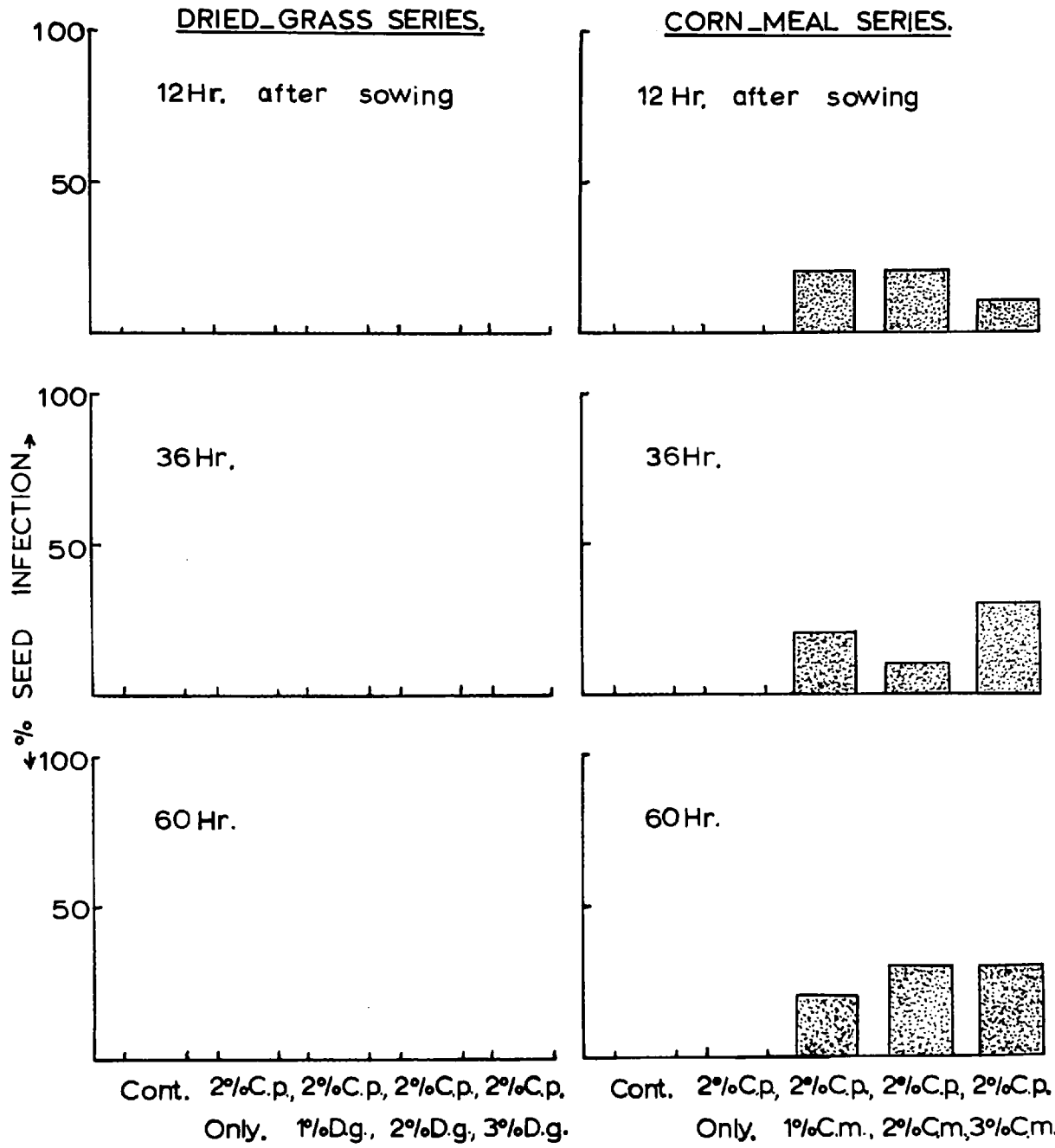
In the 12, 36 and 60 hr. series C. praticola survived in the cornmeal treated soil more than it did in the ordinary soil, originally inoculated with the same quantity of C. praticola. On the other hand, in the grass meal series the parasite was always less in amended soil than in the check treatments.

10. Persistence of C. praticola in soil 180 days after

Two pots of soil from each treatment, kept aside in Exp. 9 and previously used for Exp. 8, were kept for 90 days, i.e. for a total of 180 days after soil inoculation. They were kept watered and covered with thin polythene sheets. The procedure was then as in Exp. 9. The top inch of soil was loosened and 40 lettuce seeds were sown (5 mm. deep) per pot. The pots were watered lightly and covered again. Random samples of ten seeds were taken from each treatment at intervals of 12, 36 and 60 hr. after sowing and tested for the presence of the parasite. The survival of the parasite, expressed as the percentage of seed giving colonies of the parasite, is shown in Fig. 4.

From these results it is clear that 180 days after soil inoculation, no C. praticola was isolated from soils not inoculated with C. praticola, or from soil inoculated with C. praticola culture with or without grass meal amend-

Fig. 4. PERSISTENCE OF C. PRATICOLA IN THE SOIL 180 DAYS AFTER INOCULATION.



ment. But the parasite was isolated from each of the inoculated soils to which cornmeal had been added, although there were some irregularities in the survival figures in the different series. These results confirm the findings in Exp. 9 and imply that the adverse effect of cornmeal, in disease control, is because it stimulates growth of the parasite and prolongs its survival in the soil.

Table 10. Virulence of C. praticola in the soil 180 days after inoculation.

Final stand of seedlings as % seed sown.

Soil treatment.	A		B		C		D		E	
	Soil only		2% C.p. only		2% C.p.+1% amendment.		2% C.p.+2% amendment.		2%C.p.+ 3% amendment.	
	Emer.	F. stand	Emer.	F. stand	Emer.	F. stand	Emer.	F. stand	Emer.	F. stand
Dried grass	100	100	98	98	100	100	100	100	98	98
Corn meal	98	98	100	100	90*	64	92*	74	90*	70

C.p. = Corticium praticola, Emer. = Emerged,
F. stand = Final stand
Figures are means of two replicates
*Seedlings from which Corticium praticola was isolated

After the three seed samplings, 25 seeds left in each pot were allowed to germinate inside the green house. All seedlings which were damped-off were removed and their segments cultured on glucose nitrate agar. All of them showed characteristic infection cushions under the microscope (p.35) normally associated with Corticium praticola. The results, showing emergence and final stand as percentage seed sown are recorded (Table 10). The soil only, as well as the 2 percent C. praticola only treatments gave about 100 percent emergence and final stand, in both the cornmeal and dried grass series. The main difference was in amendment treatments, in which there was about 100 percent emergence and final stand in soils treated with 1,2 and 3% dried grass. The cornmeal series gave 90 percent emergence and 64 percent final stand at 1 percent level, 92 percent emergence and 74 percent final stand at 2 percent level and 90 percent emergence and 70 percent final stand at 3 percent level.

These results confirm the previous findings that C. praticola persisted longer in cornmeal treatments than either in ordinary soil or in soil amended with dried grass. The percentage kill of 36, 26 and 28 at 1,2 and 3 percent cornmeal levels (Table 10)were close to the percentage survival (Figure 4) which resulted from baiting with lettuce

seeds, except that the 1 percent cornmeal level gave 20 percent survival after 60 hr. which is 16 percent less than the percentage kill, and at 2 and 3 percent levels, percentage kill were 4 and 2 percent respectively lower than the results derived from seed baiting.

11. Effect of grass meal on C. praticola 1,2, 4 and 8 months after soil inoculation and amendment.

Previous experiments had revealed that grass meal amendment lessened disease caused by C. praticola when the parasite was inoculated 24 hr. before seed sowing, into soils previously amended. It was now thought that, if inoculated soil could be amended and left for longer periods, before sowing, better control of the disease could result. This could simulate natural conditions. Experiments were designed to test this.

Soil with and without grassmeal was inoculated at the rate of 2 percent by weight of cultures of C. praticola on glucose nitrate agar. These soils together with untreated soil were kept at 16°C in Silwood Park green house and watered occasionally, for one and two months and then sown with lettuce seed in the usual manner. The results, 28 days after sowing, are given in Table 11.

Table 11. Effect of grassmeal on C. praticola 1 and 2 months after soil inoculation and amendment.

Soil treatment	A	B	C	D	E
	Soil only	2% C.p. only	2%C.p.+ 0.5%G.m.	2% C.p.+ 2% G.m.	2% C.p.+ 2% G.m.
1. 1 month series	91.3±1.3	86.3±2.4	90.0±2.0	88.8±2.4	82.5±5.2
2. 2 month series	87.5±4.3	97.5±1.4	93.8±3.2	90.0±3.5	91.3±3.8

C.p. = Corticium praticola G.m. = grass meal

Figures are means of four replicates.

They show that inoculated in this way, the parasite no longer caused disease after one month. This experiment which was meant to last much longer was therefore abandoned. Other experiments were then designed to determine how long the effectiveness of grassmeal treatment could persist in the soil.

12. Persistence of the grassmeal effect 1 month after amendment.

Because of the non-persistence of C. praticola, in

Table 12. Persistence of the grassmeal effect 1 month after amendment.

Soil treatment.	Final stand of seedlings as 1% of seed sown.
A. Control soil only	87.5 ± 4.3
B. 2% *C. praticola* only	53.5 ± 4.7
C. 2% *C. praticola* + 0.5% grassmeal	86.3 ± 9.0
D. 2% *C. praticola* + 1% grassmeal	91.3 ± 4.3
E. 2% *C. praticola* + 2% grassmeal	97.5 ± 1.5

Figures are means of four replicates.

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this experiment, soil was amended with 0.5, 1 and 2 percent grassmeal as in Exp.11, but cultures of the parasite were added 24 hr. before sowing seed. Treated pots were kept aside and watered as necessary for one month before use. Before sowing, the top inch of the soil was thoroughly mixed and seeds were placed about 5 mm. below the soil level.

The final stand of 4 week seedlings is shown in Table 12.

From these results it is evident that disease was greatly reduced by the grassmeal treatment. At 0.5 percent the final stand was the same as that in uninoculated soil.

Damping-off of lettuce seedlings was decreased by about 33, 38 and 44 percent at 0.5, 1 and 2 percent of grassmeal. The final stand with 1 percent grassmeal was about 4 percent higher than in untreated soil and at 2 percent the increase was about 10 percent. Seedlings in amended soil emerged more quickly and grew faster. Disease was not severe in inoculated soil without grass meal.

13. Persistence of the grass meal effect 2 months after amendment.

The experiment was the same as Exp. 12 except that the soil was inoculated 2 months after amendment. The results are summarized in Table 13.

Table 13. Persistence of the grassmeal effect 2 months afteramendment.

Soil treatment	Final stand of seedlings as % of seed sown
A. Control soil only	96.3 ± 2.4
B. 2% *C. praticola* only	41.3 ± 5.5.
C. 2% C.p. + 0.5% grassmeal	51.3 ± 5.5
D. 2% C.p. + 1% grassmeal	75.0 ± 4.1
E. 2% C.p. + 2% grassmeal	81.3 ± 6.0

Figures are means of four replicates.

Grassmeal was still effective, reducing disease at 0.5, 1 and 2 percent by about 10, 34 and 40 percent respectively. The amendment was now less effective than after one month. In the next experiment amended soil was kept for 4 months before use.

14. Persistence of the grassmeal effect 4 months after amendment.

Treated soil (Exps. 12 & 13) was kept for 4 months before inoculation and seed sowing. The results, taken 28 days later, are recorded in Table 14. Grassmeal was still effective 4 months after soil treatment, although disease was more severe in the controls in this experiment, compared with the earlier experiments.

Table 14. Persistence of the grassmeal effect 4 months after amendment.

Soil treatment	Final stand of seedlings as % of seed sown.
A. Control soil only	80.0 ± 4.0
B. 2% C. praticola only	25.0 ± 6.1
C. 2% C.p. + 0.5% grassmeal	60.0 ± 4.1
D. 2% C.p. + 1% grassmeal	62.5 ± 5.2
E. 2% C.p. + 2% grassmeal	75.0 ± 4.0

Figures are means of four replicates.

After storage (in pots) for four months the general microbial activity in unamended soil may well have been reduced, thus allowing the parasite to spread and kill more seedlings.

There were about 35, 38 and 50 percent more seedlings than in control (2% C.p. only) with 0.5, 1 and 2 percent grassmeal. These figures compare well with those in the 2 and 1 month series and show similar increases with the higher levels of grassmeal. Seedlings did not emerge more quickly in amended soil as happened in Exp. 12.

15. Grassmeal effects in sterile and non-sterile soil.

The disease control achieved with grassmeal amendment probably depends on the soil microorganisms, which multiply on it, and antagonize C. praticola. Evidence on this was sought by comparing the effects of grass meal in sterile and non-sterile soil.

Sieved soil was steamed for 2 hr. and spread thinly on a polythene sheet for four days, before use. Each experiment was done with sterile and non-sterile soil. The soil was amended with 0.5, 1 and 2 percent grassmeal, and was inoculated with glucose nitrate agar cultures of the parasite, 0, 1, 2 and 4 weeks later; seeds were sown 24 hr. after inoculation. The experiment was done at Silwood Park green house in January.

Table 15. Grassmeal effects in sterile and non-sterile soil.

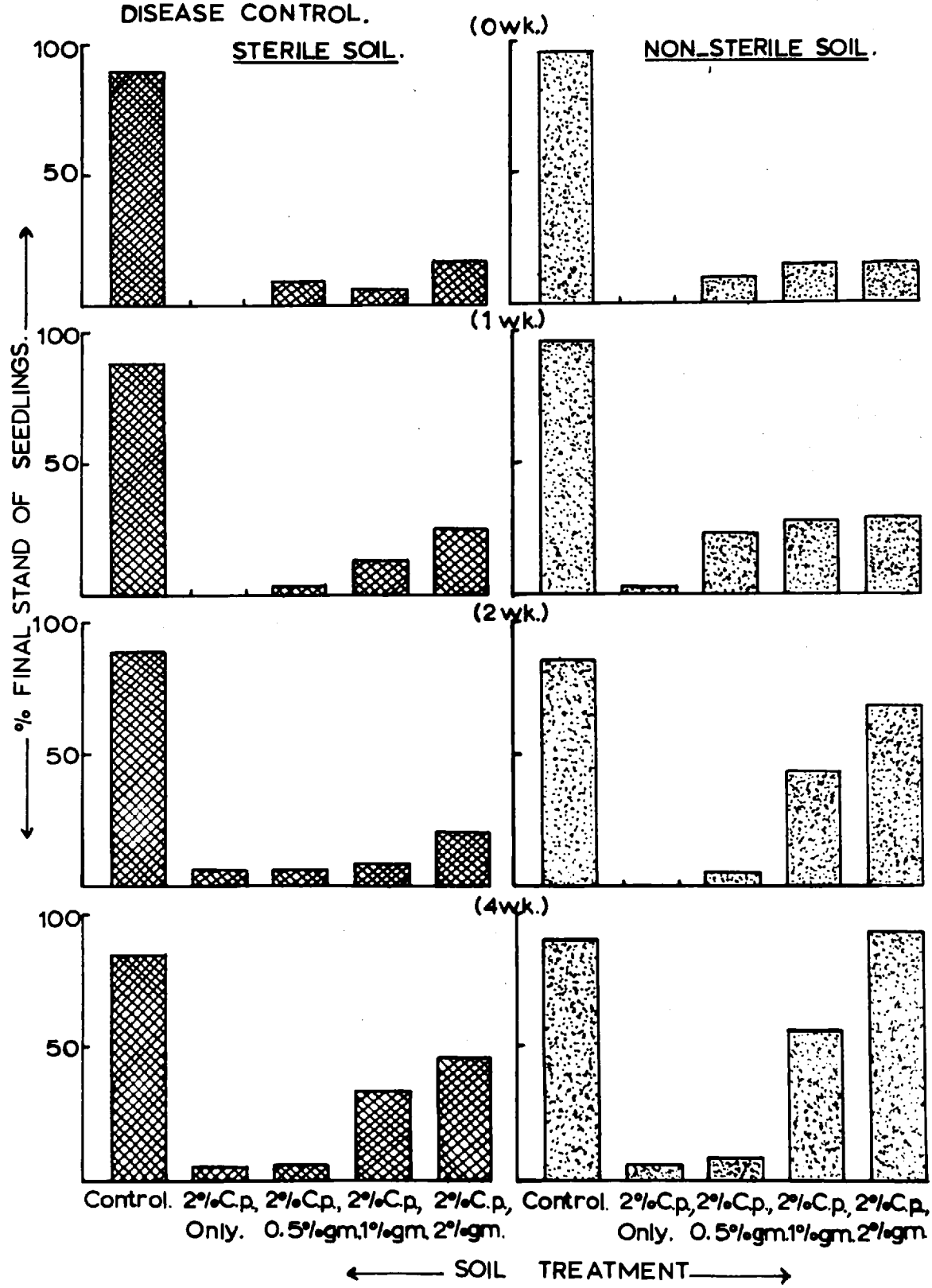
Final stand of seedlings as % of seed sown.

Soil treatment	Soil only	2% C.p. only	2%C.p. + 0.5%G.m.	2%C.p. + 1% G.m.	2%C.p.+ 2% G.m.
1. 0 week series					
Sterile soil	88.8±4.3	0.0±0.0	8.8±4.3	6.3±3.8	16.3±5.5
Non-sterile soil	96.3±3.8	1.3±1.3	10.0±4.1	15.0±3.5	15.0±6.1
2. 1 week series					
Sterile soil	87.5±2.5	1.3±1.3	2.5±2.5	12.5±2.5	25.0±2.0
Non-sterile soil	96.3±2.4	2.5±1.4	22.5±4.3	27.5±7.2	28.8±7.7
3. 2 week series					
Sterile soil	88.8±5.5	6.3±3.8	6.3±2.4	7.5±3.2	20.0±7.3
Non-sterile soil	85.0±3.5	1.3±1.3	5.0±3.5	42.5±4.3	67.5±9.6
4. 4 week series					
Sterile soil	83.8±6.2	5.0±3.5	6.3±3.1	32.5±4.3	46.3±4.3
Non-sterile soil	90.0±5.4	6.3±3.8	8.8±3.8	56.3±6.2	82.5±6.0

C.p. = Corticium praticola G.m. = Grassmeal

Figures are means of four replicates.

Fig.5. GRASS MEAL EFFECTS IN STERILE AND NON-STERILE SOIL ON DISEASE CONTROL.



The results are shown in Table 15 and Fig. 5. In the 0 week series, there was not much difference between the final stands, in sterile and non-sterile soil. Disease was severe in each series. The 1 week series showed some contrast between the two types of soil, at 0.5 percent amendment, which gave 20 percent more control in non-sterile than sterile soil. The 2 week series gave bigger differences at the 1 and 2 percent levels, with 35 and 48 percent more control in non-sterile than sterile soil. In the 4 week series, the difference between the two types of soil at 1 and 2 percent levels were 21 and 37 percent, and the sterile soil had begun to give control at 1 and 2 percent grassmeal. Thus, there were about 28 percent disease control in sterile soil at 1 percent and about 41 percent control at 2 percent amendment.

It was only in the four week series that reasonable disease control occurred in the sterile soil. This was probably caused by recolonization of the soil, by air and water-borne microorganisms, which then multiplied on the amendment.

In non-sterile soil, the amendment became increasingly effective, as the time between soil treatment and inoculation increased. These results support the hypothesis that control given by grassmeal is dependent on the activity of

soil microorganisms and that this requires some time to develop.

16. Temperature effects on disease control and soil microorganisms.

It has been shown that damping-off of lettuce is reduced by grassmeal amendment, and this requires time to become significant. Experiments were now done to see which group or groups of soil microorganisms were important in suppressing C. praticola, when grassmeal is added to soil.

