'DAMPING-OFF OF WHEAT BY FUSARIUM CULMORUM (W.G.SM.) SACC., AND ITS CONTROL'

by

Ahmed Mohamed Baghdadi
B.Sc. (Agric.) Ain Shames, Cairo, D.I.C.

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From the Department of Botany,
Imperial College of Science and Technology,
Field Station,
Silwood Park,
Sunninghill, Ascot,
Berkshire.

ABSTRACT

The present work is concerned with the study of the damping-off of wheat seedlings by Fusarium culmorum (W.G.Sm.) Sacc.; particularly the effects on the host, the factors influencing the severity of the disease and how it is controlled by seed dressings with ceresan [Methoxyethyl mercuric chloride] and PP781 [4-(2-chlorophenylhyrazono)-3-methyl-5-isoxazolone] and by soil applications of the organo-chlorine insecticide, aldrin.

F. culmorum caused a considerable reduction in seedling stand and in root and shoot growth of seedlings which survived. The severity of the disease was found to be affected by soil temperature and moisture, size and position of the fungal inoculum, age of the seedlings and the variety of wheat.

Ceresan seed dressings control the disease by exerting a fungitoxic effect (1) at the seed surface and possibly in a zone around the seed when particles are washed off and (2) in the roots to which it appears to be translocated. Seed dressings of PP781 were equally effective but only a fungitoxic effect at the seed surface could be demonstrated.

Aldrin had no effect on the fungus and little effect on seedling growth was shown. The most likely explanation for the control of F. culmorum by this compound is that when

applied to soil, a small amount is converted to dieldrin, which itself was shown to inhibit the growth of F. culmorum. The long-term effects of ceresan-seed dressings and soil application of aldrin were demonstrated.

In a preliminary investigation of biological control of this disease, two bacterial isolates, both spore formers, gave some control of the damping-off when the seed was soaked in suspensions of these organisms and then planted in infested soil.

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CONTENTS

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INTRODUCTION

In recent years there have been a number of reports which indicate, that some soil-applied insecticides are capable of reducing certain soil-borne diseases, although these materials have no effect on the growth *in vitro* of the pathogens concerned.

Specifically the starting points of this investigation were the reports that aldrin reduced root-rot of barley caused by *Helminthosporium sativum* (Richardson, 1957) and wilt of tomatoes caused by *Fusarium oxysporum* f. sp. *lycopersici* (Richardson, 1959); club-root of cabbage caused by *Plasmodiophora brassicae* (Keyworth, 1959; Channon & Keyworth, 1960) and take-all of wheat caused by *Ophiobolus graminis* (Grossmann & Steckhan, 1960; Slope *et al.*, 1962, Slope & Last, 1964).

In these instances there was no indication of how aldrin was effecting control and it was, therefore, decided to investigate this with reference to the damping-off of wheat by *F. culmorum*. This appeared, at that time, to be a relatively simple disease for experimental purposes. It soon became clear, however, that features of this disease particularly the mechanism of the control by seed dressings, needed further study before the effects of aldrin could be reasonably tackled. This thesis is concerned with these studies as well as those with aldrin.

REVIEW OF LITERATURE

The Disease:

Damping-off of wheat is common wherever the crop is grown. The symptoms of this disease are both striking and simple; either seedlings fail to emerge from seed of high germinative capacity or shortly after emergence plants appear unthrifty, then collapse and die. The cause of the disease is less simple to determine. In many situations a number of pathogenic fungi are involved, frequently species of Fusarium, and often Fusarium culmorum is one of these.

Bennett (1928, 1932, 1933a, 1935) isolated and identified 14 Fusarium spp. from diseased wheat seedlings in the North of England and showed that Fusarium culmorum and Fusarium avenaceum were to a great extent responsible. Of the two Fusarium culmorum appeared to be the more virulent. Russell (1932) similarly found Fusarium culmorum to be one of the most common fungi associated with damping-off and seedling - blight of wheat in the Cambridge area. Broadfoot (1934) in North America, made isolations from many thousands of wheat plants; for individual rotations plots between 20 and 60% of the isolates were Fusarium culmorum.

There are now many reports which indicate that damping-off by Fusarium culmorum is common in all cereal

producing countries, for example those by Doyer (1921), Guyot (1921), McDonald (1922), Lundegårdh (1923), Stakman (1923), Appel (1924), Simmonds (1926, 1928), Gram & Rostrup (1927), Schaffnit (1930), Vilkaitis (1932), Geach (1932), Schmidt (1933), Fuchs (1935), Marland (1935), Pissareff (1939), Slykhuis (1948), and Bochkareva (1964).

Whilst the present investigation is concerned with Fusarium culmorum on seedling wheat, mention should be made here of two other aspects to give an overall picture of the activities of this fungus.

Firstly, Fusarium culmorum can attack older wheat plants. This results usually from the steady encroachment by the fungus of the tissues of its developing host. At different stages of host growth there are characteristic disease symptoms which in the past were often considered to be distinct diseases. The principle phases have been summarised by Simmonds (1928) for oats and are substantially the same for wheat (Bennett, 1928), viz:-

a. Damping-off: Killing of young seedlings before shoots appear above the ground.

b. Seedling Blight: Death of the seedling after emergence.

c. Spring yellows: The young leaves of older seedlings become a paler green than normal, turn yellow at the tips, and finally die.

d. Foot-rot: The most destructive stage of all since it results in a complex of symptoms such as "thining out" between earing and harvest, in "premature ripening" and in "Whiteheads" and "deaf ears".

The second noteworthy feature of Fusarium culmorum is its ability to live as a saprophyte in the soil; frequently it is the dominant organism in the early stages of straw colonization (Sadasivan, 1939; Walker, 1941; Butler, 1953). It is a typical 'Soil-inhabiting' fungus, as defined by Garrett (1939); an unspecialised parasite with a wide host range. Thus isolates from oats, barley and maize (Simmonds, 1928), several grasses (Blair, 1937) and even lucerne, sweet clover (Cromack, 1937) and peas (Padwick, 1938) can infect wheat.

Although much is known of the saprophytic activities of Fusarium culmorum and the disease which it produces, there is comparatively little information on the initiation of infection on seedlings. Simmonds (1928) studied the infection of oats seedlings by Fusarium culmorum, and he showed that penetration took place through the cortical tissues of the mesocotyl and coleoptile and that the mycelium collects between the coleoptile and the plumule. The cortex of the root was readily invaded, with some evidence that entrance may occur through the root hair.

Russell (1932) reported that Fusarium culmorum gained entry into wheat seedlings through the root by penetrating the cuticle.

Factors Influencing Disease Development:

Several factors have been shown to influence damping-off of wheat seedlings by Fusarium culmorum, in particular, soil moisture and temperature, inoculum size and position, age of wheat seedlings and wheat variety.

There are conflicting views on the effects of soil moisture and temperature. Most evidence suggests that damping-off by Fusarium culmorum is greatest at low soil moisture and high soil temperatures. Simmonds (1928), showed that with soils at 20-35% moisture and a temperature of 18-30°C. there was an increase in disease development, while at lower soil temperatures (8-15°C.) there was a decrease in the disease. Tupenevich (1936), reported that wheat seedlings grown in soil artificially infested with Fusarium culmorum or other species of Fusarium developed a more vigorous root system at 8° to 10°C. than at 18° to 24°C. Shen (1940), found that infection of wheat seedlings by Fusarium culmorum was most severe at a low soil moisture content (30%). Johnston and Greaney (1942) were unable to demonstrate any effect of soil moisture on the virulence

of Fusarium culmorum, but they did show that the pathogenicity of this fungus increased with increasing soil temperature. Further evidence recently was given by Colhoun and Park (1964) that damping-off of wheat by Fusarium culmorum was most marked in dry soil and at higher soil temperature.

The results of Bennett (1928) on the other hand conflict with the above. He found that Fusarium culmorum caused more damage to wheat plants in wet soils than in dry ones, and at soil temperatures below 10°C.

There are few records of the effect of inoculum size and position on disease incidence. However, Shen (1940), has shown that infection of wheat seedlings by Fusarium culmorum increases with density of spore suspension used as inoculum. In an attempt to find a suitable method of inoculating oats seed with Fusarium culmorum, Simmonds (1928), found that when inoculum was placed at seed level, mixed with sand at seed level or mixed through the soil the results were approximately the same, but when placed one inch below seed level or two inches above, the infection was less severe.

Little also has been published on the influence of age of wheat plants to infection by Fusarium culmorum. Broadfoot (1931), showed that the wheat plant was more

susceptible to infection by Fusarium culmorum, during the first thirty or forty days than it was later and in later experiments (Broadfoot, 1933), emphasized that the seedling stage was most susceptible to infection.

There is comparatively little information available about varietal susceptibility to Fusarium culmorum in particular, and to cereal root rot fungi in general. Greaney et al., (1938), emphasized that though it is possible to separate varieties into those which are particularly susceptible and those which show some resistance, generally the differences are less than can be achieved by modifying the environmental conditions such as soil moisture and temperature.

Tyner and Broadfoot (1943), tested a large number of wheat varieties for their reaction to Fusarium culmorum. They also found that while these could be placed into groups with consistently different degrees of resistance, there were many factors that did appear to have a great influence on the results. They concluded that testing for varietal resistance should be carried out only under field conditions and in naturally infested soil.

So far there has been little attempt to produce varieties resistant to damping-off fungi such as Fusarium culmorum. Pisarev and Malinovskaya (1945) however, have

found that in the wheat varieties 'Prelude', 'Miltrum 321' and 'Diamond' infection by Fusarium culmorum, Fusarium avenaceum and other Fusarium spp. occurs at the base of the plants, but never extends beyond the coleoptile and does not involve the roots, whereas in susceptible varieties, the plants are either killed or make poor growth without tillers.

Control:

(a) Seed dressings.

Several groups of compounds have been used to treat cereal seed for the control of pre-and post-emergence killing of seedlings. The most important of these are the organomercurials. Many individual compounds of this type have been tested and found to give effective control of Fusarium culmorum, for example, uspulum [20% mono-chloromercurophenolate, $Cl-C_6H_4-O-Hg$] (Simmonds, 1926); semesan [35% hydroxy - mercuro - chlorophenol sulphate], germisan [mercury - cresol - sodium cyanide] (Simmonds & Scott, 1928; Simmonds, 1928); ceresan [methoxyethyl mercuric chloride, C_3H_7CLHgO], new improved ceresan [5% ethyl mercuric phosphate] (Machacek & Greaney, 1935); and fixton [phenyl mercuric dinaphthymethane disulphonate] (Hopf et al., 1951).

The mechanism of disease control obtained by treating seed with these compounds is not entirely clear. Inoculum of Fusarium culmorum can be either seed-borne (as spores or chlamydospores on the seed-surface) or soil-borne (in straw residues). There seems little doubt that seed-borne inoculum is killed by direct contact of fungus and seed dressing on the seed surface. The toxicity of these compounds to

Fusarium culmorum in vitro is well known (Machacek & Greaney, 1935; Tolba & Salah, 1958). Where the inoculum is soil-borne it may be some time after seed germination before contact between fungus and host is established and in this instance it is less obvious how control is achieved. That the use of these materials might involve at least two phases, one at the seed surface and another during germination was pointed out by Gassner (1927). He showed, for example, that uspulun was more effective than germisan against seed-borne parasites but less effective against soil-borne ones.

Several attempts have been made to explain the control of soil-borne pathogens by mercurial seed dressings. Booer (1951) suggested that while mercury in the soil retards the growth of both plants and fungi, it affects the causal fungus more than the host so that it 'disturbs the relationship between the host and parasite, possibly a symbiosis, which is the pre-requisite of infection'. This is a variation of the general statement of Leach (1947) that damping-off is most severe when conditions favour growth of the pathogen but not the host. He showed for several host-parasite combinations that the ratio, velocity of seedling emergency/growth rate of pathogen, was inversely related to pre-emergence kill.

A stimulation of host growth by mercurial seed dressing is one way in which the host-parasite relationship could be affected. There is some evidence for this. Garbowshi & Leszczenko (1924), andKempski (1925), both found that uspulun caused a temporary stimulation of wheat growth, and Pichler (1932) reported that ceresan, and abavit B enhanced the germination of wheat seed. Stimulation by uspulun of the germination of various vegetable seeds has also been reported by Csete (1921), Kreuzpointer (1922), and Scheinpflug (1924). On the other hand, some investigators were unable to find any stimulation of germination or growth in comparable experiments. (Schaffnit, 1925; Lindfors, 1926; Kiesselbach, 1927; Niethammer, 1929).

The uptake of mercury from seed dressings and its redistribution within the tissues of the developing seedling is another possible factor in the control of soil-borne parasites. Lundegårdh (1924) was one of the first to demonstrate mercury uptake by germinating wheat seed. De Paolis (1931) also found that wheat seedlings grown from seed treated with mercuric chloride, uspulun or abavit B contained mercury in the roots and stem. More recently, and using more refined techniques Pickard and Martin (1960) have demonstrated that mercury may be absorbed by young root systems and translocated within the plant, and Vir and

Bajaj (1964) that uptake and translocation of mercury occurs in wheat, oat and maize seedlings raised from treated seed.

(b) Soil application of organo-chlorine compounds:

In recent years there have been reports of combined fungicide and insecticide preparations being particularly effective in controlling certain soil-borne diseases. The insecticides used were organo-chlorine compounds such as DDT, Aldrin, Dieldrin, gamma-BHC and Heptachlor. (Duffield, 1952; Young, 1954; Leach et al., 1954; Tarr, 1954, 1954a, 1955; Forsberg, 1955; Bremer, 1957; Grogan et al., 1959; Burrage & Tinline, 1960; Richardson, 1960; Bazan, 1960; Clinton, 1960, 1962; and Schultz, 1962). In these instances one possible explanation is that an insect pest which either facilitates entry of the fungus or in other way contributes to the disease complex is also controlled by this treatment.

However, there is probably more to it than this because some investigators have shown that applications of these insecticides alone can give effective disease control in situations where interference by insects can be discounted.

For example, Richardson (1957, 1959) found that root-rot of barley seedlings caused by Helminthosporium sativum and

wilt of tomatoes caused by Fusarium oxysporum f.sp. lycopersici were reduced by aldrin and endrin, yet found these materials had no effect on the fungi in vitro. Keyworth (1959), and Channon and Keyworth (1960) reduced club-root of cabbage caused by Plasmodiophora brassicae by applying aldrin as a dust to soil or watering the plants with an aldrin emulsion. Grossmann and Steckhan (1960) found that take-all of wheat caused by Ophiobolus graminis was reduced by soil treatment with chlordane and aldrin, and similar results with aldrin, dieldrin, chlordane and heptachlor were obtained by Slope et al., (1962), and Slope & Last (1963).

In these examples there is no clear indication of how the insecticides act to give disease control. While these materials may not inhibit the growth of the pathogens in vitro (Simkover and Shenefelt, 1951; Richardson, 1957, 1959; Grossmann and Steckhan, 1960), it is possible they do so in soil either directly or because they are converted to other materials which are themselves fungitoxic. Richardson and Miller (1960), ascribed the fungitoxicity of these organo-chlorine compounds to their physical properties; they found that the ones with high water solubility or high vapour pressure were highly fungitoxic in vitro.

Alternatively, these materials may stimulate plant growth and thus enable the young seedling to escape severe attack. Several workers, (Allen and Casida, 1951; Stone and Smith, 1951; Rodrigues *et al.*, 1957; Richardson, 1957, 1959; Grossmann and Steckhan, 1960) have, in fact, shown that stimulation of plant growth sometimes occurs.

Biological Control:

Because it is difficult and expensive to control soil-borne diseases by chemical means other than seed dressings, the possibility of biological control has been examined predominantly with diseases of this type.

Several investigators have produced evidence of biological control of soil-borne pathogens, by the direct or indirect use of soil micro-organisms.

Methods involving a direct application of antagonistic micro-organisms are, (1) dipping or soaking the seed in a suspension of spores and mycelial fragments or in extracts of the organisms (2) pouring a spore or mycelial suspension over the seed in the soil, (3) adding cultures of the antagonists to soil before or at the time of planting, and (4) dusting the seed with spores and mycelial fragments.

For example, Khudiakoff (1935) found that two bacteria, a Pseudomonas sp. and an Achromobacter sp., were capable of inducing lysis in Fusarium culmorum and other Fusarium spp. Control of Fusarium graminearum (G. saubinetii) on wheat was achieved when the fungus and the lytic bacteria were added to soil simultaneously or when the bacteria were incorporated with the soil 24 hours before sowing and inoculation with the pathogen. Damping-off of Pinus sylvestris seedlings caused by seed or soil-borne species of Fusarium has also been controlled by treating the seed with suspension of

known bacteria from pure cultures. Isolates of Pseudomonas and Achromobacter were the most effective (Krasilnikov, 1946). Thomas (1948) added to soil, cultures of ten organisms selected for their antagonism to Fusarium culmorum and then a month later introduced the pathogen. He found that two isolates of Actinomyces scabies significantly reduced disease incidence, measured after a 9 month period. Mitchell and Alexander (1961) reported that the addition of a lytic Bacillus strain to sterile soil containing Fusarium oxysporum resulted in disgestion of the fungus, but control of the pathogen was not obtained in non-sterile soil.

An indirect use of antagonists is to add substances to soil which encourage the growth of a large population of micro-organisms. One hopes here that some of these micro-organisms will antagonize the soil-borne pathogens. Several diseases have been controlled by such soil amendments. For example, potato scab caused by Actinomyces scabies can be controlled by ploughing-in green manures (Millard, 1923; Millard & Taylor, 1927), so can Phymatotrichum omnivorum which causes cotton root-rot (King & Loomis, 1926; King et al., 1934; Clark 1942). More recently, it has been reported that bean root-rot caused by Fusarium solani f. phaseoli, wilt of radishes

caused by *Fusarium oxysporum* f. *conglutinans* (Mitchell & Alexander, 1961 a, b), and pea wilt caused by *Fusarium oxysporum* f. *pisi* (Buxton *et al.*, 1965; Khalifa, 1965) can be controlled by the addition of chitin to soil infested with these pathogens.

MATERIALS AND METHODS

(1) The Fungus:

The isolate of Fusarium culmorum was obtained from the I.C.I. Research Station, Jealott's Hill, Berks, and maintained at room temperature on V8 juice agar slopes under sterile liquid paraffin (B.P. grade).

(2) Culture media:

The fungus was grown on the following media.

V8 juice agar (V8)

V8 juice (Campbell's soups Ltd.)	10 ml.
Agar	2 g.
Distilled water	90 ml.

Potato-dextrose agar (P.D.A.):

300 g. peeled potatoes were cut into small pieces, covered with tap water and heated for 20 mins. Then both potatoes and liquid were strained through muslin, 5g. glucose and 20g. agar added to the extract, and the volume made up to one litre with tap water.

Both the V8 juice agar and potato-dextrose agar were sterilized by autoclaving at 120°C. for 20 mins.

Oatmeal-sand mixture

This was prepared in 500 ml. Erlenmeyer flasks each with the following mixture:

Ground oatmeal (Scott's porage Oats)	8 g.
Dry sand, passed through 0.1 in sieve	392 g.
Tap water.	40 ml.

Sterilized by autoclaving at 120°C. for one hour (Shepherd and Wood, 1963).

(3) Wheat variety:

Most experiments were carried out with the variety 'Svenno' which, in preliminary experiments, was found to be markedly susceptible to damping-off by Fusarium culmorum. Untreated seed of this variety was obtained in bulk from E. Dixon and Sons (Ware) Ltd., in April 1964. This was stored in a cool room and used throughout the investigation. The percentage germination was checked at intervals.

(4) Soil:

Soil was obtained from the Walled Garden at Imperial College Field Station, Silwood Park. This soil is humus stained to a depth of one foot and appears grey brown when dry.

Immediately preceding this investigation (summers of 1962 and 1963) it received a dressing of muriate of potash,

superphosphate and sulphate of ammonia, and was planted with wheat (Simon, 1964).

Soil was taken mainly at the 3-6 in. level. This sample was air dried in a heated greenhouse (15-18°C.), and then passed through a ¼" mesh sieve. The pH. of the soil thus treated was 6.5.

(5) Inoculum and soil inoculation:

Inoculum was prepared by introducing into each flask of oatmeal-sand (p. 19) 16 disks cut from the edge of a colony on V8 agar with a sterile cork-borer (4 mm. diam.), and then incubating at 25°C. Flasks were shaken every 2 days to obtain a uniform growth.

Preliminary experiments indicated that age of inoculum had little effect on disease incidence (see Appendix p. 190), a result similar to that obtained by Tyner (1941). The amount of inoculum added, however, significantly affected disease incidence (p. 190).

For soil inoculation, except where stated otherwise, soil was seeded with Fusarium culmorum by adding to it 5% (w/w) of a 10 - day old culture on oatmeal-sand, and then thoroughly mixing the culture and soil.

Most experiments were carried out with 5 in. unglazed earthenware pots, which were carefully washed between each change of soil. Soil moisture was maintained at the required

level (50 to 60% M.W.H.C.) by adding water twice a week to earthenware saucers in which the pots were placed. The pots were randomized on benches in a heated greenhouse (15-18°C.). During winter time the greenhouse was illuminated by means of "Mercury Vapour" lamps for 12 hours daily.

Other materials and methods use in connexion with particular experiments will be described in the appropriate sections.

EXPERIMENTAL

PART I THE DISEASE

1. Effects of Fusarium culmorum on the development of wheat seedlings

Experiment 1:

Seedlings grown in infested soil

The aim of this experiment was to investigate the effect of F. culmorum added to soil on seedling stand, and on root and shoot development.

Forty, 5 in. pots were filled with untreated soil and another 40 with soil infested with F. culmorum by the method described on p. 20. Fifteen wheat seeds were then sown in each pot, and the pots randomized on the greenhouse bench.

The first estimate of disease effects was made 3 days after sowing and further estimates at 2-day intervals until 21 days after sowing. On each sampling date 3 pots were selected at random and the following assessments carried out:-

1. Seedling stand: a direct count of seedlings emerged.
2. Height of plants: the distance from the seed to the end of the longest leaf was measured for each seedling. The mean shoot height of all emerged seedlings was then calculated.

3. Length of roots: the lengths of the primary root and the first pair of lateral roots were measured and the mean length determined per root system. The mean root length of all emerged seelings was then calculated.

Analyses of the results (Appendix Tables 1, 2 & 3) show that seedling stand is significantly reduced in infested soil and that root and shoot growth of those seedlings which do emerge is significantly less than in uninfested soil. The results are summarized in Table 1, and in Figure 1 and 2.

Table 1. Effect of F. culmorum added to soil on seedling stand

Time (days)	Mean number of seedlings		Differences
	Soil + F. culmorum	Untreated soil	
3	4	12	8**
5	8	13	5**
7	11	14	3*
9	9	14	5**
11	10	14	4**
13	7	14	7**
15	8	13	5**
17	10	14	4**
19	12	14	2 n.s.
21	10	14	4**
Mean total seedling emergence	89	136	

L.S.D: *at P. = 0.05
**at P. = 0.01

FIGURE 1. EFFECT OF F. CULMORUM ADDED TO SOIL ON ROOT GROWTH.

⊙ UNTREATED SOIL ● SOIL + F. CULMORUM

VERTICAL LINES REPRESENT SIGNIFICANT DIFFERENCES FOR P=0·05, P=0·01

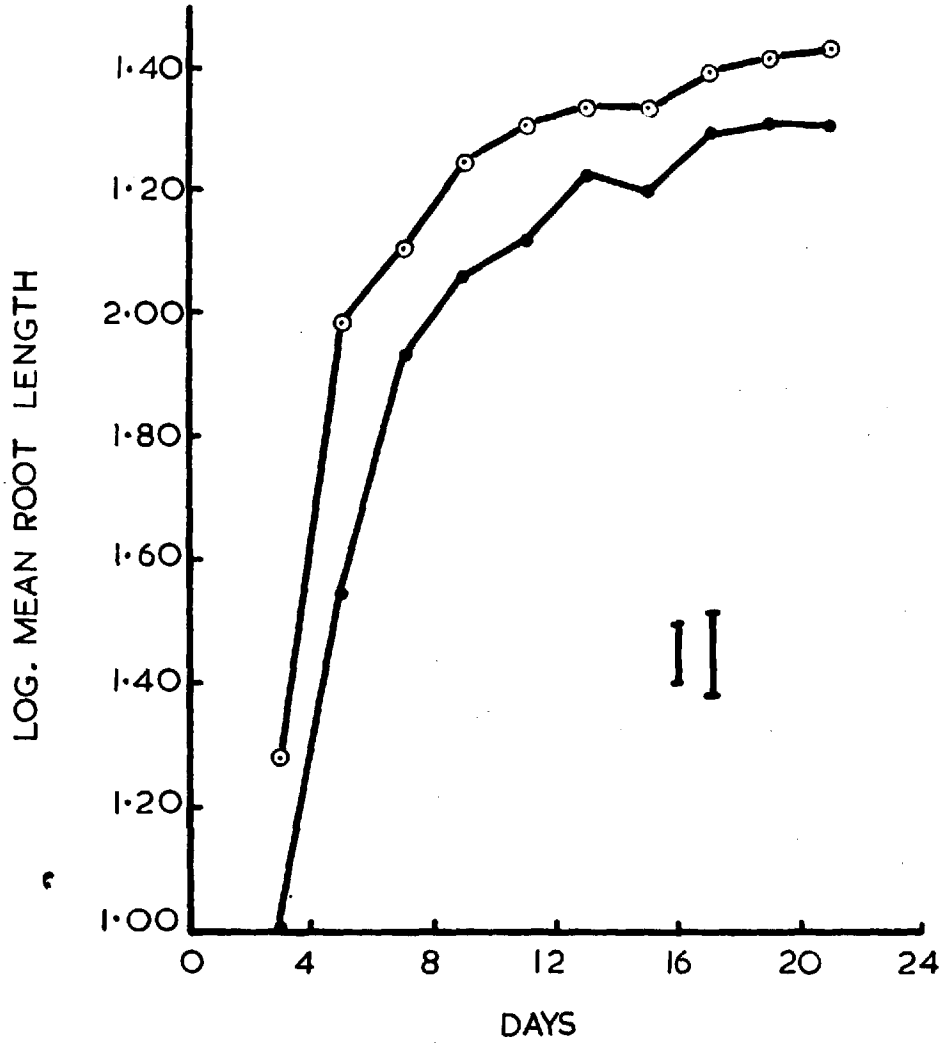
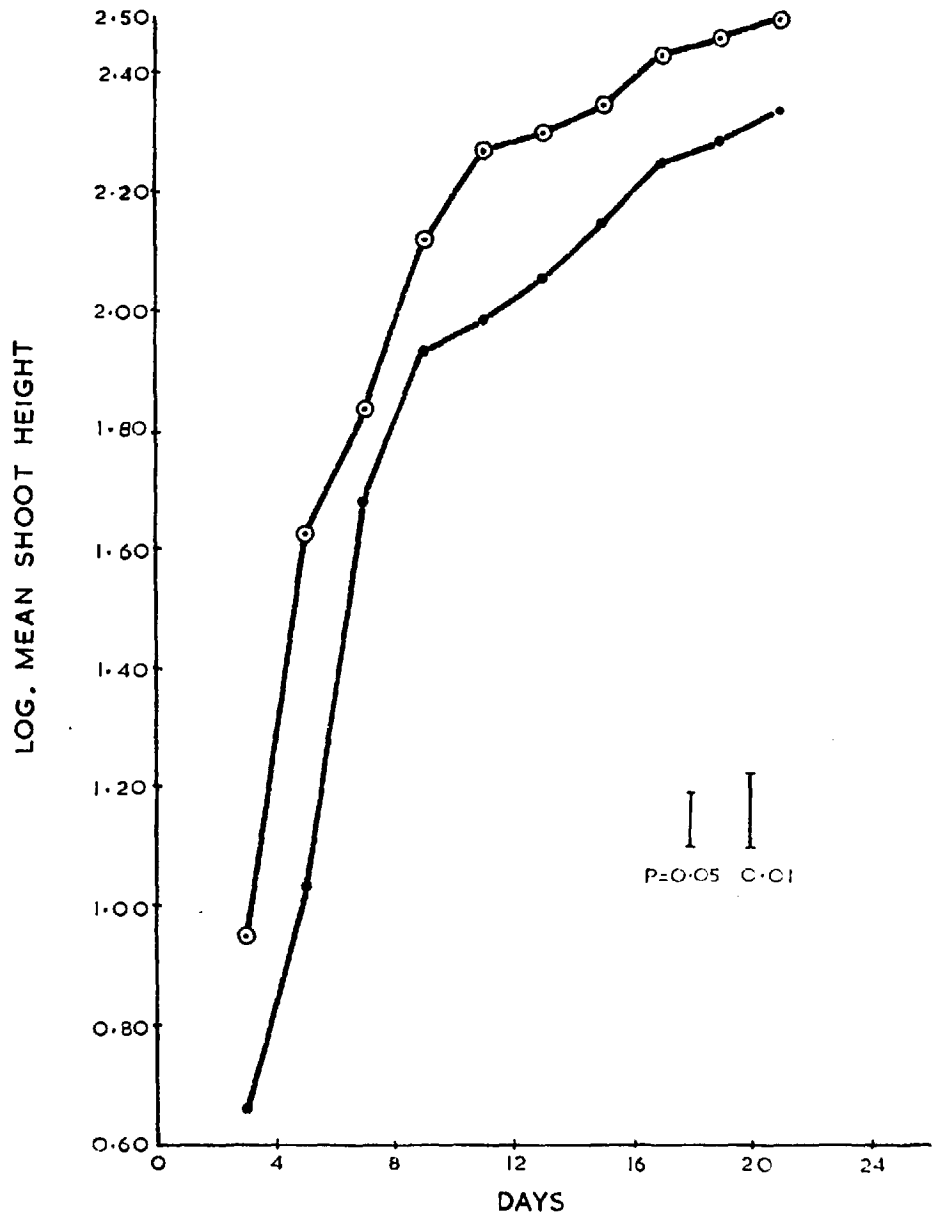


FIGURE 2. EFFECT OF F.CULMORUM ADDED TO SOIL ON SHOOT GROWTH

⊙ UNTREATED SOIL • SOIL + F.CULMORUM

VERTICAL LINES REPRESENT SIGNIFICANT DIFFERENCES FOR P = 0·05, P = 0·01



Experiment 2:

A comparison between seedlings grown from infested seed and seedlings grown in infested soil

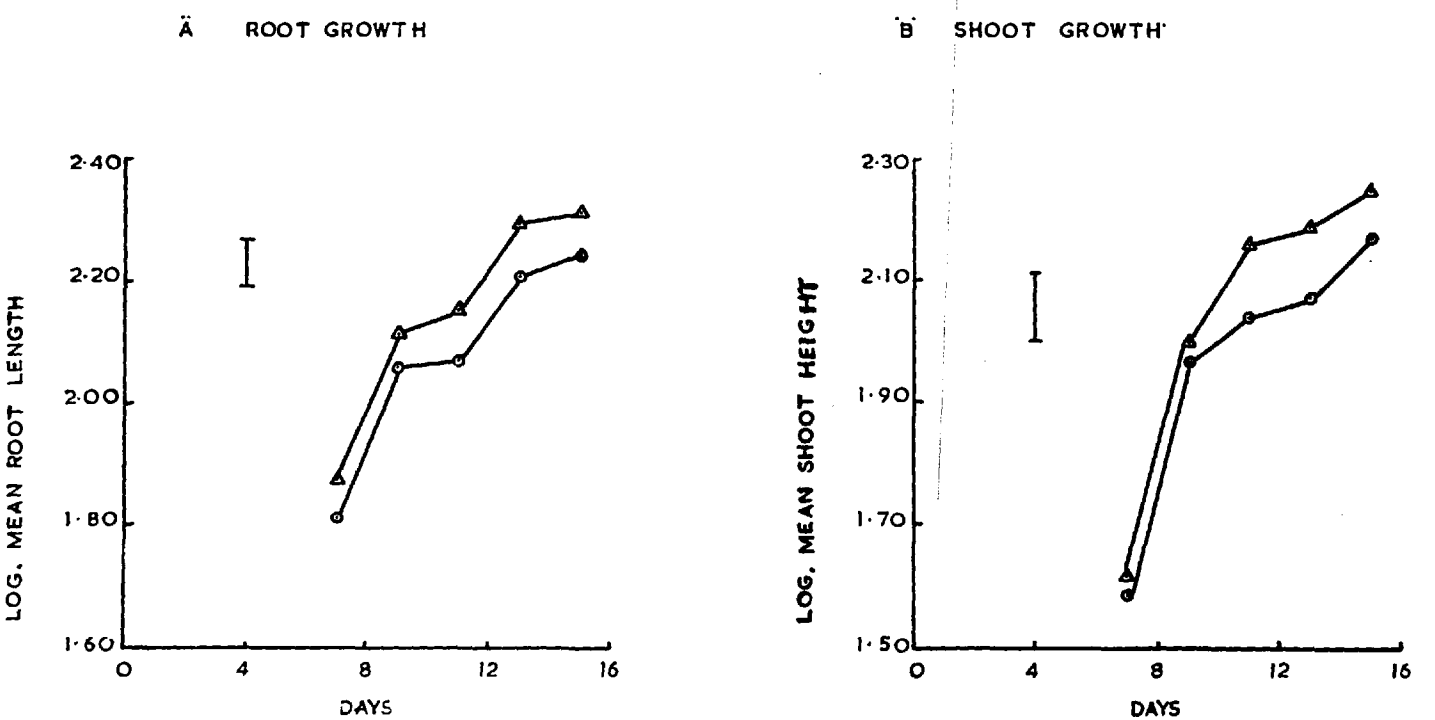
Seed was infested with F. culmorum in a manner similar to that described by Colhoun & Park (1964). A suspension containing 10^6 spores/ml. was prepared from a 6 day-old culture on a potato-dextrose agar slope. One ml. of this was added to 25 g. seed in a flask and mixed by thoroughly shaking for 5 min. Fifteen seeds so treated, were planted in each of fifteen pots of untreated soil. A similar number of pots with soil plus F. culmorum was also prepared and 15 untreated seeds were planted in each of them. Assessments on seedling stand, and root and shoot growth were carried out on two pots on the 7^{th}, 9^{th}, 11^{th}, 13^{th} and 15^{th} day after sowing in the manner described for Exp. 1. (p. 22).