Ten inch plastic pots were partly filled with air-dried and sieved soil. One lot was amended with 2 percent grassmeal, and the other left untreated. One treated and one untreated pots were kept in a constant temperature room at 10°C. Other pairs were kept at 20°C. and 30°C. All were kept suitably moist. The six lot of soil, were removed individually every seven days and thoroughly mixed before replacing in the original containers. After two and four weeks, each soil was sampled to determine the soil microbial population, and some of the soil was placed in 3 inch pots for experiments on disease control. There were four treatments in each series as follows: A. ordinary soil, B. ordinary soil inoculated with 3 percent C. praticola cornmeal/sand culture, C. amended soil + 3 per-

Table 16. Temperature effects on disease control and soil microorganisms.

	2 week series				4 week series			
	Microorganisms /g of soil			Final stand of seed lings (% seed sown)	Microorganisms /g of soil			Final stand of seed lings (% seed sown)
	Actino x10^6	Bact. x10^6	Fungi x10^3		Actino x10^6	Bact. x10^6	Fungi x10^3	
1. 10°C.								
A. Soil only				73.8				93.8
B. 3%C.p only	30	22	50	0.0	8	40	160	2.5
C. 3%C.p +2%G.m.	50	31	650	2.5	231	67	570	12.5
D. 2%G.m. only				58.8				100.0
2. 20°C.								
A. Soil only				86.3				88.8
B. 3%C.p only	33	18	3o	0.0	12	32	210	1.3
C. 3%C.p. +2%G.m.	536	230	1,120	25.0	191	632	940	41.3
D. 2%G.m only				82.5				87.5
3. 30°C.								
A. Soil only				86.3				98.8
B. 3%C.p. only	76	10	40	0.0	11	56	160	1.3
C. 3%C.p. +2%G.m	232	207	1,300	63.8	247	700	1030	62.5
D. 2%G.m. only				75.0				92.5

Figures are means of four replicates

C.p. = Corticium praticola G.m = Grassmeal

cent C. praticola and D. Amended soil only.

After inoculation, th pots were kept in the green house at about 16°C (p.32) for 24hr. before sowing 20 lettuce seeds per pot. The final stand of seedlings were counted 4 weeks later.

The six soil samples were kept in sterile sampling bottles with loose fitting covers and kept for 24hr. before use. Each sample was used separately to determine the numbers of actinomycetes, bacteria and fungi present. The actinomycetes were isolated on glycerol asparaginate agar, the bacteria on modified Thornton's medium, and the fungi on peptone dextrose agar plus rose bengal and streptomycin, by dilution plates.

The results of the dilution plate counts of the soil microorganisms and the final stand of seedlings are shown in Table 16 and Figures 6A-D.

These results show clearly that in both the two and four week series, disease was more reduced at 30°C. than at 20°C., and that there was lest effect at 10°C. The 3 least percent and 10 percent disease reduction in the second and fourth week series are much less than the 25 and 40 percent control at 20°C., and 64 and 63 percent in the second and fourth week series at 30°C. In the controls (3 percent C. praticola only) all or almost all seedlings were killed

Fig.6.

Fig. 6. TEMPERATURE EFFECTS ON DISEASE CONTROL AND SOIL MICRO_ORGANISMS.

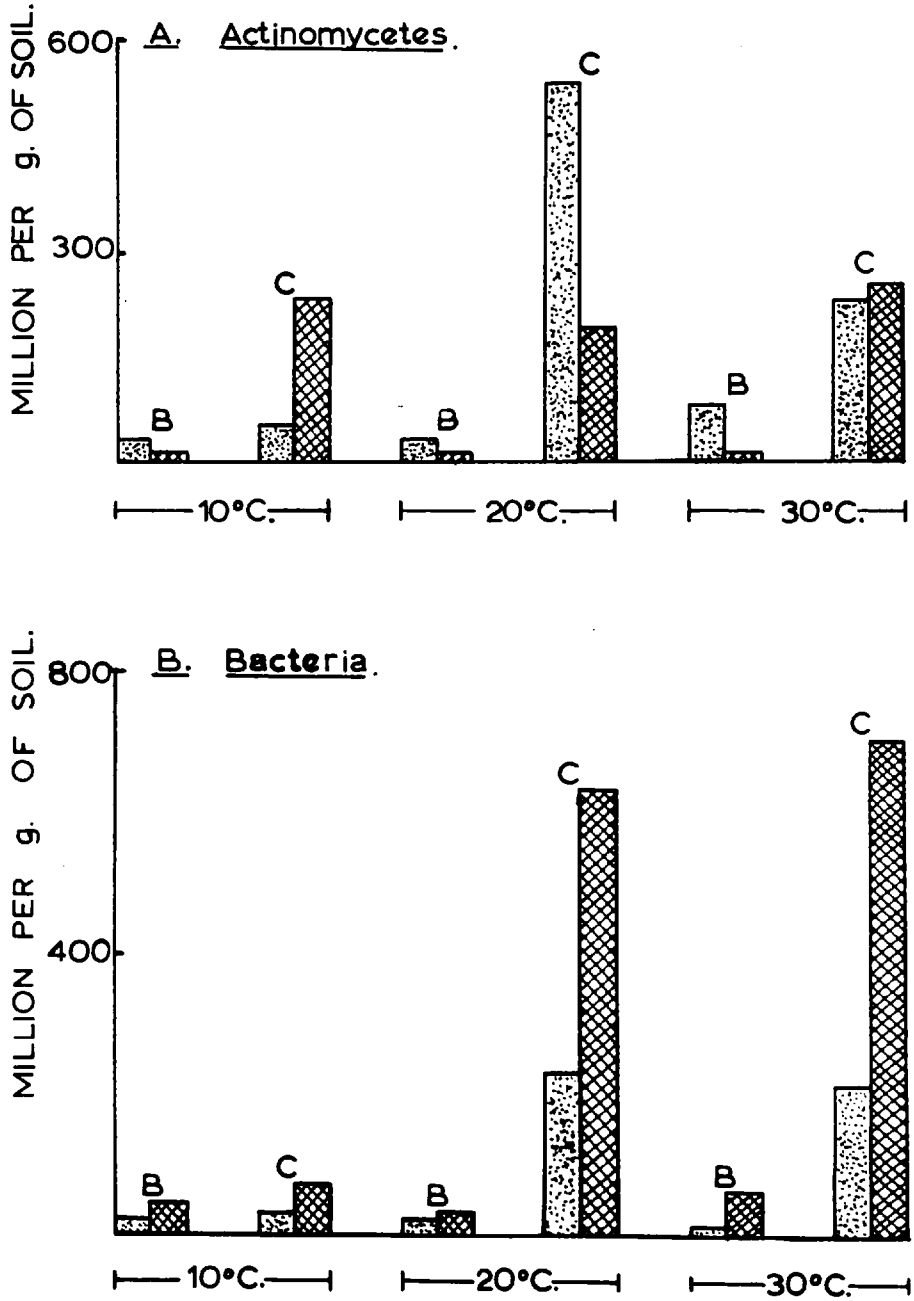
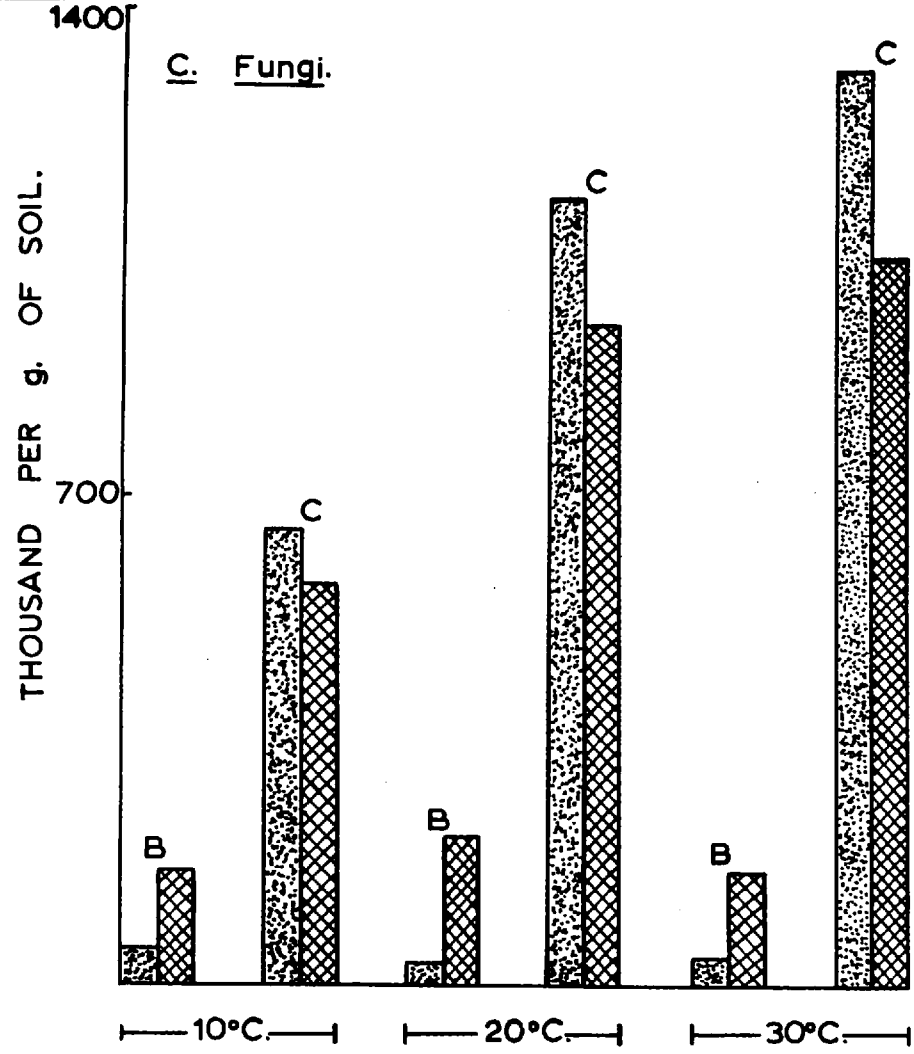
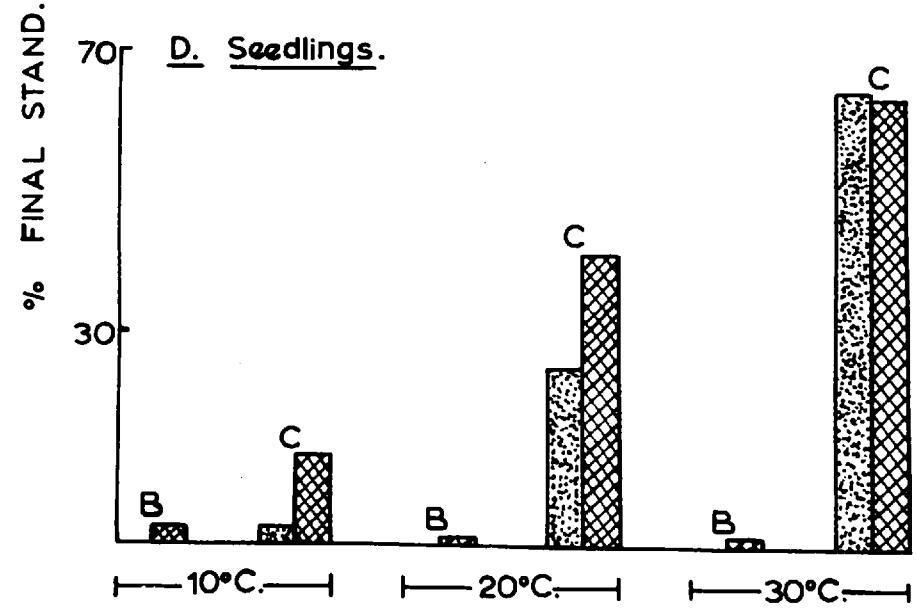


Fig. 6. Continued





in all treatments.

In ordinary soil the actinomycetes numbers were less in the fourth than in the second week series. There were variations in the treated soil at 10° C, 20°C and 30°C in the second week series, where there were 536 millions per g. of dry soil at 20°C, but only 50 and 232 at 10°C and 30°C respectively. But there was better disease control at 30°C than at 20°C (64 percent and 25 percent respectively). In the 4 week series there were 231, 191 and 247 million actinomycetes per g. of soil at 10°C, 20°C and 30°C respectively. The disease control were 10, 40 and 63 percent in that order. The actinomycetes numbers could not therefore be related logically to disease control.

The bacterial numbers in the 2 week series were 31, 230 and 207 million per g. of soil at 10°C, 20°C, and 30°C, disease being reduced by about 3, 25 and 65 percent. Here there is some indication that the higher bacterial numbers are associated with disease control, but it was not the highest numbers of colonies that gave the most disease reduction. In the 4 week series, the bacterial colonies were 67, 632 and 700 million per g. of dry soil at 10°C, 20°C and 30°C and the disease was reduced by 10,

40 and 63 percent. Damping-off was therefore related to increase in bacterial populations.

There were more fungi generally in the fourth than the second week series in the ordinary soil treatments at the three temperatures. In the amended soil, there were more in the second than in the fourth week. This was the opposite of what happened with the bacteria. In the second week, the fungi were 650, 1,120 and 1,300 thousand per g. of dry soil at 10°C, 20°C and 30°C with disease reduction of about 3,25 and 64 percent respectively, so disease control improved as the number of fungi became larger. In the 4 week series, there were 570, 940 and 1030 thousand fungi per g. of soil at 10°C, 20°C and 30°C with disease control of 10, 40 and 63 percent.

17. pH effects on disease control and soil microorganisms.

The pH of air dried and sieved soil determined with a pH meter was 5.0. Some of the soil was adjusted to pH 7 with powdered calcium hydroxide (0.15g. per 150 g. of soil). Half of each type of soil was amended with 2 percent grassmeal. Each type of soil was in two 10 inch plastic pots and kept moist throughout the experimental period. The soil was turned over and mixed every 14 days.

to give adequate soil aeration and homogeneity. Each soil type was sampled at intervals of 0(3 days after treatment), 2,4, 8 and 16 weeks after amendment. Part of the sample was put in a bottle with loose fitting top and kept for 24 hr. before use. Some of the soil was weighed into 3 inch plastic pots and some were inoculated with 2 percent C. praticola culture on glucose nitrate agar. Seeds were sown 24 hr. later. Actinomycetes were isolated on glycerol asparaginate agar, the bacteria on Thornton's medium and the fungi on peptone dextrose agar plus rose bengal and streptomycin. The results of the colony counts and for disease control are shown (Table 17 and Figure 7A-D) as mean number per g. of dry soil and as final stand as percentage seed sown.

From these results, it is clear that in ordinary soil (pH.5) treated with grassmeal, disease is reduced significantly from the 2nd week to the 16th week, whereas in the adjusted soil (pH.7) there is no disease control until the 8th week (Figure 7D.). In the 0 week series there was 100 percent kill in all inoculated soils at both pH values whether the soil was amended or not (Figure 7D.). The control treatments (2% grassmeal only) also had few final stand of seedlings, which were 4 and 9

Table 17. pH effects on disease control and soil micro-organisms.

Soil treatment	pH = 5				pH = 7			
	Microorganisms per g. of soil			Final stand (% seed sown)	Microorganisms per g. of soil			Final stand (% seed sown)
	Actino. x10^6	Bact. x10^6	Fungi x10^3		Actino. x10^6	Bact. x10^6	Fungi x10^3	
1. 0 wk. series								
A, Soil only				66.0				69.0
B, 2%C.p. only	12	6	142	0.0	11	11	92	0.0
C, 2%C.p.+ 2%G.m.	11	41	1,127	0.0	14	53	710	0.0
D, 2%G.m. only				4.0				9.0
2. 2wk. series								
A, Soil only				81.0				86.0
B, 2% C.p. only	26	8	133	0.0	20	37	147	0.0
C, 2% C.p.+ 2% G.m.	76	152	898	71.0	392	105	512	1.0
D, 2%G.m. only				81.0				74.0
3. 4wk. series								
A, Soil only				85.0				94.0
B, 2% C.p. only	32	12	66	0.0	41	13	71	0.0
C, 2% C.p.+ 2% G.m.	61	125	813	80.0	284	186	514	5.0
D, 2%G.m. only				94.0				90.0

Table 17. pH effects on disease control and soil micro-organisms. (contd.).

Soil treatment	pH = 5				pH = 7			
	Microorganisms per g. of soil			Final stand (% seed sown)	Microorganisms per g. of soil			Final stand (% seed sown)
	Actino. x10^6	Bact. x10^6	Fungi x10^3		Actino. x10^6	Bact. x10^6	Fungi x10^3	
4. 8wk. series								
A, Soil only				98.0				89.0
B, 2% C.p. only	11	11	138	11.0	24	23	125	9.0
C, 2% C.p. + 2% G.m.	16	123	870	86.0	71	364	943	80.0
D, 2%G.m.only				90.0				91.0
5. 16wk. series								
A, Soil only				96.0				95.0
B, 2% C.p. only	9	16	274	9.0	22	51	263	5.0
C, 2% C.p. + 2% G.m.	14	161	1,215	79.0	32	184	740	61.0
D, 2% G.m.only				100.0				83.0

4 replicates per treatment.

percent at pH 5 and 7 respectively, although no parasite was added in these treatments. This was probably caused by phytotoxins which are produced in soils treated with organic materials in the first few weeks (Figure 7D.).

There was not much difference in actinomycetes numbers in treated and untreated soils at pH 5 and 7 (Figure 7A.). Bacteria numbers were higher in treated than in untreated soil and at pH 7 compared with pH 5 (Figure 7B.). There were more fungi in treated than untreated soil at pH 5 and at pH 7. They were more at pH 5 than at pH 7 (Figure 7C). Most of them were fast growing Mucorales such as Zyporhynchus species.

In the 2 week series, grassmeal decreased damping-off by 7 percent at pH 5 but by only 1 percent at pH 7 (Figure 7D). The outstanding differences between the microbial polulations in the two soil types are for actinomycetes and fungi. There were 76 and 392 million actinomycetes per g. of soil at pH 5 and 7 respectively in amended soils (Plate 2)and the fungi numbered 898 and 512 thousands in the same order. Where the actinomycetes were 392 million, there was no disease control but there was control when they were 76 million. The difference in fungal numbers was probably not large enough to account

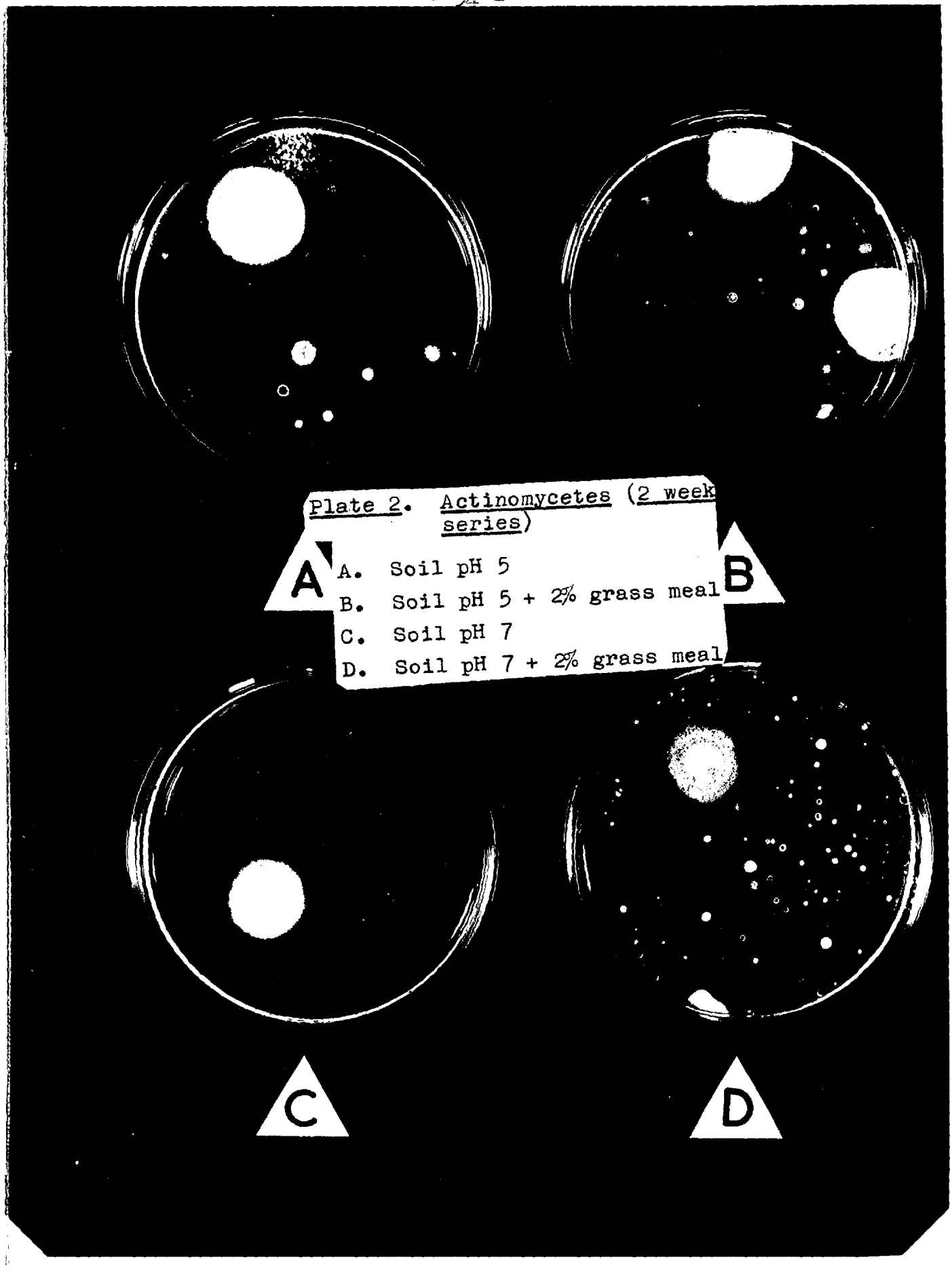


Plate 2. Actinomycetes (2 week series)

A. Soil pH 5
B. Soil pH 5 + 2% grass meal
C. Soil pH 7
D. Soil pH 7 + 2% grass meal

for the 70 percent difference in disease control in the two treatments. There was no striking difference in bacterial counts, though there were more in ordinary amended soil (pH 5) than in adjusted and amended soil (pH 7) (Figure 7B).

In the 4 week series a similar pattern of disease control was obtained. There was 80 percent disease reduction at pH 5 and only 5 percent at pH 7. The actinomycetes were respectively 61 and 284 million per g. of soil. The fungi were 813 and 514 thousand per g. of soil in the same order. Again there was no significant disease control where actinomycetes dominated the soil microflora, but there was striking control when fungi dominated the soil microflora (pH 5.). Bacterial numbers did not alter so much as did actinomycetes and fungi. It may also be noted that numbers of both actinomycetes and fungi had declined, the former from 76 to 61 at pH 5 and 392 to 284 million per g. of soil at pH 7. and the fungi declined from 898 to 813 at pH 5, and there was a slight increase from 512 to 514 thousand per g. of soil at pH 7 (Figure 7A and C).

In the 8 week series, actinomycetes were now 16 and

Fig. 7.

Fig.7. pH EFFECTS ON DISEASE CONTROL AND SOIL MICRO-ORGANISMS.

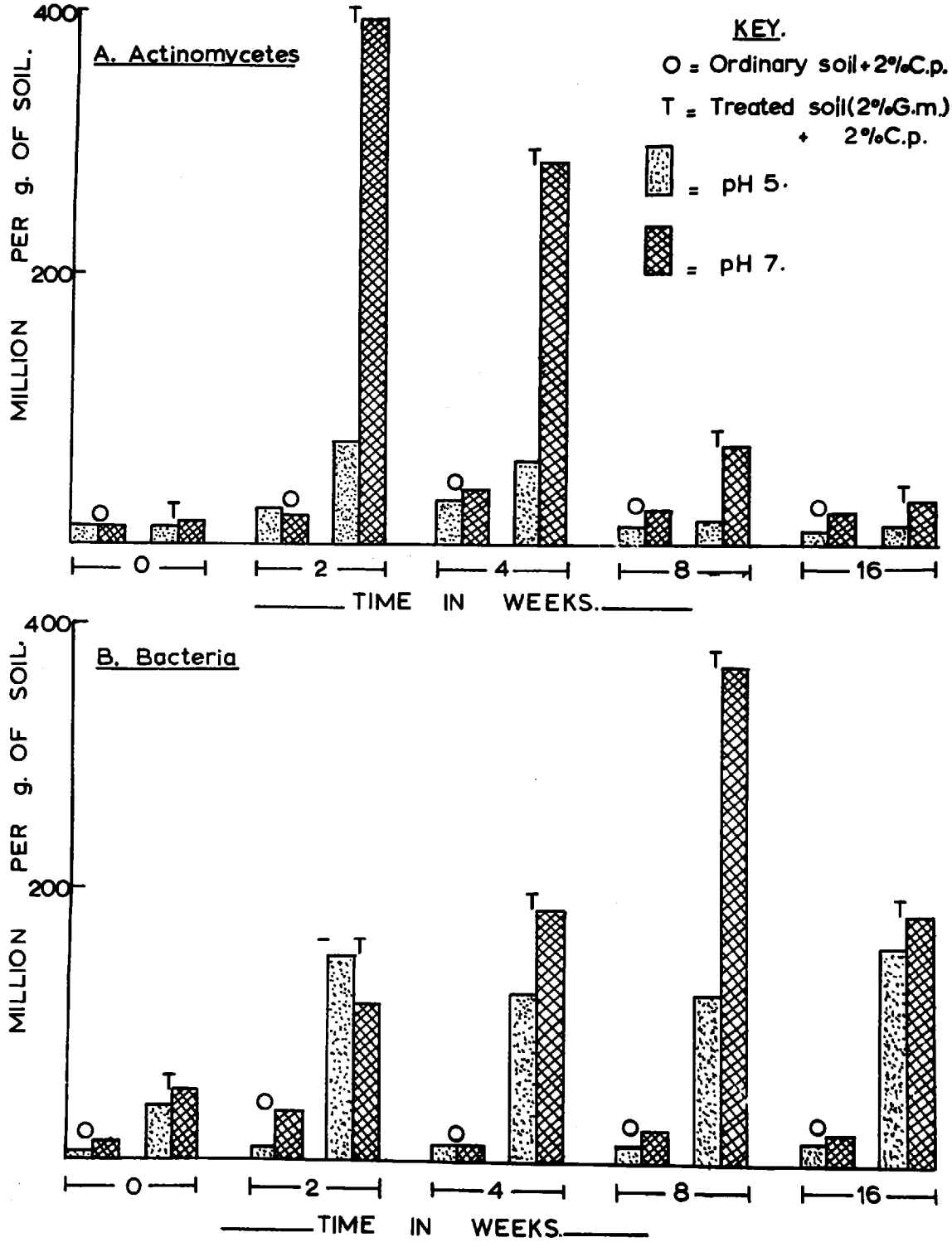
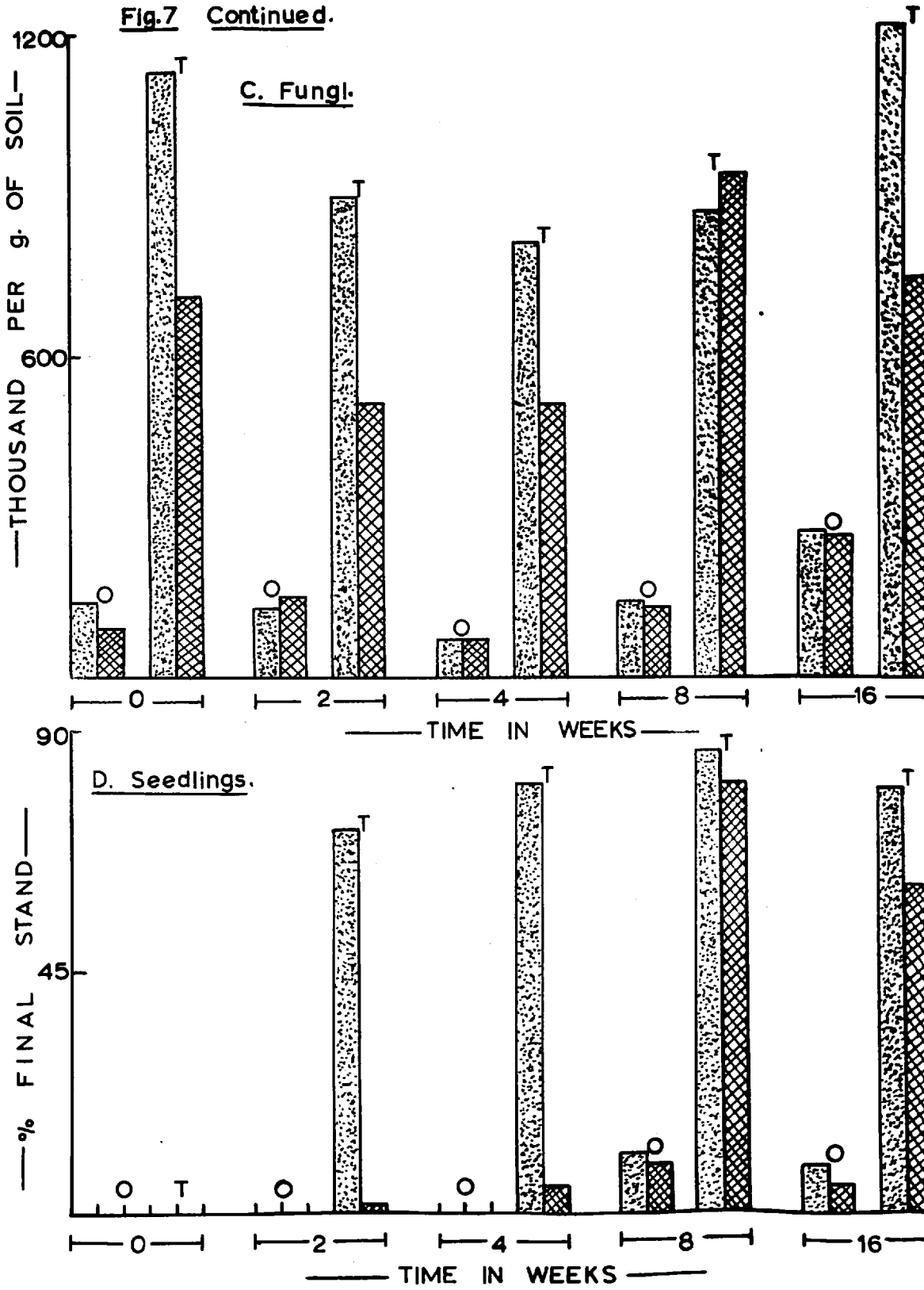


Fig.7 Continued.



71 million at pH 5 and 7. The bacterial number had dropped at pH 5 to 123 but ha increased to 364 million per g. of soil at pH 7. The fungal numbers were about equal, being 870 and 943 thousand at pH 5 and 7, and disease was reduced in both soils by 86 and 80 percent.

The pH of the four types of soil was determined at the same time as the serial experiments were being set. The results are given in Table 17 . The asterisks indicate, where significant disease control was obtained. The ordinary soil series (pH 5) treated with grassmeal recorded pH, 4.9, 5.0, 5.0 and 4.6 at the 2,4,8 and 16 week readings. These were means of five repeats and disease was reduced in all of them. On the other hand, the adjusted soil (pH 7) started giving disease control in the 8th week when the pH had dropped to 5.8 and finally reached 5.2 in the 16th week. There was a quicker pH drop in amended than in unamended soil. No significant disease control was achieved at pH above 5.8.

At the 16th week, the microorganisms had become fewer than they were in the 8th week series. The actinomycetes were about the same number as in the 0 week counts. The bscteria were about equal in the two types of soil (amended).

Table 17 . pH changes in amended and unamended soils.

Soil Treatment	pH.				
	0 wk.	2 wk.	4 wk.	8 wk.	16 wk.
A. Ordinary soil	5.0	5.0	5.2	5.2	5.0
B. Soil + 2% g.m. only	5.0	4.9*	5.0*	5.0*	4.6*
C. Soil + $Ca(OH)_2$	7.0	6.8	6.6	6.4	6.0
D. Soil + $Ca(OH)_2$ + 2% g.m.	7.0	6.7	6.2	5.8*	5.2*

*pH at which there was significant disease reduction

G.m. = grassmeal

The main difference was in fungi which were 1,215 and 740 thousand per g. of soil at pH 5 and 7. Damping-off was decreased by about 70 and 56 percent respectively.

Generally, fungi were the first to increase in population after the soil amendment (Figure 7C.). They declined a little about the fourth week and rose again from the 8th week. At pH 5, this increase continued till the 16th week in amended treatments, but fell again in the pH 7 treatment. This was the converse of what occurred in the

actinomycetes population (Figure 7A). They rose from the 2 week series and declined after the 4th week. They suddenly flared up in the adjusted soil and declined gradually. The bacteria were less ephemeral, reaching their highest numbers about the 8th week, and declined at the 16th week, while the fungi were the most consistent, and remained relatively high in numbers throughout the 4 months. As from the 2nd week sampling, Penicillium, Aspergillus, Trichoderma and Pullularia species were dominant, particularly the first two species. The fungi were not studied in details, but those which appeared in large numbers were looked at under the microscope.

18. Antagonism of C.practicola by some actinomycetes.

It is evident from Exp.16 and 17 that soils in which the actinomycetes dominated the flora, failed to check disease caused by C. practicola. This is inconsistent with current opinion by various workers in this field, who have suggested that actinomycetes check some soil-borne pathogens on the basis of antagonism tests in pure cultures. Experiments were therefore done to find the effects of simultaneous inoculation, on agar plates, of some actinomycetes and the parasite.

The actinomycetes were isolated in the standard way on soybean-meal glucose agar, from soil amended with 1 per cent grass meal and kept watered for 2 weeks before use. Twenty different (colour and culture configurations) colonies of actinomycetes which had clear zones of inhibition around them, were selected. They were grown in test tubes on Soybean-meal glucose agar at 24°C for 3 weeks. The medium used for antagonism was 50 per cent glycerol agar and 50 per cent glucose nitrate agar, the normal media for culturing the actinomycetes and the parasite respectively. All grew well in this mixed medium.

Four Petri dishes were used for each actinomycetes colony. The actinomycetes were taken from the test tubes into about 2 ml. of sterile distilled water and each agar

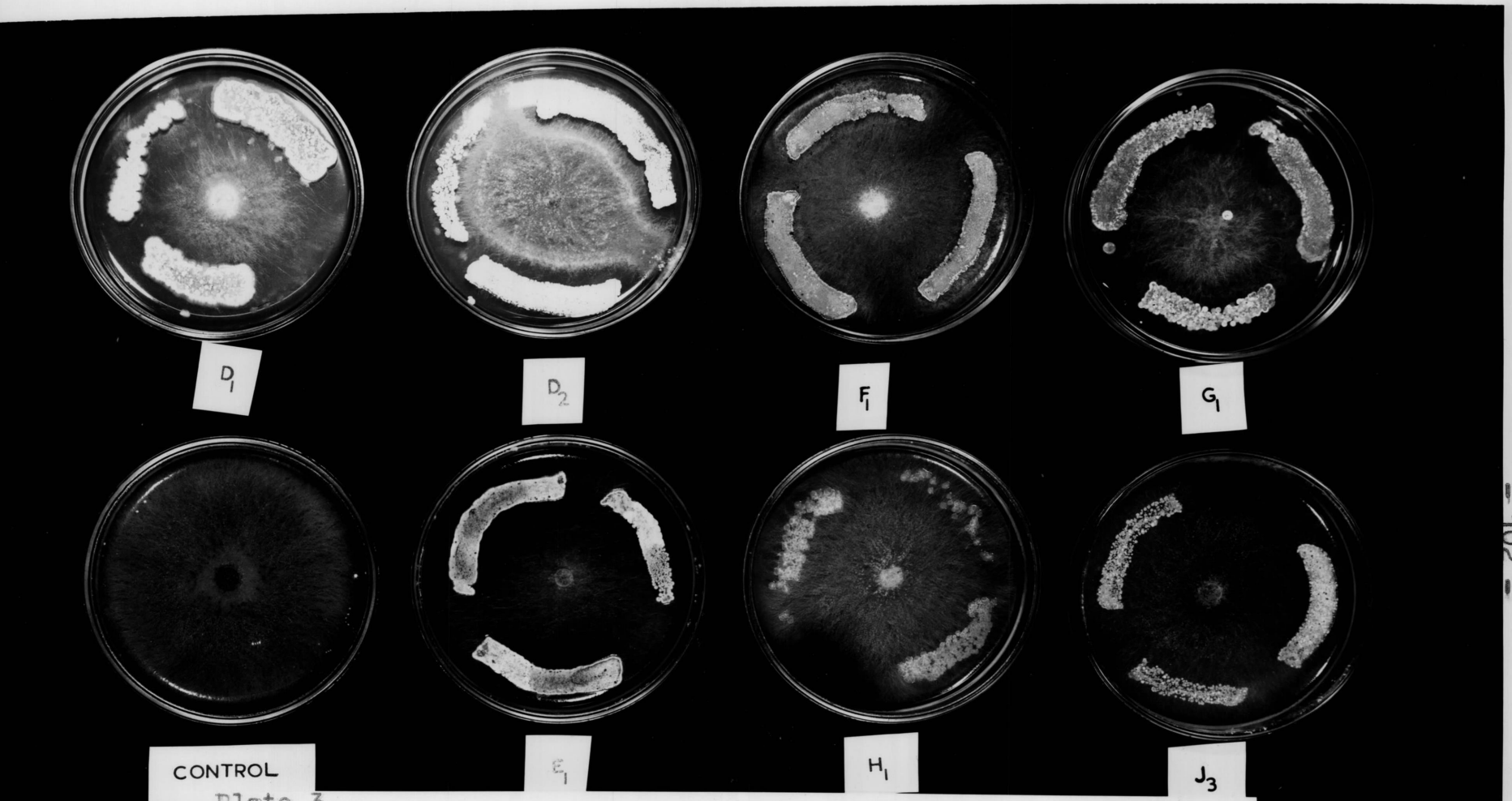


Plate 3.

THE EFFECT OF SOME ACTINOMYCETES ISOLATED FROM A POT OF SOIL, PREVIOUSLY AMENDED WITH 1% GRASS MEAL ON CORTICIUM PRATICOLA.

Agar medium —— 50% Glycerol agar & 50% Glucose nitrate agar.
The Actinomycetes were inoculated three days before Corticium praticola.

plate was inoculated at three places near the edge of the agar.

In the first experiment, a 5 mm. agar plug of C. practicola was placed at the centre of the plate immediately after inoculation with actinomycetes. The parasite covered the agar in four to five days. Some actinomycetes which were antagonistic to it, only limited the thickness of the parasite where its huphae grew over the actinomycetes.

In the second experiment the parasite was inoculated three or four days after the antagonists. The results are shown on (Plate 3). Of the twenty actinomycetes tested, seven were antagonistic to C. practicola in various degrees. Thirteen neither showed clear zones of inhibition nor decreased mycelial thickness. Of the seven antagonistic colonies, F_1 was the most powerful. About 6 days after C. practicola inoculation only very few hyphae were seen to be growing out of the agar plug. The Petri dish cover was yellow and there was a powerful smell. After the 8th day, scanty hyphae grew out of the agar plug (Picture was taken 12 days after C. practicola inoculation). D_1 and G_1 were the next best. In the first 6 days, there were very clear zones of inhibition between them and the parasite but later hyphae made some growth but were distorted (Plate 3).

In the control, the parasite covered the agar surface by the fifth day after inoculation and by the 7th day the Petri dish cover (inside) had been covered too. D_2 had very clear zones of inhibition around it, but C. practicola grew more thickly at its edge. Later some hyphae grew from this thick mycelium and approached the antagonist colonies. F_1, H_1, and J_3 significantly lessened the mycelial thickness of the parasite but did not produce zones of inhibition. In most cases C.practicola, growing among the antagonists did not form microsclerotia. The hyphae were mainly the thin runner type and under the microscope only very few stout hyphae(known to be the infection type) occurred.