There was no significant difference in seedling stand between the two treatments (Appendix Table 4), but infesting soil with F. culmorum appeared to cause a greater retardation of root and shoot growth during the later stages of the experiment (Figure 3 a & b and Appendix Tables 5 & 6). It is possible that with infested seed some roots are able to grow away from the F. culmorum inoculum and are thus less affected than roots in soil in which the fungal inoculum is evenly distributed.

FIGURE 3

COMPARISON BETWEEN SEEDLINGS GROWN FROM INFESTED SEED △
AND SEEDLINGS GROWN IN INFESTED SOIL ⊙

VERTICAL LINES REPRESENT SIGNIFICANT DIFFERENCES FOR P = 0·05



Experiment 3:

A comparison of seedlings grown from infested seed, seedlings grown in infested soil, and seedlings grown in soil inoculated with a spore suspension after sowing.

The aim of this experiment was to compare inoculation with a spore suspension after sowing with the methods used in Exp. 1. and Exp. 2.

Fifteen untreated seeds were sown in each of 5 pots of clean soil. After sowing, 10 ml. of a suspension containing approximately 10^6 spores of F. culmorum per ml. were poured over the surface of each pot.

Five pots were also set up with:-

untreated seed - untreated soil (control)

untreated seed - soil plus F. culmorum (as in Exp. 1.)

seed treated with F. culmorum spores - untreated soil (as in Exp. 2).

The pots were randomized on the greenhouse bench, and 21 days after sowing seedling stands, root lengths and shoot heights were assessed. The full results are given in Appendix Tables 7, 8 & 9 and summarized in Table 2. These confirm the finding of Exp. 2 that the effects of F. culmorum are most severe where the fungal inoculum is evenly distributed throughout the soil. Adding inoculum as

a spore suspension after sowing had least effect on seedling stand and growth (Plate 1). This suggests that under the circumstances contact between pathogen and host is delayed to a point where the seedling is less susceptible to attack. However, some caution is necessary in interpreting the results in such terms since equality of inoculum cannot be established with the three treatments used.

Table 2. Effects of F. culmorum on seedling stand and growth

Treatments	Mean		
	Seedling stand (no.)	Root length (mm.)	Shoot height (mm.)
A. No F. culmorum - untreated soil (control)	11.2	295	307
B. Soil + F. culmorum	5.2	220	236
C. Seed treated with F. culmorum	7.6	263	285
D. Soil treated with spore suspension F. culmorum after planting	8.0	258	268
L.S.D: P. = 0.05	2.2	30.2	24.1
P. = 0.01	3.1	43.8	33.8

Plate 1.

Comparison of methods of inoculating wheat seed with F. culmorum.

A. Untreated seed - untreated soil (control).
B. A spore suspension after sowing.
C. Seed treated with F. culmorum spores - untreated soil.
D. Untreated seed - infested soil.

2. Colonization of seedling roots and coleoptiles by Fusarium culmorum

The results obtained in the previous section suggest that when wheat seed is planted in infested soil, F. culmorum rapidly colonizes the tissues of the developing seedling either killing it or severely reducing root and shoot growth. When seed is infested with spores of F. culmorum and planted in clean soil the results are essentially similar but somewhat less drastic. The present section is concerned with studies on the colonization of seedling roots and coleoptiles, both externally and internally. Most studies have been made on seedlings grown in infested soil but some observations of seedlings grown from infested seed are also included.

Experiment 4:
Colonization of the root and coleoptile surfaces from soil-borne inoculum.

The distribution of mycelia on roots and coleoptiles was investigated by washing roots and coleoptiles thoroughly and plating out on agar media in a manner similar to that described by Harley and Waid (1955), and by direct, microscopical examination of washed roots and coleoptiles.

Root washing/plating technique: A number of pots containing soil infested with F. culmorum were sown with untreated wheat seeds. At days 3, 5, 7, 9 and 11 after sowing 20-25 germinated seeds were removed, gently shaken free of large soil aggregates and washed under the tap to remove small particles. From these, plants were selected at random for detailed study, viz:- 4 plants on day 3, 2 plants on day 5 and day 7, and 1 plant on day 9 and day 11. A reduction in the number of plants sampled with time was necessary because only a limited amount of tissue could be examined.

Each root system and coleoptile was cut from the plant at the point of attachment to the seed and transferred to a labelled McCartney bottle (28 ml. capacity) containing sterile distilled water. The excised tissues were then given 3 preliminary washings, cut into 4 cm. lengths (or less on day 3), and each segment placed in a fresh bottle of sterile distilled water, labelled to indicate the position within the root or coleoptile from which the segment was taken. Each segment was then washed in 20 changes of sterile, distilled water. For each washing the bottles were placed on a Griffin flask shaker for 3 mins. After the 1^{st}, 5^{th}, 10^{th}, 15^{th} and 20^{th} washings the segments were transferred to fresh bottles.

After washing each segment was placed in a sterile

Petri dish with sterile filter paper to remove excess moisture and then cut into 3 mm. pieces with a sterile scalpel. These pieces were plated in order on Rose-Bengal-Streptomycin agar (See Appendix, p.191) and incubated at 25°C. After 3 days the number of root and coleoptile pieces showing growth of F. culmorum was recorded. The plates were then left for a further 4 days at room temperature and again examined for F. culmorum. The results are given in Appendix Table 10 and summarized in Figure 4. The percentage root surface colonized increases rapidly with time but the corresponding figure for coleoptiles does not. For the period of observation growth of F. culmorum in the coleoptile appeared not to extend beyond soil level.

Figure 5 a-e shows the distribution of F. culmorum on representative seedlings of various ages, based on the data obtained from plating root and coleoptile pieces. The tissues adjacent to the seed are first attacked but the fungus also quickly becomes established at other points behind the tip as the root elongates. The root tips (with one exception on day 11) remain free of F. culmorum, and indeed of any fungal growth.

Direct microscopical examination: Observations were made on roots and coleoptiles on day 9 which provide further information on their colonization by F. culmorum.

The tissue was washed as described above for the plating method but examinations were made only on the first 3 cm. growth from the seed, both of root and coleoptile.

There were 3 sets of observations:-

(a) Ten 3 cm. root segments were mounted individually in cotton-blue/lactophenol (see Appendix 19) and examined under the microscope after 30 mins. Hyphae were visible on all parts of the root. They were most dense on parts of the root close to its attachment to the seed and least dense 3 cm. from this point. Branching occurred in all directions. Hyphae were aggregated between and in the cortical cells, large swellings of hyphae result in the formation of mycelial chlamydospores in chains or clusters.

Some of the hyphae were found in the root-hairs. (Plates 2, 3, 4 and 5 a-b).

(b) A second sample of washed roots and coleoptiles was kept at 4°C. for 12 hours before examination. These were then cut into 3 mm. pieces and also stained in cotton-blue/lactophenol. The results (Tables 3, A & B) give a more detailed picture of colonization along a 3 cm. length, and again illustrate the preponderance of hyphae on tissue adjacent to the seed.

(c) A third sample was dried on sterile filter paper after washing, cut into 3 mm. pieces, and plated onto

Rose-Bengal-Streptomycin. The plates were incubated at 25°C. for 12 hours and the tissue pieces then mounted in cotton-blue/lactophenol and examined microscopically. The results are given in Tables 4 (A & B). As far as distribution is concerned these add little to that already described in (a) and (b), but the hyphal growth observed on the plates after removing the root and coleoptile tissue confirmed the presence of F. culmorum.

FIGURE 4.

COLONIZATION OF ROOTS • AND COLEOPTILES ⊙
IN SOIL INFESTED WITH F. CULMORUM

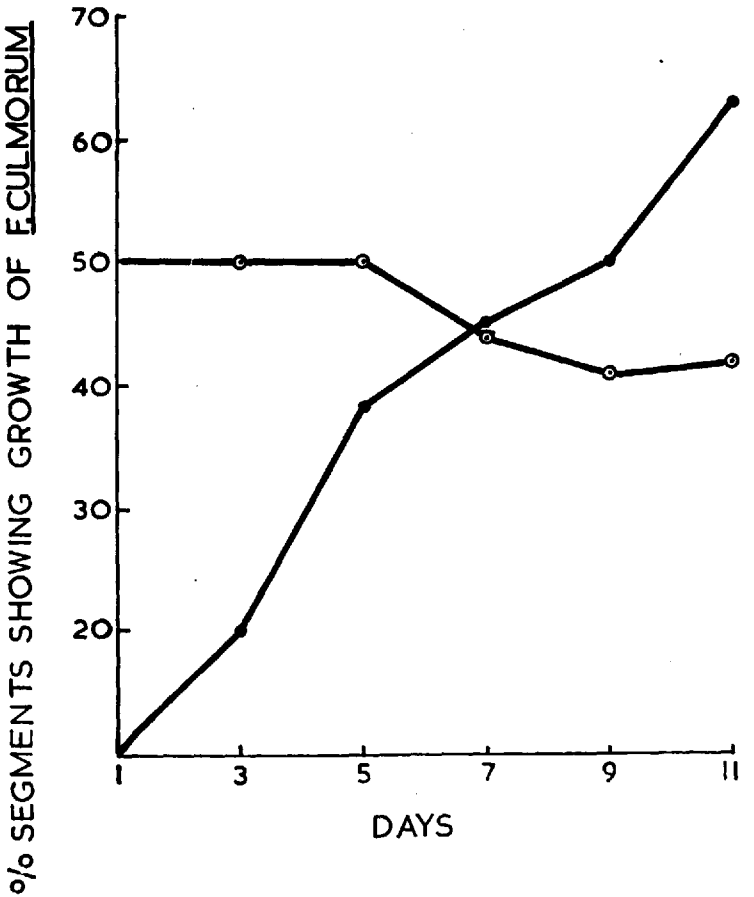


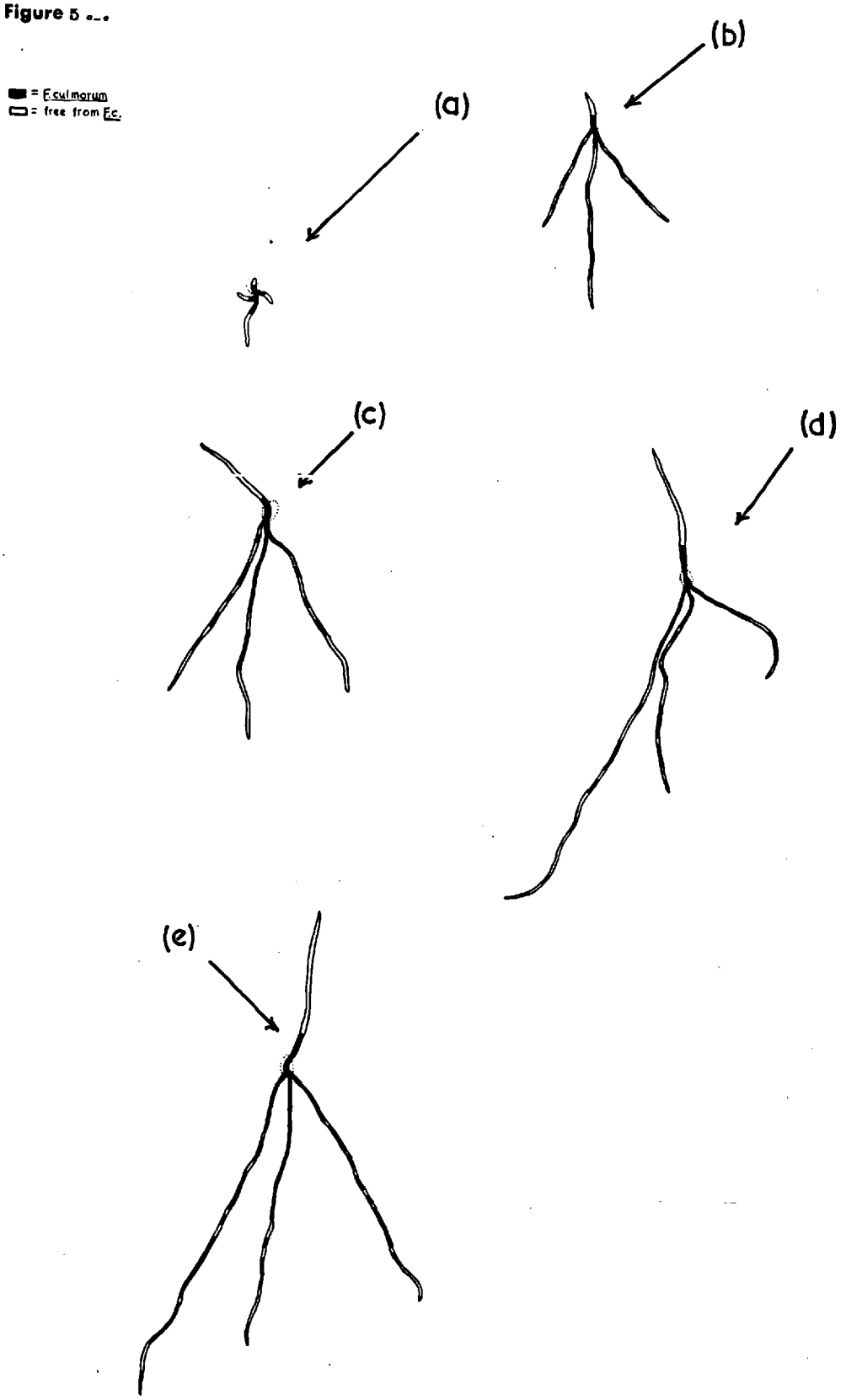
Figure 5: Distribution of F. culmorum on seedlings grown in infested soil.

(seedlings drawn to natural size, after photographing they were reduced to half the size).

(a) Day 3
(b) Day 5
(c) Day 7
(d) Day 9
(e) Day 11

Figure 5 ...

■ = E.culmorum
▭ = free from Ec.



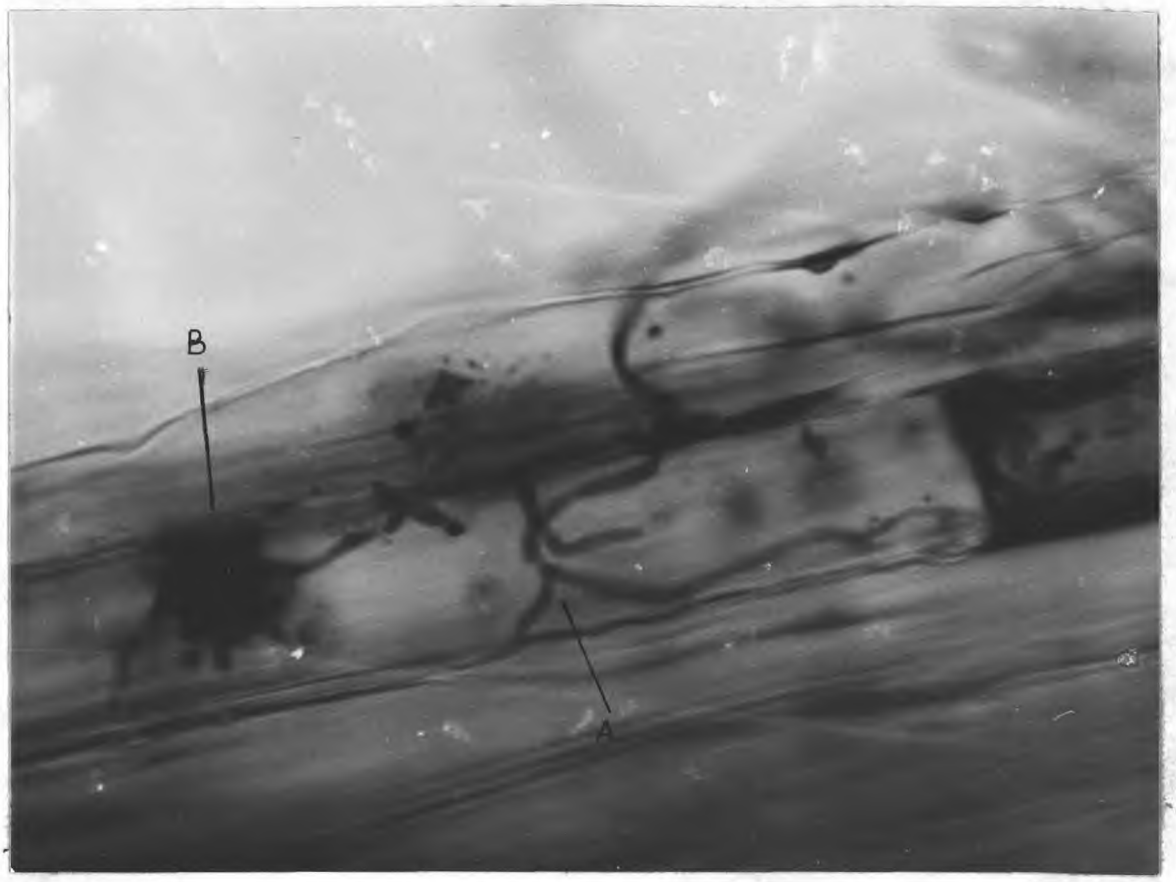


Plate 2: Surface view of part of a root segment, showing:

A. Branching of hyphae.

B. An area beneath the hyphal tip had taken the stain deeply.

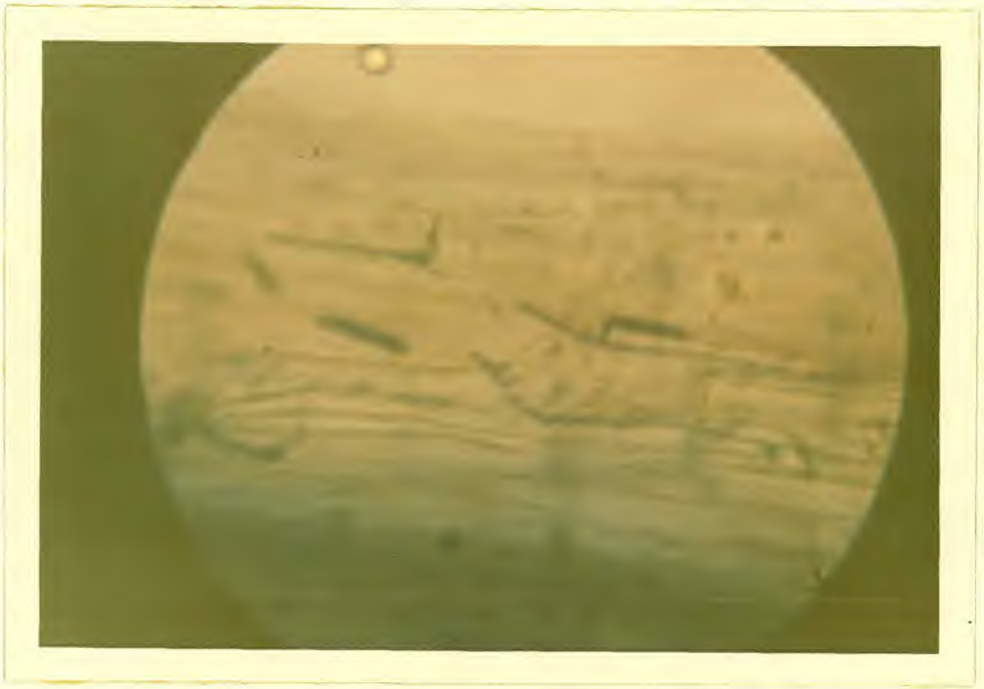
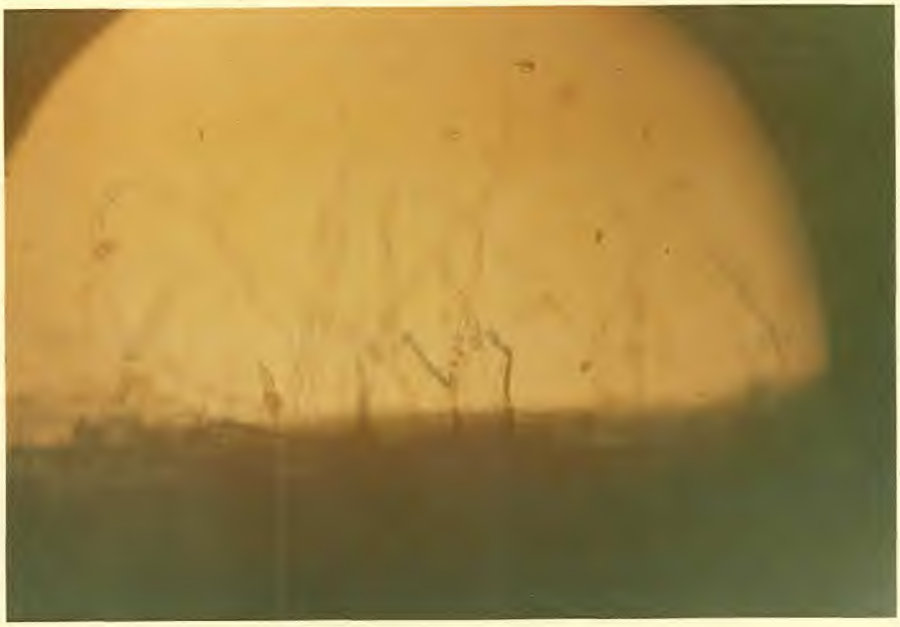


Plate 3: Surface view of part of a root segment showing the hyphae in the cortical cells.

Plate 4: Surface view of part of a root segment showing the formation of mycelial clamydospores in chains.

a.

X 100



b.

X 800

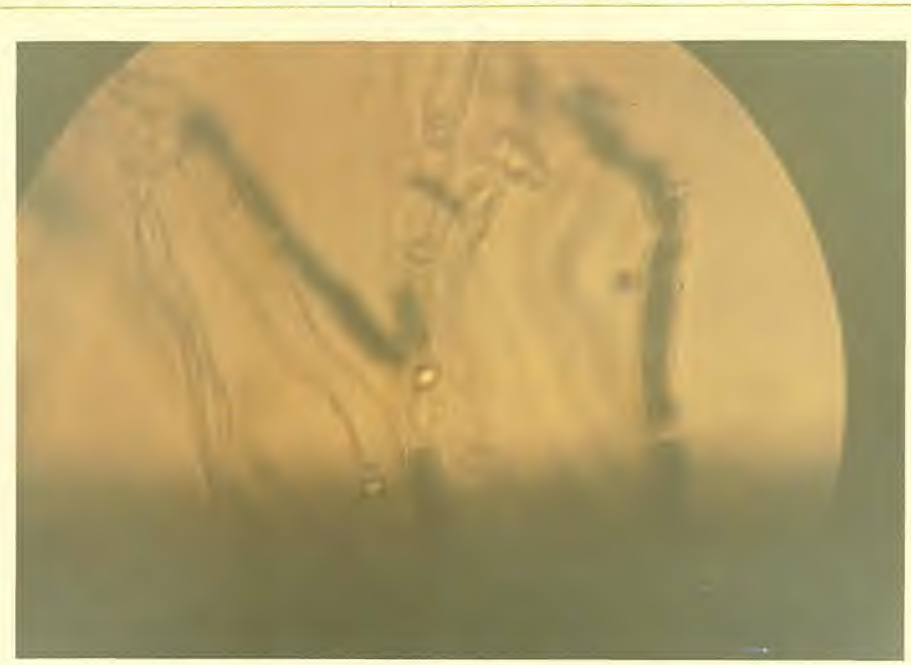


Plate 5: Surface view of part of a root segment showing the hyphae in the root hairs.

Table 3. Distribution of hyphae on seedlings grown in infested soil

(Treatment (b) - see text)

A. Roots

Max. distance from seed (cm.)	Hyphae visible on staining (+)	Position of hypae on root piece
0.3	+	Mass of hyphae on surface and inside cortical cells.
0.6	+	
0.9	+	
1.2	+	
1.5	+	
1.8	-	None
2.1	-	None
2.4	+	On the surface and inside cell.
2.7	-	None
3.0	-	None

B. Coleoptiles

Max. distance from seed (cm.)	Hyphae visible on staining (+)	Position of hyphae on coleoptile piece
0.3	+	Mass of hyphae collected around the pieces and some inside cells.
0.6	+	
0.9	+	
1.2	-	None
1.5	-	None

Table 4. Distribution of hyphae on seedling grown in infested soil

(Treatment (c) - see text)

A. Roots:

Max. distance from seed (cm.)	Growth of F. culmorum on plate (+)	Hyphae visible on staining (+)	Position of hyphae on root piece
0.3	+	+	Hyphae aggregated between and in the cortex cells.
0.6	+	+	
0.9	+	+	
1.2	-*	-	None
1.5	-	+	On the surface
1.8	+	+	" " "
2.1	+	+	" " "
2.4	+	+	" " "
2.7	-	-	None
3.0	-	+	On surface

*Bacteria.

B. Coleoptiles

Max. distance from seed (cm.)	Growth of F. culmorum on plate (+)	Hyphae visible on staining (+)	Position of hyphae on coleoptile piece
0.3	+	+	Mass of hyphae collected around the pieces and inside cells.
0.6	+	+	
0.9	+	+	
1.2	+	+	
1.5	+	+	

Experiment 5:

A comparison of the colonization of the root and coleoptile surfaces by F. culmorum from soil-borne and seed-borne inocula

Seedlings were raised in infested soil and from seed treated with a spore suspension of F. culmorum, as described in the previous sections (pp. 27 & 29). Roots and coleoptiles were examined 7, 9 and 11 days after sowing by washing and plating on Rose-Bengal-Streptomycin agar as described (p. 34). Only the first 3 cm. of root and the first 1.5 cm. of coleoptile (i.e. nearest the seed) were used in this instance. The full results are given in Appendix Table 11 a-b and summarized in Table 5. Colonization of root and coleoptile by F. culmorum was more rapid from infested soil than from infested seed, a result in accord with the previously found for disease effects (Exp. 3, p. 29).

Table 5. Colonization of seedlings grown in soil and from seed infested with F. culmorum

Day number	Percentage colonization by F. culmorum			
	Infested Soil		Infested Seed	
	Root	Coleoptile	Root	Coleoptile
7	58.75	47.5	3.75	7.5
9	85.0	62.5	16.25	15.0
11	82.5	65.0	23.75	22.5

Experiment 6:

Internal colonization of roots and coleoptiles by F. culmorum

A number of pots containing soil infested with F. culmorum were prepared and 15 wheat seeds planted in each. Ten days after sowing the seedlings were removed and washed in tap water. The first 2 cm. of root and coleoptile (i.e. nearest the seed) were taken and washed in 5 changes of distilled water and then prepared for sectioning as follows:-

(1) The segments were fixed in Formalin - Acetic - Alcohol (50% Ethyl alcohol 90 ml., Glacial acetic acid 5 ml., Formalin 5 ml.).

(2) They were then dehydrated by the method described by Johansen (1940).

(3) The first centimetre (nearest the seed) from both root and coleoptile were separately embedded in paraffin wax, M.pt. 60° to 63°C. (Johansen, 1940).

Sections were cut at 10 µ with a Cambridge Rocking microtome, mounted on slides with egg-albumin and then transferred in turn to xylol, absolute ethyl alcohol, a series of ethanol/water mixtures of increasing water content and finally water. The slides were then stained in cotton-blue/lactophenol for 10 minutes and examined under the microscope. Drawings were made with a camera lucida.

The extent and type of colonization by F. culmorum is illustrated in Figures 6, 7 A & B and Plates 6 a-c.

Fig. 6.

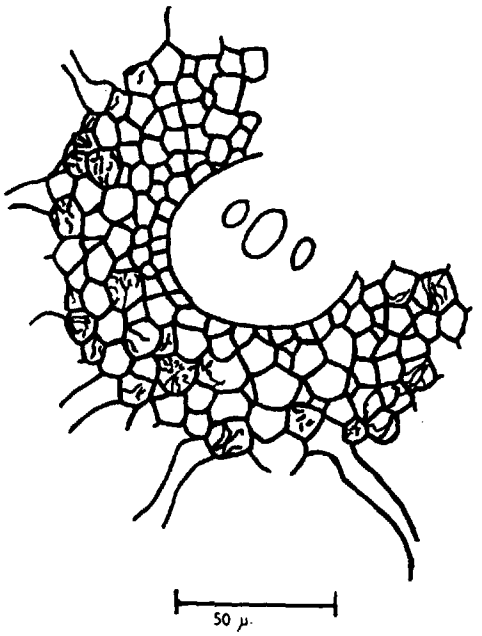


Figure 6: Transverse section of a root showing the presence of hyphae in the cortex.

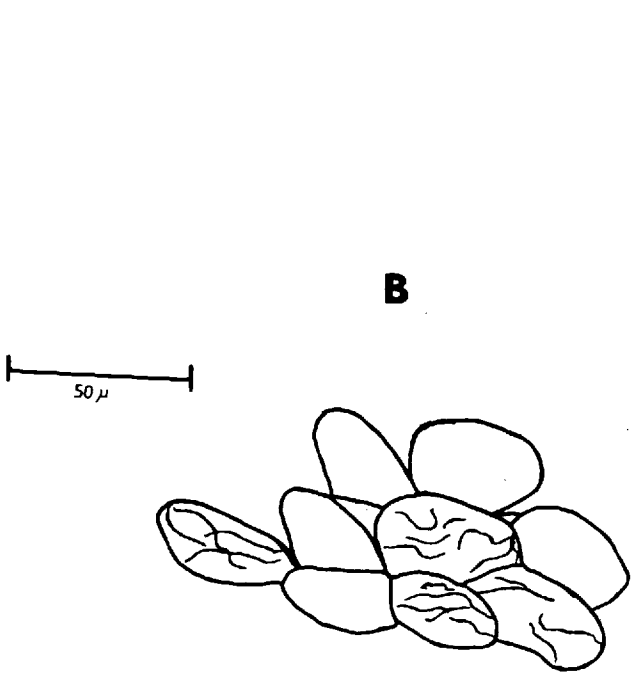
Figure 7:

A longitudinal section of coleoptile of wheat seedling showing:

A. Invasion of the coleoptile by by F. culmorum (basal part xxxxx)

B. Part of the invaded cortical cells.

Figure 7.



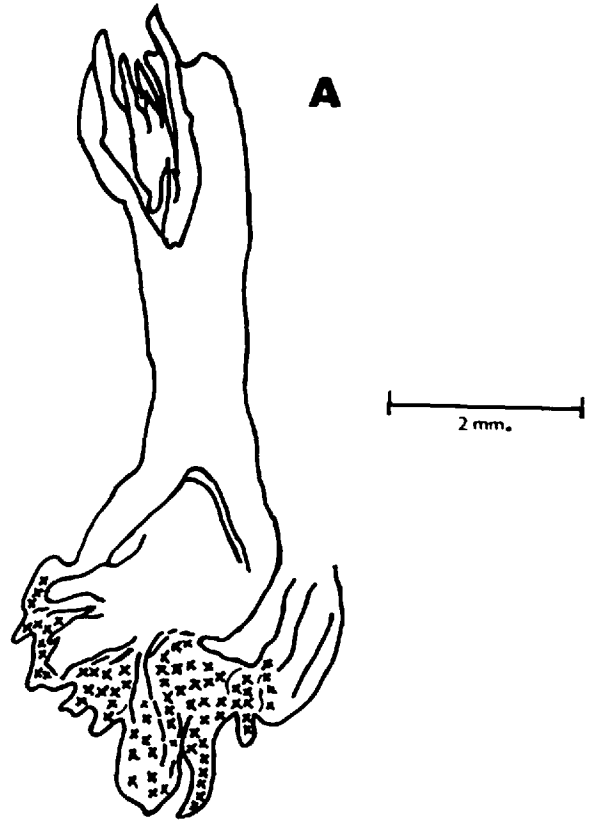


Plate 6 a-c.

A longitudinal section of base of the coleoptile.

a - Showing a mass of hyphae blocking the cortical cells. X800.

Plate 6: (Continued)

b - Showing the invasion is well established. X800

Plate 6: (Continued)

c - Showing the cells are disorganised and breaking down. A mass of hyphae collected between the broken down cells (⟶). X800

3. Factors influencing disease development:-

(a) Soil moisture and temperature

Experiment 7:

Effects of soil moisture

The water holding capacity (W H C) of air-dried Walled Garden soil was determined by the method of Coutts described by Piper (1950). Thirty 3½in. plastic pots sealed at the bottom to stop drainage were each filled with the same amount of the air-dried soil. Half of the pots were inoculated with F. culmorum by introducing a standard weight of an oat-meal/sand culture of known moisture content. Ten wheat seeds were then sown in each pot and water added so that for each set of pots (infested with F. culmorum and not infested) five were brought to 30%, five to 50% and five to 70% of the W H C judged by weight. The pots were randomized on the greenhouse bench and the initial moisture levels were maintained by reweighing the pots at 2-day intervals and adding enough water to bring them back to their original weight.

Pre-emergence damping-off was assessed 10 days after sowing and post-emergence damping-off 21 days after sowing, both from a direct count of seedling stand. Daily counts of seedlings emerged were made between days 1 & 10. On day 21 shoot height and root length was measured as described

on p. 22 . The full results are given in Appendix Tables 12 to 16, and summarized in Table 6 and Figures 8, 9, & 10 a-b.

Both pre-and post-emergence damping-off were most severe at the lowest soil moisture level (30% W H C) and least at the highest soil moisture level (70% W H C). Most seedlings were killed before emergence and this may result in part from the adverse effect of low soil moisture on the germination process (Figure 8). Those seedlings which did develop in soil of low moisture showed a corresponding reduction in root and shoot growth compared with those in soil of high moisture content (Plate 7).

Table 6. Effects of soil moisture on damping-off (F. culmorum)

% soil moisture	% pre-emergence damping-off		% post-emergence damping-off	
	Infested soil	Uninfested soil	Infested soil	Uninfested soil
30	62	4	21.1	0
50	42	2	13.8	2
70	14	4	4.8	0

FIGURE 8

EFFECT OF SOIL MOISTURE ON SEEDLING EMERGENCE IN INFESTED ⊙ & UNINFESTED △ SOIL.

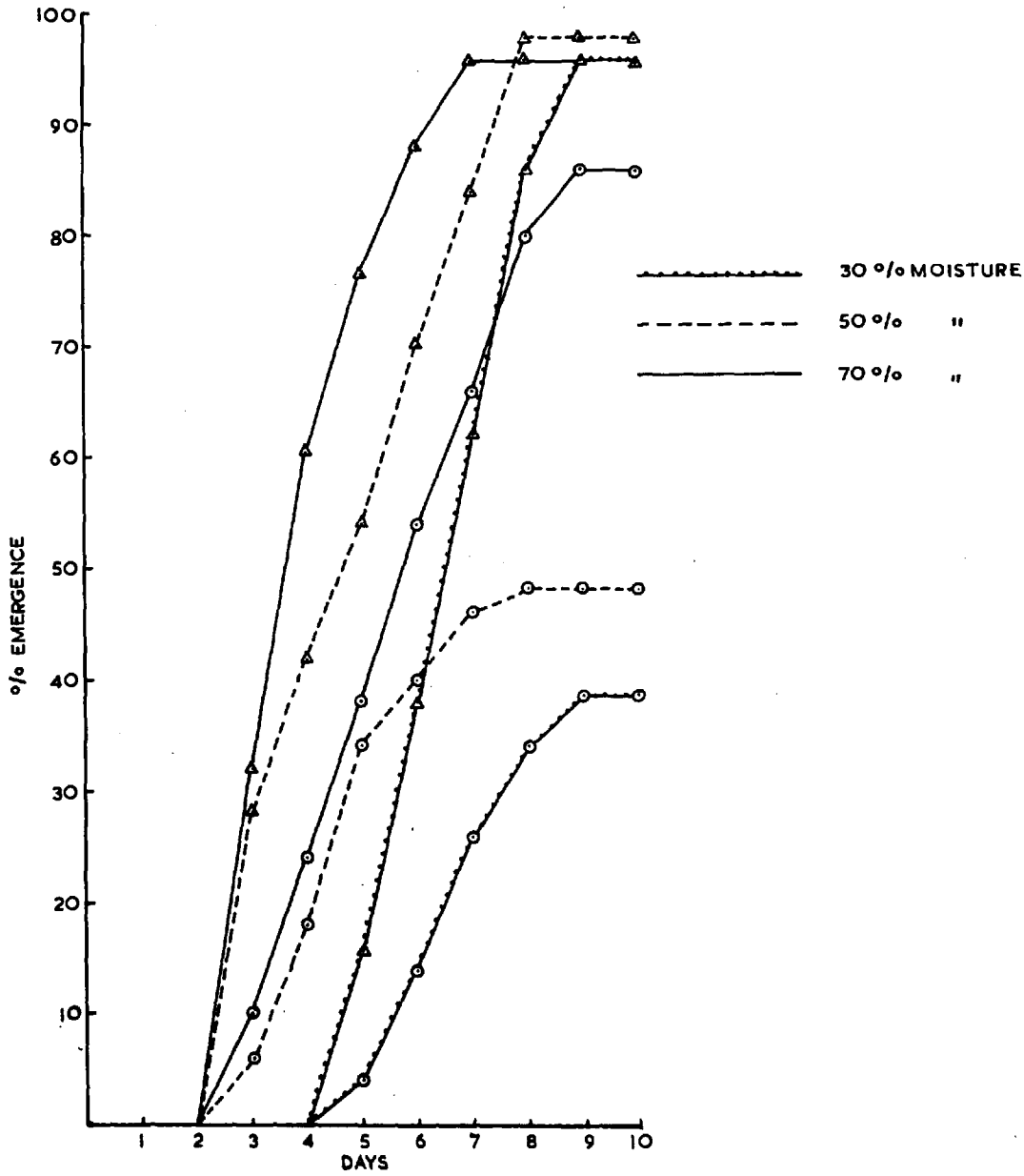


FIGURE 9.

EFFECTS OF SOIL MOISTURE ON FINAL STAND IN SOIL INFESTED ⊙ WITH F. CULMORUM & IN UNINFESTED △ SOIL.

VERTICAL LINES REPRESENT SIGNIFICANT DIFFERENCES FOR P=0·05 & 0·01

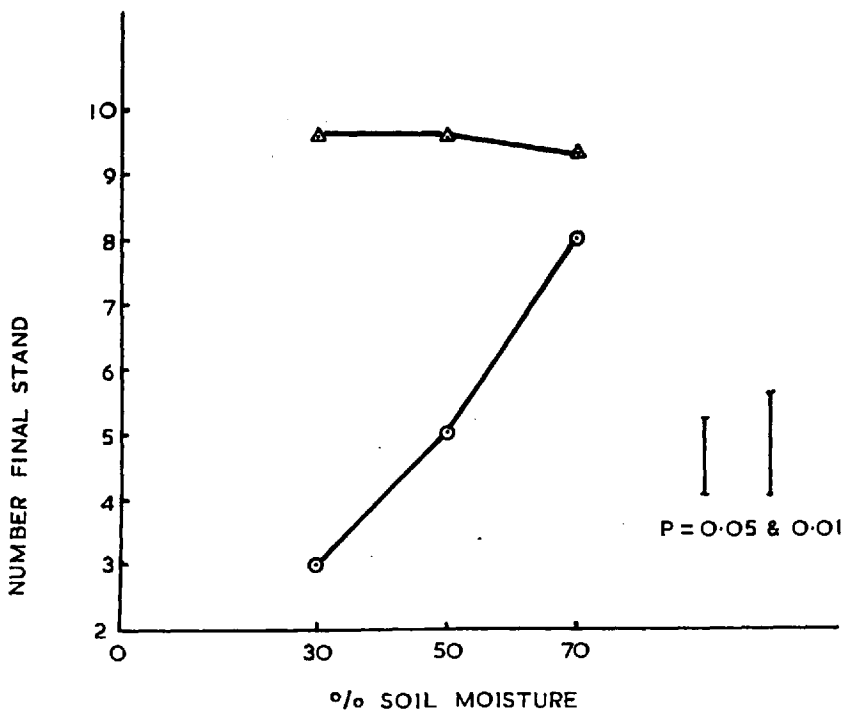
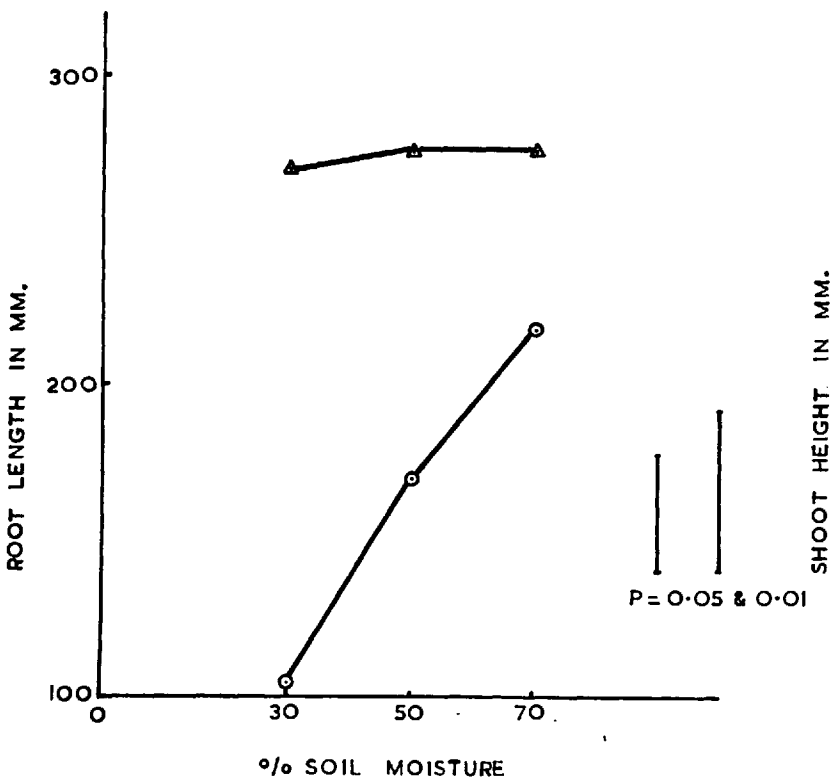


FIGURE 10 a & b

EFFECTS OF SOIL MOISTURE ON SEEDLING GROWTH IN SOIL INFESTED ⊙ WITH F. CULMORUM & UNINFESTED △ SOIL

VERTICAL LINES REPRESENT SIGNIFICANT DIFFERENCES P = 0·05 & 0·01

'A' ROOT GROWTH



'B' SHOOT GROWTH

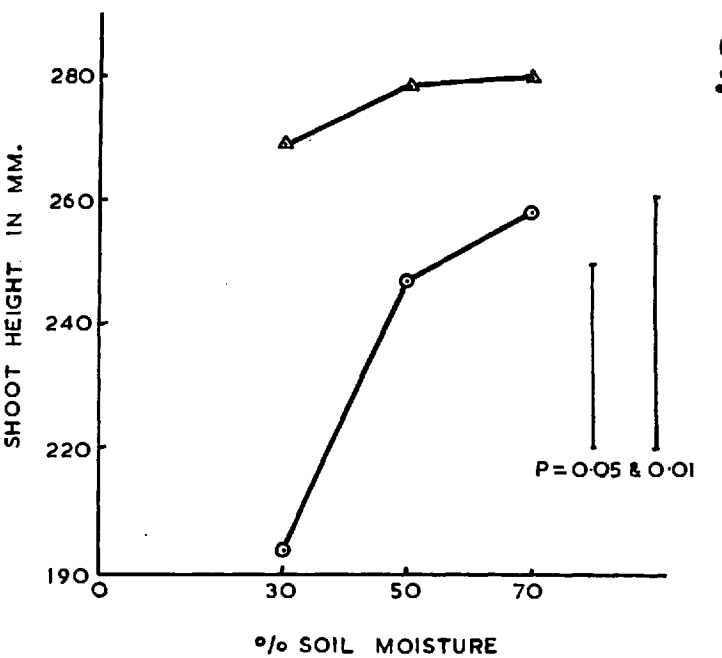


Plate 7: Effect of soil moisture on the growth of seedlings

A. 30% moisture.
B. 50% moisture.
C. 70% moisture.

Experiment 8:

Effects of different soil moisture/temperature regimes

Pots of infested soil were prepared as in Experiment 7 at three moisture levels, 30, 50 and 70% W H C. Five pots of each moisture level were then placed in each of the following temperatures conditions:

(i) low, 2-8°C. in an unheated greenhouse.

(ii) medium, 15-18°C. in a heated greenhouse.

(iii) high, 25°C. in an illuminated growth chamber.

The temperatures were checked by thermographs throughout the experiment. Estimates of disease effects were carried out as in Experiment 7, except daily seedling emergence for which no assessments were made. The results are given in Appendix Tables 17 to 20 and summarized in Tables 7 and 8. With one exception, these are quite clear-cut and straightforward. The effects of soil moisture were similar to those obtained in Experiment 7. At each soil moisture level an increase in temperature gave a corresponding increase in damping-off. Seedling death was greatest at low soil moisture/high temperature and least at high soil moisture/low temperature. Root growth of those seedlings which survived was correspondingly greatest at high soil moisture/low temperature and least at low soil moisture/high temperature. The figures for shoot height fit this pattern

only for the low and medium temperature regimes. At 25°C. shoot growth was fairly uniform at all moisture levels and was greater than that of plants maintained at lower temperatures. It appears that the illumination provided at 25°C. offset the temperature and moisture effects.

Table 7. Effects of various soil moisture/temperature regimes on damping-off by F. culmorum

% Soil moisture	% pre-emergence damping-off			% post-emergence damping-off		
	Temperature regimes			Temperature regimes		
	2-8°C.	15-18°C.	25°C.	2-8°C.	15-18°C.	25°C.
30	20	58	70	7.5	19.1	46.7
50	12	46	40	14.5	11.1	23.3
70	8	16	24	2.2	7.1	15.8

Table 8. Effects of soil moisture and temperature on seedling growth in soil infested with F. culmorum

Treatments		Mean	
% soil moisture	Ranges of temperature	Root length (mm.)	Shoot height (mm.)
30	2-8°C.	224	235
	15-18°C.	153	174
	25°C.	79	273
50	2-8°C.	242	262
	15-18°C.	187	222
	25°C.	157	266
70	2-8°C.	277	268
	15-18°C.	230	252
	25°C.	209	271
L.S.D: P. = 0.05		36.1	33.7
P. = 0.01		48.2	45.0
P. = 0.001		63.5	59.3

(b) Inoculum size and position:

Experiment 9:

Effects of varying the amount of inoculum in infested soil

Pots of soil were infested with oatmeal/sand cultures of F. culmorum to obtain five replicates of the following levels of inoculum: 5, 10, 20, 30 and 50% w/w. Fifteen seeds were planted in each pot and the pots then randomized on the greenhouse bench. Disease effects were assessed 21 days after sowing by counting the seedlings emerged and measuring shoot height and root length (see p. 22). The results are detailed in Appendix Tables 21 to 23, and summarized in Table 9. An increase in inoculum size resulted in a corresponding decrease in seedling stand and root growth, but shoot growth of those seedlings which survived appeared not to be affected.

Table 9. Effects of inoculum size on seedling stand and growth

Treatments % inoculum	Mean		
	Seedling Stand (no.)	Root length (mm.)	Shoot height (mm.)
5	6.4	193	230
10	6.0	201	216
20	3.6	175	190
30	2.8	151	181
50	1.8	101	205
L.S.D.			
P. = 0.05	2.2	50.1	45.7
P. = 0.01	2.9	69.5	62.9

Experiment 10:

Effects of placing inoculum in different position relative to the germinating seed

Five pots were set up for each of the treatments detailed in Figure 11, and fifteen seeds were planted per pot, and the pots randomized on the greenhouse bench.

The amount of inoculum (in each instance except treatment A & F) was calculated to give 5% w/w relevant to the soil infested. The pots were watered carefully to ensure least movement of inoculum from its initial position. Treatments A, B & F were watered from above, the remainder by placing the pots in saucers of water. After 21 days disease effects were assessed as in Experiment 9. The results are presented in Appendix Tables 24 to 26, and summarized in Table 10.

The effects on seedling stand are quite striking. Generally seedling stand was significantly reduced only where the inoculum of F. culmorum was initially in close contact with the seed. The effects on root growth were more variable: treatment means for D, E & F are not all significantly different from those of B & C, but there are enough differences between individual pairs to suggest that inoculum near the seed is more effective in reducing root growth. Shoot height was less affected by inoculum than root growth but here again there are similar indications (e.g.D, E cf.B & C).

FIGURE II : PLACING OF INOCULUM IN DIFFERENT POSITIONS RELATIVE TO THE GERMINATING SEED

INOCULUM

SEEDS

A UNTREATED SEED - NON-INFESTED SOIL

B INOCULUM MIXED WITH BOTTOM 2" OF SOIL ONLY

C INOCULUM MIXED WITH SOIL ABOVE THE SEEDS ONLY

D INOCULUM MIXED WITH SOIL AROUND THE SEEDS

E INOCULUM MIXED WITH ALL THE SOIL

F SEEDS INOCULATED WITH SPORE SUSPENSION- NON-INFESTED SOIL

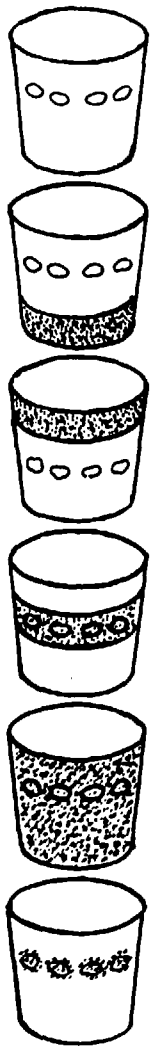


Table 10. Effects of inoculum position on seedling stand and growth

Treatments (inoculum position)	Mean		
	Seedling stand (no.)	Root length (mm.)	Shoot height (mm.)
A. Untreated seed/ non-infested soil	13.0	281	293
B. Inoculum mixed with bottom 2" of soil only	12.8	270	280
C. Inoculum mixedwith soil above seeds only	12.4	276	275
D. Inoculum mixed with soil around seeds	6.6	216	244
E. Inoculum mixed with all the soil	6.2	198	232
F. Seeds only treated with spore suspension	8.2	240	262
L.S.D.			
P. = 0.05	2.4	32.4	34.4
P. = 0.01	3.3	44.2	46.9
P. = 0.001	4.5	59.8	-

(c) Age of seedlings:

Two experiments were carried out:

Experiment 11:

Twenty-four 3½ in. plastic pots were filled with untreated soil and ten wheat seeds planted per pot. The pots were then randomized on the greenhouse bench and watered. Immediately after this, and then daily until the 7th day 3 pots were taken at random and inoculated with 10 ml. of a sporesuspension of F. culmorum prepared as described on p.27. Seedling stand was assessed 21 days afer sowing. Detailed results are presented in Appendix Table 27, and summarized in Table 11.

Only the inoculation on day 1, 2 & 3 significantly reduced seedling stand, and it thus appears that by the 4th day seedlings had already developed some resistance to attack. The lack of any effect of the inoculation after sowing is peculiar and in the light of the results of the next experiment appears anomalous.

Table 11. Effect of age of seedling at time of inoculation with F. culmorum on final stand

Age of seedling at inoculation	Final mean seedling stand
0	7.0
1	5.0**
2	4.3***
3	5.3**
4	7.3
5	8.0
6	8.7
7	8.3

L.S.D	P.0.05 =	2.1
	P.0.01 =	2.8
	P.0.001 =	3.8

Experiment 12:

Forty-eight 3½ in. plastic pots were filled with untreated soil and ten wheat seeds planted per pot as in Experiment 11. Immediately and then daily until the 7th day 6 pots were taken at random; 3 were inoculated with spore suspension of F. culmorum as described in Experiment 11, and the other 3 were treated with sterile water to serve as controls. Assessments of seedling stand and growth were carried out on both treatments 7 days after inoculation.

The detailed results are presented in Appendix Tables 28-30, and summarized in Figures 12 & 13 a-b. With the exception of day 0, the effects on seedling stand were similar to those of Experiment 11. The results indicate that after the 4th day the seedlings are no longer so severely attacked that they are killed. Similarly, root growth is markedly reduced only in those seedlings inoculated during the first 4 days after planting. The effects on shoot growth were less striking but basically similar.

FIGURE 12

EFFECTS OF AGE OF SEEDLING AT TIME OF INOCULATION WITH F.CULMORUM ON FINAL STAND.

INFESTED ⊙ & UNINFESTED X SOIL

VERTICAL LINES REPRESENT SIGNIFICANT DIFFERENCES FOR P = 0·05 & 0·01

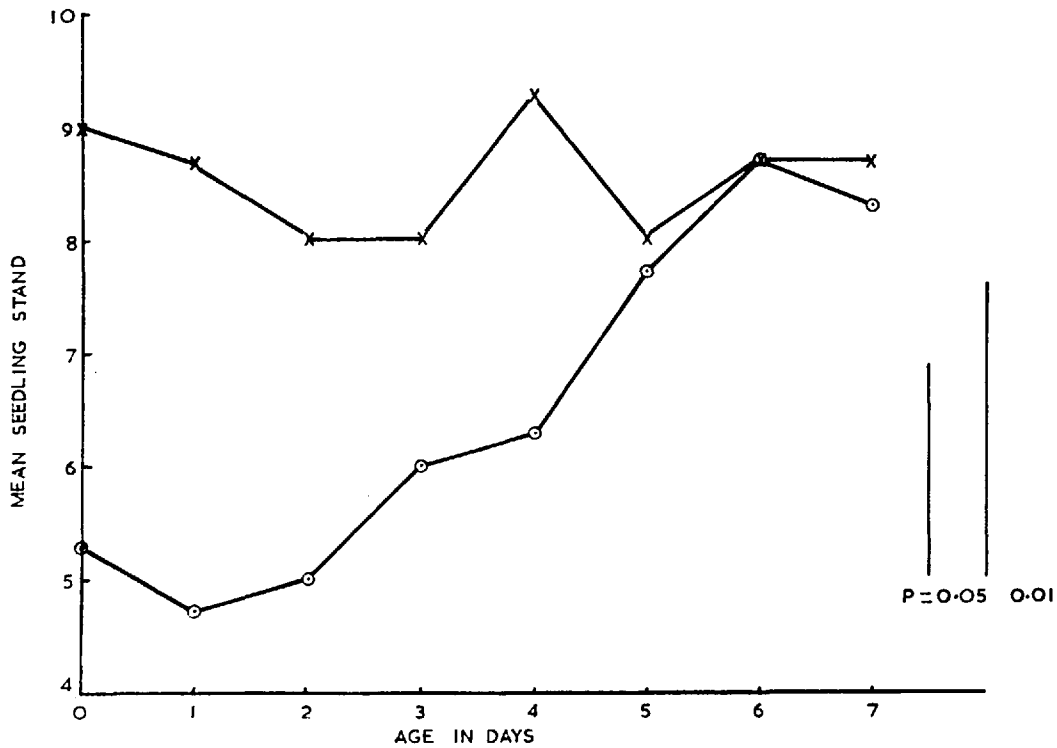
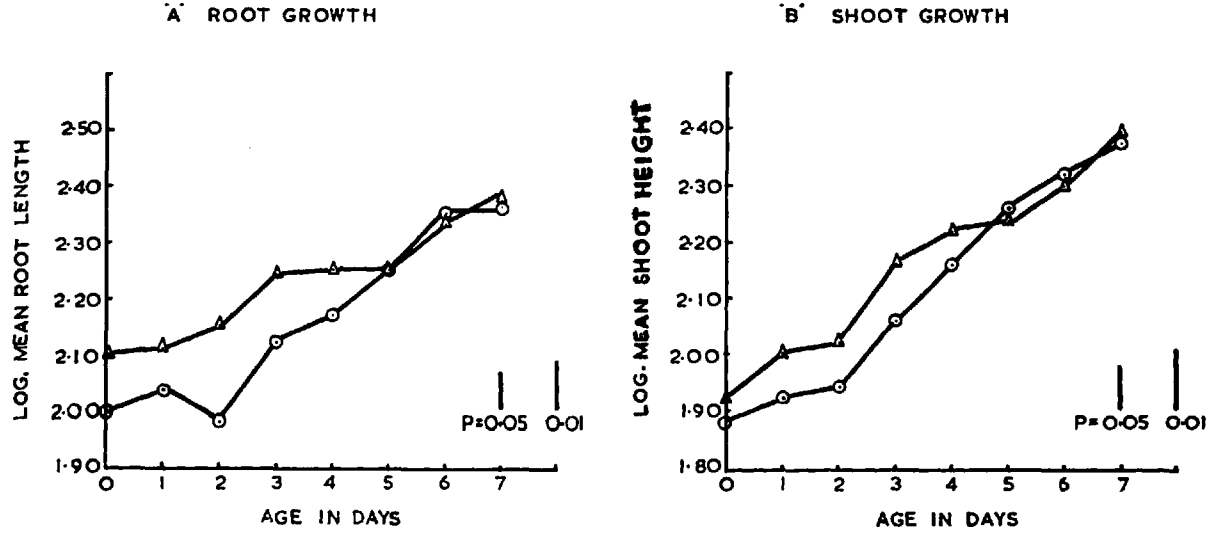


FIGURE 13

EFFECTS OF AGE OF SEEDLING AT TIME OF INOCULATION WITH F.CULMORUM, ON ROOT & SHOOT GROWTH. INFESTED ⊙ & UNINFESTED △ SOIL.