19. Control of established parasite by grass meal treatment.

The main drawback of this work has been the failure of C. practicola to persist in natural soil. All that has been achieved so far, with grass meal is the prevention of the parasite from establishing itself in amended soil. It was desirable to investigate whether grass meal could control established mycelium of C. practicola. It had been seen that C. practicola persisted in soils for up to four months, if amended with high nitrogen materials such as corn meal. Use was made of this in establishing the parasite for two weeks before applying the amendment.

Table 19. Control of established parasite by grass meal treatment.

Final stand of seedlings as % seed sown

Soil Treatment	A Soil only control	B 3%C.p. only control	C 3%C.p.+ Thiram (seed treatment	D 3%C.p.+ 1% grass meal	E 3%C.p.+ 2% grass meal	F 2% grass meal only control
I. Owk. series	91.0 ± 3.6	5.0 ± 3.2	80.0 ± 5.4	10.0 ± 4.6	9.0 ± 4.3	4.0 ± 2.4
II. 2 wk. series	95.0 ± 1.4	36.0 ± 3.8	70.0 ± 5.6	96.0 ± 2.4	95.0 ± 2.0	95.0 ± 1.5

C.p. = Corticium practicola

Figures are means of four replicates.

A bulk of soil was amended with 0.5 per cent corn meal and inoculated with 3 per cent C. practicola cultured on corn meal/sand. The soil was then, thinly spread (about 3 inch deep) in a metal tray, watered occasionally and turned over and mixed every seven days. Another bulk of inoculated soil was similarly treated. Both were kept for two weeks in a green house (in August) before use. Two weeks later, the inoculated soil was divided into two lots and a half was amended with 2 per cent grass meal. The inoculated soil was divided into three portions:

one quarter was amended with 1 per cent grass meal and the other quarter with 2 per cent, and the remaining half was unamended. They were used 24 hr. later. There were six treatments including one in which seeds were treated with thiram. Each treatment was quadruplicated and healthy seedlings were counted four weeks after sowing. There were two series: I. seeds were sown 24 hr. after amendment, i.e. about 2 weeks after inoculation and II. seeds were sown 2 weeks after amendment, i.e. 4 weeks after inoculation.

The results are shown in Table 19 and Plate 4 which belongs to the 2 week series.

0 week series: Disease was very severe in the control B. resulting in 95 per cent kill. The parasite remained longer here than in any other previous experiments because in some cases, C. practicola failed to cause disease if left in the soil for two weeks before sowing seeds. Thiram gave good protection to the tune of 30 per cent final stand. Grass meal did not give reasonable control at either 1 or 2 per cent where final stands were 10 and 9 per cent. The F. treatment (2 per cent grass meal only) gave only 4 per cent final stand in the absence of the parasite. Thus suggesting that the low final stand in D, E. and F. treatment (Table 19) was due to the phytotoxins which are normally present in the first

Plate 4. Control of established parasite by grass-meal treatment.



A.	B.	C.	D.	E.	F.
Control, Soil only.	3% C.p. only.	3% C.p. + Thiram, (seed tretment)	3% C.p. +1% grass-meal.	3%C.p.+2% grass-meal.	2% Grass-meal only.

few weeks after soil amendment.

Two week series. There was less disease in control B. by about 30 per cent, but it was still active resulting in 64 per cent kill, four weeks after soil inoculation. This was much higher than in previous experiments, where the parasite almost disappeared completely if seeds were sown four weeks after inoculation. One per cent grass meal decreased disease by about 60 per cent and 2 per cent by about 59 per cent. Phytotoxins had disappeared and there was 95 per cent final stand in soil treated with grass meal only. Thiram protected seeds almost completely during emergence, but thereafter, there was about 30 per cent post-emergence killing. Consequently there was 26 per cent more seedlings in 1 per cent grass meal treatment than in Thiram treatment. The 2 per cent grass meal gave about 25 per cent more disease control than Thiram. The final stand of seedlings was about equal in the ordinary soil, inoculated soil treated with 1 and 2 per cent grass meal and ordinary soil with 2 per cent grass meal. Surviving seedlings in all pots treated with grass meal were bigger than those in ordinary soil, Plate 4.

20. Control of established parasite by grass meal treatment.

A second method was used to establish C. practicola in the soil. Lettuce seeds were sown (20 per pot) immediately after soil inoculation with 3 per cent corn meal/ sand culture of the parasite. The soil was then watered and pots covered with thin polythene and kept in the green house at Silwood Park in November. Most of the few seedlings that emerged were killed and those that survived were removed with little disturbance to the soil three weeks later. Some pots were partly filled with ordinary soil and given similar treatments. Seven days after removing the seedlings, some of the inoculated soil was amended with 1 per cent grass meal for one treatment and 2 per cent grass meal for another treatment. Some of the ordinary soil was amended with 2 per cent grass meal. There were six treatments, each with four replicates. A treatment in which Thiram treated seeds were sown in inoculated soil, was included. There were two series: 0 week series seeds were sown 24 hr. after amendment, i.e. 4 weeks after inoculation and 4 week series in which seeds were sown 4 weeks after amendment, i.e. 8 weeks after inoculation. The result of final stand of seedlings are given (Table 20) .

Table 20. Control of established parasite by grass meal treatment.

Final stand of seedlings as % seed sown.

Soil Treatment	A Soil only control	B 3% C.p. only control	C 3%C.p.+ Thiram (seed treatment	D 3%C.p.+ 1%grass meal	E 3%C.p.+ 2%grass meal	F 2% grass meal only control
0 wk. series	93.0 ± 3.2	40.0 ± 5.0	93.0 ± 3.2	15.0 ± 6.1	19.0 ± 5.5	26.0 ± 4.3
4 wk. series	95.0 ± 3.5	53.0 ± 3.2	85.0 ± 2.0	93.0 ± 3.2	90.0 ± 5.4	89.0 ± 5.5

0 week series: There was about 60% kill in the control (3 per cent C. practicola only). This was unusual, as the parasite had been in the soil for four weeks before the second seed sowing. The parasite has survived more here than in previous experiments in ordinary soil. Thiram seed treatment gave very good seedling protection with 93 per cent final stand which was the same as in ordinary soil. Grass meal at 1 and 2 per cent failed to control disease. Instead, it rather worsened disease by about 25 per cent at 1 per cent and 21 per cent at 2 per cent level. Two per cent grass meal to which no parasite culture was added gave about 74 per cent kill. So that grass meal and the parasite together, produced

more diseases than either.

4 week series. There was about 47 per cent kill in the control, so a marked improvement over earlier experiments. Here, the parasite persisted in soil for 8 weeks. Thiram gave about 32 per cent more seedling than the control, 1 per cent grass meal about 40 per cent and 2 per cent grass meal about 37 per cent. There was 89 per cent final stand in the 2 per cent grass meal only, treatment, showing that the toxin which was present in the 0 week series had disappeared.

In both experiments 18 and 19, Thiram gave better seed protection if used immediately after seed treatment than when used some weeks later. In both experiments, seeds were treated to last for the two series in each experiment.

21. Effects of mixed grass meal and cellulose on C.practicola disease and soil microorganisms.

The effect of an organic material in lessening disease of C. practicola appears to be determined by its carbon-nitrogen content. Grass meal has proved suitable, perhaps because it contains the right ratio of these elements but cellulose which gave control at 16°C., caused stunting of lettuce seedlings. It was of interest, therefore, to see how both together, in different proportions would act.

The experiment was done in a green house at 28°C. Air dried and sieved soil was weighed into wooden boxes, and some were inoculated with 3 percent culture of C. praticola. Some were amended with various combinations of grass meal and wood cellulose (Table 21). There were nine treatments each with four replicates. They were watered as required for two weeks before use. The various treatments were sampled for determining the microbial populations in the normal way. Some soil was weighed into 3 inch plastic pots and lettuce seeds sown. Surviving seedlings were counted 4 weeks after sowing and percentage kill was determined. The results are shown in Table 21, together with the dilution plate counts of actinomycetes, bacteria and fungi. These results are summarized in Figure 8A-C. and Plates 5 and 6. It is clear that least disease (Fig. 8C) occurred in soil with 2 percent grass meal to which no cellulose was added and disease increased thereafter as grass meal decreased and cellulose increased.

The bacterial numbers (Fig. 8A) were highest at 2 percent grass meal and lowest in the ordinary soil. Considering the amended soils alone, the bacteria declined with decrease in grassmeal and increase in wood cellulose.

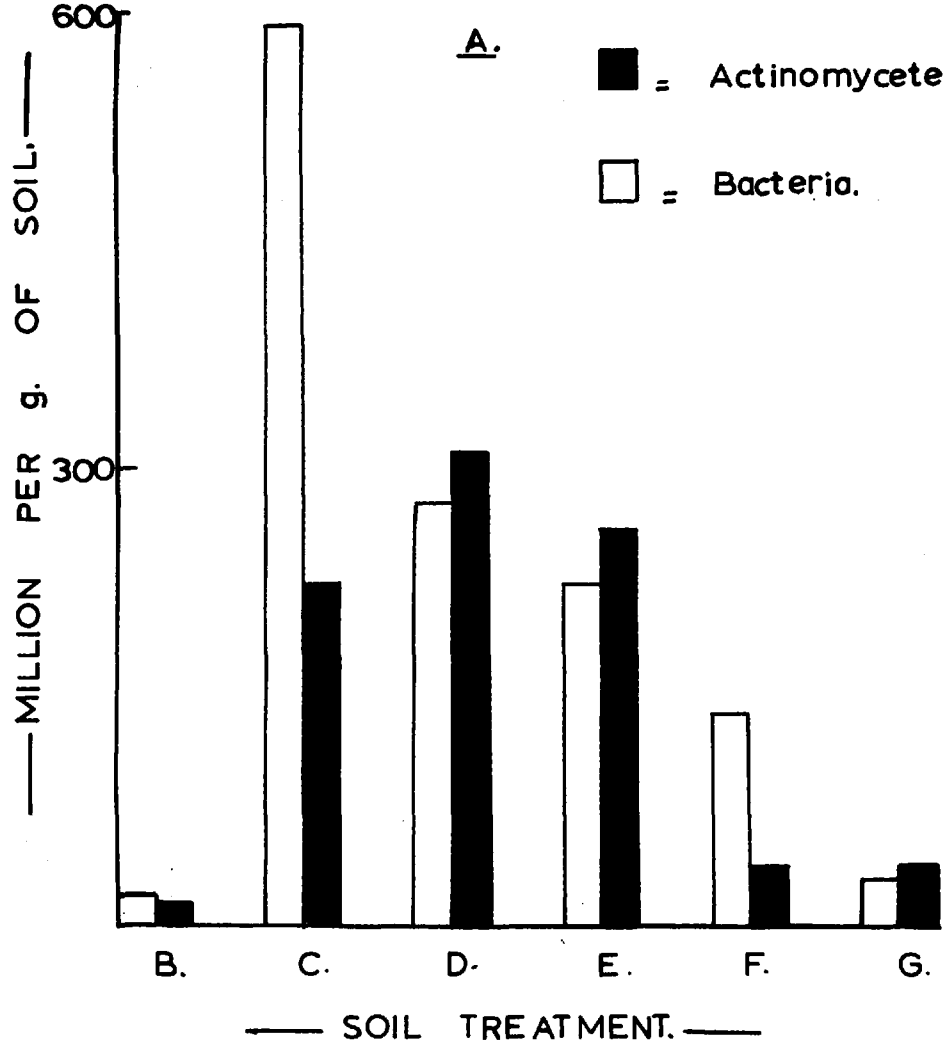
Table 21. Effects of mixed grass meal and cellulose on C. praticola disease and soil microorganisms.

Soil treatment	Microorganisms per g. of dry soil			Percentage kill (% of seed sown
	Actino x 10^6	Bacteria x 10^6	Fungi x 10^3	
A. Control soil only				0.0
B. 3% C. praticola only	14	21	160	45.0
C. 3% C.p+(2%G.m. + 0% W.c.)	227	584	500	9.0
D. 3% C.p+(1.5% G.m+0.5% W.c.)	311	279	390	19.0
E. 3% C.p.+(1% G.m. +1%W.c.)	262	225	355	19.0
F. 3% C.p.+(0.5% G.m+1.5%W.c.)	38	142	285	35.0
G. 3% C.p.+(0%G.m + 2% W.c.)	43	30	152	44.0
H. 2% G.m. only				4.0
I. 2% W.c. only				0.0

G.m. = grass meal W.c. = Wood cellulose
C.p. = Corticium praticola

Fig. 8.

Fig.8. EFFECTS OF MIXED GRASS_MEAL AND CELLULOSE ON C.PRATICOLA AND SOIL MICROORGANISMS.



KEY.

B. Control, 3% C.praticola only.

C. 3% C.p. + (2%G.m.+ 0.0%W. c.)

D. „ „ + (1.5% „ +0.5% „)

E „ „ + (1.0% „ + 1.0% „)

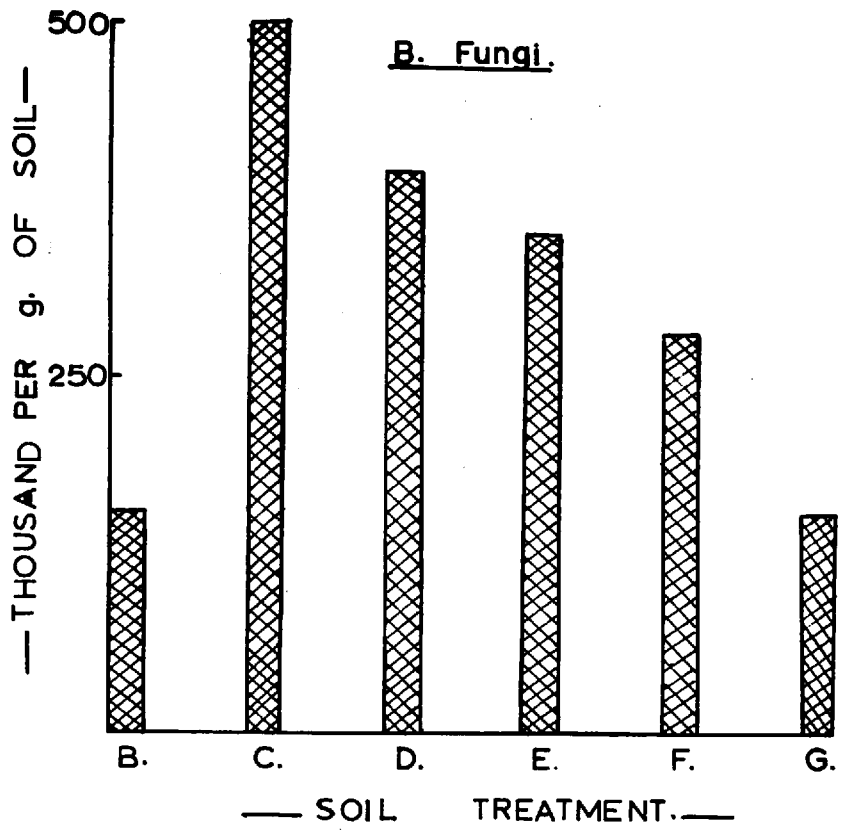
F. „ „ + (0.5% „ +1.5% „)

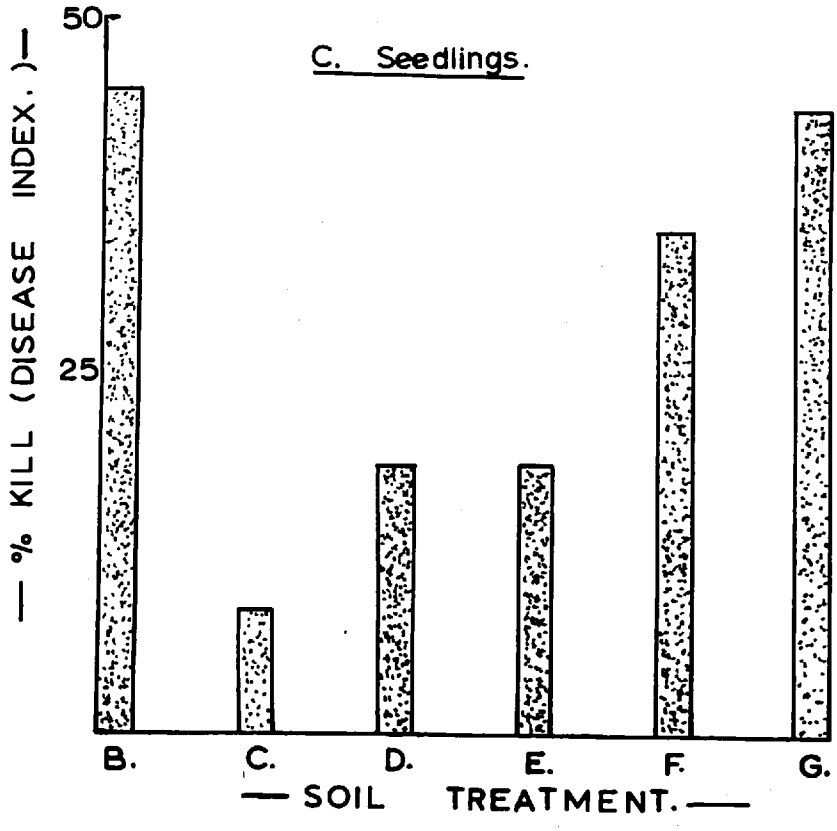
G. „ „ + (0.0% „ + 2.0% „)

G.m. = Grass_meal.

W.c. = Wood cellulose

Fig. 8 Continued.





Percentage kill was the converse, being least with 2 percent grass meal and highest with 2 percent wood cellulose (Fig. 8C). The bacterial numbers are therefore related to the effectiveness of each grass meal/wood cellulose combination. The actinomycetes (Fig. 8A) could not be correlated with disease control. The 2 percent grass meal which afforded the best control had 227 million per g. of soil whereas the D and E treatments with (1.5 percent grass meal + 0.5 percent wood cellulose) and (1 percent grass meal + 1 percent wood cellulose) respectively had 311 and 262 million per g. of soil, but had higher percentage kill than when the actinomycetes were less in number (B treatment). The fungal colonies (Fig. 8B) could be related to disease control. They, like the bacteria, were highest at 2 percent grass meal and least at 2 percent wood cellulose, and disease were 9 percent and 45 percent kill respectively.

Generally the microorganisms, except the actinomycetes were highest at 2 percent grass meal (Plates 5 and 6) which gave the best disease reduction. But this control declined as the bacterial and fungal number became less and actinomycetes became more (Treatments D and E).