VERTICAL LINES REPRESENT SIGNIFICANT DIFFERENCES FOR P= 0·05 & 0·01



(d) Wheat variety:

Experiment 13:

The effects of F. culmorum on germination and growth were examined for the following five varieties (see also Appendix p.193): 'Svenno', 'Prestige', 'Lineg', 'Capelle Desprez', and 'Atson'. Fifteen seeds of each were sown in 5 in. pots containing soil infested with F. culmorum (p. 20). There were 5 replicates for each variety, and the pots were randomized on the greenhouse bench.

An assessment of pre-emergence damping-off was made 10 days after sowing by counting the seedlings emerged. On day 21, a second count was made and an estimate of post-emergence damping-off thus obtained. On the same day, plants were removed, shoot height and root length measured and the fresh weight of the roots and shoots determined. The full results are given in Appendix Tables 31 to 38, and summarized in Tables 12 & 13.

By and large the varieties Svenno, Lineg, and Capelle Desprez as a group were more affected by F. culmorum than Prestige and Atson. While differences between individual varieties as regards seedling stand, root growth and fresh weight for both root and shoot per pot, are not all significant, the general order of susceptibility is:

Svenno → Lineg → Capelle Desprez → Prestige → Atson

Most susceptible — Least susceptible

Although the figures for shoot height and both root & shoot fresh weight per plant appear to fit this general pattern, in fact there are significant differences only between Svenno on the one hand and Prestige & Atson on the other for shoot height. The results of other experiments in this section also indicate that shoot height is the one aspect of growth that is least affected by F. culmorum.

Table 12. Effects of F. culmorum on the germination and seedling stand of five varieties of wheat

Wheat varieties	% pre-emergence damping-off	%post-emergence damping-off
A. Svenno	48.0	15.3
B. Prestige	26.6	5.5
C. Lineg	42.6	9.3
D. Capelle Desprez	40.0	8.8
E. Atson	20.0	6.6

Table 13. Effects of F. culmorum on the seedling growth of five wheat varieties

Treatments wheat varieties	Mean					
	Root Growth			Shoot Growth		
	Fresh weight (g)		Length (mm.)	Fresh weight (g)		Height (mm.)
	per pot	per plant		per pot	per plant	
A. Svenno	2.4	0.35	177	2.5	0.39	241
B. Prestige	4.8	0.44	243	6.0	0.57	272
C. Lineg	2.9	0.36	218	3.2	0.41	258
D. Capelle Desprez	3.1	0.37	229	4.4	0.54	254
E. Atson	6.2	0.54	255	6.4	0.59	277
L.S.D. P. = 0.05	2.3	0.16	46.5	2.4	0.25	28.2

Part II CONTROL

1. Seed dressings and their mode of action

Seed-borne pathogens are often controlled by treating the seed with a chemical. This method is generally less effective for soil-borne pathogens but may be of value in improving seedling emergence where the pathogens concerned attack the host chiefly at the seedling stage as in the damping-off diseases. The investigations here deal with the relative effeciency and modes of action of two seed dressings in preventing pre - and post-emergence damping-off of wheat by F. culmorum. The two seed dressings are:

'Ceresan' containing 1.5% mercury w/w as:
Methoxyethyl mercuric chloride.

'PP781' 5%, [4 (2-chlorophenylhydrazono) -3-methyl-5- isoxazolone]

A - 'Ceresan'

Experiment 14:

Effect of 'Ceresan' seed dressings on seedling growth in soil infested with F. culmorum

Ceresan was applied at the rate of 0.1g./100g. seed by thoroughly shaking seeds and chemical for 10 minutes in a flask. This rate of application corresponds approximately to the 2 oz./bushel recommended by the manufacturers.

Soil was infested with F. culmorum as described previously (p. 20). Five replicates were set up of each of the following treatments:-

A. Untreated seed/infested soil.

B. Treated seed/infested soil.

C. Treated seed/non-infested soil.

D. Untreated seed/non-infested soil.

Fifteen seeds were planted per pot and the pots randomized on the greenhouse bench.

Assessments of disease effects were carried out 21 days after sowing by counting the seedlings emerged and by measuring shoot height and root length (see p. 22). The results are given in full in Appendix Tables 39 to 41 and summarized in Table 14.

Treating the seed with ceresan significantly improved seedling stand and growth in soil infested with F. culmorum to a level comparable with that of untreated seeds grown in non-infested soil (Plate 8).

Table 14. Effect of ceresan seed dressings on seedling stand and growth

Treatments	Mean		
	Seedling stand (no.)	Root length (mm.)	Shoot height (mm.)
A. Untreated seed/ infested soil	7.0	198	226
B. Treated seed/ infested soil	12.6	283	280
C. Treated seed/ non-infested soil	12.0	292	283
D. Untreated seed/ non-infested soil	11.6	276	275
L.S.D: P. = 0.05	2.3	23.4	27.3
P. = 0.01	3.3	32.8	38.3
P. = 0.001	-	46.4	-

Plate 8: Effect of ceresan on stand and growth in infested soil

A. Untreated seed/infested soil.
B. Treated seed/infested soil.
C. Treated seed/non-infested soil.
D. Untreated seed/non-infested soil.

Experiment 15:

Effect of ceresan on the growth of F. culmorum in vitro

It is reasonable to suppose that the effects of ceresan demonstrated in Exp. 14 stem from the direct action of the chemical on the growth of the fungus. The efficiency of ceresan in limiting growth of F. culmorum in culture was examined as follows:-

A small quantity of ceresan (0.4 g.) was dissolved in 50 ml. acetone and from this a dilution series prepared with acetone so that in each 5 ml. aliquot there was respectively 40, 20, 10, 5 and 2.5 mg. ceresan. Five ml. of each ceresan dilution were then mixed with 95 ml. sterile V8 agar in a 250 ml. Erlenmyer flask, and this was distributed amongst 5 Petri dishes to give final concentrations of 400, 200, 100, 50 & 25 p.p.m. ceresan. Plates were also poured with V8 agar alone and V8 agar plus acetone to serve as controls. After solidifying plates were incubated at 35°C. for 24 hours to allow the acetone to evaporate. Following that the Petri dish lids were replaced with fresh ones. Two diameters were marked on the back of each Petri dish for centering the inoculum. This was a 3 mm. disk cut from the edge of a 3-day old culture of F. culmorum and placed with mycelium in contact with the agar in the Petri dish. The plates were incubated at 25°C. and growth estimated daily by measuring

colony size along the two diameters drawn previously. The results are summarized in Table 15 and given in full in Appendix Table 42.

Table 15. Effect of ceresan on the linear growth of F. culmorum

Time after inoculation (days)	Mean colony diameter (cm.)			% inhibition of growth	
	Level of ceresan p.p.m.			Low and high level	
	Nil	25	400	25	400
1	1.6	0.9	0.3	43.8	81.3
2	3.5	2.1	0.7	36.8	78.9
3	6.3	3.9	1.4	35.7	75.0
4	7.7	4.9	2.2	28.6	42.9
5	8.4	5.7	2.8	-	14.3

Percentage inhibition of growth was calculated as:

$$\frac{(C-T)\ 100}{C}$$ (Priest, 1960)

Where T = daily increase in diameter of treated inoculum.

C = daily increase in diameter of untreated inoculum (control).

Clearly ceresan markedly inhibits the growth of F. culmorum in culture and similar effect could be expected during germination in the vicinity of a seed treated with this chemical. This was demonstrated as follows:-

Experiment 16:

Two flasks each containing 100 ml. sterile, Potato-dextrose agar were cooled to 40°C. and both seeded with 5 ml. of a spore suspension of F. culmorum (10^3 spore/ml.) prepared from a 6 day-old culture. After mixing each flask of medium was distributed amongst 5 sterile Petri dishes. Four wheat seeds were placed on the agar surface of each plate, two of the seeds had been treated with ceresan, the other two were untreated. The plates were then incubated at 25°C. Two days after inoculation fungal growth was clearly visible over the untreated seeds but the treated seeds were surrounded by clear zones in which no mycelium can be found. The diameters of these zones were measured on days 2 & 10 after inoculation (Appendix Table 43); there was no change in their size between these two dates; suggesting that treatment with ceresan protects the seed from attack for at least 10 days (Plate 9).

A treated seed in soil, however, is under very different conditions from the seeds in the above experiment. Part of the chemical deposit on the seed may be washed off by percolating rain water and the seed be protected from fungal attack for a much shorter period. Some effects of washing treated seed were examined in the following experiment.

Plate 9: Zone of inhibition caused by ceresan-treated seeds in a seeded plate with F. culmorum. (10 days after inoculation).

Experiment 17:

Twenty treated seeds and twenty untreated seeds were selected at random and each separately transferred to a labelled McCartney bottle containing 10 ml. sterile distilled water. The seeds were then washed by placing the bottles on a flask shaker for 5 mins. The washing was repeated 5 times with changes of sterile water and after each washing the water was collected and kept for further study. The untreated seeds were then discarded, but the treated ones were placed on sterile filter paper (in sterile Petri dishes) to remove excess moisture.

Two of these treated seeds were finally transferred to each of 10 plates seeded with F. culmorum together with 2 treated but unwashed seeds for comparison. The plates were incubated at 25°C. for 2 days when the zones of inhibition around the seeds were measured and compared (Appendix Table 44). The mean diameter of this zone for washed seeds 8 mm.; that for unwashed 15.8 mm., indicating that, although a considerable amount of ceresan was removed by the washing, there was treatment enough remained to limit fungal growth.

A check on the water collected from seed washing was also carried out. Twenty ml. from both treated and untreated seeds were separately added to 2 flasks containing 180 ml. molten V8 agar at 45°C. After shaking the contents of each

flask were distributed amongst 10 Petri dishes. The poured plates were inoculated with F. culmorum as described in Exp. 15, incubated at 25°C. and the colony diameters then measured daily.

Incorporating the water used to wash treated seeds in the agar checked the growth of the fungus initially but thereafter, had little effect. It would appear that, although a certain amount of ceresan was removed by washing, this was diluted in the bulked sample of washing water to a level where it had little fungitoxicity (Table 16).

Table 16. Effect on F. culmorum of incorporating the washings from ceresan - treated seed in an agar medium

Time after inoculation (days)	Mean colony diameter (cm.)		% inhibition of growth
	Treated	Untreated	
1	0.6	1.7	64.7
2	1.8	3.9	45.5
3	3.8	5.9	5.0
4	5.9	7.6	-
5	7.2	8.4	-

Percentage inhibition of growth was culculated as described in Exp. 15 (p.80)

Experiment 18:

Effect of seed dressings with ceresan on the colonization of wheat root and coleoptile surfaces by F. culmorum

Experiments 15-17 indicate that the growth of F. culmorum is inhibited near germinating seeds treated with ceresan. The extent to which colonization of the root and coleoptile is affected when treated seeds are planted in soil infested with F. culmorum was next examined.

Untreated seed and seed treated with ceresan were sown separately in pots of soil infested with F. culmorum as

described on p. 20. Roots and coleoptiles were examined 5, 7, 9, & 11 days after sowing, as follows.

Twenty seedlings grown from both treated and untreated seeds were carefully removed from the pots and washed in tap water. The root systems and coleoptiles were then cut from the plants at their points of attachment to the seeds. Each root system was cut into 3 cm. length and for the coleoptile into 1.5 cm. length. From the material available 8 segments from each (root and coleoptile) were selected at random. These were washed and plated on Rose-Bengal-Streptomycin agar as described on p. 34.

The results (Table 17 and Appendix Table 45), show that colonization of the root surfaces of 5 - and 7-day old seedlings grown from treated seed was significantly less than on comparable seedlings grown from untreated seed. On 9 - and 11-day old seedlings, however, the differences were no longer significant and no significant difference in the colonization of coleoptiles could be found with any age of seedling (Appendix Table 46).

A picture of the distribution of F. culmorum on the roots & coleoptile of both sets of seedlings was also obtained from data treated in similar manner to that described in Exp. 4 (p. 34). This is illustrated in Figure 14 a-d. Colonization of roots was less advanced on

seedlings grown from treated seed than on seedlings of comparable age grown from untreated seed. This was particularly so for the root tissue adjacent to the seed. The same was true of coleoptiles.

Table 17. Effect of ceresan on colonization of root surfaces by F. culmorum

Days	Mean no. segment with F. culmorum		Difference	L.S.D.
	Treated	Untreated		P. = 0.05
5	5.88	8.13	2.25*	1.79
7	6.75	8.75	2.00*	1.46
9	6.63	8.25	1.62 n.s.	1.98
11	7.13	8.50	1.37 n.s.	2.07

Figure 14 a - d:

Distribution of F. culmorum in seedling roots drived from treated (ceresan) and untreated seeds.

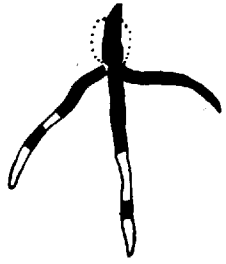
(seedlings drawn to natural size)

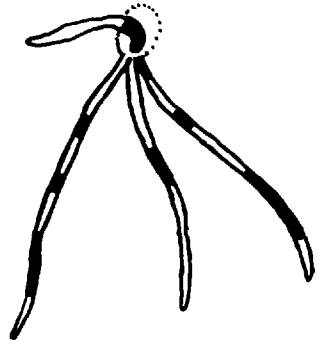
(a) Day 3
(b) Day 5
(c) Day 7
(d) Day 9

Figure 14 a-d

a.

■ = F. culmorum
□ = free from F.c.

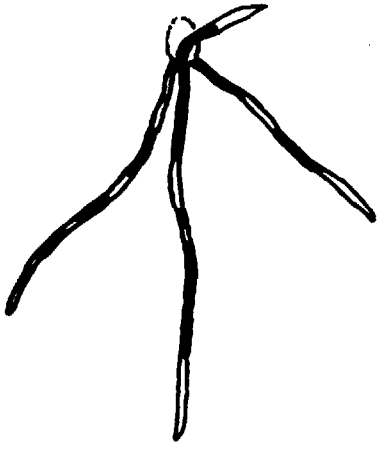




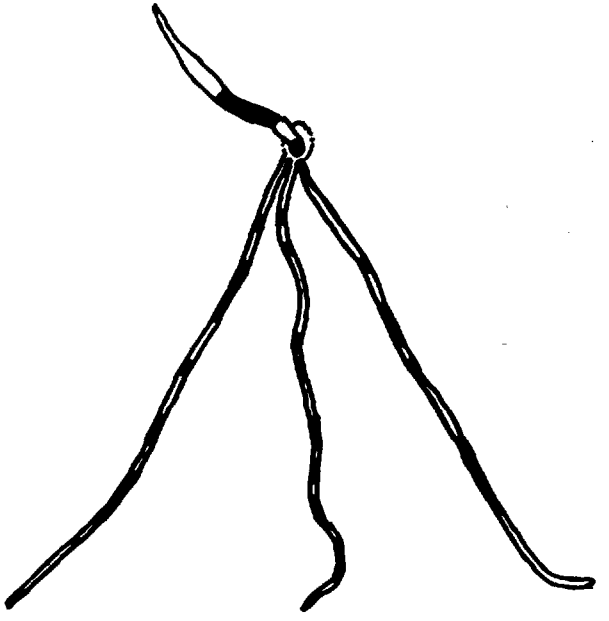
UNTREATED SEED/INFESTED SOIL

TREATED SEED/INFESTED SOIL

b.

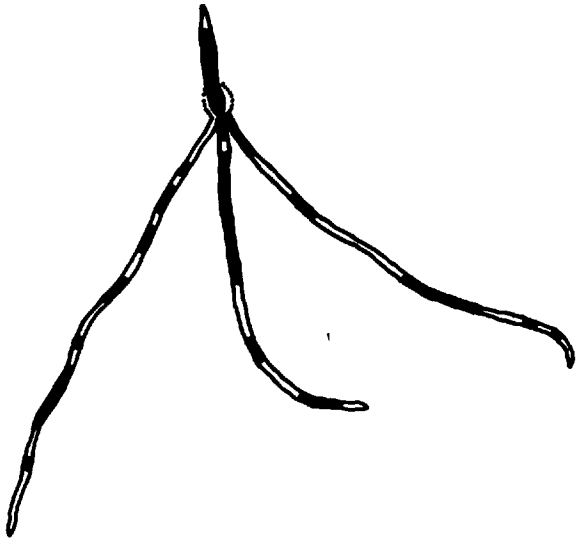


UNTREATED SEED / INFESTED SOIL



TREATED SEED / INFESTED SOIL

C.



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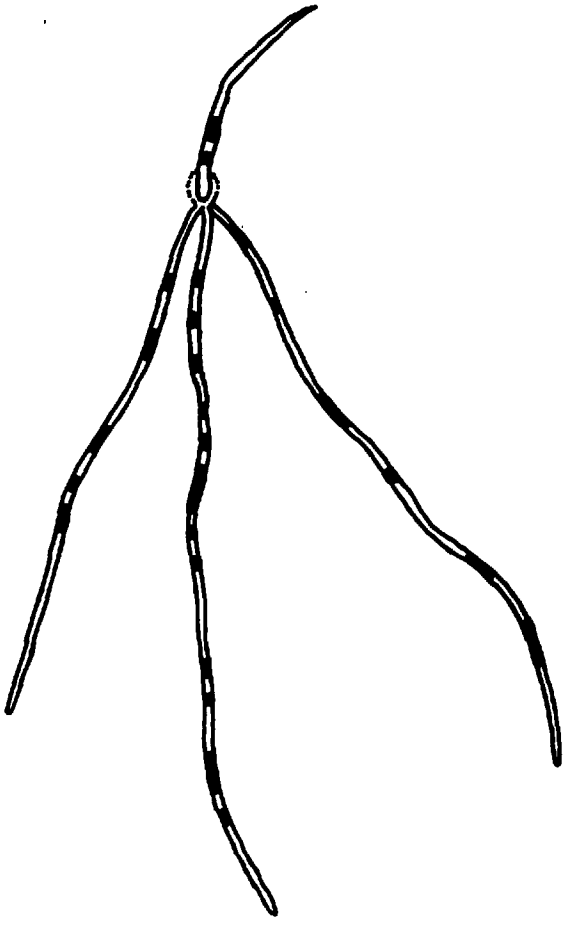
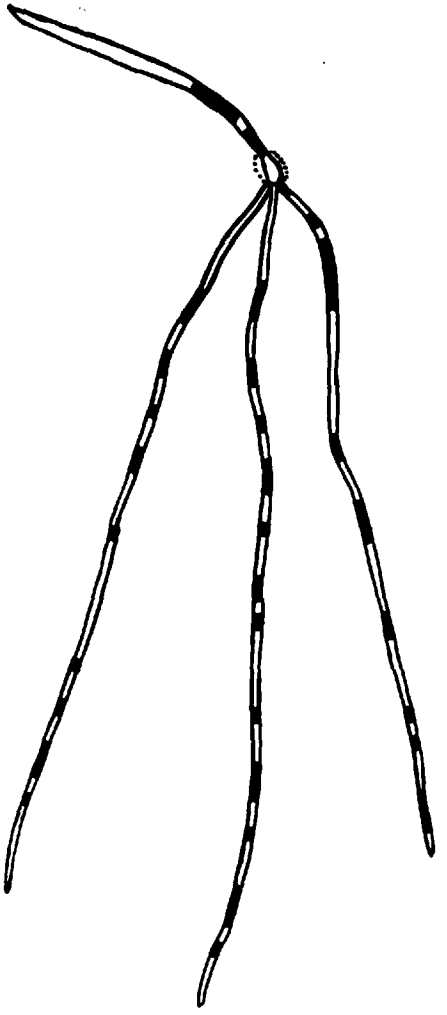


TREATED SEED / INFESTED SOIL

d.



UNTREATED SEED / INFESTED SOIL

TREATED SEED / INFESTED SOIL

Experiment 19:

Effect of seed dressing with ceresan on the internal colonization of seedling roots

In Exp. 18, growth of F. culmorum from a washed root segment could have been derived from a propagule on the root surface or from internal mycelium. The purpose of the present experiment was to determine the extent to which internal colonization was inhibited in roots of seedlings grown from treated seed.

Ten pots (5 in.) of soil were infested with F. culmorum, five planted with seed treated with ceresan and five with untreated seed at the rate of 15 seeds per pot. After 10 days, the seedlings were removed and washed in tap water. Twenty root pieces, each the 3 cm. nearest the seed, were then selected at random, 10 from seedlings derived from treated seed and 10 seedlings from untreated seed. Each root piece was washed in 20 changes of sterile distilled water (p. 34), and then transferred to a suspension of calcium hypochlorite, prepared as described by Mead (1933), for 20 mins. to kill any fungus on the root surface. The root pieces were then washed in six further changes of sterile distilled water and dried on sterile filter paper. After drying, each piece was plated on Rose-Bengal-Streptomycin agar, incubated for 5 days at 25°C. and then

examined for growth of F. culmorum.

Six of the ten root segments from the treated seeds were completely free from F. culmorum, three showed growth of F. culmorum at some points along the segment and one only was completely covered with fungal growth. In contrast, seven of the ten segments from untreated seeds were covered with F. culmorum, two showed growth of F. culmorum at points along the segments and only one was free from fungus (Table 18).

Since all the root segments were surface sterilized, it is reasonable to suppose that the differences in growth of F. culmorum refect differences in the internal colonization of the roots, (Plate10).

Table 18. Presence of F. culmorum in root tissues from treated and untreated seed after surface sterilization

No. segment	Treated	Untreated
1	+ -	+
2	-	+
3	-	+ -
4	+ -	+
5	+	+
6	-	+
7	-	-
8	-	+
9	+ -	+
10	-	+ -
Total +	4	9
Total -	6	1

\+ = Covered with fungal growth (F. culmorum)
\+ - = Fungal growth at point along the segment.
\- = Free from fungal growth.

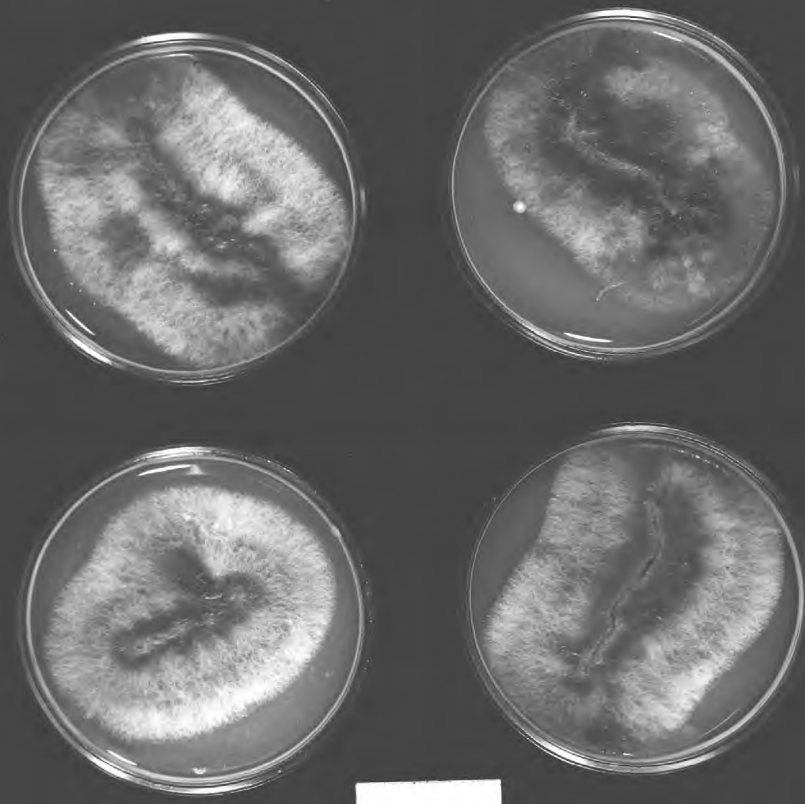
Plate 10:

Effect of seed dressing on the internal colonization of roots

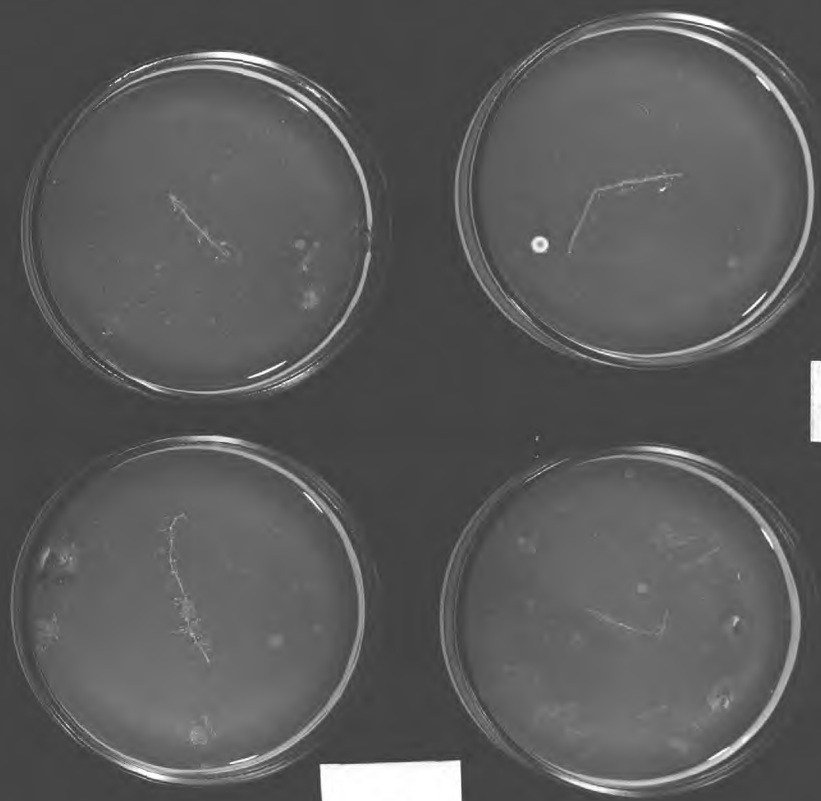
A. Root segments derived from untreated seed/infested soil - surface sterilized.

B. Root segments derived from ceresan - treated/infested soil - surface sterilized.

PLATE 10:



A



B

Experiment 20:

Effects on F. culmorum of homogenates derived from roots of seedlings grown from ceresan - treated seed

The result of Exp. 19 can be partially explained in terms of a reduced inoculum at the roots of seedlings grown from treated seed. It is also possible, however, that on germination some mercury is absorbed and translocated to the root and this limits the growth of F. culmorum within the root tissues. This was therefore investigated.

Untreated wheat seeds were thoroughly washed in 10 changes of sterile distilled water on a flask shaker. The seeds were then dried on sterile filter papers in sterile Petri dishes. Half were treated with ceresan, the remainder left untreated. Samples of both were then transferred under sterile conditions to Petri dishes containing damp filter papers. There were approximately 15-20 seeds per plate; and the plates were incubated at 25°C. for 48 hours.

At the end of this period germinated seeds (treated & untreated) were transferred to sterile growth chambers (Figure 16). These were prepared as follows: a piece of gauze was stretched over one end of a glass cylinder, diameter 6.5 cm., height 6.8 cm., and this was placed in an 800 ml. 'Tall form' Pyrex beaker. Distilled water was poured into the beaker to a level just below the gauze, the

beaker was covered with a Petri dish lid and sterilized by autoclaving at 120°C. for one hour.

Fifteen germinated seeds were placed on the gauze of each container; 20 containers were set up with treated seed and 20 with untreated seed. The lid of each container was firmly sealed with 'Sellotape' and the lower half of the beaker covered with black paper. The containers were then randomized in an illuminated growth chamber at 25°C.

After 10 days, 100 seedlings were selected from each treatment and the roots cut off at their point of attachment to the seed. The excised roots were washed (each treatment separately), in ten changes of sterile distilled water and then homogenized in 15 ml. sterile distilled water in a sterile blender (Kenwood 'Kenmix') run at full speed for 30 mins. Standard amounts of the homogenates, of roots from untreated seeds and of roots of treated seeds, were then pipetted under sterile conditions into cavities cut (with a 6 mm. sterile cork borer) in PDA plates seeded with F. culmorum (p.81) viz:

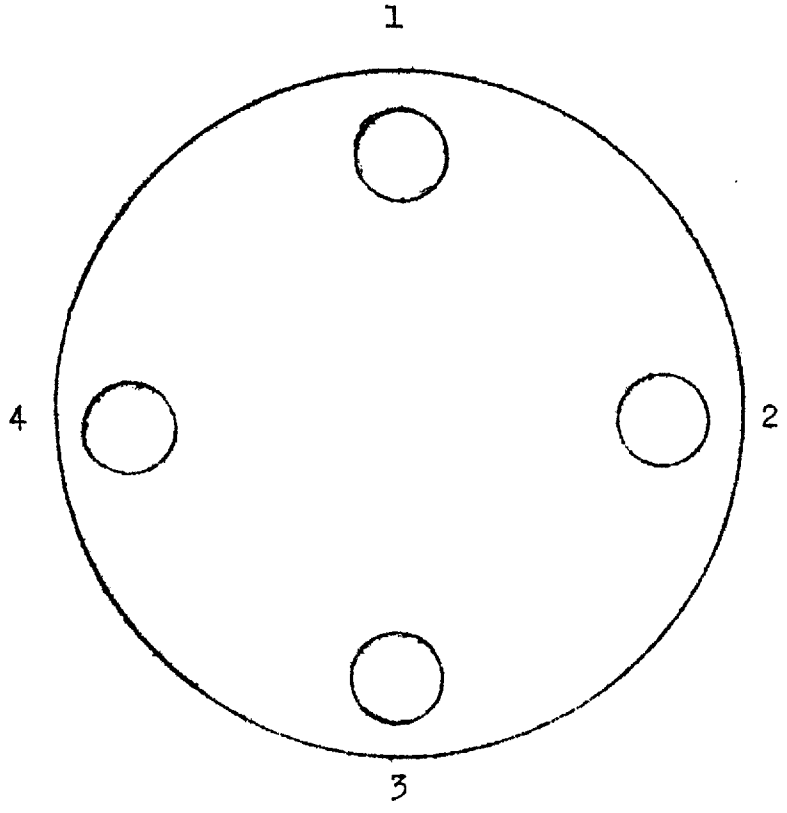


Figure 15: Diagram showing the arrangement of the cavities filled with root homogenates. 1 & 3 from treated seeds and 2 & 4 from untreated seeds, (in a plate seeded with F. culmorum).

The plates were incubated at 25°C. and were examined after 5 days. The result is given in Table 19 and illustrated in Plate 11. Clearly, homogenates of roots from treated seed are markedly inhibitory to F. culmorum, which strongly suggests that mercury is absorbed from seed coat during germination and translocated to the young root.

Table 19. Effects on F. culmorum on homogenates derived from roots of seedlings grown from ceresan-treated seed

No. plate	Homogenates-treated seed		Homogenates-untreated seed	
	Replicates		Replicates	
	1	3	2	4
1	+	- +	-	-
2	+	+	-	-
3	- +	+	-	-
4	+	- +	-	-
5	+	- +	-	-
6	+	- +	-	-
7	- +	+	-	-
8	+	+	-	-
9	+	+	-	-
10	+	+	-	-

\+ inhibition
\- + " (not clear)
\- Non-inhibition

Figure 16:

The growth chamber used for growing wheat seedlings under sterile conditions.

A = 800 ml. 'Tall form' Pyrex beaker.
B = A piece of gauze stretched over one end of glass cylinder.
C = Sterile distilled water.
D = Petri dish lid.
E = Shoot.
F = Wheat seed.
G = Root.
H = Black paper.

Figure 16 - The Growth Chamber

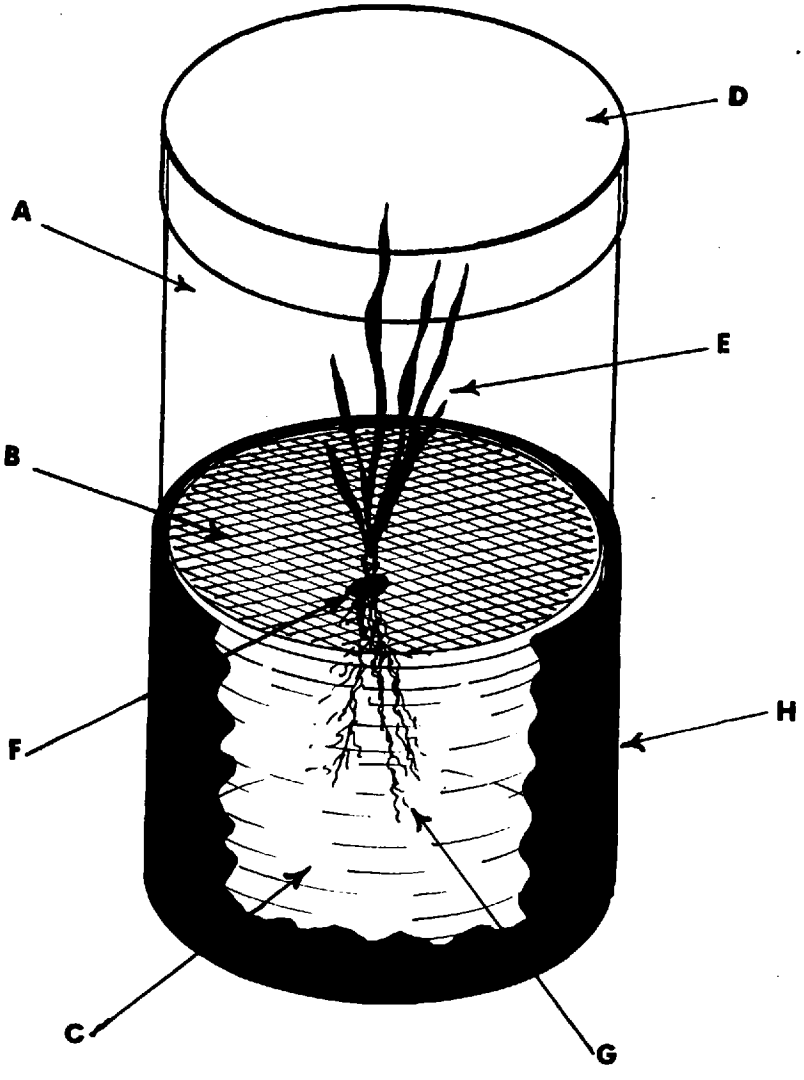


Plate 11:

Effect of root homogenates derived from seedlings grown from ceresan-treated seed on F. culmorum

A. Root homogenates/treated seed. (showing zone of inhibition).

B. Root homogenates/untreated seed. (showing non-inhibition).

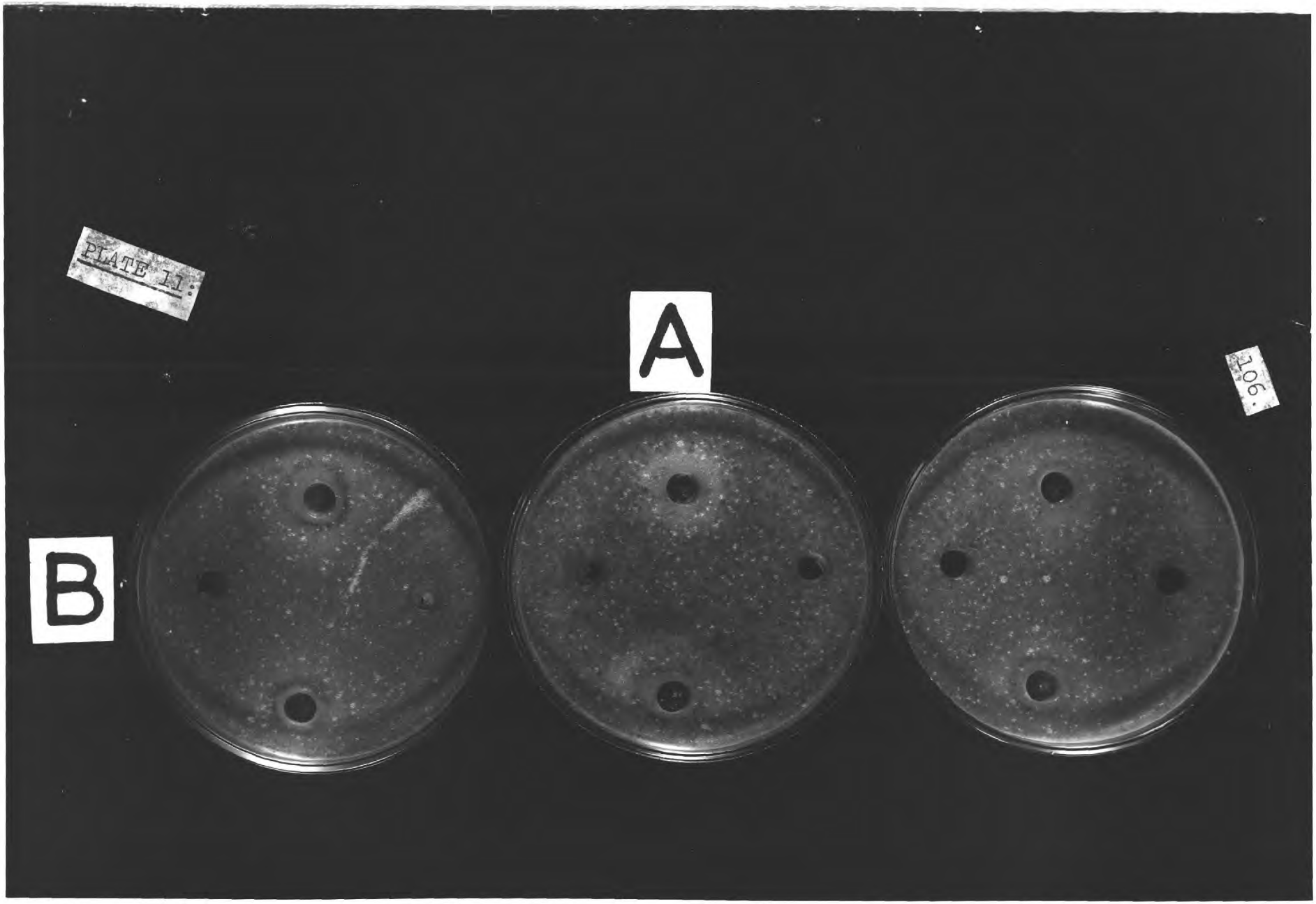


PLATE 11:
A
B
106.

Experiment 21:

Effect of ceresan on the growth of wheat seedlings

The beneficial effects of treating seed with ceresan demonstrated in Exp. 14, have so far been investigated in terms of toxicity to F. culmorum. There is the possibility that the seed treatment increases seedling vigour and contributes in this way to the disease control which is observed. This was examined.

Ten seeds treated with ceresan were sown in each of twenty-four 3½ in pots; the same number of untreated seeds were sown in another 24 pots. All the pots randomized on the greenhouse bench.

On days 1-7 after sowing 3 pots were selected at random from each treatment and the germinated seeds removed gently and washed in tap water. Root length and shoot height were measured (see p. 22). The results are given in Appendix Tables 47 & 48, and these show no significant differences in growth of seedlings from treated and untreated seed.

B - 'PP781':

One of the main disadvantages of mercury is its high mammalian toxicity and fungicide manufacturers are continually searching for less-toxic materials which are of combarable effeciency. Through the courtesy of I.C.I., a quantity of a fungicide designated PP781 was obtained. Tests atJealott's Hill Research Station indicated that this effectively controlled certain damping-off diseases. It was, therefore, compared with ceresan in a number of experiments with F. culmorum. These follow the same pattern of the previous section except that there are no data on root colonization comparable to Exp. 18. Throughout the experiments to be described PP781 was applied to seeds at the same rate as ceresan i.e. 0.1g./100g. seeds.

Experiment 22:

Effect of PP781 seed dressing on seedling growth in soil infested with F. culmorum

Five pots were set up, with 15 seeds per pot, for each of the following treatments:-

A. Untreated seed/infested soil.

B. Seed treated with PP781/infested soil.

C. Seed treated with ceresan/infested soil.

D. Seed treated with PP781/non-infested soil.

E. Untreated seed/non-infested soil.

Assessments of disease effects were carried out 21 days after sowing, as described in Exp. 14 (p. 75). The results are given in Appendix Tables 49 to 51 and summarized in Table 20. In all respects, the degree of control obtained with PP781 was comparable to that obtained with ceresan (Plate 12).

Table 20. Effect of PP781 seed dressing on seedling growth in infested soil

Treatments	Mean		
	Seedling stand (no.)	Root length (mm.)	Shoot height (mm.)
A. Untreated seed/ infested soil	6.6	207	246
B. PP781-treated seeds/ infested soil	11.6	277	280
C. Ceresan-treated seeds/ infested soil	12.6	281	299
D. PP781-treated seeds/ non-infested soil	13.2	279	285
E. Untreated seed/non-infested soil	12.4	276	285
L.S.D: P. = 0.05	2.0	27.3	29.3
P. = 0.01	2.8	37.6	-
P. = 0.001	3.9	51.7	-

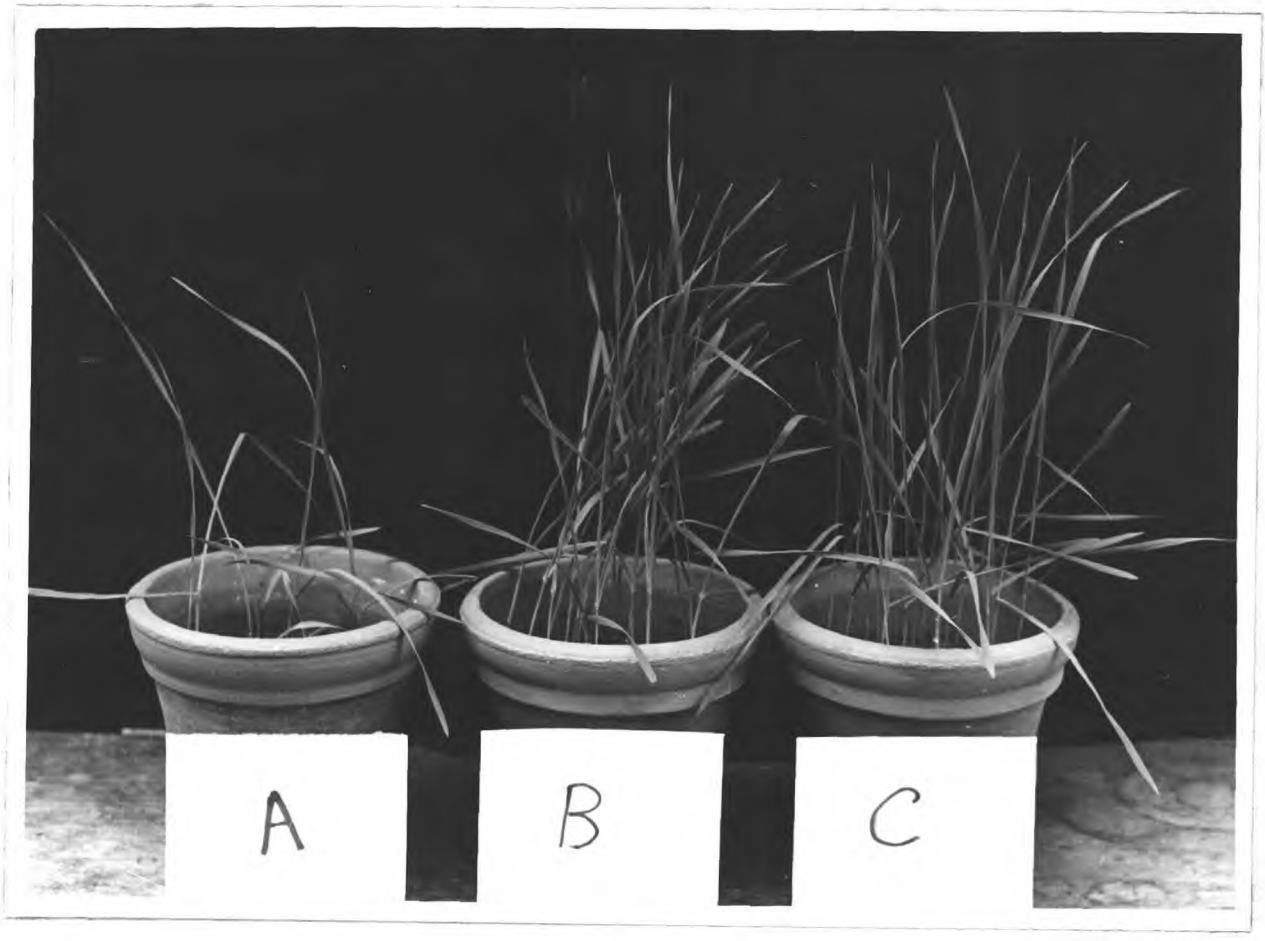


Plate 12: Effect of PP781 on stand and growth in infested soil

A. Untreated seed/infested soil.
B. PP781 - treated seed/infested soil.
C. Ceresan-treated seed/infested soil.

Experiment 23:

Effect of PP781 on the growth of F. culmorum in vitro

PP781 was incorporated into V8 agar and its effect on the growth of F. culmorum investigated in a manner similar to that described for ceresan in Exp. 15 (p. 79).

The results (Table 21 and Appendix Table 52) indicate that PP781 is only slightly less toxic to the growth of F. culmorum in culture than ceresan (Plate 13), on wt/wt basis but no allowence is made here for differences in percentage active ingredient.

Table 21. Effect of PP781 on the linear growth of F. culmorum

Time after inoculation (days)	Mean colony diameter (cm.)			% inhibition of growth	
	Level of PP781 p.p.m.			Low & high level	
	Nil	25	400	25	400
1	1.7	1.4	0.7	17.6	58.8
2	3.3	2.7	1.4	18.8	56.3
3	5.8	4.9	2.7	12.0	48.0
4	7.5	6.7	3.6	-	47.1
5	8.4	7.8	4.4	-	11.1

Percentage inhibition of growth was calculated as described in Exp. 15 (p. 80).

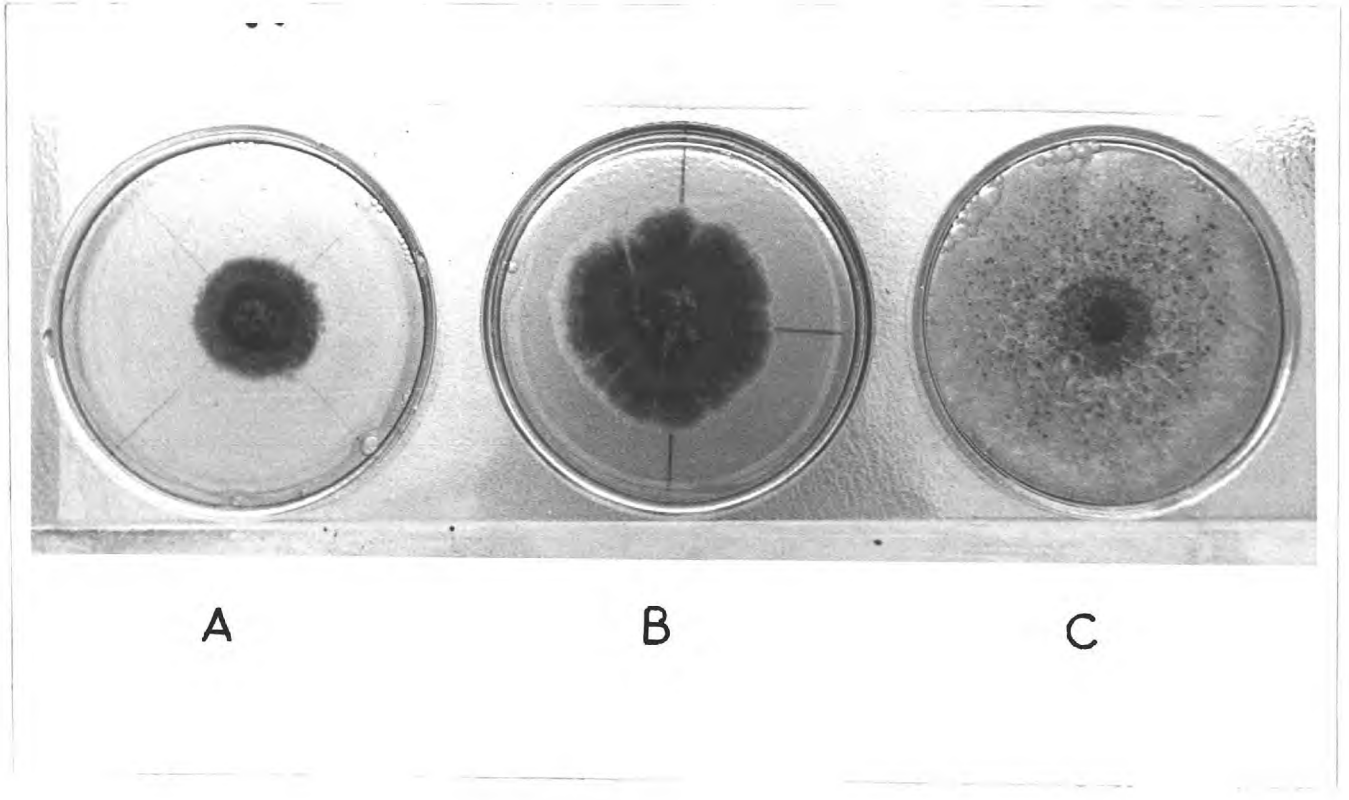


Plate 13: Effect of PP781 on F. culmorum in vitro. (after 5 days)

A. Ceresan 400 p.p.m.
B. PP781 400 p.p.m.
C. Control Nil p.p.m.

Experiment 24:

This was similar to Exp. 16 (p. 81). Ten P.D.A. plates, seeded with F. culmorum were each sown with four wheat seeds, two of which had been treated with PP781 and the other 2 with ceresan. The plates were incubated at 25°C. and the zones of inhibition measured after 2 days (Appendix Table 53a). There was a significantly greater (P. = 0.001) zone of inhibition around ceresan-treated seeds (mean diam. 16.3 mm.) than around PP781 - treated seeds (mean diam. 9.3 mm.), and this is illustrated in Plate 14.

On day 10 the zones of inhibition around PP781 - treated seeds were remeasured and compared with those for day 2 (Appendix Table 53b). That for day 10 (mean diam. 6.0 mm.) was significantly less (P. = 0.001) than that for day 2 (mean diam. 9.3 mm.).

Experiment 25:

This was similar to Exp. 17 (p. 82). Twenty PP781 - treated seed and 20 untreated seeds were selected at random, and thoroughly washed as previously described (p. 82). The water used in the washing was kept for further study. Treated and washed seeds were then plated onto P.D.A. seeded with F. culmorum and their ability to inhibit growth of the fungus compared with treated that had not been washed. The

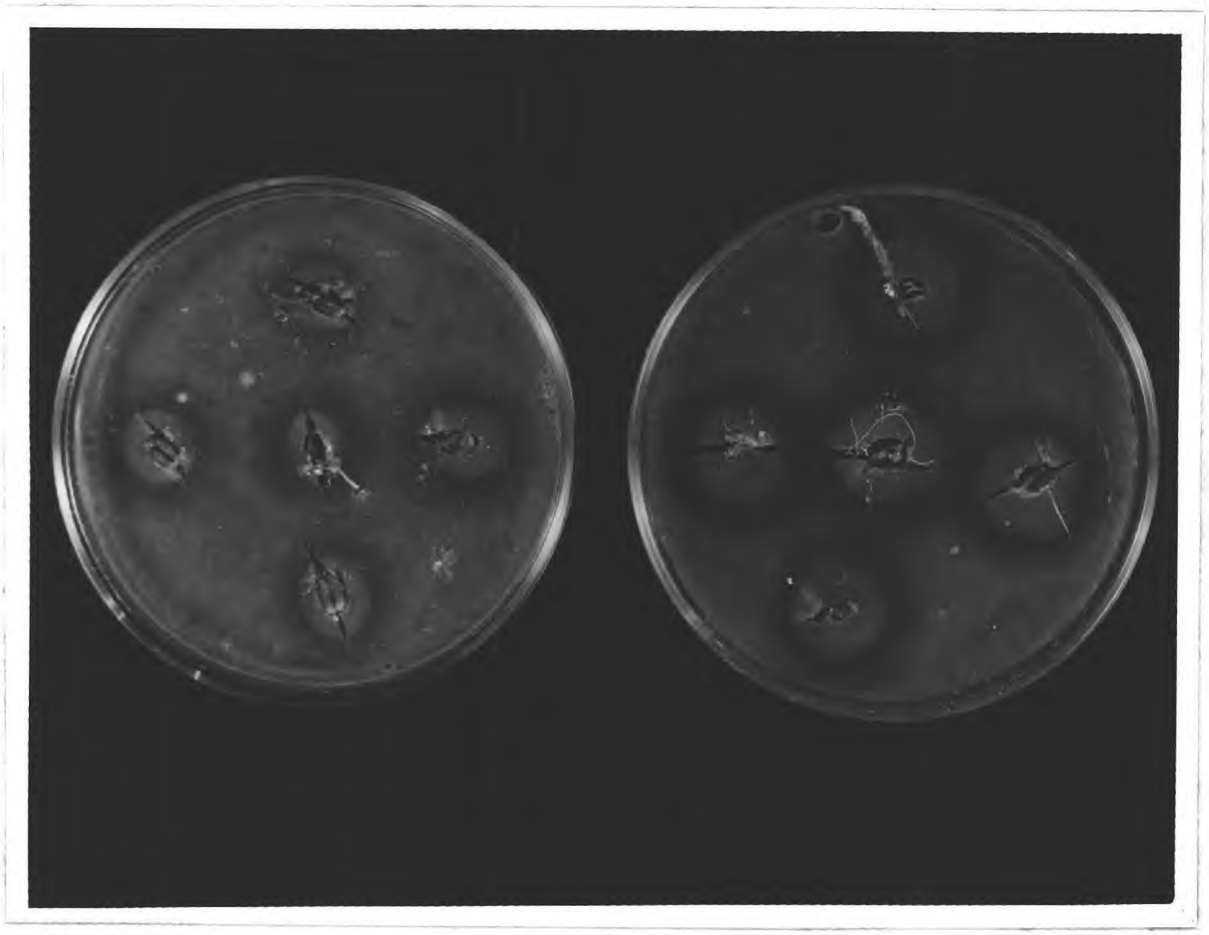
results (Plate15) show that virtually all the PP781 was removed by washing.

This was checked by incorporating the washing water into agar plates as described on p. 82 and inoculating them with F. culmorum. The results are shown in Table 22. There was some inhibition of growth where the washings from treated seed were mixed with the agar but as with ceresan Exp. 17 the dilution was too great to demonstrate this convincingly.

Table 22. Effect on F. culmorum of incorporating the washing from PP781 - treated seed in an agar medium

Time after inoculation (days)	Mean colony diameter (cm.)		% inhibition of growth
	Treated	Untreated	
1	1.4	1.8	22.2
2	2.9	3.8	25.0
3	6.1	6.3	-
4	7.5	7.6	-
5	8.4	8.4	-

Percentage inhibition of growth was calculated as described in Exp. 15 (p. 80).



1 2

Plate 14: Comparison between zone of inhibition from PP781 - and ceresan-treated seeds in a seeded plate with F. culmorum.

(1) PP781 - treated seeds.
(2) Ceresan-treated seeds.

2 1

1 2

Plate 15: Comparison between zone of inhibition from PP781 - treated washed (1) & unwashed (2) seeds in a seeded plate with F. culmorum.

Experiment 26:

Effects on F. culmorum of homogenates derived from root of seedlings grown from seed treated with PP781

This experiment was carried out in a manner similar to that described for ceresan (Exp. 20, p. 98). Although it was repeated several times, in no instance was any inhibition of F. culmorum observed near homogenates of roots, derived from PP781 - treated seed.

Experiment 27:

Effect of PP781 on the growth of wheat seedlings

This experiment was carried out in a manner similar to Exp. 21 (p.107). The results are given in Appendix Tables 54 & 55. There was no significant difference between seedlings grown from treated seed and those grown from untreated seed.

2. Soil application of aldrin

Eno (1958) showed that some insecticides, particularly organo-chlorine derivatives, reduced the population of fungi when applied to soil. This striking result led to research by other workers on the use of these compounds to control several plant diseases caused by soil-borne fungi (see p. 12).

The investigations dealt with here are concerned with the application of aldrin (1, 2, 3, 4, 10, 10-Hexachloro-1, 4, 4a-5, 8, 8a-hexahydro-1, 4-endo-5, 8-dimethanonaphthalene) as a dust to soil infested with F. culmorum.

A 5% aldrin dust was kindly supplied by Dr. A.B.P. Page of Imperial College Field Station and a 10% aldrin dust by the Shell Chemical Co. Ltd. The 5% dust was used in a preliminary experiment only; the 10% dust was used in all other experiments.

Experiment 28:

Effect on damping-off by F. culmorum of a soil application of 5% aldrin dust

Twenty, 5 in. pots were partly filled with soil infested with F. culmorum. For each of 16 pots, aldrin dust (5%) was mixed with a further 200 g. infested soil and this alrdin - soil mixture was used to fill the pot. The remaining 4 pots were topped up with infested soil only. In terms of soil

surface area the amounts of aldrin added to the pots and the corresponding applications per acre were as follows:-

Level	g. dust/pot	lb. dust/acre
A	nil	nil
B	0.22	150
C	0.44	300
D	0.66	450
E	2.20	1500

A second series of 20 pots was also set up with uninfested soil to test the effect of the chemical alone on plant growth. Fifteen wheat seeds were sown in each pot of both series and all the pots randomized on the greenhouse bench.

Assessments of disease and other effects were carried out 15 days after sowing by counting the seedlings emerged, measuring shoot heights and estimating seedling weight after washing and drying in an oven at 60°C. for 48 hours. The results are summarized in Table 23 and given in detail in Appendix Tables 56-58. These indicate that aldrin dust applied to soil significantly improved the stand and shoot height of seedlings in soil infested with F. culmorum but had no effect on dry weight.

Table 23. Effect of aldrin (5% dust) on seedling stand and growth in infested soil

Treatments	Mean		
Level of aldrin	Seedling stand (number)	Shoot height (mm.)	Plant dry weight (mg.)
A	4.3	163	31.0
B	10.3	218	37.3
C	7.8	203	39.9
D	11.8	220	39.1
E	7.3	209	39.7 n.s.
L.S.D.			
P. = 0.05	3.7	33	9.1
P. = 0.01	5.2	47	-

Experiment 29:

A comparison of a soil application of aldrin (10% dust) and seed dressings of ceresan and PP781

Aldrin (10% dust) was applied to soil in the manner previously described at the rate of 0.66 g./pot which corresponds to 450 lbs/ac. Seeds were treated with ceresan or PP781 as described in Exp. 14 (p.75). Five pots were

set up for each of the following treatments:-

A. Untreated seed/infested soil.

B. Untreated seed/infested soil + aldrin.

C. Seed treated with ceresan/infested soil.

D. Seed treated with PP781/infested soil.

E. Untreated seed/non-infested soil.

Fifteen seeds were planted per pot and the pots randomized on the greenhouse bench.

Assessments of disease effects were carried out 21 days after sowing by counting the seedlings emerged and by measuring root length and shoot height (see p. 22). The full results are given in Appendix Tables 59-61 and summarized in Table 24. These again illustrate that aldrin applied to soil gives appreciable control of damping-off by F. culmorum. Seedling stand with the seed dressings is significantly better than that in the aldrin treatment, but there is no significant difference between the 3 chemical treatments in respect of root length and shoot height (Plate 16).