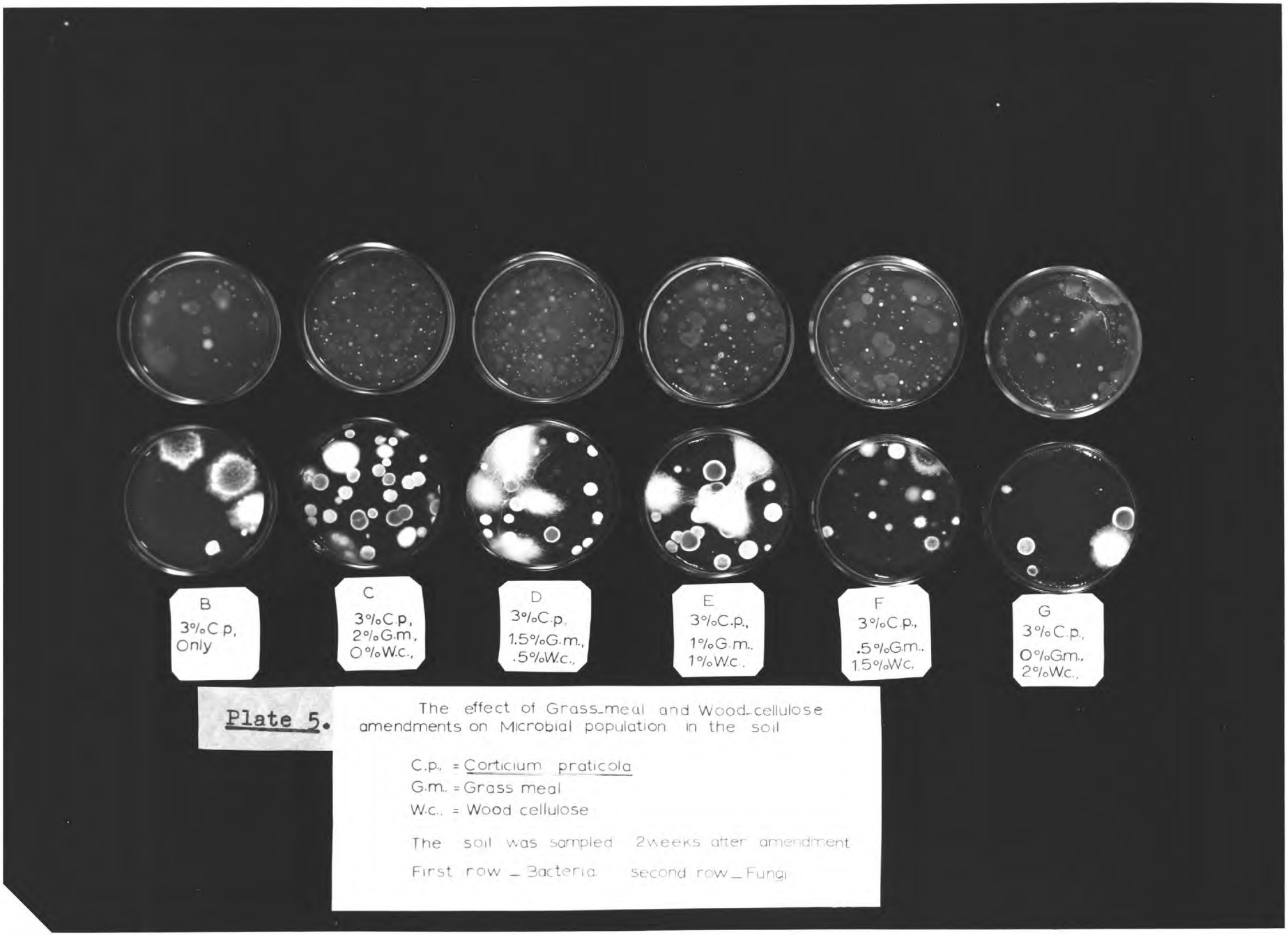


Plate 5. The effect of Grass-meal and Wood-cellulose amendments on Microbial population in the soil

C.p. = Corticium praticola
G.m. = Grass meal
W.c. = Wood cellulose

The soil was sampled 2weeks after amendment

First row – Bacteria. second row – Fungi

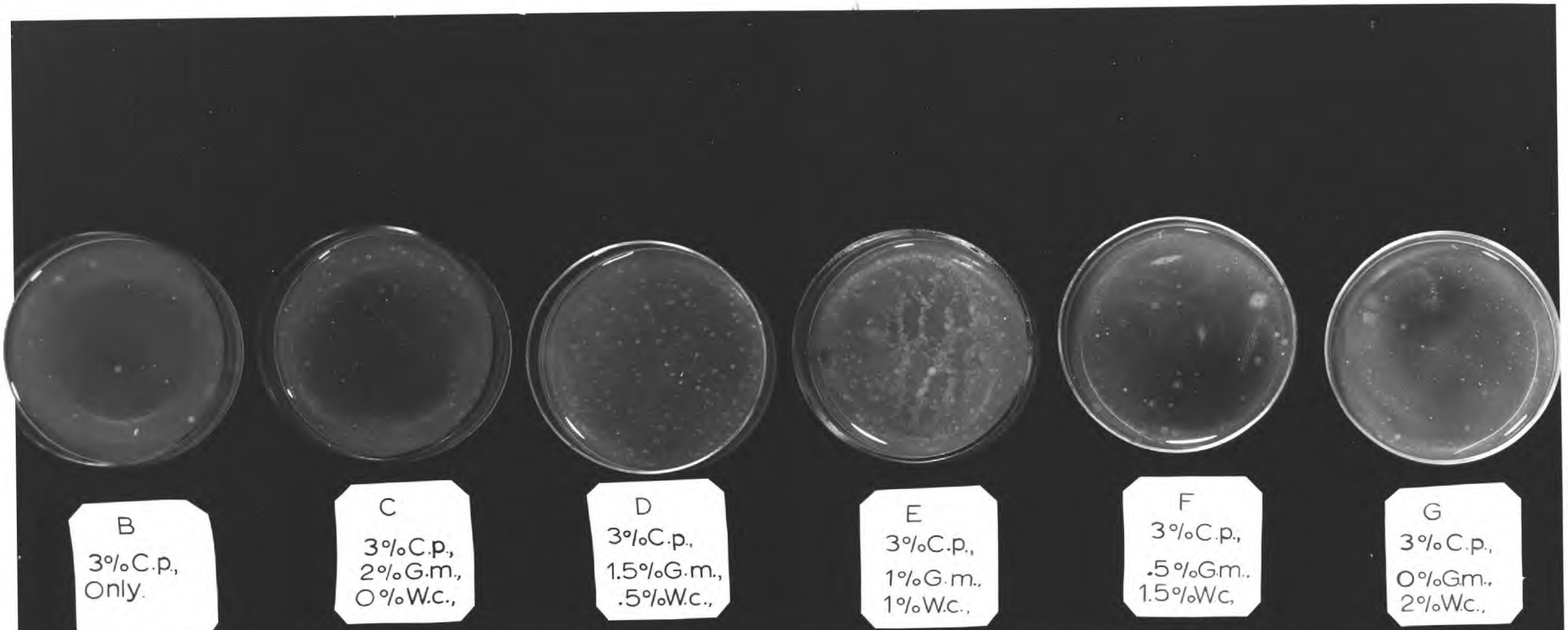


Plate 6.

Actinomycetes.

The effect of Grass-meal and Wood-cellulose amendments on Microbial population in the soil

C.p., = Corticium praticola
G.m., = Grass meal
W.c., = Wood cellulose

The soil was sampled 2weeks after amendment

Most of the fungi were Penicillium species, particularly at 2 percent grass meal. The addition of wood cellulose to grass meal altered the microbial composition of treated soil by increasing actinomycetes numbers and decreasing fungi and bacteria. In the 7 treatment (Table 21) the addition of 1.5 percent wood cellulose nullified the effect of 0.5 percent grass meal and disease became severe. This experiment done at 28°C produced results different from those done at 16°C when wood cellulose gave some control though not as good as that offered by grass meal.

Most of the seedlings in pots with high proportions of wood cellulose were stunted and watering with "Bio" 125 p.p.m. two times a week failed to restore normal growth. The seedlings were 4 weeks old before Bio treatment was started.

These results confirm the findings of Exps. 16 and 17 that actinomycetes did not play much part in disease control, and the fungi and bacteria appear to be the active antagonists to C. praticola in amended soil.

22. Temperature effects on manurial phytotoxin and disease control.

Corticium praticola was established by amending some

Table 22. Temperature effects on manurial phytotoxin and disease control.

Final stand of seedlings as % seed sown.

Soil treatment	A Soil only control	B 3% C.p. only control	C 3%C.p.+ Thiram (seed treatment)	D 3%C.p.+ 1% grass meal	E 3%C.p. + 2% grass meal	F 2%grass meal only control
1. 0 week series	97.5±1.4	31.3±5.5	80.0±3.5	71.3±5.5	77.5±7.2	70.0±7.9
2. 2 week series	96.3±2.4	58.8±3.1	75.0±6.1	95.0±2.0	95.0±2.0	96.3±2.4

Figures are means of 4 replicates

bulk of soil with 1 percent cornmeal and inoculating with 3 percent culture of the parasite on corn meal/sand medium. Two weeks later some inoculated soil was amended with 1 percent grass meal, some with 2 percent grass meal and the remainder left unamended. Ordinary soil was treated with 2 percent grass meal. Twenty four hours later, each soil was weighed into 3 inch pots and seeds sown immediately. Each treatment consisted of four replicates. The experiment was done in South Kensington green house at about 28°C. The grass meal used was a new material and appeared

to contain more cellulosic materials than the original stuff. The experiment was done in two series:- 0 week series and 2 week series, in which seeds were sown two weeks after soil amendment , i.e. 4 weeks after inoculation. Seedlings counted 4 weeks after each sowing are recorded in Table 22. 0 week series. From these results, it is clear that the high temperature (about 28°C) of this experiment give unusual results.

All treatments with grass meal give high final stand percentages whereas, at lower temperature (about 16°C) most seedlings would fail to emerge, in similar treatments, due to phytotoxins produced in the first few weeks after amendment. C. praticola persisted in the soil, giving about 69 percent kill, 2 weeks after inoculation. Thiram gave good seedling protection with 80 percent final stand, i.e. about 79 percent more than in the control. One and two percent grass meal decreased disease by about 40 and 46 percent. The final stand at 2 percent grass meal only (treatment F) was 70 percent. It appears that at a high temperature (about 28°C), the toxin from breakdown of organic materials in the soil is quickly destroyed. The control achieved with grass meal, 24 hr.



Plate 7. Temperature effects on manurial phytotoxin and disease control.

after application could be due to increased microbial activities which suppressed the parasite. Seedlings in the 2 percent grass meal only were stunted.

2 week series: Disease was less in the control (3 percent C. praticola only) than in similar treatment in the 0 week series (Table 22). Thiram improved seedling stand by about 16 percent, whereas 1 and 2 percent grass meal gave 36 percent more final stand than the control, which was equal to that in ordinary soil and soil amended with 2 percent grass meal only. Seedlings in F. (2 percent grass meal only) were very stunted (Plate 7) and did not develop above seedling stage when left longer. This could be due to the grass meal which contained much cellulosic materials and little of nitrogen. Soils previously treated with corn meal to establish the parasite, and later amended with grass meal, gave normal seedlings (Treatments D and E) (Plate 7). Nitrogen might then have been supplied by the corn meal treatment, whereas in the F. treatment (2 percent grass meal only) soil microorganisms which multiplied on the amendment at such a high temperature might have used up most of the normal soil nitrogen, resulting in seedlings suffering from nitrogen starvation.

23. Effects of fungal mycelium on C. praticola disease.

Spent mycelium of Penicillium sp. from penicillin production of Beechman's Research Laboratories was supplied autoclaved. It was then oven dried at 70°C for two days and ground into powder before storing.

Corticium praticola on cornmeal/sand culture was inoculated into soil which had been treated with 1 percent cornmeal (to establish the parasite). Powdered fungal mycelium was added to the inoculated soil at the rates of 0.5, 1 and 2 peroent (dry weight of powder/dry weight of soil). Ordinary soil was also amended with 2 percent fungal mycelium. Lettuce seeds were sown 0 week (24 hr.), 2 and 4 weeks after inoculation and amendment. Surviving seedlings counted 4 weeks after each sowing are recorded in Table 23.

From these results, it is clear that fungal mycelium did not lessen disease in the 0 week series. There was no seedling emergence at 0.5, 1 and 2 percent levels. The control (3 percent C. praticola only) gave also nearly 100 percent kill, whereas 2 percent fungal mycelium only had about 71 percent final stand, indicating that toxin effect of mycelial degradation was very

Table 23. Effects of fungal mycelium on C. praticola disease.

Final stand of seedlings as % seed sown.

Soil treatment	A Soil only	B 3% C.p. only	C 3%C.p.+ 0.5%F.my	D 3%C.p.+ 1%F.my	E 3%C.p.+ 2%F.my	F 2% Fungal mycelium only
1. 0 week series	100.0±0.0	1.3±0.6	0.0±0.0	0.0±0.0	0.0±0.0	71.3±5.1
2. 2 week series	95.0±2.0	26.3±9.8	50.0±15.9	41.3±6.2	56.2±18.3	91.3±2.4
3. 4 week series	92.5±3.2	38.8±5.9	31.3±2.4	21.3±2.4	31.3±9.6	95.0±3.7

F. my = Fungal mycelium
Each figure is a mean of 4 replicates

negligible, though this treatment had about 29 percent less seedlings than in the ordinary soil.

There was minor disease control in the 2 week series. There were about 24, 15 and 30 percent disease control at 0.5, 1 and 2 percent levels. There was a lot of variation in the final stand percentages between replicates, as shown by very high standard errors in these treatments. The little toxin effect which resulted in about 71 percent

final stand in the 0 week series has disappeared giving about 91 percent final stand in the 2 percent fungal mycelium only treatment. This was about equal to that in ordinary soil (Table 23).

In the 4 week series there was about 61 percent kill in the control, indicating the persistence of the parasite for up to 4 weeks. All amendment treatments gave more disease than the control. There were about 8, 18 and 8 percent more disease at 0.5, 1 and 2 percent levels than in the control.

Generally, fungal mycelium failed to accord significant seedling protection against C. praticola. Disease was more severe in amended than unamended soil, when seeds were sown either 24 hr. or 4 weeks later. The control achieved 2 weeks after amendment was very irregular (between replicates) and somewhat unreliable. Some pots had up to 80 percent final stand while others had nearly no stands at all. This control failed to persist in soil and disappeared 2 weeks later.

24. Group of soil microorganisms affecting C. praticola control.

Some experiments had indicated that C. praticola was

Table 24. Group of soil microorganisms affecting C. praticola control.

Final stand of seedlings as % seed sown

	Soil treatment	A Soil only control	B 3%C.p. only control	C 3%C.p. + 2.6% Act. culture	D 3%C.p. + 2.6%Bact. culture	E 3%C.p. + 2.6 % fungal culture
1	0 week series					
	(a) No grass meal	90.0±4.1	5.0±2.0	0.0±0.0	8.8±7.2	8.8±4.3
	(b) 2% grass meal in C,D+E	97.5±2.5	13.8±7.5	6.3±3.1*	8.8±5.5*	7.5±4.3*
2	2 week series					
	(a) No grass meal	97.5±1.4	23.8±4.2	2.5±2.5	7.5±3.2	7.5±3.2
	(b) 2% grass meal in C,D+E	98.8±1.2	27.5±5.9	6.3±3.1*	45.0±8.4*	53.8±2.4*

C.p. = Corticium praticola, Act = Actinomycetes,
Bact. = Bacterial.
* = C,D and E treatments with grass meal amendment.

decreased in grass meal treated soils where fungi and bacteria dominated the soil microflora, but disease often became worse where conditions favoured the actinomycetes. Antagonism experiments on sterile agar plates showed that some actinomycetes delayed the spread of C. praticola, where the parasite was inoculated three days after the antagonists. A more direct evidence on the effects of the three groups of organisms viz. Actinomycetes, Bacteria and Fungi was sought by devising experiments in which agar cultures of these microorganisms were added to soils inoculated with the parasite, with or without grass meal.

The three groups of microorganisms were isolated by the normal methods from a soil previously amended with 2 percent grass meal and kept at 28°C before use. The actinomycetes were isolated on glycerol asparaginate agar, bacteria on modified Thornton's medium and Fungi on Peptone dextrose agar plus rose bengal and streptomycin. The cultures were separately homoginized with Omni-homoginizer set at speed control no. 3 for 10 minutes. Sterile distilled water was added at the rate of 50 ml. per 100 ml. agar culture before homoginization. Account was taken of this added water in weighing the cultures.

The apparatus was steam sterilized between treating one group and another. Vermiculite "Veri-Gro" (fine grade) was used instead of soil. About 270 cc. Vermiculite was used per 3 inch plastic pot and all percentages pertaining to added materials are in volumes. There were two series, at 0 week (24 hr.) and 2 weeks after inoculation and amendment, before sowing seeds. Each series consisted of sub-series "a" without grass meal and "b" with 2 percent grass meal (Table 24).

The vermiculite was inoculated with 3 percent (v/v) culture of C. praticola on corn meal/sand medium. Treatments C, D and E were also inoculated with 2.6 percent (v/v) homoginized cultures of actinomycetes, bacteria and fungi respectively. They were amended with 2 percent grass meal (v/v) in the b sub-series (Table 24). For the 0 week series seeds were sown 24 hr. after amendment whereas for the 2 week series seeds were sown 14 days later. All pots were watered with 1200 p.p.m. "Bio" twice each week. The experiments were done in South Kensington green house (28°C). Seedlings were counted 4 weeks after each sowing and the results are recorded in Table 24.

In the 0 week series, there was no disease control

Plate 8. EFFECT OF ACTINOMYCETES, BACTERIA AND FUNGI IN THE CONTROL OF *CORTICIUM PRATICOLA* DISEASE OF LETTUCE.

	A.	B.	C.	D.	E.
First Row	Control	3%Cp. only.	3%Cp. 2.6%Act.	3%Cp. 2.6%Bac.	3%Cp. 2.6%Fun.
Second Row	"	"	" 2%Gm.	" 2%Gm.	" 2%Gm.



whether the vermiculite was amended or not in the three groups of microorganisms. The no grass meal sub-series a showed 5 percent final stand in the control treatment. No seedlings emerged in C treatment with 2.6 percent (v/v) actinomycetes culture, but there were about 9 percent final stand in the bacterial and fungal treatments (D and E). The b sub-series, with 2 percent grass meal, showed about 14 percent final stand in the control, but only 6 percent with actinomycetes and 9 and 8 percent with bacteria and fungi. It could be seen that disease was increased in all amendment treatments, and that the worst was in the actinomycetes treatment.

The 2 week series gave a very interesting result. The unamended sub-series a showed disease increase by about 21 percent with the actinomycetes and about 16 percent with both bacteria and fungi treatments. But the b sub-series, with 2 percent grass meal showed about 22 percent disease increase with the actinomycetes. The disease was lessened by about 17 percent and 26 percent with the bacterial and fungal culture respectively (Plate 8). Analysis of variance done on these figures (Table 24a) and (Table 24b Appendix) shows that the actinomycetes

Table 24 a. Analysis of variance table of final stand percentage (in angles) on the group of soil microorganisms affecting C. praticola control.

Soil treatment	A Soil only	B 3% C.p. only	C 3% C.p.+ 2.6%Act. culture	D 3%C.p. + 2.6%Bact. culture	E 3%C.p.+ 2.6%Fungal culture
4 week series					
(b) 2 percent grass meal in C, D and E	86.8	31.0	12.3**	42.0*	47.9**

Figures are means of 4 replicates. * Treatment means compared with B for significant differences.

L.S.D., P0.05 = 11.0 *
P0.01 = 15.6 **
P0.001 = 22.0

in the presence of 2 percent grass meal increased C. praticola disease significantly (P0.01) and that the bacterial culture reduced the disease significantly (P0.05) and that the most significant (P0.01) disease reduction was accorded by the fungal culture.

These results confirm earlier findings that C. praticola was more severe under conditions which favoured actinomycetes growth, and that disease was reduced under conditions which favoured fungi mainly and the bacteria in part.

25. Field work on biological control of C. praticola.

Field experiments on biological control of Corticium praticola damping-off disease of lettuce was done between the months of April and July in the "Walled garden" at Silwood Park. There were five treatments of four replicate plots which were randomized (Table 25a Appendix). Each plot was divided into three equal parts and seeds were sown 0 week (2 days), 2 and 4 weeks after soil amendment. Top 2 inch of each plot was treated with 1 percent corn meal in order to establish the parasite. Uninoculated plots were also treated with corn meal so as to generalize the effects which it might have on seedlings. Plots for C, D and E treatments were inoculated with 3 percent corn meal/sand culture of C. praticola (wet weight of soil/wet weight of culture). Plots B and E were amended with 1 percent grass meal two weeks later and seeds sown, 200 per plot, 2 days later in the 0 week series.

Table 25. Field work on biological control of C. praticola.