Table 24. Effect of aldrin, ceresan and PP781 on seedling stand and growth in infested soil

Treatments	Mean		
	Seedling stand (no.)	Root length (mm.)	Shoot height (mm.)
A	6.0	174	185
B	9.8	263	271
C	12.0	275	293
D	11.2	269	286
E	11.8	266	292
L.S.D.			
P. = 0.05	1.9	23.7	23.3
P. = 0.01	2.6	32.6	32.1
P. = 0.001	3.6	44.8	44.2

Plate 16:

A comparison of a soil application of aldrin (10% dust and seed dressings of ceresan and PP781

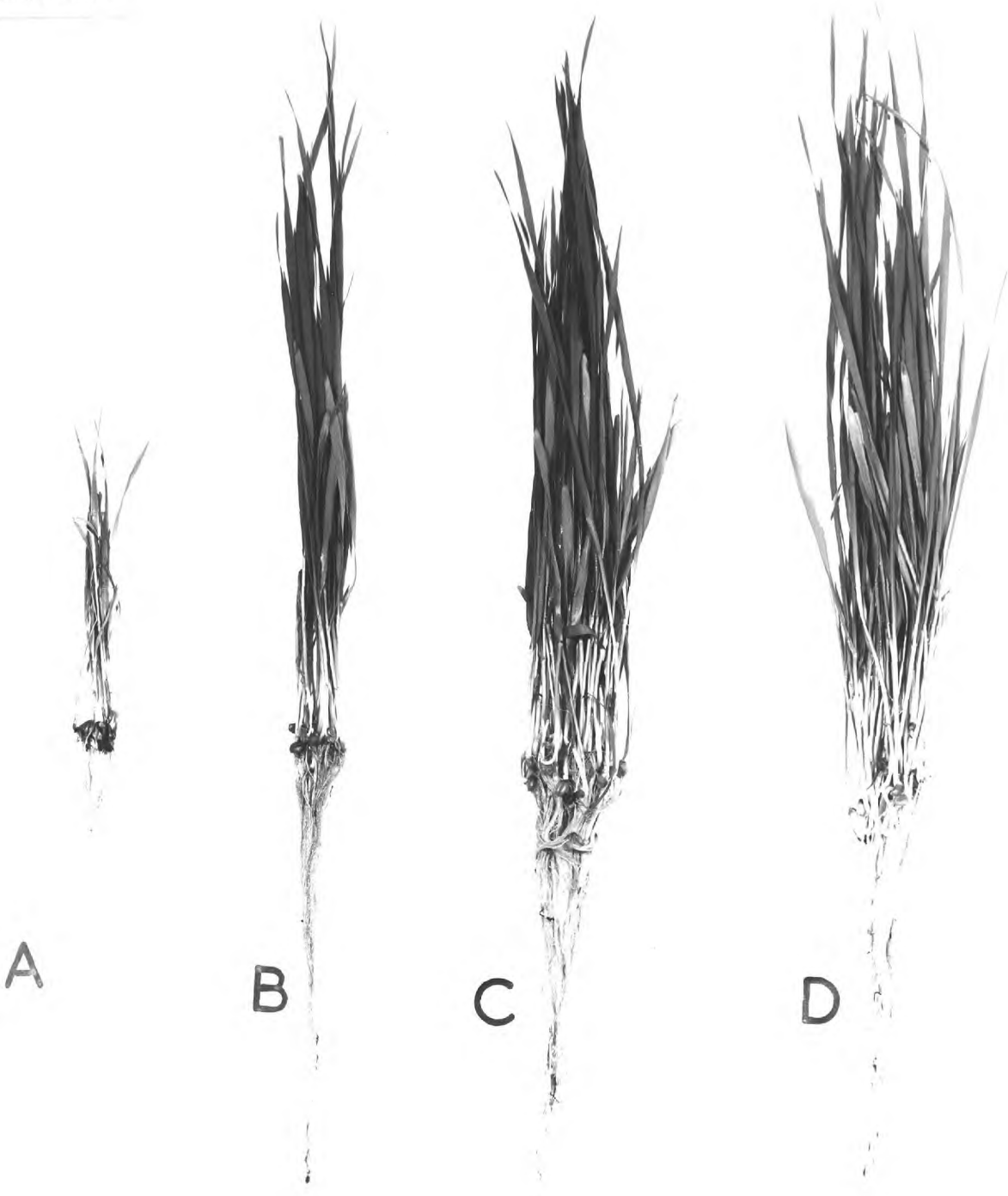
A. Untreated seed/infested soil.

B. Untreated seed/infested soil + aldrin.

C. Ceresan-treated seed/infested soil.

D. PP781 - treated seed/infested soil.

PLATE 16:



Experiment 30:

Effect of a soil application of aldrin (10% dust) on the colonization of wheat roots and coleoptiles by F. culmorum

The result of Exp. 18 showed that colonization of root surfaces by F. culmorum was less advanced on seedlings grown from ceresan-treated seed than from untreated ones. A similar experiment was therefore undertaken for soil treated with aldrin.

Twenty, 5 in. pots were filled with soil infested with F. culmorum. Ten of these were treated with aldrin by mixing 0.66g. of the 10% dust with top 200 g. soil. Fifteen seeds were then sown in each pot. At days 5, 7, 9 & 11 after planting 2 pots were selected at random (from both aldrin-treated and the controls) and between 20 and 25 seedlings removed from each treatment. The seedlings were washed in tap water and the root systems and coleoptiles were examined as described in Exp. 18 (p.85).

A summary of the results is given in Table 25 and a full analysis in Appendix Table 62a. These show that on day 11 colonization of root surfaces of seedlings grown in treated soil was significantly less than in the corresponding controls. On days 5, 7 & 9 there were no significant differences, nor was there any significant difference in the colonization of the coleoptiles of seedlings in the two treatments (Appendix Table 62b).

Table 25. Effect of aldrin on colonization of root surfaces by F. culmorum

Days	Mean number of segment with F. culmorum		Difference	L.S.D.
	Treated soil	Untreated soil		P. = 0.05
5	5.7	7.0	1.30 n.s.	2.46
7	7.3	7.8	0.50 n.s.	1.46
9	6.8	8.0	1.20 n.s.	1.22
11	5.3	8.1	2.80*	1.60

Experiment 31:

Effect of a soil application of aldrin (10% dust) on the growth of wheat seedlings

A further experiment was carried out to see if aldrin had any stimulatory effect on seedling growth which might, in part, account for the disease control observed with this material.

Forty-eight, 3½ in. plastic pots were filled with untreated soil. Half of them were treated with aldrin by mixing 0.66 g. of the 10% dust with the top 200 g. soil in each pot. Ten, untreated seeds were planted per pot and

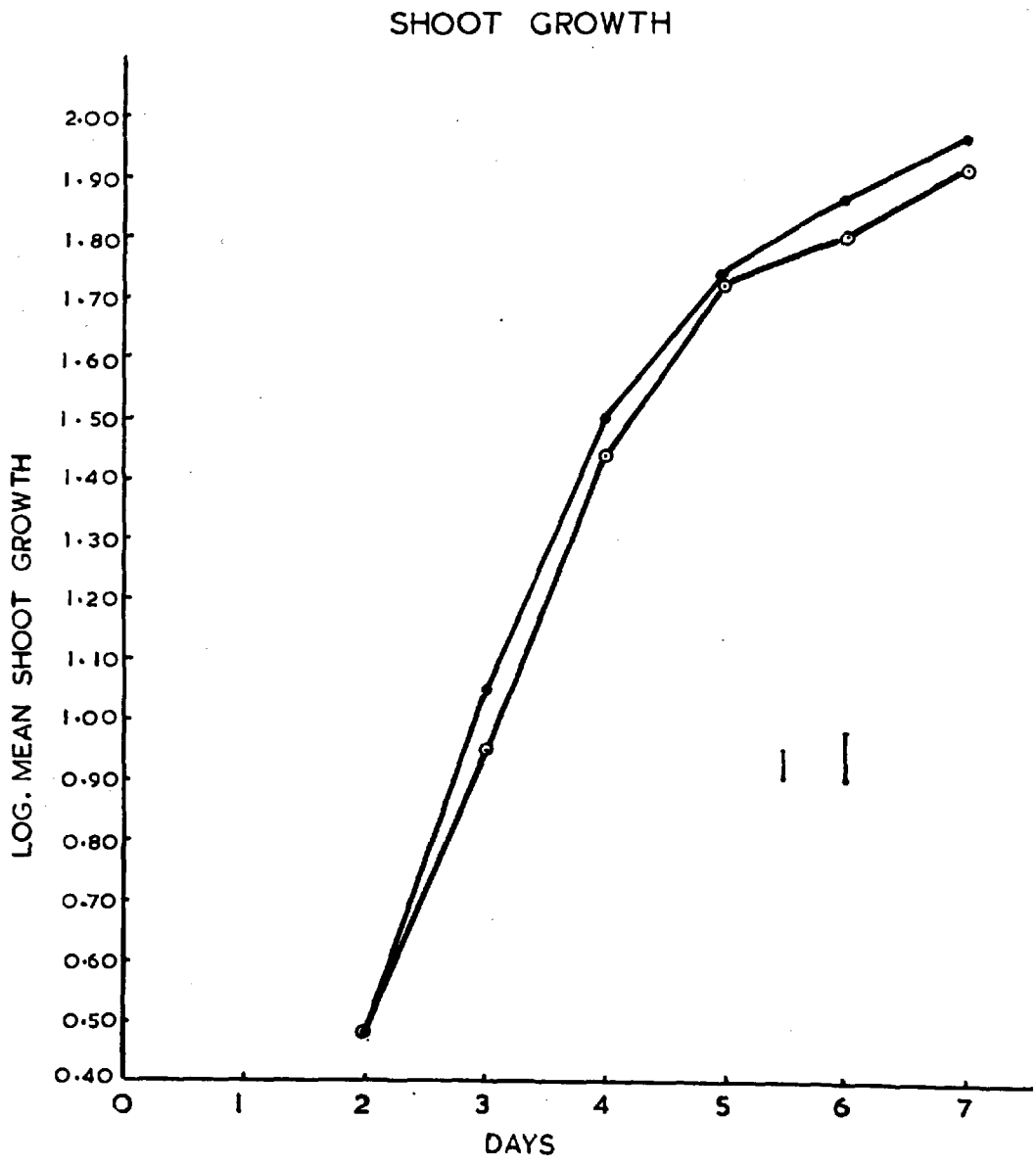
experiment otherwise carried out in a similar manner to that for ceresan (Exp. 21, p.107).

The results are given in full in Appendix Tables 63 & 64. Only shoot growth was significantly increased in aldrin - treated soil (Figure 17) but the differences do not seem sufficiently large to account for the disease control demonstrated in Exp. 28.

FIGURE 17.

EFFECT OF ALDRIN ON SEEDLING GROWTH
TREATED • & UNTREATED ⊙ SOIL

VERTICAL LINES REPRESENT SIGNIFICANT DIFFERENCES FOR P=0·05, P=0·01



Effect of aldrin (10% dust) on the growth of F. culmorum in vitro

The results of Exps. 28 & 29 show that aldrin reduces the damage caused to seedlings by F. culmorum in soil, probably by limiting the growth of the fungus on the seedling roots. The effect of aldrin on the growth of F. culmorum in culture was therefore investigated.

Experiment 32:

Effect of aldrin on the growth of F. culmorum in an agar medium

A small quantity of 10% aldrin dust (0.4g.) was suspended in 40 ml. sterile water and from this a dilution was prepared viz:- 1/20, 1/40, 1/80, 1/160. With a sterile pipette 10 ml. of each dilution were transferred to 90 ml. V8 agar cooled to 45°C. After thorough mixing this was used to pour 5 plates. In this way 5 plates of each of the following levels of aldrin were obtained:-

10,000, 5,000, 2,500, 1,250 & 650 p.p.m.

Plates of V8 agar with no aldrin were prepared for controls. Two diameters were drawn at right angles on the backs of all plates. The plates were then inoculated with F. culmorum as described in Exp. 15 (p. 79), incubated at 25°C., and growth measured daily as described on p.79. The results (Appendix Table 65a) indicate that aldrin had

no effect on the growth of F. culmorum.

The experiment was repeated but instead of preparing aqueous suspensions, equivalent amounts of aldrin were dissolved in acetone and then incorporated into V8 agar. A similar result was obtained (Appendix Table 65b).

Experiment 33:

Effect of aldrin (10% dust) on growth of F. culmorum in a liquid medium

A series of aldrin/acetone dilutions were prepared and 5 ml. of each dilution were added to 95 ml. liquid medium to give final concentrations of 650, 1,250, 2,500, 5,000 & 10,000 p.p.m. aldrin. The liquid medium had the following composition:-

Glucose	1.00 g.
Peptone	0.25 g.
$(NH4)2HPO4$	0.10 g.
$MgSO_4.7H_2O$	0.05 g.
KCL	0.05 g.
Minor elements (Appendix p. 192)	1 ml.
Distilled water	94 ml.

Twenty ml. of each dilution were placed in each of four 150 ml. Erlenmeyer flasks and sterilized by autoclaving at 120°C. for 20 min. The remainder of each dilution was kept for Exp. 34. Flasks were also prepared in a similar way with basic medium only, and basic medium plus an appropriate amount of acetone. All flasks were then inoculated with a

disk (3 mm. diam.) cut from the edge of a 3 - day old culture of F. culmorum, and incubated at 25°C. After 7 days the mycelium from each treatment was harvested and dried for 24 hours in an oven at 60°C. and then weighed. The fungal mats were then reweighed after a further 24 hrs. in the oven.

Analysis of the results is given in Appendix Table 66. This shows that there were no significant differences between the treatments.

Experiment 34:

Effect of aldrin (10% dust) on the spore germination of F. culmorum

Spore germination of F. culmorum was examined in the range of aldrin media prepared for Exp. 33, by the slide germination technique described by the American Phytopathological Society, Committee (1943). Germination was assessed after 6 hours incubation at 25°C. None of the aldrin treatments had any significant effect on germination. (Appendix Table 67).

Experiment 35:

Effect on F. culmorum of homogenates derived from roots of wheat seedlings grown in soil treated with aldrin (10% dust)

While soil application of aldrin gave substantial control of F. culmorum (Exps. 28 & 29), no effect on the growth of the fungus in culture could be demonstrated (Exps. 32-34). The possibility that aldrin is absorbed by the seedling roots and converted to substances toxic to F. culmorum was therefore examined.

Forty, 800 ml. 'Tall Form' Pyrex beakers were each partly filled with 400 g. of washed and air-dried sand, covered with a Petri dish lid, and sterilized by autoclaving at 120°C. for one hour. Then 0.66 g. of aldrin was mixed with the surface layer of sand in each of 20 beakers; the remaining beakers received no treatment. Ten to fifteen untreated seeds were sown in each beaker and sterile distilled water was added to 50% of the water holding capacity. The lid of each beaker was firmly sealed with 'Sellotape' and the lower half of the beaker covered with black paper. The beakers were then randomized in an illuminated growth chamber at 25°C.

Twelve days after planting, roots from 100 seedlings were selected from both aldrin-treated beaker and the controls. Homogenates were prepared from these roots and

the effect of these on the growth of F. culmorum in an agar medium was tested, as described on p. 99.

This experiment was repeated several times but in no instance was any inhibition of F. culmorum obtained with homogenates of roots from seedlings grown in aldrin-treated soil.

Experiment 36:

Effect of an extract of soil treated with aldrin (10% dust) on the spore germination of F. culmorum

The possibility was next examined that aldrin is broken down in soil to a substance(or substances) which itself is toxic to F. culmorum.

Ten, 5 in. pots were filled with uninfested soil and to each of 5 of them, 2.2 g. 10% aldrin dust were added; the other 5 were not treated. Fifteen untreated wheat seeds were sown in each pot and the pots placed in the greenhouse. After 21 days, 5 replicate samples of soil were taken from each pot by inserting a number 8 cork borer to a depth of 2 in. The 5 samples of soil from each pot were bulked and the resulting composite samples dried in an oven at 35°C. for 24 hours.

The dried soil was then ground in a mortor and 20 g. from each treatment were transferred separately to a screw-cap bottle containing 10 ml. acetone. The bottles

were sealed firmly and shaken for 10 mins. on a Griffin flask shaker. The resulting suspensions were left to stand for 2 hours, then filtered through muslin. The remaining soil particles were allowed to settle and the supernatant liquid (soil extract) was decanted.

A slide test (see Exp. 34, p131) was used to examine the effects of the soil extracts on spore germination. 0.025 ml. of extract was placed on each coverslip and the acetone allowed to evaporate. The spore suspension of F. culmorum was prepared in 0.1% glucose from a 6-day old culture of P.D.A. and adjusted to 10^2 spores/ml.

The full results are given in Appendix Table 68 a-b. There was a significant difference (P. = 0.001) between the germination of spores in extracts from treated soil (mean 79.9%) and that in extracts from untreated soil (mean 94.3%). Germ-tube growth was also significantly less in extracts from the aldrin-treated soil (mean 84.4 μ, compared with mean of 136.0 μ in extracts from untreated soil).

Experiment 37:

Effect of an extract of soil treated with aldrin (10 % dust) on the growth of F. culmorum in a liquid medium

(a) Soil extract from soil treated with aldrin and planted with wheat seed

The following amounts of aldrin (10% dust) were added to each of 5 pots of uninfested soil as described on p.118.

A.	nil
B.	0.22 g.
C.	0.44 g.
D.	0.66 g.
E.	2.20 g.

Fifteen wheat seeds were then planted in each pot, and the pots randomized on greenhouse bench.

Fifteen days after sowing the top 200 g. soil were taken from each pot and the 5 replicate samples of each treatment mixed together thoroughly. 200 g. of each bulked sample were then transferred to a 500 ml. Erlenmeyer flask containing 125 ml. distilled water. The flasks were shaken for one hour on a flask shaker, allowed to stand overnight, and then shaken once more. The suspensions were then filtered several times through filter paper (no. 1) and finally through bacteriological filter 'Oxoid membrane'.

Liquid media were then prepared in which the filtered extracts replaced distilled water in the medium described on p.130. Five replicate flasks were set up for each

extract/medium with 20 ml. in each together with 5 flasks of normal liquid medium (no soil extract). After sterilization 4 of the 5 flasks were inoculated with F. culmorum (see p.130) and incubated at 25°C. for one week, then the mycelium was harvested and weighed as in Exp. 33 (p.131).

The remaining flask of each medium was used for a spore germination test as described on p.131. The full results of both tests are given in Appendix Table 69 a-c, and summarized in Table 26.

It is clear from these that extracts from soils treated with the higher amounts of aldrin (treatments C, D, E) adversely affect germination and growth of F. culmorum substantiating the findings of Exp. 36.

Table 26. Effect on growth of F. culmorum of soil extracts from soil treated with aldrin and planted with wheat

Treatments	Mean		
	Mycelium dry weight (mg.)	% germination	Length of germ-tube(μ)
A. Extract from untreated soil.	97.3	95.5	150
B. Extract from soil + 0.22g. aldrin.	95.6	97.5	149
C. Extract from soil + 0.44g. aldrin	92.8	94.5	141
D. Extract from soil + 0.66g. aldrin	89.0	94.3	136
E. Extract from soil + 2.2g. aldrin	86.8	87.5	129
F. Liquid medium only (no soil extract)	94.8	95.5	149
L.S.D. P. = 0.05	7.3	4.3	7.8
P. = 0.01	10.1	5.9	10.6
P. = 0.001	-	7.8	14.2

(b) Soil extracts from soil treated with aldrin only

Experiment 37 was repeated using extracts from aldrin - treated and untreated soil, in which no wheat seedlings had grown. The results (Table 27 & Appendix Table 70 a-c) were similar to those obtained in part (a).

Table 27. Effect of soil extracts from soil treated with aldrin only on growth of F. culmorum

Treatments (as in table 26)	Mean		
	Mycelium dry weight (mg.)	% germination	Length of germ-tube (μ)
A	94.0	95.2	148
B	93.3	94.2	145
C	90.5	93.2	140
D	88.0	90.2	128
E	80.0	81.8	113
F	94.8	94.0	150
L.S.D.			
P. = 0.05	8.3	5.0	12.5
P. = 0.01	11.4	6.9	16.9

Effect of dieldrin on the growth of F. culmorum in vitro.

The results of Experiment 36 & 37 suggest that substances are formed from aldrin in soil which inhibit the growth of F. culmorum. Lichtenstein & Schulz (1959); Wheatley et al. (1962) and Lichtenstein et al. (1964) have reported that small amounts of dieldrin are formed (epoxidation) when aldrin is applied to soil so the effect of dieldrin on F. culmorum was examined.

A small quantity of pure dieldrin (1, 2, 3, 4, 10, 10 - hexachloro - exo - 6, 7 - epoxy - 1, 4, 4a, 5, 6, 7, 8, 8a - octahydro - 1, 4 - endo, exo - 4, 8 - dimethanonaphthalene) was kindly supplied by Dr. H.H. Shatoury of Imperial College Field Station.

Experiment 38:

Effect of dieldrin on the growth of F. culmorum in an agar medium

A series of media containing the following concentrations of dieldrin were prepared: 100, 50, 25, 12.5, 6.25, p.p.m. in a manner similar to that detailed for ceresan in Exp. 15 (p.79). Growth of F. culmorum on these media were compared with that on the basic media without dieldrin. The full results are given in Appendix Table 71, and summarized in Table 28. These show that dieldrin markedly inhibits the growth of F. culmorum.

Table 28. Effect of dieldrin on the linear growth of F. culmorum

Time after inoculation (days)	Mean colony diameter (cm.)			% inhibition of growth	
	Level of dieldrin p.p.m.			Low & high level	
	Nil	6.25	100	6.25 p.p.m.	100 p.p.m.
1	1.7	1.3	0.4	23.5	74.6
2	3.2	2.5	0.9	20.0	66.6
3	5.6	4.4	1.8	20.8	62.5
4	7.2	5.8	2.8	12.5	37.5
5	8.4	7.0	3.6	8.3	33.3

The percentage inhibition of growth was calculated as described in Exp. 15 (p. 80).

Experiment 39:

Effect of dieldrin on growth of F. culmorum in liquid medium and on spore germination.

The effect on F. culmorum of dieldrin incorporated in a liquid medium was investigated in the same way as that described for aldrin in Exps. 33-34 (pp. 130 & 131), and the dilutions of dieldrin used were the same as those in the previous experiment (38).

The results (Table 29 and Appendix Table 72 a-c) confirm those of Exp. 38. Mycelial dry weight, percentage

germination and germ-tube growth were all seriously decreased in the presence of dieldrin.

Table 29. Effect of dieldrin on growth of F. culmorum in liquid medium.

Level of dieldrin p.p.m.	Mean		
	Mycelium dry weight (mg.)	% germination	Length of germ-tube (μ.)
100	53.8	64.0	40.0
50	57.8	75.8	65.8
25	61.0	81.2	89.3
12.5	68.5	85.8	99.5
6.25	78.5	87.0	108.7
nil + acetone	101.8	96.7	147.3
nil	101.3	96.8	149.0
L.S.D.			
P. = 0.05	4.6	11.3	23.1
P. = 0.01	8.3	15.5	31.5
P. = 0.001	8.6	20.2	41.2

Experiment 40:

Long-term effect of ceresan seed dressings and a soil application of aldrin (10% dust).

The experiments so far have dealt only with the effects of ceresan and soil applications of aldrin on seedlings. An experiment was conducted in the spring of 1965 to find out whether these treatments would have any long-term effects on the growth of wheat.

Five, 10 in. pots were set up for each of the following treatments:-

A. Untreated seed/non-infested soil.

B. Seed treated with ceresan/infested soil.

C. Untreated seed/infested soil + aldrin.

D. Untreated seed/infested soil.

E. Seed treated with ceresan/non-infested.

F. Untreated seed/non-infested + aldrin.

Aldrin was added, where indicated, at the rate of 1.32 g. per pot which corresponds to 450 lb/ac., and mixed with the top 400 g. soil. Ten wheat seeds were planted in each pot and the pots randomized on the greenhouse bench. After 21 days the pots were transferred to the Walled Garden and randomized as before. They remained there until harvesting.

The first estimates of disease effects were made 21 days after sowing by counting the seedlings emerged. Then

the following assessments were made.

1. Tillering: A count of the number of tillers produced 70 days after sowing.
2. Height of plants: At harvesting, by measuring the distance from the soil surface to the tip of each spike and calculating the average height for each plant.
3. Length of the ears, at harvesting.
4. Weight of ears, grain, and straw, at harvesting.

The results are given in full in Appendix Table 73 a-g, and summarized in Table 30.

The long-term effects of treating seed with ceresan and applying aldrin to the soil are well-marked*. All the characters assessed at harvesting were significantly better for these two treatments than for untreated seed planted in infested soil. The figures obtained for these two treatments were infact, similar to those for untreated seed planted in non-infested soil. There is some indication that aldrin increased tillering: the figures for treatment 'F' (untreated seed/non-infested soil + aldrin) are significantly greater than those for treatment 'A' (untreated seed/non-infested soil), *(Plate 17).

At harvesting the stems of a number of plants in treatment 'D' (untreated seed/infested soil) were

discoloured at and below soil level and there was some rotting associated with this discolouration (Plate 18). Most of these plants subsequently collapsed and died, (Plate 19). A number of the fractured bases of the collapsed plants were surface sterilized and pieces plated on Rose-Bengal-Streptomycin agar (see p. 93). After 3 day's inocubation at 25°C. all these pieces yielded F. culmorum.

Table 30. Long-term effect of ceresan and aldrin on seedling stand and growth in infested and non-infested soil.

Treatments	Mean/Pot					Mean/Plant	
	Stand (no.)	Tillering (no.)	Ear wt. (g.)	Grain wt. (g.)	Straw wt. (g.)	Plant height (cm.)	Ear Length (cm.)
A. Untreated seed/non-infested soil.	8.8	59.4	93.8	65.8	104.6	95.6	10.4
B. Ceresan-treated seed/ infested soil.	9.2	63.0	99.6	66.6	113.0	99.0	10.8
C. Aldrin-treated soil/ infested.	7.2	67.8	95.6	74.8	108.4	98.0	11.0
D. Untreated seed/ infested soil.	5.0	32.8	28.6	18.4	42.6	63.8	8.2
E. Ceresan-treated seed/non-infested soil.	9.4	64.8	112.6	67.0	115.0	99.8	11.2
F. Aldrin-treated soil/non-infested.	9.0	81.6	114.6	77.8	119.0	101.0	11.6
L.S.D.							
P. = 0.05	1.2	15.2	28.1	18.2	23.4	9.0	0.98
P. = 0.01	1.7	20.7	38.4	24.8	31.9	12.2	1.33
P. = 0.001	2.3	28.0	51.9	33.6	43.1	16.6	1.81

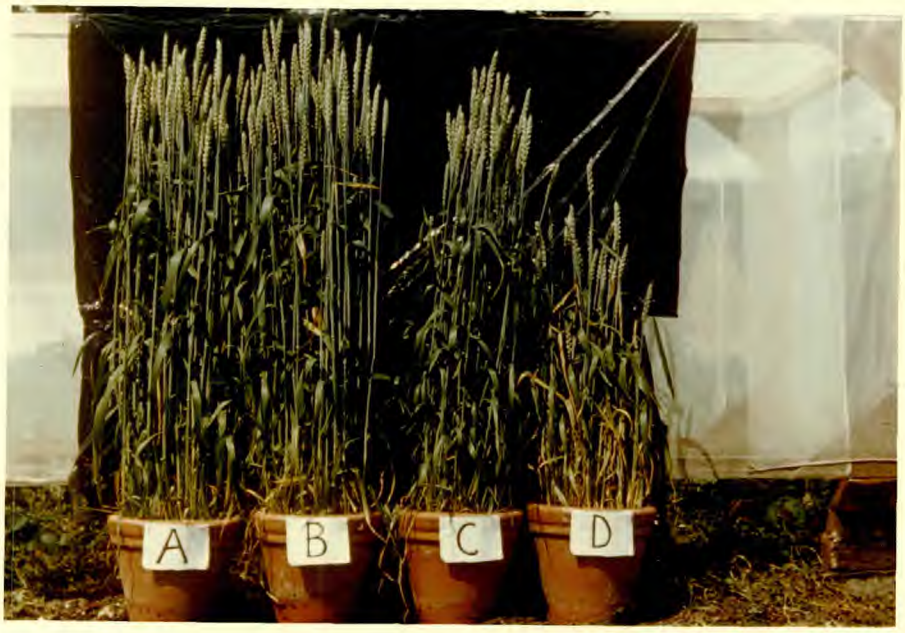


Plate 17: The long-term effects of treating seed with ceresan and soil application of aldrin (10% dust).

A. Ceresan-treated seed/infested soil.

B. Untreated seed/infested soil + aldrin.

C. Untreated seed/non-infested soil.

D. Untreated seed/infested soil.

Plate 18: Wheat plant showing a discoloured and rotting part at and below soil level, (Foot-rot).

Plate 19: Bases of premature wheat showing point of fracture.

3. Some experiments on biological control

There is now considerable evidence that the activities of many pathogenic fungi are influenced by the soil microflora. In the following experiments an attempt was made to find organisms in soil antagonistic to F. culmorum and in a preliminary way, examine the possibility of using these for controlling the pathogen.

Experiment 41:

Colonization of seedling root surfaces by F. culmorum in a sterilized and non-sterile soil

The object of this experiment was to find out the effects of soil micro-organanisms on the colonization of wheat roots by F. culmorum in non-sterile soil.

A quantity of soil sufficient to fill ten 5 in. pots was sterilized by autoclaving at 120°C. for one hour, and this was then infested with F. culmorum (see p. 20). Another ten pots were filled with non-sterile, infested soil. Fifteen seeds were planted in each pot. At days 5, 7, 9 & 11 after sowing, two pots from each treatment were selected at random and between 20-25 germinated seeds were removed from both treatments. The seedlings were washed in tap water and the root systems from each treatment were examined as described in Exp. 18 (p. 85).

A summary of the results is given in Table 31 and the full results in Appendix Table 74. Root colonization was significantly less in the non-sterile soil compared with the sterilized soil, although similar amounts of inoculum were used in each. This suggests that the soil microflora has some effect on root colonization by F. culmorum, but this is limited since even in non-sterile soil a considerable amount of root colonization occurs.

Table 31. Colonization of root surfaces by F. culmorum in a sterile and non-sterile soil

Days	Mean number of segment with F. culmorum		Difference	L.S.D.	
	Sterile soil	Non-sterile soil		P. = 0.05	0.01
5	9.3	8.0	1.3*	1.3	-
7	9.5	7.8	1.7*	1.3	-
9	9.8	8.4	1.4**	1.0	1.3
11	10.0	7.9	2.1**	1.0	1.4

Experiment 42:

Antagonism of soil micro-organisms to F. culmorum

It can be argued that the growth of micro-organisms antagonistic to F. culmorum will be stimulated by the addition of this fungus to non-sterile soil. In the absence of an appropriate host one could expect on the one hand a decline in F. culmorum and on the other an increase in the antagonists. In this connection Semeniuk & Henry (1960) concluded that the decline of F. culmorum was a degenerating process resulting from the activity of soil micro-organisms. This could be useful in searching for antagonistic micro-organisms and was investigated as follows:

Thirty-four pots were filled with infested soil. Immediately and then at weekly intervals for 7 weeks, 4 pots were planted with wheat seed. Seedling stand was assessed 14 days after sowing. The results are given in Appendix Table 75, and illustrated in Figure 18. These show clearly a falling-off with time in percentage damping-off which suggests a decline in the population of F. culmorum added.

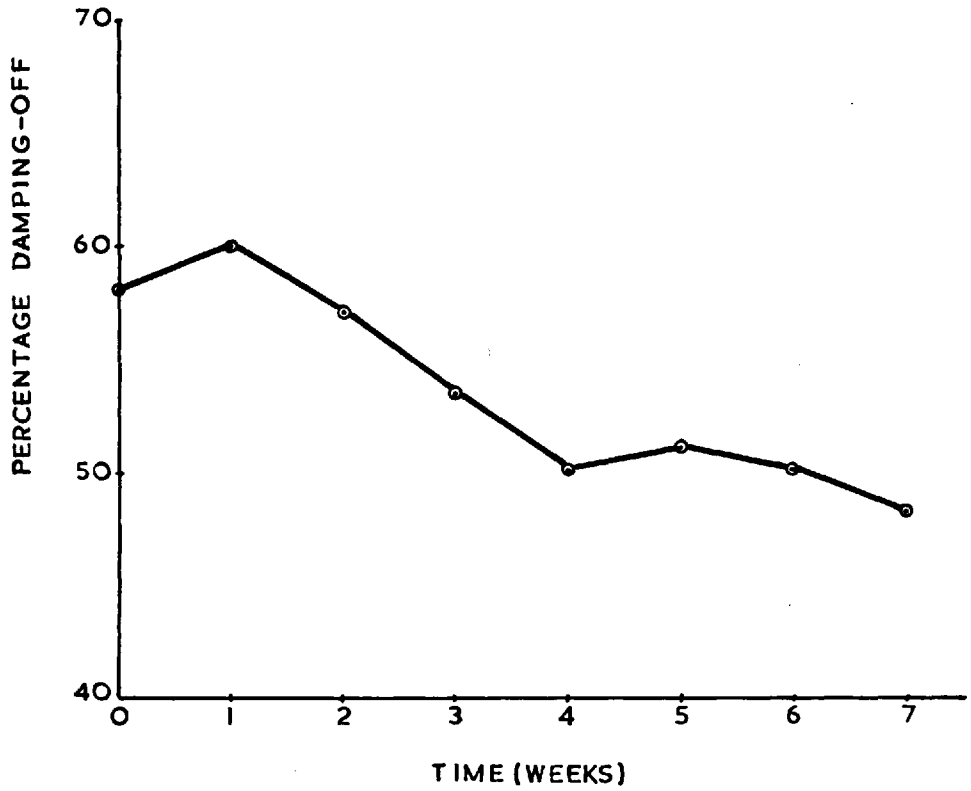
A week after sowing 25 g. of soil were collected from each pot. From the bulked sample a serial dilution of soil was prepared ($1:10^{-2}$ - $1:10^{-6}$). Five ml. of each dilution were then transferred to separate flasks containing 95 ml. P.D.A. which had been cooled to $45^{o}C$. After thoroughly

mixing, the contents of each flask were distributed amongst 5 Petri dishes. All plates were incubated at 25°C. for 3 days and then examined, after which they were kept at room temperature and re-examined at intervals. The presence of antagonists was indicated by clear zones around F. culmorum colonies.

This procedure was repeated weekly. Four organisms showing particularly marked antagonism against F. culmorum were isolated and tested in further experiments. They were:- a Penicillium sp., a Trichoderma sp. and two bacterial isolates designated 'F' & 'H'.

FIGURE 18.

DECLINE OF F. CULMORUM INOCULUM WITH TIME IN NON-STERILE SOIL



Experiment 43:

Effect on damping-off by F. culmorum in sterilized sand of treating seed with spores of an isolate of Penicillium sp.

The antagonism of the Penicillium isolated in Exp. 42, was checked by inoculating a plate of P.D.A. on opposite arcs with this fungus and with F. culmorum.

A number of medical flats each containing 20 ml. P.D.A. were kept horizontally until the agar solidified. The bottles were then inoculated with the isolate of Penicillium, and incubated at 25°C. for 15 days. After that 10 g. of wheat seeds were transferred to each bottle. These were shaken for 10 min. Four 3½ in. plastic pots were set up for the following treatments:-

A. Untreated seed/sand sterilized by autoclaving at 120°C. for one hour, then infested with F. culmorum.

B. Seed treated with the Penicillium isolate/autoclaved and infested sand.

C. Seed treated with ceresan/autoclaved and infested sand.

D. Untreated seed/autoclaved and uninfested sand.

Ten seeds were planted per pot and the pots randomized on the greenhouse bench. After 15 days the seedlings emerged were counted and their growth assessed. The results are shown in Appendix Tables 76 a-c and summarized in Table 32.

They showed that treating wheat seeds with spores of the Penicillium sp. significantly improved seedling stand and shoot growth in autoclaved and infested sand. There was however, no significant improvement in root growth.

Table 32. Effect of seed treatments with spores of Penicillium sp. on seedling stand and growth in sterile infested sand.

Treatments	Mean		
	Seedling stand (no.)	Shoot growth (mm.)	Root growth (mm.)
A. Untreated seed/sterile & infested sand.	2.5	145	108
B. Seed treated with Penicillium/sterile & infested sand.	6.3	199	110
C. Seed treated with ceresan/sterile & infested sand.	9.3	221	150
D. Untreated seed/sterile & infested sand.	9.0	223	149
L.S.D. P. = 0.05	1.3	5.2	23.8
P. = 0.01	1.9	7.5	34.3
P. = 0.001	2.8	11.1	50.4

Experiment 44:

Effects of various antagonistic micro-organisms on F. culmorum in non-sterile soil

The 4 micro-organisms (Penicillium sp., Trichoderma sp. and the 2 spore-forming bacteria F & H) isolated in Exp. 42 were re-tested on agar media for their antagonism to F. culmorum.

Separate batches of wheat seed were treated with these organisms viz:-

(1) By mixing with spores of the respective fungi as described in Exp. 43.

(2) By soaking seed in suspensions of the respective bacteria. The bacteria were grown in nutrient broth (see Appendix p. 192) for 10 days and 25 g. wheat seed were soaked in 25 ml. of these cultures for one hour. The seeds were then air-dried for 30 mins.

Five pots were set up for the following treatments:

A. Untreated seed/infested soil.

B. Seed treated with ceresan/infested soil.

C. Seed treated with Trichoderma sp./infested soil.

D. Seed treated with Penicillium sp./infested soil

E. Seed treated with bacterium 'F'/infested soil.

F. Seed treated with bacterium 'H'/infested soil.

G. Untreated seed/non-infested soil.

Fifteen seeds were planted per pot, and the pots randomized on the greenhouse bench.

The assessments of disease effects were carried out 21 days after sowing by counting seedling emerged and measuring shoot height and root length. The summarized result (Table 33) indicate that bacterium 'H' significantly improved seedling and shoot growth to a degree similar to that obtained with ceresan-treated seed. Bacterium 'F' also significantly improved seedling stand and shoot growth (Plate 20), but no effect could be shown for Trichoderma sp. and Penicillium sp. None of the 4 micro-organisms had any significant effect on root growth. The full results are presented in Appendix Table 77 a-c.

Time did not allow development of this line of investigation. However, the above results indicate the possibility of controlling F. culmorum by antagonistic organisms.

Table 33. Effect of seed treatments with various antagonistic micro-organisms on seedling stand and growth in non-sterile and infested soil

Treatments	Mean		
	Seedling stand (no.)	Shoot growth (mm.)	Root growth (mm.)
A. Untreated seed/infested soil.	6.8	208	189
B. Seed treated with ceresan/ infested soil.	12. 4	284	266
C. Seed treated with *Trichoderma* sp./infested soil.	7.8	234	194
D. Seed treated with *Penicillium* sp./infested soil.	7.0	214	183
E. Seed treated with bacterium 'F'/infested soil.	9.4	238	192
F. Seed treated with bacterium 'H'/infested soil.	10.4	265	198
G. Untreated seed/non-infested soil.	12.0	285	270
L.S.D.			
P. = 0.05	1.8	25.5	23.6
P. = 0.01	2.4	34.5	32.0
P. = 0.001	3.2	46.2	42.9

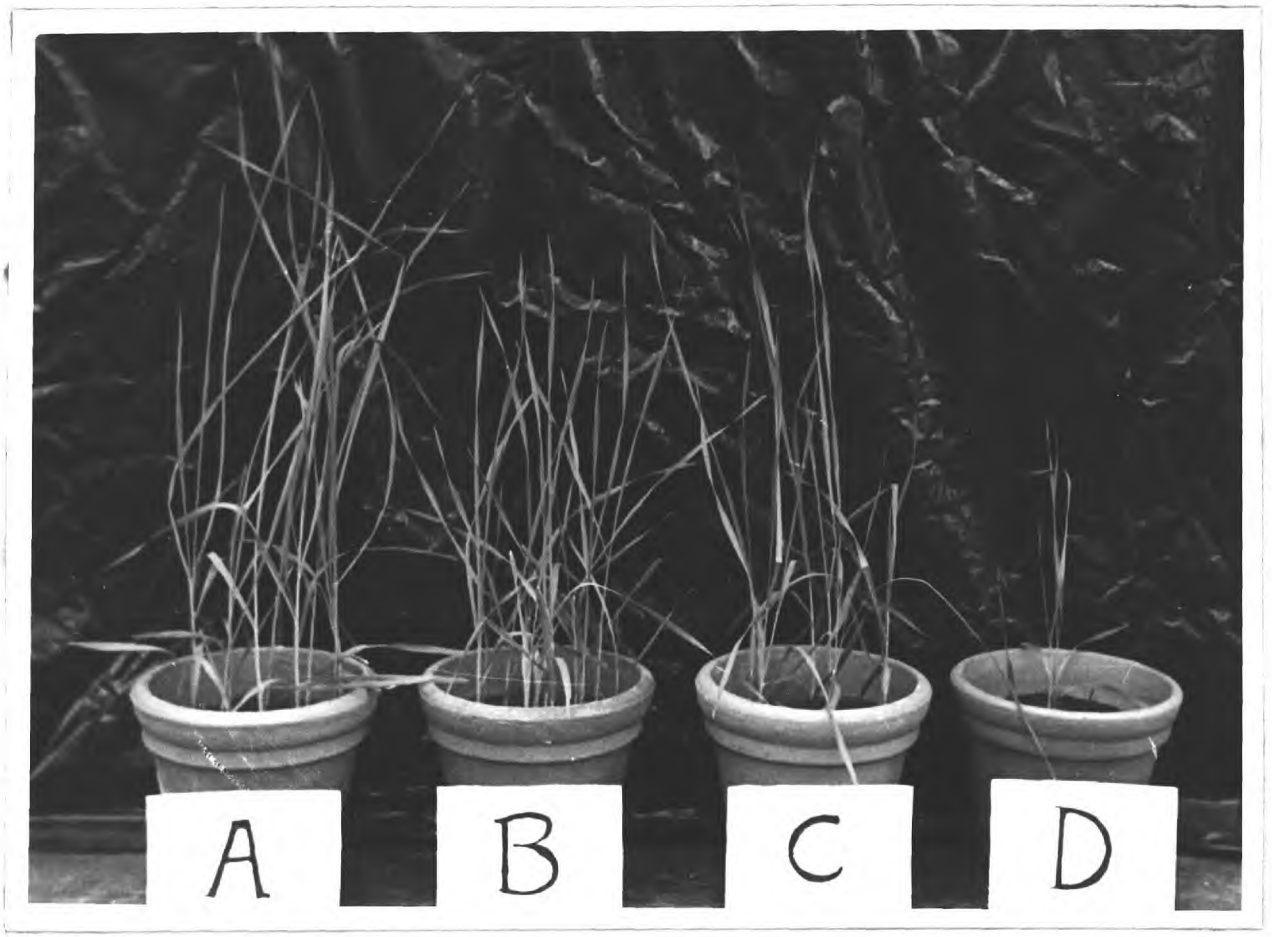


Plate 20: Effects of treating wheat seed with antagonistic micro-organisms on stand and growth in infested soil.

A. Ceresan-treated seed/infested soil.
B. Seed treated with bacterium 'F'/infested soil.
C. Seed treated with bacterium 'H'/infested soil.
D. Untreated seed/infested soil.

DISCUSSION

F. culmorum has three main effects on the development of wheat seedlings: it reduces the stand by killing the germinating seedling either before or after emergence and it reduces the shoot height and the root length of those seedlings which survive. These effects were readily demonstrated by sowing seed in soil infested with the fungus or by soaking seed in a spore suspension of the fungus before sowing (Exp. 1 & 2). The visible symptoms are preceded and are caused by, the growth of the fungus on the young root and coleoptile. F. culmorum was found to have colonized the root and coleoptile surfaces, particularly those adjacent to the seed, only 3 days after planting (Exp. 4 & 5). As the roots elongate they are further colonized, both on the surface and internally (Exp. 6); only the root tip remains free of F. culmorum and indeed of any fungus. This apparent sterility of the root tip has been noted by other workers, e.g. Stenton (1958) on pea root.

A number of factors influence the severity of the disease, and the effects of some of them have been demonstrated experimentally in this investigation. Briefly, the disease is most severe:

(1) At high temperatures (25°C.) in soils of low (30% W H C) moisture content (Exp. 7 & 8).

(2) When large amounts of fungal inoculum are added to soil (Exp. 9).

(3) When the inoculum is placed near the germinating seed (Exp. 10).

(4) On very young seedlings (Exp. 11 & 12).

In addition, there are indications that some varieties are more adversely affected than others, eg. 'Svenno' is more susceptible than 'Prestige' and 'Atson' (Exp. 13).

The most striking feature of these results is the vulnerability of the seedlings in the first few days of growth and conversely, the development of a certain measure of resistance in older seedlings. Experiments 11 & 12 illustrate this most clearly. Most losses in seedlings occurred when the inoculum was added to the soil either at sowing or up to 4 days after; beyond that the effects of the fungus on the seedlings were much less severe.

The study of root and coleoptile colonization suggests that the tissues adjacent to the seed are the most susceptible and there is further evidence of this from Exp. 10 in which inoculum was placed in different positions relative to the germinating seeds. Here, seedling stand was significantly reduced only where the inoculum of F. culmorum was initially in close contact with seed. It is presumably these first formed tissues of the seedlings which with time

develop some resistance.

The damping-off phase of this disease can be viewed in terms of a competition between the growth of the pathogen and the maturation of the seedlings and, as Leach (1947) has suggested, is severe when conditions favour growth of the pathogen, not the host. Thus when the inoculum is placed near the seed there is an opportunity for the fungus to become established before the tissues develop any resistance and a large number of seedlings are killed. When inoculum is placed at some distance from the seed,contact between pathogen and host is delayed, the host tissues become somewhat more resistant and less seedlings are killed. Similarly, damping-off is severe in soils of low moisture in which the germination process appears to be slowed down.

It would have been interesting to examine the growth rates in non-infested soil of the 5 varieties tested in Exp. 13. It is possible that the differences in susceptibility observed are directly related to the growth rate, i.e. the least susceptible variety is the one with the most rapid germination and growth.

In the light of these results it becomes clear that even a limited restriction of the pathogen's activities during the very earliest stages of seedling growth is likely to give some measure of control. In this respect it is

hardly surprising that substantial control was obtained by dusting with ceresan (Exp. 14), since this material markedly inhibited the growth of F. culmorum in vitro (Exp. 15). The extent to which the fungus colonizes the surfaces of roots from treated seed is remarkable however, in view of the degree of control obtained in infested soil (Exp. 18). It is true that colonization is somewhat less rapid than that on roots from untreated seed but the apparent differences are scarcely large enough to provide a satisfactory explanation. The method used to determine colonization may itself be misleading. For each washed root segment, growth or not of F. culmorum on an agar plate indicates colonization or lack of it. The method gives no indication of the extent to which the root-piece is colonized. It is thus possible that substantial differences in the degree of colonization of roots from treated seed and those from untreated seed have been obscured. In particular, the method gives no indication of the extent to which the inner tissues of the root are colonized. This was partly overcome by surface sterilizing the root pieces before plating (Exp. 19). While again the results give no quantitative estimate they do, at least, indicate rather more striking differences between the roots from treated seed and those from untreated seed. It may well be that the degree

of control obtained stems from the failure of the fungus to penetrate tissues derived from treated seed. The results of Exp. 20 lend weight to this argument. There is evidence, here, that homogenates of roots from treated seed inhibit the growth of F. culmorum in vitro, which suggests that mercury is absorbed by the germinating seed and translocated to the root tissues. That mercury can be absorbed by plants and translocated is well established particularly from the experiments of Lundegårdh, (1924), De Paolis, (1931), Pickard & Martin (1960) and Vir & Bajaj (1964).

The control of F. culmorum by ceresan seed dressings is thus envisaged as:-

(1) A direct fungitoxic effect at the surface and possibly in a zone around the seed as particles of the material are washed off.

(2) An effect at a distance, in which colonization is restricted by mercury translocated to the roots.

There was no evidence that treating seed enhanced the growth of seedlings(Exp. 21) which itself might lead to some control. Possibly measurements of root length and shoot height are too crude. The critical zone for infection is near the seed and it may be that changes leading to resistance occur more rapidly in the tissues derived from treated seed.

In experiments with infested soil, seed dressings of PP781 appear as effective as ceresan (Exp. 22) yet other results (Exp. 24) suggest that *in vitro* the amount of PP781 on treated seed is less toxic to *F. culmorum* than the equivalent dressing (wt./wt.) of ceresan. Moreover, there is no evidence that PP781 is translocated to the roots (Exp. 26) or advantageously affects seedling growth (Exp. 27). In view of these results, the degree of control obtained with PP781 is remarkable, and is worth investigating further. An examination of root colonization in relation to seed treatment with PP781 would be useful in this connexion.

The degree of control obtained with soil applications of aldrin (Exps. 28 & 29) and the effects on root colonization (Exp. 30) are even more striking in view of the lack of effects on the growth of *F. culmorum* *in vitro* (Exps. 32, 33, 34). Experiments with root homogenates (Exp. 35) gave no support to the hypothesis that aldrin is absorbed by the seedlings and converted to substances which are fungitoxic. It is true that shoot growth is increased in aldrin-treated soil but this alone seems insufficient to account for the control obtained. The most plausible explanation is that some aldrin is converted in soil to a fungitoxic substance. There is some circumstantial evidence

for this. Extracts of aldrin-treated soil inhibit spore germination of F. culmorum (Exp. 36) and also the growth of the fungus in a liquid medium (Exp. 37). If any breakdown compound is involved then dieldrin appears the most likely. Several investigators have reported that small amounts of this compound are formed when aldrin is applied to soil (Lichtenstein & Schulz, 1959; Wheatley et al., 1962) and the results of Exps. 38 & 39 clearly show that low levels of dieldrin markedly inhibit F. culmorum in vitro.

The experiments on biological control can only be regarded as preliminary ones. While it is normally not too difficult to isolate from soil, micro-organisms which in vitro inhibit the growth of a pathogen, these are seldom found to do so in experiments with soil. In this respect the control obtained with the bacterial isolates in non-sterile soil (Exp. 44) is particularly encouraging and merits further investigation.

SUMMARY

1. When wheat seed was planted in soil infested with F. culmorum there was a considerable reduction in seedling stand and in the root length and shoot height of seedlings which survived. Similar effects were obtained (i) by treating seed with a suspension of F. culmorum before sowing and (ii) pouring a suspension of F. culmorum over soil in which seeds had been planted.

2. An examination of seedlings grown in infested soil showed that F. culmorum begins to colonize the root and the coleoptile surfaces within 3 days. After 11 days most of the available surface appears to be colonized; only the root tips remain free of fungus.

3. A number of factors influencing the disease were investigated, viz:- soil moisture and temperature, inoculum size and position, age of the seedlings and the varietal susceptibility. The main results were:

(a) The disease was most severe at high temperatures, in soils of low moisture content and least at low temperatures in soils of high moisture content.

(b) The disease became progressively more severe as the inoculum was increased from 5 to 50% by weight of soil. Seedling stand was significantly reduced only

where the inoculum was placed adjacent to the seeds.

(c) The seedlings were most severely attacked when inoculations were carried out during the first 4 days after sowing, and after this the seedlings were less affected.

(d) The general order of susceptibility of the 5 varieties tested was:

Svenno → Lineg → Capelle Desprez → Prestige → Atson

Most susceptible — Least susceptible

4. Ceresan (Methoxyethyl mercuric chloride) was markedly toxic to F. culmorum in vitro and treating seed with ceresan significantly improved the seedling stand and growth in soil infested with F. culmorum. Colonization of the surface of roots from treated seeds was slightly less rapid than on roots from untreated, and internal colonization also appeared to be considerably less. Homogenates of roots from treated seeds inhibited the growth of F. culmorum in vitro. Ceresan had apparently no effect on seedling vigour.

5. The chemical PP781 [4 (2-chlorophenylhydrazone)-3-methyl-5-isoxazolone] also inhibited F. culmorum in vitro and damping-off of wheat in infested soil. Root homogenates from treated seeds had no effect on the growth of F. culmorum in culture. Seedlings derived from treated

seed were apparently no more vigorous than those grown from untreated seed.

6. Applications of a 10% aldrin dust improved seedling stand and growth in soil infested with F. culmorum though not to the same degree as ceresan or PP781. Root colonization of seedlings grown in aldrin-treated soil was less extensive than on corresponding controls. Seedlings grown in aldrin-treated soil had slightly better shoot growth than seedlings grown in untreated soil. Aldrin had no effect on the growth of F. culmorum in vitro nor had root homogenates derived from seed grown in soil treated with aldrin; but extracts of this soil (with or without wheat seedlings grown in it) inhibited spore germination and growth in a liquid medium.

7. Small quantities (100 - 6.25 p.p.m.) of dieldrin markedly inhibited spore germination of F. culmorum and growth of the fungus both in a liquid and on an agar medium.

8. In an experiment to examine the long-term effects of seed dressings with ceresan and a soil application of aldrin, marked effects of the treatments on stand, tillering and yield were apparent at harvesting.

9. Four micro-organisms (a Penicillium sp., a Trichoderma sp. and 2 spore-forming bacteria) were isolated from soil previously infested with F. culmorum. These organisms

markedly inhibited the growth of this fungus *in vitro*. In a test in non-sterile infested soil with *F. culmorum* the two bacteria gave some control of damping-off.

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APPENDICES

Appendix 1: General

Table a

Effect of age of inoculum on disease incidence

Replicates	Age of inoculum in (days)		
	5	10	15
i	7	9	10
ii	8	5	4
iii	10	7	9
iv	9	8	8
Total stand	34	29	31
%D.O.	43.3	51.6	48.3

Total out of 60

D.O. = Damping-off

The amount of inoculum used was 5% w/w.

Rose-Bengal-Streptomycin Agar

This was prepared as follows:

	KH_2PO_4	1.0 g.
	$MgSO_4.7H_2O$	0.5 g.
	Peptone	5.0 g.
	Dextrose	10.0 g.
	Agar	20.0 g.
	Water	980 ml.
x	Rose-Bengal	10 ml.
xx	Streptomycin	10 ml.

x Rose-Bengal: 33 p.p.m. in the final medium, was added before autoclaving.

xx Streptomycin: 30 p.p.m. in the final medium, it was autoclaved separately and added to rest of medium afterwards. The basic medium was autoclaved at 120°C for 20 min.

Cotton-blue/lactophenol (Linder, 1929)

Cotton-blue	1.0 g.
Lactophenol	100 ml.

Then, 10 ml. of the mixture was dissolved in 90 ml. of lactophenol.

Lactophenol

Phenol (pure crystals)	20 g.
Lactic acid	20 ml.
Glycerine	40 g.
Distilled water	20 ml.

Nutrient Broth

5 gms. 'Lab. Lemco' were dissolved in 150 ml. of hot tap water, 10 gms. peptone and 5 gms. NaCl were mixed in a mortar. Then the mixture added to the hot lemco solution and the final volume was made up to one litre. This was filtered hot and neutralized to pH 7.0.

Minor elements solution:

500 ml. containing

$FeSO_4.7H_2O$	0.125 g.
$ZnSO_4.7H_2O$	0.110 g.
$CuSO_4.5H_2O$	0.020 g.
$MnSO_4.4H_2O$	0.020 g.
$Na_2MoO_4.2H_2O$	0.025 g.

and acidified with of H_2SO_4 to clarify.

Wheat Varieties:

'Svenno' (see p. 19)

'Prestige', from C. W. Masters Ltd., Norfolk.

'Lineg', from Nickersons, Field House, Grimsby.

'Capelle Desprez', from Elsoms (Spalding) Ltd. Seeds.

'Atson', from Dixons & Sons, (Ware) Herts.

The five varieties were obtained in April 1964.

Appendix 2: Tables of the Results.

In the analysis of variance several symbols have been used, which represent the following:

D.F.	Degrees of freedom
S.S.	Sums of squares
M.S.	Mean square
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.

L.S.D.	Least Significant Difference

Table 1: Effect of F.culmorum on seedling stand.

Time (days)	Treatments							
	Soil + F.culmorum				Soil alone (Control)			
	Replicates			Mean	Replicates			Mean
	i	ii	iii		i	ii	iii	
3	3	4	7	4	13	14	9	12
5	8	10	8	8	14	12	14	13
7	12	11	10	11	14	15	14	14
9	6	11	10	9	14	13	15	14
11	13	8	9	10	13	14	15	14
13	5	9	9	7	15	14	13	14
15	6	8	12	8	14	14	12	13
17	13	10	9	10	15	13	15	14
19	14	12	10	12	14	15	13	14
21	10	11	9	10	14	15	13	14

Analysis of variance:

Source	D.F.	S.S.	M.S.	F.
Treatments (Ts.)	1	303.76	303.76	135.00***
Time (Te.)	9	96.49	10.72	4.76**
Interaction (Ts.X Te.)	9	30.74	3.41	1.51 n.s.
Error	40	90.00	2.25	
Total	59	520.99		

Table 2: Effect of F.culmorum on root growth.
(Log. transformation of root lengths)

Time (days)	Treatments							
	Soil + F.culmorum				Soil alone (Control)			
	Replicates			Mean	Replicates			Mean
	i	ii	iii		i	ii	iii	
3	1.114	0.954	0.954	1.007	1.447	1.322	1.079	1.283
5	1.519	1.544	1.602	1.555	1.969	1.909	2.093	1.990
7	1.924	1.973	1.949	1.949	2.167	2.033	2.134	2.111
9	2.061	2.114	2.009	2.061	2.236	2.290	2.233	2.253
11	2.161	2.093	2.127	2.127	2.320	2.330	2.296	2.316
13	2.241	2.230	2.225	2.232	2.354	2.346	2.324	2.342
15	2.274	2.146	2.207	2.209	2.340	2.344	2.360	2.348
17	2.295	2.292	2.318	2.302	2.389	2.407	2.410	2.402
19	2.277	2.316	2.313	2.302	2.447	2.430	2.408	2.429
21	2.337	2.286	2.316	2.313	2.457	2.446	2.433	2.445

Analysis of variance:

Source	D.F.	S.S.	M.S.	F.
Treatments (Ts.)	1	0.52	0.52	137.0***
Time (Te.)	9	7.91	0.88	231.5***
Interaction (Ts.X Te)	9	0.14	0.015	3.9**
Error	40	0.15	0.0038	
Total	59	8.72		

Table 3: Effect of F.culmorum on shoot growth.
(Log. transformation of shoot hieghts)

Time (days)	Treatments							
	Soil + F.culmorum				Soil alone (Control)			
	Replicates			Mean	Replicates			Mean
	i	ii	iii		i	ii	iii	
3	0.699	0.602	0.699	0.667	0.903	1.000	0.954	0.952
5	1.041	1.146	1.255	1.148	1.580	1.602	1.724	1.635
7	1.623	1.763	1.699	1.695	1.851	1.748	1.949	1.850
9	1.924	2.004	1.909	1.946	2.083	2.137	2.127	2.122
11	1.982	1.987	2.021	1.997	2.308	2.270	2.253	2.277
13	1.987	2.104	2.117	2.069	2.303	2.308	2.314	2.308
15	2.121	2.167	2.176	2.155	2.328	2.340	2.403	2.357
17	2.290	2.215	2.246	2.250	2.425	2.433	2.446	2.435
19	2.281	2.272	2.324	2.292	2.477	2.468	2.464	2.470
21	2.344	2.335	2.369	2.349	2.502	2.487	2.500	2.496

Analysis of variance:

Source	D.F.	S.S.	M.S.	F.
Treatments (Ts.)	1	0.81	0.81	289.3***
Time (Te.)	9	14.36	1.60	571.4***
Interaction (Ts.X.Te)	9	0.14	0.016	5.7***
Error	40	0.11	0.0028	
Total	59	15.42		

Table 4: Effect of seed inoculum & soil inoculum on seedling stand.

Time (days)	Treatments					
	Seed inoculum			Soil inoculum		
	Replicates		Mean	Replicates		Mean
	i	ii		i	ii	
7	11	7	9	10	9	9
9	8	12	10	7	8	7
11	10	9	9	7	9	8
13	11	11	11	10	7	8
15	9	11	10	7	9	8

Analysis of variance:

Source	D.F.	S.S.	M.S.	F.
Treatments (Ts.)	1	12.80	12.80	$4.57^{n.s.}$
Time (Te.)	4	2.80	0.70	$0.25^{n.s.}$
Interaction (Ts.X Te.)	4	6.20	1.55	$0.55^{n.s.}$
Error	10	28.00	2.80	
Total	19	49.80		

Table 5: Effect of seed inoculum & soil inoculum on root growth. (Log. transformation of root lengths).