No. of seedlings per 200 seeds sown per plot

Soil treatments	A Soil only control + 1%Corn meal	B Soil only +1% grass meal+1% corn meal	C 3%C.p. + 1% cornmeal	D 3%C.p.+ Thiram (seed)1% cornmeal	E 3%C.p.+ 1%grass meal+1% cornmeal
Plots 0week I	8	0	0	0	0
II	0	0	0	0	0
III	0	0	0	0	0
IV	0	0	0	0	0
Total out of 800 seeds	8	0	0	0	0
Percentage	1.0%	0.0%	0.0%	0.0%	0.0%
2week I	91*	58*	2*	109	0*
II	128	78*	0*	135	46*
III	14*	119	15	126	131
IV	125	36*	27	108	102
Total out of 800 seeds	358	291	44	478	299
Percentage	44.8%	36.4%	5.5%	59.8%	37.4%
4week I	144	28*	51*	100	27*
II	181	96*	24*	126	51*
III	148	136	17	135	108
IV	112	161	18	134	139
Total out of 800 seeds	585	421	110	497	325
Percentage	73.1%	52.6%	13.8%	62.1%	40.6%

*Plots severely attacked by ants. C.p. = Corticium praticola

When the plots were inspected 2 weeks later, some ants, which moved in fror the grassy foot paths at both ends of the plots, were seen making holes at the sites of the furrows in which seeds were sown. They did not invade D plots where seeds treated with Thiram were sown (Table 25a Appendix). These ants were probably attracted by corn meal which had been applied to all plots. Healthy seedlings were counted about 6 weeks after each sowing. The results are given in Table 25 and the plan of the plots showing those invaded and disturbed by ants is given (Table 25a Appendix).

From these results, it is clear that only 8 seedlings survived in one A plot, whereas all other plots with or without the parasite had no emergence at all. This is due to phytotoxins which had been noticeable in the green house experiments as resulting from organic materials when freshly applied to the soil. In the 2 week series, the ordinary soil (Treatment A) gave only 44.8 percent final stand (Table 25). Due to ants activities two plots which were severely invaded gave only 14 and 91 seedlings out of 200 seeds sown per plot and two unattacked plots gave 128 and 125. In the B treatment where there was 1

percent grass meal and no parasite culture, one unattacked plot gave 119 out of 200 seeds, the remaining three plots were severely invaded. The final stand was 36.4 percent. The control plot C with 3 percent C. praticola only had two attacked plots which gave 0 and 2 seedlings out of 200 seeds each and two uninvaded plots gave 15 and 27. The final stand was 5.5 percent. The D treatment was not attacked by ants, perhaps due to the Thiram which might have either repulsed or killed them. Percentage final stand was 59.8. In the E treatment with the parasite culture and grass meal amendment, two invaded plots gave 0 and 46 seedlings whereas two unattacked plots gave 131 and 102 per 200 seeds sown in each case. The final stand was 37.4 percent.

Grass meal lessened disease by about 32 percent, whereas Thiram did so by about 54 percent.

The final stand in B and E treatments, both having been amended with 1 percent grass, but E was inoculated with the parasite, were about equal. In the 4 week series the ordinary soil (A treatment) gave 73.1 percent final stand and the B, with 1 percent grass meal only, gave about 52.6 percent whereas the control C with 3 percent C. praticola only had 13.8 percent final stand. Thiram gave

reasonable seedling protection and decreased disease by about 48 percent whereas grass meal did so by about 27 percent.

Generally Thiram had one advantage over grass meal in this experiment. It also protected seeds and seedlings against ants, which grass meal did not do, thereby resulting in Thiram giving much higher protection. But if plots which were free from ants are considered separately, it is evident that grass meal was as effective as Thiram in protecting lettuce seeds and seedlings against C. praticola damping-off.

26. Effect of time on control of Fusarium culmorum by soil amendment.

Some air dried soil was amended with 2 percent grass meal. A portion was inoculated with 2 percent cultures of Fusarium culmorum grown on oatmeal/sand medium. Ordinary soil was also inoculated with the parasite culture. The remaining amended soil was kept regularly watered for 2 and 4 weeks before use. There were four treatments each replicated four times. Treatments A and B were ordinary soil to which F. culmorum was inoculated, and ceresan treated wheat seeds were sown in B. D was ordinary soil alone while C was inoculated and amended. For the 0 week series 20 wheat seeds were sown about 1cm deep per 5 inch clay pot 24hr. after inoculation and amendment. Pots were occasionally watered and there was no rigid regulation about this. Three to four week old seedlings were carefully removed from each pot and washed under running tap water and disease assessed (p.36) Root and short lengths of individual seedlings were measured. All seedlings from one pot were then weighed together after drying for about 5 minutes between two filter papers. Disease was assessed by a standard design (p.38) with

Table 26a. 0 week series

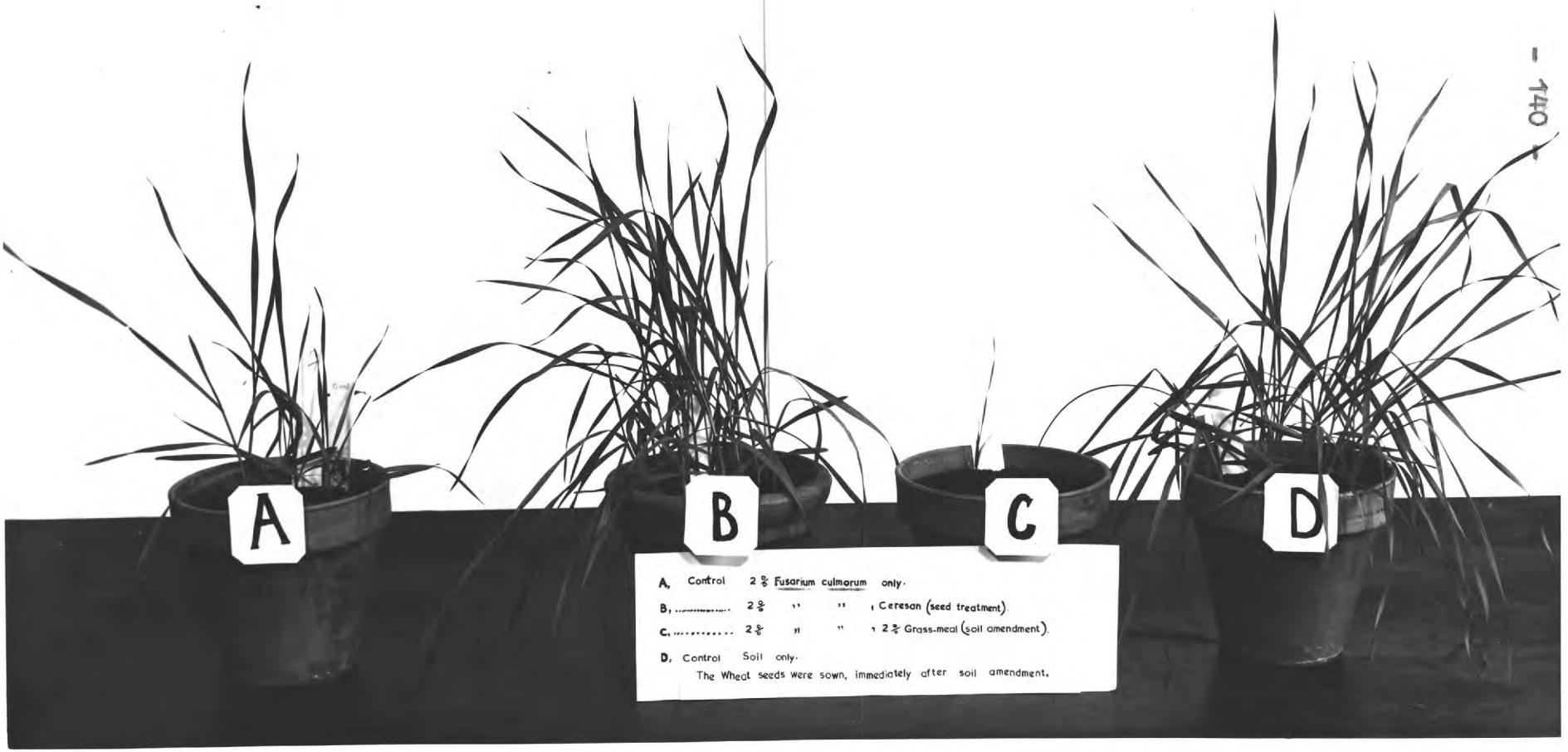
Soil treatment	A 2% F.c only	B 2% F.c.+ ceresan (seed treat)	C 2% F.c + 2% grass meal	D Soil only
Disease Index	74	31	94	26
Mean shoot length	33.9cm.	33.5cm.	34.5cm.	35.4cm.
Mean root length	28.3cm.	28.6cm.	28.2cm.	30.6cm.
Mean fresh weight	1087.5mg.	1072.5mg.	1136.0mg.	1093.4mg.
No. of seedlings out of 80 seeds sown.	32	68	5	61

F.c. = Fusarium culmorum

minimum value of 0 (Disease index) and maximum value of 100 (Disease index). These numbers will be mentioned just as numerical figures. The results are shown (Tables 26a,b,c) corresponding to 0,2 and 4 week series.

In the 0 week series, F. culmorum only (Treatment A) resulted in severe attack with 74 (Disease index). Ceresan lessened this by about 43, whereas grass meal

Plate 9. Effect of time on control of F. culmorum by soil amendment.



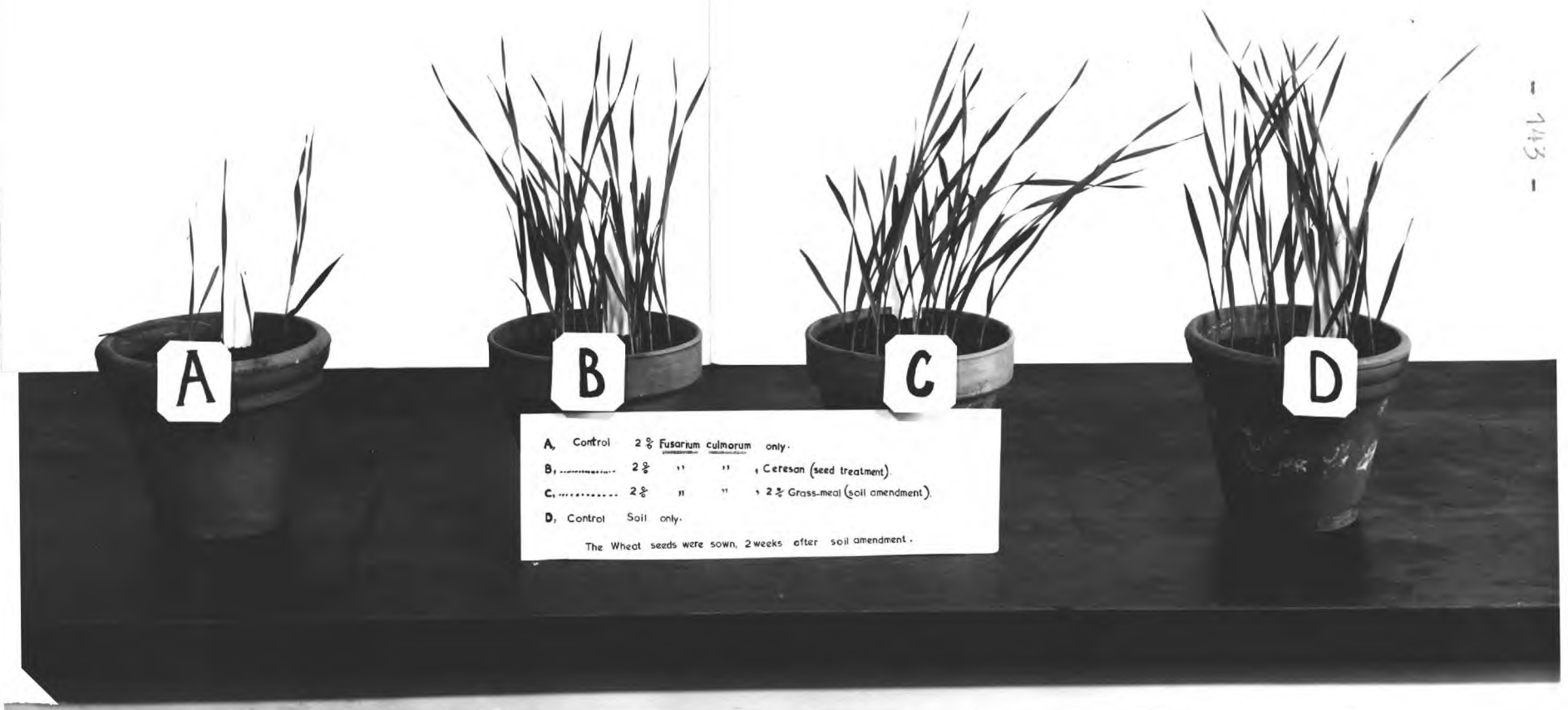
increased the disease by about 20 (Plate 9). There were only 5 seedlings out of 80 seeds sown in the grass meal treatment. Surprisingly the ordinary soil which was not inoculated with F. culmorum was diseased and only 61 seedlings survived resulting in disease index of 26. Fusarium culmorum and other unidentified Fusaria were isolated from seedlings killed in the ordinary soil treatment. It is clear that the soil had some natural infection. At this stage nothing definite could be said about the effect of grass meal, but the two possibilities were either that it increased F. culmorum propagule in the soil, resulting in increased disease in amended soil or that wheat was affected by toxins produced in the initial phase of grass meal degradation.

Table 26b. 2 week series

Soil treatment	A 2% F.c. only	B 2%F.c.+ ceresan (seed treat)	C 2% F.c. + grass meal	D Soil only
Disease index	80	14	35	24
Mean short length	18.7cm.	27.4cm.	27.7cm.	25.1cm.
Mean root length	26.8cm.	29.3cm.	30.2cm.	31.8cm.
Mean Fresh weight	285.2mg.	577.2mg	700.0mg.	525.2mg
No. of seedlings out of 80 seeds sown.	32	71	54	64

Grass meal amendments showed some inprovement when treated soil was used two weeks later. Disease index was high in the A treatment at 80. This was lessened by ceresan and grass meal by about 66 and 45 respectively. There were 22 more seedlings in the grass meal treatment than in the control (2% F. culmorum only). Wheat seedlings in the ceresan and grass meal treatments were about 9 cm taller than in the control (Plate 10) and weighed about 2 times as much as in the control.

Plate 10. Effect of time on control of F. culmorum by soil amendment.

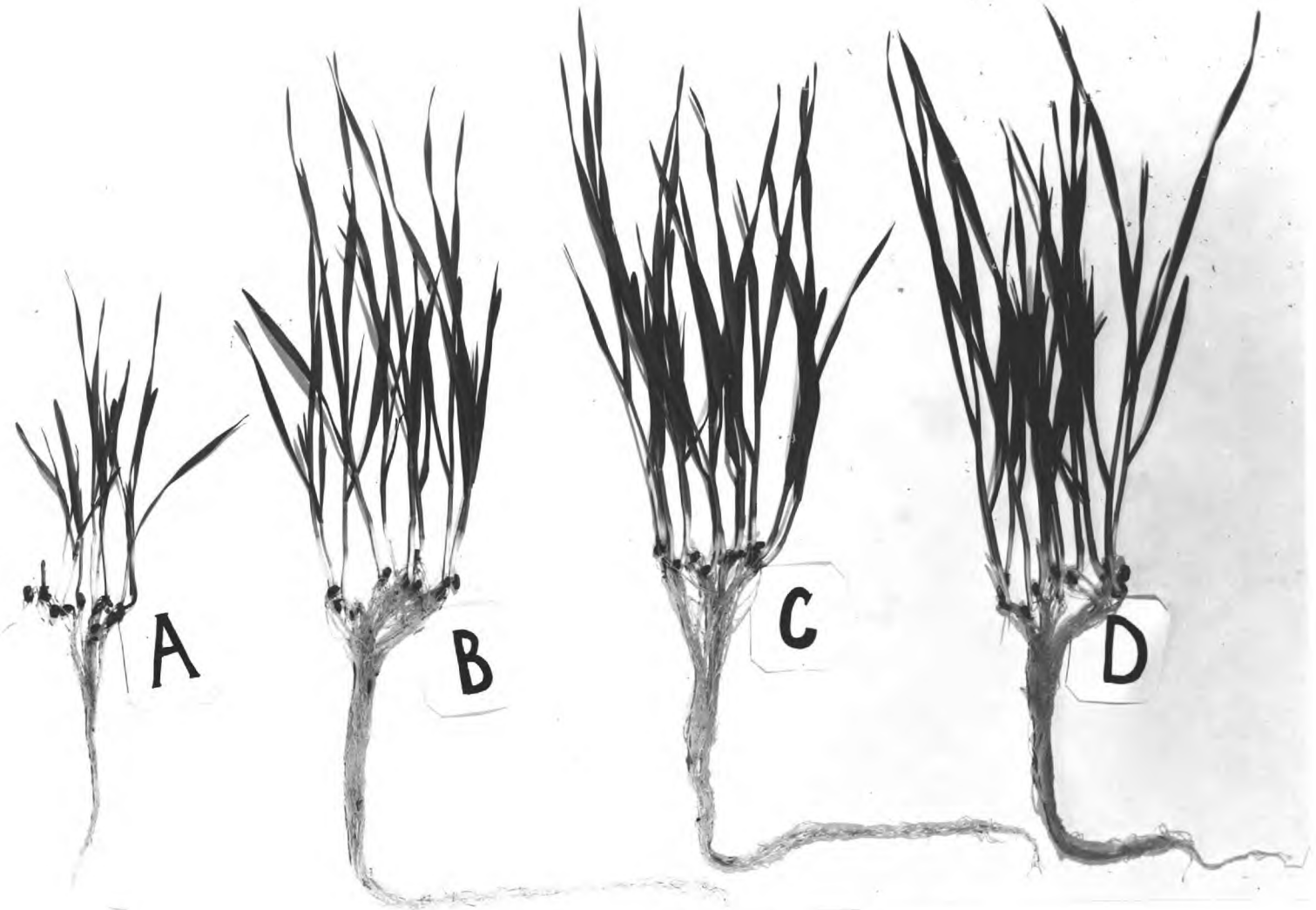


Fusarium culmorum appears to affect the shoot more than the root length (Plate 11). It could be seen that most seedlings in the A treatment with the parasite only had roots about equal in length with those of healthy seedlings in treatments B,C and D. But the shoots of attacked seedlings are much shorter than the normal seedlings.

Table 26c. 4 week series.

Soil treatment	A 2%F.c only	B 2%F.c. + ceresan (seed treat	C 2% F.c.+2% grass meal	D soil only
Disease index	74	21	15	40
Mean shoot length	20.8cm.	26.3cm.	29.2cm.	27.4cm.
Mean root length	35.9cm.	40.9cm.	37.5cm.	45.9cm.
Mean fresh weight	552.6mg.	776.4mg.	704.0mg.	705.2mg.
No. of seedlings out of 80 seeds sown	38	72	75	58

Grass meal treatment gave the highest final stand in the four week series. There were 75 seedlings out of 80 sown and disease had gone down to 15 as compared with 74



The Wheat seeds were sown, 2 weeks after soil amendment.

A, Control 2% Fusarium culmorum only.

B, ... 2% " " + Ceresan (seed treatment).

C, ... 2% " " + 2% Grass-meal (soil amendment).

D, Control Soil only.

Plate 11.

Effect of time on control of F. culmorum by soil amendment.

in the control. Ceresan was also effective with 72 seedlings as compared with 38 in thecontrol. But grass meal was slightly better here than ceresan. Disease in uninoculated soil was very high with index of 40 and only 58 seedlings. It would appear that both ceresan and grass meal had some effect on the natural infection. In the three series ceresan gave higher seedling stand than in ordinary soil to which no parasite culture was added.

By these results it appears that the effect of grass meal improved with time, worsening disease in the 0 week, and giving some control after 2 week and the best if not complete control in the 4th week. In effect, what grass meal had done was preventing the parasite from establishing in amended soil, rather than control the disease. An attempt to control established disease was made in the next experiment.

27. Persistence and control of Fusarium culmorum by soil amendment.

Bulk of air dried soil was inoculated with 2 percent cultures of Fusarium culmorum on oatmeal/sand medium. It was watered as required for two weeks, before a part was amended with 2 percent grass meal. Wheat seeds were sown

Table 27a. 0 week series

Soil treatment	A 2%F.c.only	B 2% F.c. + ceresan (seed treat)	C 2% F.c.+2% grass meal	D soil only
Disease index	88	23	91	43
Mean shoot length	21.7cm.	25.4cm.	38.0cm.	24.9cm.
Mean root length	23.2cm.	29.5cm.	25.0cm.	29.0cm.
Mean Fresh weight	497.2mg.	733.8mg.	2211.1mg.	723.5mg.
No. of seedlings out of 80 seeds sown.	36	71	9	51

in the 0 week series, 24 hr. after amendment. In two other series treated soil was used 2 and 4 weeks after amendment. Each of the four treatments was quadruplicated in each series. Four to three week old seedlings were lifted from the pots, carefully washed without much damage to the roots, and root and shoot lengths measured. All seedlings from one pot were weight together and disease assessed in the standard way (p.38), with maximum disease index of 100 and

minimum ot 0. The results are shown in Tables 27a,b,c, corresponding to 0,2 and 4 week series.

In the 0 week series grass meal increased the disease by about 3 (Disease index). There were only 9 surviving seedlings as compared with 36 seedlings out of 80 seeds in the control (2% F.c. only). Ceresan gave good control, the disease was mild and 71 seedlings survived out of 80 seeds sown. The abnormally high weight of seedlings in the grass meal treatments could be due to growth stimulating substances from the amendment and also due to few seedlings occupying the whole pot whereas in other treatments overcrowding of seedlings could result in slower growth due to competition for light and nutrients from the limited soil. Ordinary soil gave very high disease despite there being no parasite inoculation to it. It had 51 seedlings out of 80 seeds and disease index of 43.

Table 27b. 2 week series.

Soil treatment	A 2%F.c. only	B 2%F.c.+ ceresan seed treat	C 2%F.c.+2% grass meal	D soil only
Disease index	63	16	29	31
Mean shoot length	24.4cm.	27.9cm.	35.0cm.	26.2cm.
Mean root length	35.8cm.	38.4cm.	36.9cm.	35.2cm.
Mean fresh weight	489.8mg.	680.3mg.	945.2mg.	700.0mg.
No. of seedlings out of 80 sown	59	71	62	60

The two week series shows that F. culmorum persisted in soil for up to 4 weeks after inoculation though disease was less severe here than in the 0 week series when inoculation was two weeks old. There were 59 surviving seedlings which were nearly all attacked by the parasite resulting in disease index of 63. Their mean fresh weight was about half of those in grass meal treatment. Grass meal gave good seedling protection reducing the index by about 34 when compared with the control. Ceresan gave much better protection and reduced disease by about 47 and had 71 surviving seedlings.

Table 27c. 4 week series

soil treatment	A 2%F.c.only	B 2%F.c. + ceresan (seed treat)	C 2% F.c.+2% grass meal	D soil only
Disease index	83	29	47	39
Mean shoot length	23.4cm.	28.9 cm.	33.6cm.	28.4cm.
Mean root length	37.2cm.	33.2 cm.	29.5cm.	41.6cm.
Mean fresh weight	580.9mg.	769.3mg.	883.6mg.	769.8mg.
No. of seedlings out of 80 seeds sown	42	62	55	53

The control treatment had 83 (disease index) showing severe rot of most seedlings whereas the ceresan and grass meal treatment had 29 and 47 respectively. The severe rot in the control treatments show clearly that F. culmorum persisted in soil for up to 6 weeks. There were only 42 seedlings in the control out of 80 seeds sown. Grass meal gave less protection here than in the second week series. Disease index was about 18 higher here than in the 2nd week and the seedlings about 7 less. It appears that grass meal is less effective in controlling established F. culmorum than freshly introduced inoculum.

28. Temperature effects on Fusarium nivale rot of oats

It was thought that root and foot rot disease of oats caused by Fusarium nivale developed during winter months. In this experiment, development of the disease in naturally infected oat seeds was observed at a low temperature of 36°F and a high one at 62°F. It was presumed that more rotting would show up at the lower temperature. Some oat seeds with about 20-30 percent infection were sown about 1cm. deep, 25 per 5 inch clay pot. In another treatment similar seeds were treated with ceresan at the rate of 0.36g. per 100g. of seed and sown. Two lots of each treatment were prepared. One lot was kept at 36°F and the second lot in an adjoining green house at 62°F. Each treatment had four replicates. The low temperature varied a lot between 30°F and about 45°F depending upon how long the connecting door between it and that of 62°F remained open. After four weeks all seedlings were carefully removed and washed under running water. Shoot and root lengths were measured and disease assessed as described (p.38) with minimum value at 0 and maximum value of 100 (Disease index). The results are given (Table 28).

Table 28. Sizes of 4 week old oat seedlings.

treatment	Low temperature 36°F		High temperature 62°F	
	ceresan treated	untreated	ceresan treated	untreated
Disease index	11	46	16	48
Mean shoot length	29.5cm.	21.9cm.	39.3cm.	36.7cm.
Mean root length	14.7cm.	9.5cm.	18.0cm.	13.3cm.
Mean fresh weight	458.0mg.	278.0mg.	665.8mg.	584.0mg.
No. of seedlings out of 100 seeds sown.	100	100	96	88

From these results, it is clear that F. nivale caused no appreciable pre- or post-emergence killing in both temperatures. It was surprising that the little killing that occurred was in the high temperature treatment with 4 in ceresan treatment and 12 in the untreated. In effect the temperature did not seem to make much difference, rather it appears that disease was slightly more severe at the higher temperature. This was unexpected. Only heights and fresh weights of seedlings were affected. Ceresan treatment at low temperature lessened the disease

by 35, but at higher temperature by 32. Ceresan treated seedlings were taller and weighed more than untreated seedlings. It seems that disease spread in the soil by root contact because of the high disease indices of 46 and 48 recorded in ordinary soil (Table 28). These were much higher than percentage infection as determined on filter papers which was about 30 percent (p.30).

29. Grass meal effects on Fusarium nivale rot of oats.

Some soil was amended with 2 percent grass meal and weighed into 5 inch pots. Two other treatments were similarly set up with ordinary soil. In one treatment, ceresan treated infected seeds were sown and in the other treatment untreated infected seeds were sown. There were three series and seeds were sown 0,2 and 4 weeks after soil amendment. Each treatment consisted of four replicates and disease assessment was done on four to three week old seedlings in the standard way. The results are shown (Table 29a,b,c).

Table 29a. 0 week series

Treatment	A soil only	B 2% grassmeal	C ceresan (seed treat)
Disease index	45	14	16
Mean shoot length	30.7cm.	32.3cm.	30.0cm.
Mean root length	17.8cm.	21.0cm.	23.2cm.
Mean fresh weight	479.4mg.	638.3mg.	617.6mg.
No. of seedlings out of 80 sown	71	77	75

There was not much difference in the number of surviving seedlings in the three treatments, neither was there much difference in seedling hights but roots were shorter in the ordinary soil than in either grass meal or ceresan treatment. The main difference was in mean fresh weight of seedlings in grass meal and control treatments. In most cases oat seedlings with severe rotting in the ordinary soil failed to die but looked very unhealthy. Absence of seedling killing by F. nivale suggests that this parasite might have been adjusted with the host. It was also surprising that freshly added grass meal did not diminish oat germination as it did with wheat and lettuce. It now seems that toxins from decaying grass meal have no effect on oat seeds.

Table 29b. 2 week series

Treatment	A soil only	B 2% grassmeal	C ceresan (seed treat)
Disease index	36	9	21
Mean shoot length	29.0cm.	29.6cm.	32.4cm.
Mean root length	21.7cm.	23.5cm.	24.3cm.
Mean fresh weight	737.5mg.	793.7mg.	870.3mg.
No. of seedlings out of 80 seeds sown.	72	79	74

When seeds were sown two weeks after grass meal treatment there was about complete emergence and final stand and most seedlingswere free from rots. Disease index was 9. as compared with 36 in the control where 72 seedlings emerged out of 80 seeds. Ceresan gave less seedling protection with disease index at 21 and had 74 seedlings as compared with 79 in grass meal treatment. Here there was not much contrast between the three treatments in both hights and weights of seedlings, though seedlings in grass meal and ceresan treatments were slightly better.

Table 29c. 4 week series

Treatment	A soil only	B 2% grass meal	C ceresan (seed treat)
Disease index	42	9	27
Mean shoot length	24.7cm.	28.9cm.	24.3cm.
Mean root length	21.5cm.	21.4cm.	22.1cm.
Mean fresh weight	580.7mg.	961.8mg.	748.7mg.
No. of seedlings out of 80 seeds sown.	75	76	76

Final stand of seedlings was about equal in the three treatments but disease was much lessened by both grass meal and ceresan. Disease index for the control was 45, grass meal and ceresan treatments were 9 and 27. The only measurement which appeared to be much affected by the disease here is in fresh weights which were much higher in the grass meal and ceresan treatments. Most seedlings in the grass meal treatment had clean roots and shoots and there was much clear contrast between them and those in ordinary soil.

DISCUSSION

Studies made with Corticium praticola causing lettuce damping-off, Fusarium culmorum and Fusarium nivale which cause foot and root rots of wheat and oat respectively, have shown that these parasites could be controlled by the application of certain organic soil amendments. Most of the work was done on C. praticola. Grass meal is the most effective amendment, and in some cases gave about 100 percent disease check. Suitability of organic amendments was found to depend upon their carbon-nitrogen contents. Those with high nitrogen such as corn-and fish meals increase C. praticola disease, whereas those with high carbohydrate, such as grass meal, bran and cellulose decrease the disease. Cellulose caused stunting of seedlings and is considered unsuitable. Grass meal amendment compared very favourably with some standard fungicides and in some instances proved slightly better.

The first two experiments showed that, virulence of C. praticola depended upon, (1) inoculum concentration, (11) microbial population in inoculated soil and (111) growth vigour of the parasite acquired from the nutrient medium. Virulence was found to increase with increase in inoculum concentration. C. praticola was more pathogenic in sterile than non-sterile soil, which could be due to

partial elimination of antagonistic soil microorganisms, and virulence was higher when the parasite was cultured on G.N.A (Glucose nitrate agar) than when cultured on G. N.A/10 (Exp.2). On G.N.A/10 mainly thin runner hyphae known to be non-infectious were produced, whereas in G. N.A. stout infection hyphae were more common. Food for supplying growth vigour appears an important factor in the spread of the parasite in soil. With adequate food base as supplied in G.N.A. C.praticola overcame the resistance offered to it by antagonistic soil microorganisms and caused disease in non-sterile soil equally as it did in sterile soil. But when cultured on G.N.A/10 it failed to cause disease in non-sterile soil but did so in sterile soil. The removal of this resistance by steaming, allowed the parasite to cause disease even when cultured in poor medium-G.N.A/10.

It was then thought that C. praticola would cause more disease and stay longer in soil if extra nutrients were added to its cultures just before inoculating (Exp.3). This proved wrong. C. praticola persisted longer in soil when cultured on G.N.A. alone than when extra G.N.A was poured over the original culture before inoculation.

This surprising result might have been due to suppression by soil saprophytes which multiplied on the fresh G.N.A. at a high temperature (about 28°C). The parasite inoculated in this way might have been at a disadvantage being introduced into a new environment without fully occupying the freshly introduced food, which might have been quickly occupied by soil microorganisms that were more adjusted to the soil conditions than the parasite.

Attempts to control C. praticola by treating lettuce seeds with organic materials failed (Exp.4). Only cellulose and ammonium sulphate gave slight control. This is similar to reports by Small (1927) on Rhizoctonia solani. It would have been surprising if the attempt succeeded because of the time lapse normally necessary for the build up of soil microorganisms after amendment. With respect to cellulose, C. praticola being a poor cellulose decomposer, (Blair 1943) might have been suppressed by cellulose decomposing microbes which increased around the treated seeds. Ammonium sulphate was less effective, perhaps because the parasite as well could metabolize it. Other materials which might have been utilized by the parasite such as starch, casein hydrolysate worsened the disease. Grass meal made no significant difference.

When grass meal was applied to the soil at 0.5, 1 and 2 percent there was significant disease control at 0.5 percent (Exp.5). The control declined as the amendment increased and at 2 percent there was hardly any control. The results appeared irregular and unexpected at first. Later this was found to be due to temperature effect. The experiment was done in September when green house heating was not started before the beginning of cold spells, which might have led to slower degradation of higher quantities of the amendment, resulting in phytotoxin accumulation which diminished seed germination. Similar results are reported in Exp. 8 where dried grass was used in April, as a result of the green house low temperature when cold spells returned after heating had been stopped. Here control fell off with increase in dried grass amendment and at 3 percent there were less seedlings than in the control. Baiting the parasite with lettuce seeds revealed that there was less survival at higher than lower amendment doses (Exp. 9 and 10). The low seedling stands at high grass meal doses could be due to phytotoxins from the amendment. These toxins were found to persist longer at low temperatures (about 16°C) and dissipate quickly at high temperatures (about 28°C) (Exp. 22).

Seedlings which survived in the dried grass treatments (Plate 1) were stunted. Stunting increased with amendment dose. Its effects were similar to those of starch and cellulose (Exp. 7). These amendments caused seedling stunting, perhaps due to soil microorganisms which multiplying upon them utilized most of the normal soil nitrogen. The symptoms on seedlings resembled those normally associated with nitrogen deficiency. The grass used here was cropped from an old bush, as such its contents might have been mainly cellulosic and little nitrogen.

Corn meal amendment made the disease worse. When the effects of corn meal were compared with those of grass meal (Exp. 8), corn meal worsened the disease by about 25 percent and there was no emergence at all at 1 percent level. Further investigation with seed baiting (Exp. 9 and 10) showed that C. praticola multiplied in the corn meal treatment and persisted for up to 6 months after inoculation. C. praticola persisted more at 3 percent than at 1 percent corn meal, whereas the parasite had decreased in both ordinary soil and grass meal treatments three months after inoculation. The decline was more in grass meal treatments than in ordinary soil. Six months after inoculation the parasite could not be isolated from both ordinary soil and

grass meal treatments. It survived in corn meal treatments, which could be due to high nitrogen content of corn meal. This will be dealt with more fully.

Grass meal was found to have prolonged beneficial effects on treated soil (Exp. 12,13 and 14). When the soil was used one month after amendment, the final stand of seedlings at 0.5 percent equalled that in ordinary soil, and at 2 percent seedlings were 10 percent more than in ordinary soil. In effect there was about complete control of the disease. The experiment was done in summer and seedlings emerged quicker and grew faster in amended than unamended soil. These support the work of Patrick et.al. (1962,1963) who showed that the toxicity of amended soils declined 25 days after amendment and that about 35 days after treatment soil extracts stimulated germination and growth of seedlings. It may be suggested that grass meal appears to have lessened the disease in two ways:(I) It may have increased soil microorganisms which suppressed the parasite and (II) the degradation products of the amendment, by stimulating both seed germination and seedling growth, might have caused the seedlings to escape from the parasite. C. praticola attacks mainly young and supple seedlings which acquire resistance after about three or four weeks, under normal conditions.

With the beneficial effects of grass meal the seedlings emerged sooner than others and perhaps escaped pre-emergence killing and their quicker growth could make them acquire earlier resistance. Though contributory to biological control, the second effect of grass meal appears to have caused the host to escape from the parasite physically - rather a non-biological disease control.

Grass meal still checked C. praticola 2 and 4 months after soil treatment, but seedlings neither emerged before those in untreated soil nor did they grow significantly quicker than others. It would appear that the microorganisms responsible for suppressing C. praticola in amended soil are persistent at least for up to four months.

When sterile and non-sterile soils were amended with grass meal (Exp. 15) disease was severe in both soils in the 0 week series when seeds were sown 24 hr. after amendment and inoculation. Two weeks later the non-sterile soil gave better disease control. In the fourth week non-sterile soil still gave more control than the sterile soil, but surpringly the sterile soil gave significant disease reduction, which might have been due to recolonization by air- and water-borne microorganisms. Better control in non-sterile soil indicated that soil microorganisms might be a main

factor in the mechanism whereby grass meal acts.

There are three types of soil microorganisms which could antagonize C. praticola in amended soil. Many workers suggested that organic soil amendments which control disease in unsterile soil substantially increase the total soil microflora and CO_2 evolved from them is responsible for suppressing parasites which are sensitive to it (Garret 1937, 1950; Durbin, 1959; Papavizas, 1960 and Papavizas et. al. 1960, 1962). Attempts to determine whether actinomycetes, bacteria or fungi or a combination of them was involved in disease control gave interesting results. Striking correlation in microbial populations and disease control was obtained when amended soil was kept at 10°C, 20°C and 30°C before use (Exp. 16). The soil so treated was used at 16°C to avoid non-biological disease reduction which high temperatures might give. Disease control occurred in soil where fungi dominated the microflora. The fungi were least at 10°C, more at 20°C and most at 30°C and the parasite declined most at 30°C, less at 20°C and least at 10°C. The actinomycetes were dominant at 10°C, followed by 20°C and were least at 30°C. The bacteria were rather intermediate but slightly increased with the fungi.

Similar correlations between disease reduction in

treated soil with high fungal population and non-disease reduction in soil where actinomycetes dominated the microflora were obtained when amended soil was adjusted to pH 7 with $Ca(OH)_2$ from pH 5 of ordinary soil. In the two week series (Exp. 17) grass meal decreased the disease by about 71 percent in ordinary soil but by only 1 percent in the alkaline soil (pH 7). There were 76 and 392 million actinomycetes and 898 and 512 thousand fungi per g. of dry soil respectively. The difference in fungal numbers does not appear enough to account for the difference of 70 percent in disease reduction. The difference in actinomycetes counts appears outstanding. In the fourth week the ordinary soil gave about 75 percent more control than the alkaline soil and the microbial populations were about similar to the second week series. Striking disease reduction occurred in the alkaline soil in the 8th week series when the pH had dropped to 5.8 and the actinomycetes were about one quarter of the 2nd and 4th week counts. The fungi were nearly equal in both soils and disease decreased by 86 percent in ordinary soil and 80 percent in alkaline soil. By the 16th week microbial numbers had dropped to nearly what they were in the 0 week counts, except the fungi which still remained high, and disease was equally controlled in both soil. pH of the alkaline soil was 5.2 in the 16th week.

Various workers have suggested that certain soil-borne parasites persist in alkaline soils because such soils are good carbon-dioxide acceptors and because those parasites are sensitive to CO_2, they spread less in acidic and poorly aerated soils. These views are supported by results of Exp. 17 in which C. praticola was unchecked in alkaline soil but was reduced in normal soil by grass meal, but it may also be suggested that the effects of the two types of soil on actinomycetes, bacteria and fungi may determine the persistence and disappearance of C. praticola in alkaline and non-alkaline soils. This suggestion is supported by results obtained when cultures of actinomycetes, bacteria and fungi, isolated from grass meal treated soil, were simultaneously inoculated with C. praticola cultures, in vermiculite experiments (Exp. 24). In the series without amendment neither of the microorganisms checked disease, but with 2 percent grass meal amendment the fungal cultures reduced C. praticola by about 26 percent which was significant at 1 percent level of probability. Disease reduction by bacterial cultures by about 18 percent was less significant (at 5 percent level of probability) whereas the actinomycetes cultures increased the disease by about 21 percent (significant at 1 percent level of probability).

When grass meal and cellulose mixed in various proportions were used as amendment, study of the groups of soil microorganisms and disease reduction at 28°C showed correlations similar to those of Exp. 16 and 17. Two percent grass meal only gave the best control and the soil contained the highest number of fungi and bacteria. Addition of cellulose increased the actinomycetes, reduced the fungal and bacterial numbers and gave poorer control. This was a surprising result, for it had been thought that by increasing the carbon and decreasing the nitrogen with cellulose, disease would be better reduced. It now appears that there is a minimum and maximum limit of C:N ratio beyond which an amendment becomes less effective.

Some suggestions may be put forward to explain the persistence of C. praticola in alkaline soil and at low temperatures and its non-persistence in normal soil and at high temperatures. It is evident that soil fungi reduce this parasite whereas actinomycetes appear to worsen the disease. Soil fungi dominate the microflora at high temperatures and in acidic or normal soil but actinomycetes are dominant in alkaline soils and at low temperatures. Failure of amended alkaline soil to reduce the parasite appears due to dominance by actinomycetes which seem to excite the disease and

absence of fungi which appear the main antagonists. Low temperatures have similar effects. Vermiculite is a porous material, as such CO_2 may not have played much part in suppressing C. praticola in the fungal culture series. It is not clear how the parasite is suppressed. Some Penicillium, Aspergillus and Trichoderma species were in the fungal cultures, and these fungi known to antagonize another related parasite R. solani were often isolated from grass meal treated soils. Because of the effects of actinomycetes in increasing C. praticola damping-off in soil, they were tested on agar plates with the parasite. Of twenty actinomycetes colonies selected because of clear zones of inhibition around them during their isolation, only seven were antagonistic to C. praticola in various degrees, under special conditions. When both parasite and actinomycetes were inoculated simultaneously, none prevented the spread of the parasite on agar plate. A few affected its colony thickness. But when C. praticola was inoculated three days after the actinomycetes, , isolate E_1, completely suppressed the parasite. Others had clear zones of inhibition around them where the parasite hyphae did not grow. It may be suggested that antagonism on agar plates is not a suitable criterion for assaying the suppression of one organism by

another in soil. The method of giving the actinomycetes some days growth advantage over the parasite, could give a misleading result and in fact appears far removed from what may actually occur in nature.

One major difficulty in working with C. praticola was due to its failure to persist long in soil. This was overcome by treating the soil with corn meal which might have acted through its high nitrogen contents. When the parasite was established (Exp. 19)there was about 64 percent kill four weeks after inoculation, which was very striking because in normal soil C. praticola would in many cases fail to cause disease two weeks after inoculation. Grass meal amendment at 1 and 2 percent gave about complete control. There was about equal seedling stand in ordinary soil and amended soil to which C. praticola was inoculated. Two percent grass meal gave about 26 percent more seedling protection. than thiram. In the thiram treatment there was about complete emergence, but 30 percent were killed after. This was not surprising as thiram treated seeds were sown about 5mm. below soil surface, thereby leaving the vulnerable hypocotyl exposed to attack, but grass meal being mixed with the whole top soil gave general seedling protection. Similar better control was accorded by grass

meal than thiram when C. praticola was established by sowing lettuce seeds. immediately after inoculation (Exp. 20).

When grass meal was applied to established C. praticola at a high temperature (28°C) the results were surprising. Seedlings emerged equally in both ordinary and amended soils in the 0 week series (seeds sown 24hr. after amendment) (Exp. 22). Phytotoxins which often diminished seedling emergence at lower temperatures (about 16°C) in freshly treated soil had disappeared, thus suggesting that at high temperatures these toxins are either not produced or are quickly destroyed.

Attempts to control C. praticola with fungal mycelium failed. Some workers (Mitchell and Alexander 1961,1962; Khalifa, 1965 and Buxton, 1965) reporting on control of certain fusarial disease with chitin suggested that it encourages growth of mycolytic actinomycetes and Maurer and Baker (1963) failed to control F. solani with chitin alone. Fungal mycelium failed to check C. praticola perhaps because actinomycetes do not appear to antagonize the parasite in soil; rather their presence in large numbers seems to nullify the effect of soil fungi which significantly reduce C. praticola.

In field experiments done in Summer, grass meal was

less effective than thiram in protecting seedlings against C. praticola (Exp. 25). This may be due to ants which migrated from nearby grass paths and affected most plots except those in which thiram treated seeds were sown. Thiram might have been unpleasant to the ants. There was still significant disease reduction by grass meal. Phytotoxins, which had been reported in green house experiments, also affected seedling stands when seeds were sown 2 days after amendment.

Grass meal increased Fusarium root and foot rot of wheat by about 20 (disease index) when seeds were sown 2hr. after amendment, whereas, ceresan reduced it by about 43. But two weeks later there was about 22 (disease index) reduction by grass meal. In the fourth week series grass meal was as effective as ceresan with about equal seedling protection. Grass meal proved less effective in checking established Fusarium culmorum

With Fusarium nivale on oats there was little or no seedling killing. Disease showed up in the rotting of roots and shoots and affected heights and weights of seedlings. Grass meal and ceresan were about equally effective in checking the disease, but seedlings from grass meal treatments grew faster and weighed more and showed less disease symptoms. Oat seeds and seedlings were not affected

by toxins from the amendment even when sown 24hr. after amendment. Why these toxins affected lettuce and wheat but not oat is not clear. It is possible that some of the toxins act on contact. Oat seeds are covered by husks which might have excluded these toxins from the seeds. The protection accorded by grass meal against Fusarium nivale suggests that part of the mechanism involved here might be antibiotic because of inner seated nature of F. nivale infection. The antibiotic substances may have suppressed the parasite by diffusing into the seeds or being absorbed by the seedlings. This aspect was not investigated.

Work on Fusarium culmorum and Fusarium nivale is still at its initial stage. Nothing definite could be said about them till a more thorough investigation has been done.

On the whole, this work is more extensive than intensive. The role of the actinomycetes in grass meal treatment is not very clear, but there are evidences suggesting that they worsen C. praticola in soil. They might do this by, either enhancing the growth of the parasite, or acting on lettuce seeds in such a way that they become predisposed to parasite attack or they might be responsible for degrading the antibiotics produced by soil fungi and bacteria.

SUMMARY

1. Eleven organic materials were tested by seed treatment for control of C. praticola on lettuce. Two only slightly reduced the parasite.

2. Ten other materials were tested by soil amendment. Three were effective against C. praticola, but four increased its virulence on lettuce.

3. The best soil amendment was grass meal which in some instances gave about complete seedling protection. The other two amendments, bran and cellulose, were less effective.

4. Cellulose caused seedling stunting, probably by the depletion of normal soil nitrogen by microorganisms which multiplied upon it.

5. The effect of an amendment on C. praticola in soil was found to depend upon its Carbon-nitrogen content. Materials such as grass meal, bran and cellulose with high C:N. ratio lessened the disease, whereas, those with low C:N. ratio such as corn-, barley- and fish-meal incresed the disease.

6. Corn meal caused C. praticola to multiply and persist in soil for up to 6 months and longer.

7. Reduction of disease by grass meal is thought due to its effects on soil microorganisms which multiply upon it, and depending upon other environmental conditions such as temperature and soil reaction, which determine the type and composition of microbial populations.

8. C. praticola reduction by grass meal at high temperature and in acid soil conditions was correlated with dominance of soil microflora by fungi. Its persistence at low temperatures and in alkaline soil was correlated with dominance of soil microflora by actinomycetes.

9. Grass meal has prolonged beneficial effects in treated soil, for up to 4 months or more when C. praticola failed to cause disease if introduced into treated soil. In such soils, bacterial and actinomycetes were rather ephemeral, flaring up at the beginning and declining after a short period but soil fungi were more persistent and remained in large numbers for up to 4 months.

10. There was a pattern of succession of fungi in amended soil. Initially, fast growing Mucorales such

as Zygorhynchus and Rhizopus species were common but later they were succeeded by Penicillium, Aspergillus, Trichoderma and Pullularia species.

11. Grass meal was as effective as thiram - a standard fungicide against C. praticola, but was at times better than the fungicide which failed to protect the vulnerable hypocotyl regions of lettuce seedlings.

12. The effects of grass meal compared favourably with ceresan against F. culmorum on wheat and F. nivale on oat. It was less effective in checking F. culmorum when established in soil but gave oat seedlings better protection than thiram against F. nivale.

13. Toxins produced in the decay of grass meal initially diminished seedling emergence and increased the disease caused by C. praticola, but about 28 days later substances which stimulate seed germination, causing quicker growth of seedling are produced. These stimulatory materials are considered to reduce disease by causing lettude seedlings escape parasite attack.

14. The phytotoxins persist longer at low temperatures and either dissipate quickly or are not produced at high temperatures.

15. Mechanism of disease control with grass meal is not yet fully known, but in inoculation experiments fungal cultures isolated from amended soil reduced C. praticola when grass meal was added in vermiculite, bacterial cultures were less effective, but actinomycetes cultures increased disease significantly.

16. Because of the porosity of vermiculite, CO_2 effects are considered not very important in C. praticola suppression in amended vermiculite.

17. Actinomycetes failed to antagonize C. praticola on agar plates when inoculated simultaneously, but some did so when given three days growth advantage.

18. Because of the inner seated nature of F. nivale in oat seeds, its check with grass meal is thought to be antibiotic, at least in part.

19. How the actinomycetes increase the disease caused by C. praticola is not clear, but they might do this by, either stimulating C. praticola growth in soil, or causing lettuce seeds to be predisposed to parasite attack or they might be responsible in degrading antibiotic materials produced by fungi and bacteria.

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APPENDIX I

In the appendix I. several symbols have been used in the analysis of variance tables which represent the following quantities

D.F.	Degrees of freedom
S.S.	Sums of squares
M.S.	Mean sqare
F.	Variance ratio
*	Significant difference, with a fiducial probability P = 0.05 or 5 per cent.
**	Significant difference - P = 0.01 or 1 per cent.
***	Significant difference - P = 0.001 or 0.1 per cent.
n.s.	No significant difference - P = 0.05 or 5 per cent.

Table 1a. Effects of soil sterilization and inoculum concentration on the virulence of Corticium praticola.

TREATMENT	Disease Index (Percentage kill in angles) REPLICATES			
	I	II	III	IV
Series I				
NORMAL SOIL				
A_1, control soil only	0.0	0.0	12.9	0.0
B_1, 1% Corticium praticola	53.7	42.1	60.0	36.3
C_1, 2% " "	56.8	90.0	56.8	60.0
D_1, 3% " "	71.6	67.2	56.8	77.1
E_1, 4% " "	90.0	77.1	67.2	90.0
F_1, 5% " "	90.0	90.0	90.0	90.0
Series II				
STERILE SOIL				
A_2, Control soil only	0.0	18.4	0.0	0.0
B_2, 1% Corticium praticola	67.2	71.6	63.4	67.2
C_2, 2% " "	90.0	67.2	67.2	71.6
D_2, 3% " "	77.1	90.0	90.0	77.1
E_2, 4% " "	90.0	90.0	90.0	90.0
F_2, 5% " "	90.0	90.0	90.0	90.0

Table 1b. Analysis of Variance table of Percentage kill (in angles) in the effects of soil sterilization and inoculum concentration on the virulence of Corticium praticola. (Derived from Table 1a).

Source of Variation	D.F.	S.S.	M.S.	F.
Replicates	3	134.97	44.99	0.59 n.s.
Treatments	11	41243.26	3749.38	49.16***
Error	33	2516.90	76.26	
Total	47	43895.13		

L.S.D. Percentage kill (in angles)

for P = 0.001 L.S.D. = 22.31 angles ***

Table 2a. Effects of soil inoculum concentration and nutrient

SOIL TREATMENT	Pots	Percentage kill G.N.A./10 A CONTROL SOIL ONLY	B 1 per cent C.p.	C 2 per cent C.p.	D 3 per cent C.p.	E 5 per cent C.p.
NORMAL SOIL	1	18.4	18.4	0.0	12.9	26.6
	2	0.0	12.9	0.0	18.4	30.0
	3	18.4	12.9	18.4	18.4	26.6
	4	18.4	18.4	18.4	12.9	12.9
STERILE SOIL	1	26.6	36.3	33.2	39.2	45.0
	2	22.8	39.2	47.9	39.2	50.8
	3	22.8	30.0	39.2	33.2	45.0
	4	33.2	26.6	36.3	39.2	56.8
STERILE PEAT/ SAND	1	18.4	22.8	47.9	39.2	50.8
	2	0.0	26.6	47.9	53.7	56.8
	3	18.4	30.0	39.2	53.7	45.0
	4	0.0	26.6	45.0	45.0	45.0
EFFECT OF NUTRIENT	TOTAL FOR 60 POTS = 1768.2 MEAN = 29.5					

G.N.A. = Glucose nitrate agar

status on the virulence of Corticium praticola.

(in angles)						
G.N.A.					Total percentage kill for 40 pots	Mean Percentage kill for soil type (in angles)
A CONTROL SOIL ONLY	B 1 per cent C.p.	C 2 per cent C.p.	D 3 per cent C.p.	E 5 per cent C.p.		
12.9	45.0	56.8	90.0	71.6		
0.0	33.2	60.8	71.6	90.0		
0.0	45.0	53.7	67.2	77.1		
12.9	39.2	63.4	67.2	77.1	1348.0	33.7
0.0	71.6	67.2	77.1	90.0		
0.0	63.4	67.2	67.2	77.1		
0.0	60.0	63.4	71.6	77.1		
0.0	77.1	63.4	90.0	90.0	1915.9	47.9**
18.4	67.2	63.4	90.0	77.1		
12.9	53.7	56.8	90.0	71.6		
12.9	42.1	47.9	71.6	71.6		
0.0	60.0	60.0	63.4	90.0	1832.6	45.8**
TOTAL FOR 60 POTS = 3328.7 MEAN = 55.5***						

Table 2b. Analysis of variance table of percentage kill (in angles) in the effects of soil inoculum concentration, and nutrient status on the virulence of Corticium praticola. (derived from table 2a).

Source of Variation	D.F.	S.S.	M.S.	F.
TREATMENTS				
(a) Nutrient	1	20303.40	20303.40	a/b 9.95*
(b) Inoculum concentration	4	35560.18	8890.04	b/d 4.36*
(c) Soil type	2	4702.38	2351.19	c/d 1.15n.s.
(d) Interaction	8	16309.14	2038.64	a/e 441.2 b/e 193.2***
(e) Error	105	4831.93	46.02	c/e 51.1*** d/e 44.3***
TOTAL	119	81707.03		

Least significant difference (percentage kill in angles).

L.S.D. between two treatment means of 4 replicates each

P = 0.05 = 9.5 angles *

P = 0.01 = 12.6 " **

P = 0.001 = 16.2 " ***

Table 3a. The effect of extra nutrients on the persistence of Corticium praticola in soil.

SOIL TREATMENT	Percentage kill					
	REPLICATES				TOTAL	MEAN
0wk series						
A, CONTROL	0.0	0.0	0.0	0.0	0.0	0.0
B, 2% C.p., (G.N.A.)	100.0	100.0	100.0	100.0	400.0	100.0
C, 2% C.p.,(2G.N.A.)	100.0	100.0	100.0	100.0	400.0	100.0
1wk series						
A, CONTROL	20.0	0.0	15.0	15.0	50.0	12.5
B, 2% C.p., (G.N.A.)	90.0	85.0	85.0	90.0	350.0	87.5
C, 2% C.p.,(2G.N.A.)	65.0	85.0	55.0	80.0	285.0	71.25
2wk. series						
A, CONTROL	0.0	5.0	0.0	0.0	5.0	1.25
B, 2% C.p., (G.N.A.)	65.0	55.0	35.0	45.0	200.0	50.0
C, 2% C.p.,(2G.N.A.)	35.0	25.0	15.0	45.0	120.0	30.0
4wk series						
A, CONTROL	5.0	10.0	15.0	0.0	30.0	8.5
B, 2% C.p., (G.N.A.)	25.0	5.0	25.0	30.0	85.0	21.25
C, 2% C.p.,(2G.N.A.)	35.0	5.0	5.0	5.0	50.0	12.5

C.p., = Corticium praticola
G.N.A. = Glucose Nitrate Agar

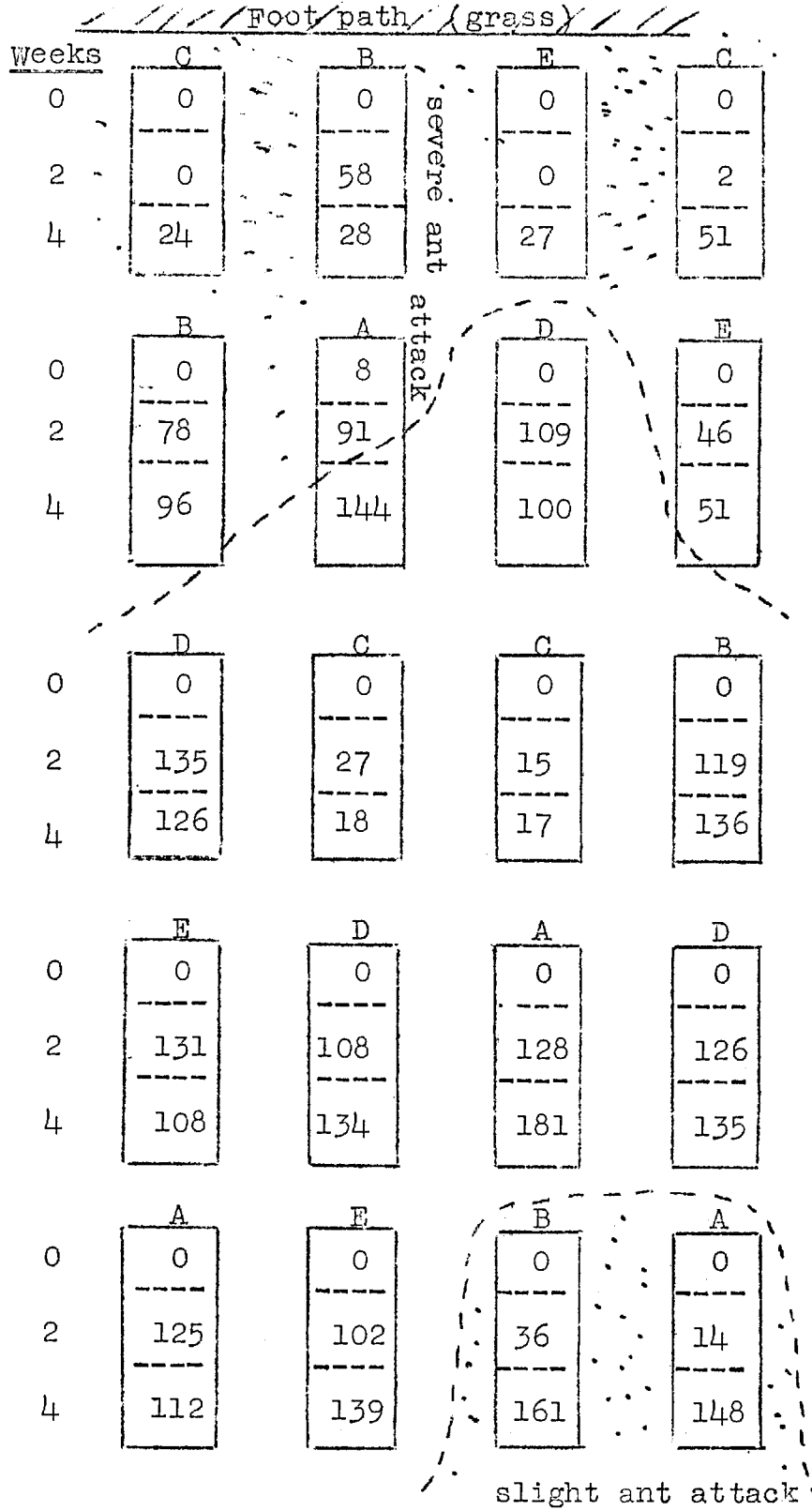
Table 24b. Analysis of variance table of final stand percentage (in angles) on the group of soil microorganisms affecting C. praticola control.

Source of variation	D.F.	S.S.	M.S.	F.
(a) Block (Replicates)	3	303.94	101.31	1.90n.s.
(b) Treatments	4	12073.74	3018.43	58.06***
(c) Errors	12	634.88	52.90	
Total	19	13012.56		

Least significant difference (percentage final stand[in angles]).

L.S.D. between 2 treatment means of four replicates for P = 0.001 = 9.63 angles.

Table 25a.
Field work on biological control of C. praticola



Plots

A

Control, soil only + 1% corn meal

B

Control, soil + 1% corn meal + 1% grass meal

C

3% C. praticola + 1% corn meal

D

3 % C. praticola + 1% Corn meal Thiram (seed treatment)

E

3% C. praticola + 1% Corn meal + 1% Grass meal

Figures are surviving seedlings out of 200 seeds sown per plot.