Time (days)	Treatments					
	Seed inoculum			Soil inoculum		
	Replicates		Mean	Replicates		Mean
	i	ii		i	ii	
7	1.851	1.909	1.880	1.756	1.881	1.818
9	2.124	2.111	2.117	2.068	2.053	2.061
11	2.140	2.176	2.158	2.100	2.041	2.071
13	2.283	2.303	2.298	2.220	2.201	2.211
15	2.290	2.334	2.312	2.212	2.272	2.242

Analysis of variance:

Source	D.F.	S.S.	M.S.	F.
Treatments (Ts.)	1	0.026	0.026	17.3^{**}
Time (Te)	4	0.465	0.116	77.3^{***}
Interaction (Ts.X Te.)	4	0.001	0.0003	$0.2^{n.s.}$
Error	10	0.015	0.0015	
Total	19	0.507		

Table 6: Effect of seed inoculum & soil inoculum on shoot growth. (Log. transformation of shoot heights.)

Time (days)	Treatments					
	Seed inoculum			Soil inoculum		
	Replicates		Mean	Replicates		Mean
	i	ii		i	ii	
7	1.544	1.699	1.622	1.544	1.634	1.589
9	2.009	2.000	2.004	1.978	1.973	1.975
11	2.188	2.140	2.164	2.038	2.049	2.044
13	2.170	2.225	2.198	2.045	2.107	2.076
15	2.248	2.250	2.249	2.227	2.127	2.177

Analysis of variance:

Source	D.F.	S.S.	M.S.	F.
Treatments (Ts.)	1	0.027	0.027	10.8^{**}
Time (Te.)	4	0.921	0.230	92.0^{***}
Interaction (Ts.X Te.)	4	0.010	0.0025	$1.0^{n.s.}$
Error	10	0.025	0.0025	
Total	19	0.983		

Effect of growing seedlings from infested seed, in infested soil and in soil inoculated with a spore suspension after planting, on:

1. Seedling stand:

Table 7:

Replicates	Treatments			
	A	B	C	D
i	11	6	8	9
ii	10	6	7	8
iii	13	4	9	5
iv	13	6	6	9
v	9	4	8	9
Mean	11.2	5.2	7.6	8

Analysis of variance:

Source	D.F.	S.S.	M.S.	F.
Replicates	4	3.5	0.87	
Treatments	3	91.2	30.40	11.64**
Error	12	31.3	2.61	
Total	19	126.0		

2. Root lengths: (in mm.)

Table 8:

Replicates	Treatments			
	A	B	C	D
i	318	209	264	290
ii	293	214	257	264
iii	303	192	258	252
iv	279	256	252	210
v	284	231	285	273
Mean	295	220	263	258

Analysis of variance:

Source	D.F.	S.S.	M.S.	F.
Replicates	4	1,484.2	371.0	
Treatments	3	14,169.2	4,723.0	9.23**
Error	12	6,137.8	511.5	
Total	19	21,791.2		

3. Shoot heights (in mm.)

Table 9:

Replicates	Treatments			
	A	B	C	D
i	321	244	288	287
ii	303	222	270	288
iii	302	210	295	227
iv	292	261	259	260
v	317	243	312	279
Mean	307	236	285	268

Analysis of variance:

Source	D.F.	S.S.	M.S.	F.
Replicates	4	2,855.5	713.8	
Treatments	3	13,884.4	4,628.1	15.10**
Error	12	3,682.1	306.4	
Total	19	20,422.0		

Table 10: Percentage colonization of:

a. Root surfaces

Days									
3		5		7		9		11	
+	-	+	-	+	-	+	-	+	-
3	15	16	26	34	40	50	49	77	45
20.0		38.0		45.9		50.5		63.1	

b. Coleoptile surfaces

Days									
3		5		7		9		11	
+	-	+	-	+	-	+	-	+	-
1	1	2	2	4	5	5	7	6	8
50.0		50.0		44.4		41.6		42.8	

+ = Number of segments with F.culmorum

- = Number of segments without F.culmorum

Table 11: Percentage colonization of:

a. Root surfaces

Seed inoculum						Soil inoculum					
Days						Days					
7		9		11		7		9		11	
+	-	+	-	+	-	+	-	+	-	+	-
3	77	13	67	19	61	47	33	68	12	66	14
3.75		16.25		23.75		58.75		85.00		82.50	

b. Coleoptile surfaces

Seed inoculum						Soil inoculum					
Days						Days					
7		9		11		7		9		11	
+	-	+	-	+	-	+	-	+	-	+	-
3	37	6	34	9	31	19	21	25	15	26	14
7.5		15.0		22.5		47.5		62.5		65.0	

+ = Number of segments with F.culmorum

- = Number of segments without F.culmorum

Table 12: Effect of soil moisture on rate of seedling emergence in infested (+) and non-infested (-) soil.

Days	% soil moisture					
	30		50		70	
	% emergence		% emergence		% emergence.	
	+	-	+	-	+	-
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	6	28	10	32
4	0	0	18	42	24	60
5	4	16	34	54	38	76
6	14	38	40	70	54	88
7	26	62	46	84	66	96
8	34	86	48	98	80	96
9	38	96	48	98	86	96
10	38	96	48	98	86	96

+ Infested soil.

- Non-infested soil.

Table 13: Effect of soil moisture on seedling stand (after 10 days).

%W.H.C. of soil	Treatments	Replicates					Mean
		i	ii	iii	iv	v	
30	+	4	7	3	2	3	3.8
	-	10	9	9	10	10	9.6
50	+	5	8	5	4	7	5.8
	-	10	9	10	10	10	9.8
70	+	9	7	9	10	8	8.6
	-	10	10	8	10	10	9.6

(+) infested & (-) non-infested soil

Analysis of variance:

Source	D.F.	S.S.	M.S.	F.
Treatments (Ts.)	1	97.20	97.20	64.8***
Moisture levels (M.)	2	28.87	14.43	9.6***
Interaction (Ts.X M.)	2	29.40	14.70	9.8***
Error	24	36.00	1.50	
Total	29	191.47		

Table 14: Effect of soil moisture on seedling stand after 21 days.

% W.H.C. of soil	Treatments	Replicates					Mean
		i	ii	iii	iv	v	
30	+	4	4	2	2	3	3.0
	-	10	9	9	10	10	9.6
50	+	5	4	4	5	7	5.0
	-	9	9	10	10	10	9.6
70	+	9	7	8	9	7	8.0
	-	10	10	10	8	9	9.4

(+) infested & (-) non-infested soil

Analysis of variance:

Source	D.F.	S.S.	M.S.	F.
Treatments (Ts.)	1	132.30	132.30	161.3***
Moisture levels (M.)	2	29.07	14.53	17.7***
Interaction (Ts.X M.)	2	34.40	16.20	19.8***
Error	24	19.60	0.82	
Total	29	215.37		

Table 15: Effect of soil moisture on root growth in infested (+) and non-infested (-) soil.

% W.H.C. of soil	Treatments	Replicates					Mean
		i	ii	iii	iv	v	
30	+	79	88	104	162	85	104
	-	279	305	255	249	263	270
50	+	195	234	146	165	115	171
	-	282	288	309	258	245	276
70	+	220	199	216	201	258	219
	-	245	284	267	293	291	276

Root lengths in (mm.)

Analysis of variance:

Source	D.F.	S.S.	M.S.	F.
Treatments (Ts.)	1	90,310.5	90,310.5	101.3***
Moisture levels (M.)	2	18,587.3	9,293.6	10.4***
Interaction (Ts.X M.)	2	15,030.9	7,515.4	8.4**
Error	24	21,396.0	891.5	
Total	29	145,324.7		

Table 16: Effect of soil moisture on shoot growth in infested (+) and non-infested (-) soil.

% W.H.C. of soil	Treatments	Replicates					Mean
		i	ii	iii	iv	v	
30	+	172	221	161	220	195	194
	-	250	269	288	273	266	269
50	+	238	283	274	218	221	247
	-	279	257	269	305	280	278
70	+	249	267	223	264	286	258
	-	298	313	262	272	255	280

Shoot heights in (mm.)

Analysis of variance:

Source	D.F.	S.S.	M.S.	F.
Treatments (Ts.)	1	13,824.5	13,824.5	25.9***
Moisture levels (M.)	2	7,986.1	3,993.0	7.5**
Interaction (Ts.X M)	2	4,454.1	2,227.0	4.2*
Error	24	12,837.2	534.9	
Total	29	39,101.9		

Table 17: Effect of soil moisture and Temperature on seedling stand (after 10 days)

Treatments		Replicates					Total
% WHC	Temperature	i	ii	iii	iv	v	
30	2-8°C	7	10	6	9	8	40
	15-18°C	4	5	6	3	3	21
	25°C	2	5	3	3	2	15
50	2-8°C	8	7	10	10	9	44
	15-18°C	6	3	4	7	7	27
	25°C	4	7	4	6	9	30
70	2-8°C	10	9	10	8	9	46
	15-18°C	9	8	8	7	10	42
	25°C	8	7	6	8	9	38

Total out of 50 seeds.

Table 18: Effect of soil moisture and temperature on seedling stand (after 21 days)

Treatments		Replicates					Total
% WHC	Treatments	i	ii	iii	iv	v	
30	2-8°C	6	9	6	8	8	37
	15-18°C	4	3	6	2	2	17
	25°C	1	2	1	2	2	8
50	2-8°C	8	6	8	7	9	38
	15-18°C	5	3	4	7	5	24
	25°C	4	6	4	3	6	23
70	2-8°C	9	9	10	7	10	45
	15-18°C	9	7	8	6	9	39
	25°C	6	7	3	8	8	32

Total out of 50 seeds.

Table 19: Effect of soil moisture and temperature on root growth.

Treatments		Replicates					Mean
% WHC	Temperature	i	ii	iii	iv	v	
30	2-8°C	230	247	230	214	203	224
	15-18°C	132	193	188	104	147	153
	25°C	81	62	113	70	69	79
50	2-8°C	267	260	240	230	223	242
	15-18°C	213	179	244	107	191	187
	25°C	195	140	166	146	136	157
70	2-8°C	270	293	266	254	301	277
	15-18°C	262	246	225	212	207	230
	25°C	193	214	190	243	206	209

Root lengths in (mm.)

Analysis of variance:

Source		D.F.	S.S.	M.S.	F.
Treatments					
Temperature	(T.)	2	76,106.1	38,053.1	48.6***
Moisture	(M.)	2	56,242.3	28,121.1	35.9***
Interaction	(TXM.)	4	8,704.0	2,176.0	2.8n.s.
Error		36	28.184.4	782.9	
Total		44	169,236.8.		

Table 20: Effect of soil moisture and temperature on shoot growth.

Treatments		Replicates					Mean
% WHC	Temperature	i	ii	iii	iv	v	
30	2-8°C	220	197	259	260	241	235
	15-18°C	193	130	122	231	193	174
	25°C	282	271	267	273	273	273
50	2-8°C	279	230	281	251	269	262
	15-18°C	260	228	200	188	233	222
	25°C	292	220	288	243	290	266
70	2-8°C	282	263	277	265	254	268
	15-18°C	229	280	230	246	274	252
	25°C	274	287	255	275	264	271

Shoot height in (mm)

Analysis of variance

Source		D.F.	S.S.	M.S.	F.
Treatments.					
Temperature	(T.)	2	23,729.9	11,864.9	17.4 **
Moisture	(M.)	2	10,036.9	5,019.5	7.4 **
Interaction	(TXM.)	4	8,592.4	2,148.1	3.2
Error		36	24,510.4	680.8	
Total		44	66,869.7		

Table 21: Effect of inoculum size on seedling stand.

Replicates	% level of inoculum				
	5	10	20	30	50
i	4	6	6	4	1
ii	8	4	2	1	2
iii	5	7	4	2	3
iv	9	5	3	3	1
v	6	8	3	4	2
Mean	6.4	6.0	3.6	2.8	1.8

Analysis of variance:

Source	D.F.	S.S.	M.S.	F.
Replicates	4	3.84	0.96	
Treatments	4	80.64	20.16	7.6**
Error	16	42.16	2.64	
Total	24	126.64		

Table 22: Effect of inoculum size on root growth.

Replicates	% level of inoculum				
	5	10	20	30	50
i	204	184	203	186	93
ii	220	209	100	150	102
iii	214	173	120	171	80
iv	148	190	221	109	109
v	179	248	231	138	123
Mean	193	201	175	151	101

Root lengths in (mm.)

Analysis of variance:

Source	D.F.	S.S.	M.S.	F.
Replicates	4	3,902.0	975.6	
Treatments	4	32,045.2	8,011.3	5.7**
Error	16	22,374.8	1,398.4	
Total	24	58,322.0		

Table 23: Effect of inoculum size on shoot growth.

Replicates	% level of inoculum				
	5	10	20	30	50
i	215	221	198	147	261
ii	233	244	243	236	205
iii	220	200	186	221	130
iv	258	217	174	190	247
v	226	196	150	111	180
Mean	230	216	190	181	205

Shoot height in (mm.)

Analysis of variance:

Source	D.S.	S.S.	M.S.	F.
Replicates	4	10,664.6	2,666.1	
Treatments	4	7,753.4	1,938.3	$1.8^{n.s.}$
Error	16	18,589.8	1,161.9	
Total	24	37,007.8		

Table 24: Effect of inoculum position on seedling stand.

Replicates	Inoculum position					
	A	B	C	D	E	F
i	11	13	14	7	4	8
ii	13	14	12	6	9	7
iii	14	12	9	7	8	6
iv	13	11	14	7	7	11
v	14	14	13	6	3	9
Mean	13.0	12.8	12.4	6.6	6.2	8.2

Analysis of variance:

Source	D.F.	S.S.	M.S.	F.
Replicates	4	5.5	1.4	
Treatments	5	258.7	51.7	15.4***
Error	20	67.3	3.4	
Total	29	331.5		

Table 25: Effect of inoculum position on root growth.

Replicants	Inoculum position					
	A	B	C	D	E	F
i	309	264	254	235	189	260
ii	263	282	280	200	218	188
iii	266	270	263	218	223	231
iv	289	281	291	222	220	244
v	277	255	292	204	140	279
Mean	281	270	276	216	198	240

Roct lengths in (mm.)

Analysis of variance:

Source	D.F.	S.S.	M.S.	F.
Replicates	4	1,501.8	375.5	
Treatments	5	29,744.7	5,948.9	9.9***
Errcr	20	12,062.2	603.1	
Total	29	43,308.7		

Table 26: Effect of inoculum position on shoot growth.

Replicates	Inoculum position					
	A	B	C	D	E	F
i	283	284	277	203	198	291
ii	313	299	303	240	212	278
iii	281	298	273	250	243	244
iv	272	250	291	250	261	229
v	315	271	226	277	246	268
Mean	293	280	275	244	232	262

Shoot height in (mm.)

Analysis of variance:

Source	D.F.	S.S.	M.S.	F.
Replicates	4	1,237.1	309.3	
Treatments	5	13,232.9	2,646.6	3.9*
Error	20	13,584.9	679.3	
Total	29	28,054.9		

Table 27 : Effect of age of seedling at time of inoculation on final stand.

Replicates	Time of inoculation (in days)							
	0	1	2	3	4	5	6	7
i	7	6	2	4	7	8	7	10
ii	7	4	5	7	8	8	10	8
iii	7	5	6	5	7	8	9	7
Mean	7.0	5.0	4.3	5.3	7.3	8.0	8.7	8.3

Analysis of variance:

Source	D.F.	S.S.	M.S.	F.
Replicates	2	2.25	0.3	
Treatments	7	57.16	8.2	4.9**
Error	14	23.09	1.7	
Total	23	82.50		

Table 28 : Effect of age of seedling at time of inoculation on final stand.

Time days	Treatments							
	Infested soil				Non-infested soil			
	Replicates			Mean	Replicates			Mean
	i	ii	iii		i	ii	iii	
0	4	6	6	5.3	9	9	9	9.0
1	5	4	5	4.7	9	8	9	8.7
2	3	7	5	5.0	7	9	8	8.0
3	6	4	8	6.0	7	9	8	8.0
4	6	8	5	6.3	10	10	8	9.3
5	8	8	7	7.7	7	8	9	8.0
6	8	9	9	8.7	10	8	8	8.7
7	10	7	8	8.3	8	9	9	8.7

Analysis of variance:

Source		D.F.	S.S.	M.S.	F.
Time "days"	(Ti)	7	27.81	3.9	
Treatments	(Ts)	1	50.02	50.0	37.60***
Interaction	(TiXTs)	7	27.48	3.9	
Error		32	42.67	1.3	
Total		47	147.98		

Table 29: Effect of age of seedling at time of inoculation on root growth (Log. transformation of root lengths.)

Time (days)	Treatments							
	Infested soil				Non-infested soil			
	Replicates			Mean	Replicates			Mean
	i	ii	iii		i	ii	iii	
0	1.949	2.053	2.000	2.001	2.152	2.111	2.041	2.101
1	2.017	2.009	2.076	2.034	2.127	2.093	2.111	2.110
2	1.954	1.954	2.029	1.979	2.188	2.140	2.143	2.157
3	2.127	2.137	2.086	2.117	2.267	2.238	2.215	2.240
4	2.233	2.137	2.152	2.174	2.267	2.210	2.258	2.245
5	2.250	2.238	2.265	2.251	2.215	2.267	2.258	2.247
6	2.346	2.324	2.366	2.345	2.358	2.339	2.286	2.327
7	2.366	2.354	2.360	2.360	2.377	2.354	2.367	2.366

Analysis of variance:

Source	D.F.	S.S.	M.S.	F.
Time 'days'(Ti.)	7	0.62	0.0886	55.6
Treatments (Ts.)	1	0.06	0.0600	37.5***
Interaction (Ti. X Ts.)	7	0.04	0.0057	
Error	32	0.05	0.0016	
Total	47	0.77		

Table 30: Effect of age of seedling at time of inoculation on shoot growth. (Log. transformation of shoot heights).

Time (days)	Treatments							
	Infested soil				Non-infested soil			
	Replicates			Mean	Replicates			Mean
	i	ii	iii		i	ii	iii	
0	1.690	1.996	1.954	1.880	1.969	1.892	1.909	1.923
1	1.869	1.851	2.045	1.922	2.013	1.949	2.025	1.996
2	1.857	1.982	1.991	1.944	2.041	2.021	2.009	2.024
3	2.021	2.086	2.090	2.066	2.140	2.179	2.146	2.155
4	2.079	2.248	2.161	2.163	2.212	2.182	2.260	2.218
5	2.253	2.248	2.274	2.262	2.185	2.263	2.267	2.238
6	2.312	2.342	2.301	2.318	2.292	2.318	2.286	2.299
7	2.378	2.358	2.305	2.347	2.386	2.335	2.362	2.361

Analysis of variance:

Source	D.F.	S.S.	M.S.	F.
Time 'days'(Ti.)	7	1.22	0.174	
Treatments (Ts.)	1	0.02	0.020	9.1**
Interaction (Ti. x Ts.)	7	0.02	0.0029	
Error	32	0.07	0.0022	
Total	47	1.33		

Table 31: Effect of varietal susceptibility on the seedling stand after 10 days.

Rep.	Varieties				
	A	B	C	D	E
i	9	12	11	11	12
ii	7	13	12	12	13
iii	10	13	7	9	13
iv	8	9	6	10	10
v	6	8	9	6	12
Total	39	55	43	45	60

Table 32: Effect of varietal susceptibility on the seedlings stand after 21 days.

Rep.	Varieties				
	A	B	C	D	E
i	4	9	9	6	11
ii	8	10	6	9	12
iii	6	13	8	8	12
iv	8	8	9	11	10
v	7	12	7	7	12
Total	33	52	39	41	57

Total out of 75 seeds.

Table 33: Effect of varietal susceptibility on the root growth.

Rep.	Varieties				
	A	B	C	D	E
i	140	253	267	245	253
ii	147	210	248	238	270
iii	227	282	173	219	212
iv	158	208	217	228	261
v	215	260	186	219	277

Root lengths in (mm.)

Analysis of variance:

Source	D.F.	S.S.	M.S.	F.
Replicates	4	1,036.24	259.1	
Treatments	4	17,599.04	4,399.8	3.66*
Error	16	19,242.96	1,202.7	
Total	24	37,878.24		

Table 34: Effect of varietal susceptibility on fresh weight of roots / pot.

Rep.	Varieties				
	A	B	C	D	E
i	1.2	3.6	3.2	1.6	7.0
ii	3.3	2.7	2.2	4.2	3.3
iii	1.8	7.8	3.4	3.2	5.2
iv	3.8	3.3	2.6	4.2	5.8
v	1.9	6.4	3.1	2.5	10.5
Mean	2.4	4.8	2.9	3.1	6.2

Roots weight in (g.)

Analysis of variance:

Source.	D.F.	S.S.	M.S.	F.
Replicates	4	10.00	2.5	
Treatments	4	49.58	12.4	3.9*
Error	16	50.45	3.2	
Total	24	110.03		

Table 35: Effect of varietal susceptibility on fresh weight of root / plant.

Rep.	Varieties				
	A	B	C	D	E
i	0.30	0.40	0.36	0.27	0.64
ii	0.41	0.27	0.37	0.47	0.37
iii	0.30	0.60	0.43	0.38	0.43
iv	0.48	0.41	0.29	0.38	0.50
v	0.27	0.53	0.39	0.36	0.81
Mean	0.35	0.44	0.37	0.37	0.55

Root weight in (g.)

Analysis of variance

Source	D.F.	S.S.	M.S.	F.
Replicates	4	0.03	0.008	
Treatments	4	0.14	0.04	$2.5^{n.s.}$
Error	16	0.22	0.014	
Total	24	0.39		

Table 36: Effect of varietal susceptibility on shoot growth.

Rep.	Varieties				
	A	B	C	D	E
i	277	275	248	240	308
ii	260	280	253	232	265
iii	210	263	270	255	294
iv	240	254	257	273	261
v	217	288	264	269	255
Mean	241	272	258	254	277

Shoot heights in (mm.)

Analysis of variance

Source.	D.F.	S.S.	M.S.	F.
Replicates	4	545.84	136.5	
Treatments	4	4,143.44	1,035.9	$2.3^{n.s.}$
Error	16	7,088.16	443.0	
Total	24	11,777.44		

Table 37: Effect of varietal susceptibility on weight of shoot / pot.

Rep.	Varieties				
	A	B	C	D	E
i	2.7	6.5	4.4	2.7	9.0
ii	4.9	8.7	2.9	3.1	3.7
iii	1.4	6.1	3.6	5.1	4.9
iv	2.4	2.9	2.5	4.7	6.6
v	1.0	5.6	2.7	6.2	8.0
Mean	25	6.0	3.2	4.4	6.4

Shoot weights in (g.)

Analysis of variance

Source	D.F.	S.S.	M.S.	F.
Replicates	4	4.34	1.1	
Treatments	4	57.36	14.3	4.4*
Error	16	51.96	3.3	
Total	24	113.66		

Table 38: Effect of varietal susceptibility on fresh weight of shoot / plant.

Rep.	Varieties				
	A	B	C	D	E
i	0.68	0.72	0.49	0.45	0.82
ii	0.61	0.87	0.48	0.34	0.41
iii	0.23	0.47	0.45	0.64	0.41
iv	0.33	0.36	0.28	0.43	0.66
v	0.14	0.47	0.39	0.89	0.67
Mean	0.39	0.58	0.42	0.55	0.59

Shoot weight in (g.)

Analysis of variance.

Source	D.F.	S.S.	M.S.	F.
Replicates	4	0.15	0.038	
Treatments	4	0.17	0.043	$1.2^{n.s.}$
Error	16	0.59	0.037	
Total	24	0.91		

Table 39: Effect of ceresan seed dressings on seedling stand in infested soil.

Rep.	Treatments			
	A	B	C	D
i	9	14	16	11
ii	6	12	14	13
iii	7	10	13	12
iv	5	13	12	12
v	8	14	11	10
Mean	7.0	12.6	12.0	11.6

Analysis of variance.

Source.	D.F.	S.S.	M.S.	F.
Replicates	4	1.7	0.4	
Treatments	3	98.8	32.9	11.4**
Error	12	34.7	2.9	
Total	19	135.2		

Table 40: Effect of ceresan seed dressing on root growth in infested soil

Rep.	Treatments			
	A	B	C	D
i	175	290	301	273
ii	229	276	293	289
iii	187	310	284	260
iv	196	280	309	300
v	201	260	273	258
Mean	198	283	292	276

Root lengths in (mm.)

Analysis of variance:

Source	D.F.	S.S.	M.S.	F.
Replicates	4	1,538.2	384.6	
Treatments	3	28,463.2	9487.7	31.8**
Error	12	3,579.8	298.3	
Total	19	33,581.2		

Table 41: Effect of ceresan seed dressings on shoot growth in infested soil.

Rep.	Treatments			
	A	B	C	D
i	244	284	268	258
ii	238	256	312	266
iii	197	299	287	281
iv	209	270	269	293
v	240	292	280	275
Mean	226	280	283	275

Shoot heights in (mm.)

Analysis of variance:

Source	D.F.	S.S.	M.S.	F.
Replicates	4	305.3	76.3	
Treatments	3	11,017.8	3,672.6	9.4**
Error	12	4,700.7	391.7	
Total	19	16,023.8		

Table 42: Effect of ceresan on F. culmorum in vitro.

Days	level of ceresan						
	1	2	3	4	5	6	7
i	0.3	0.5	0.5	0.7	0.9	1.6	1.6
ii	0.7	0.9	1.6	1.7	2.1	3.4	3.5
iii	1.4	1.8	2.5	3.1	3.4	6.3	6.3
iv	2.2	2.9	3.5	4.1	4.6	7.7	7.7
v	2.8	3.7	4.5	5.1	5.7	8.3	8.4

Mean colony diameter in (cm.) of 5 replicates

1 = 400, 2 = 200, 3 = 100, 4 = 50, 5 = 25,

6 = nil + acetone & 7 = nil p.p.m.

Table 43: Measurements of zone of inhibition caused by ceresan-treated seed, zone diameter in (mm.) on day 2 & 10

Rep.	Treatments		Dev. $\bar{X}_1$	Dev. $\bar{X}_2$
	Day 2 (x1)	Day 10 (x2)		
1	16	16	-0.1	+0.2
2	16	16	-0.1	+0.2
3	15	14	-1.1	-1.8
4	16	16	-0.1	+0.2
5	17	16	+0.9	+0.2
6	16	16	-0.1	+0.2
7	17	16	+0.9	+0.2
8	16	16	-0.1	+0.2
9	16	16	-0.1	+0.2
10	16	16	-0.1	+0.2
Mean	16.1	15.8		

Dev. X_1^2 = 2.9

Dev. X_2^2 = 3.6

$$S^2 = \frac{X_1^2 + X_2^2}{n_1 + n_2 - 2} = \frac{2.9 + 3.6}{18} = 0.361$$

$$SD = \sqrt{\frac{0.361}{5}} = \sqrt{0.0722} = 0.26870$$

LSD P. = 0.05 = 2.09 X 0.26870 = 0.6

Treatments mean		Difference
(X_1)	16.1	
(X_2)	15.8	0.3 n.s.

Table 44: Measurements of zone of inhibition caused by ceresan-treated washed and unwashed seed after 2 days.

Teatments	Replicates										Mean
	1	2	3	4	5	6	7	8	9	10	
U.	18	15	16	14	16	16	15	15	17	16	15.8
W.	8	7	8	10	7	8	9	9	7	7	8.0

Zone diameter in (mm.)

U. treated seed unwashed

W. " " washed

Treatments	Mean	Difference
U.	15.8	7.8 ***
W.	8.0	

L.S.D. P = 0.001 = 1.9

(See Table 43 p.236).

Table 45 : Effect of ceresan-seed treatments on the colonization of wheat root surfaces

Days	Treatments	Replicates								Mean
		1	2	3	4	5	6	7	8	
5	T	7	7	8	8	10	7	10	8	8.1
	U	5	3	5	8	6	4	9	7	5.9
7	T	8	8	9	10	9	8	10	8	8.8
	U	5	5	6	8	6	8	10	7	6.8
9	T	9	10	10	5	5	9	9	9	8.3
	U	7	7	9	4	5	9	5	7	6.6
11	T	10	10	8	7	10	7	10	6	8.5
	U	9	9	10	8	6	6	4	5	7.1

T. = ceresan-treated seed.

U. = untreated seed.

(See Table 43 p.236)

L.S.D.

P. = 0.05 = 1.79

Table 46: Effect of ceresan - seed treatments on the colonization of coleoptile surfaces.

Days	Treatments	Replicates								Mean
		1	2	3	4	5	6	7	8	
5	T.	5	4	5	5	5	3	3	5	4.4
	U	4	5	5	5	3	4	4	4	4.3
7	T.	5	3	5	5	4	4	5	5	4.5
	U.	4	4	5	5	5	4	5	5	4.6
9	T.	4	4	3	5	5	5	5	4	4.4
	U.	4	5	5	3	4	5	5	5	4.5
11	T.	5	5	5	4	5	3	4	5	4.5
	U.	5	4	4	5	5	5	5	3	4.5

T. ceresan-treated seed.

U. Untreated seed.

L.S.D:

P. = 0.05 = 0.8

(see Table 43 p.236)

Table 47: Effect of ceresan-seed treatments on wheat root growth in non-infested soil (Log. transformation of root lengths)

Days	Treatments	Replicates			Mean
		i	ii	iii	
2	T.	0.903	0.903	0.699	0.835
	U.	1.146	0.903	0.954	1.001
3	T.	1.362	1.255	1.146	1.254
	U.	1.343	1.380	1.230	1.318
4	T.	1.708	1.708	1.699	1.705
	U.	1.887	1.732	1.699	1.773
5	T.	1.863	1.903	1.929	1.898
	U.	1.949	1.908	1.898	1.918
6	T.	2.053	1.996	2.004	2.018
	U.	2.076	2.000	2.029	2.035
7	T.	2.121	2.140	2.086	2.116
	U.	1.973	2.100	2.086	2.053

T. treated seed U. untreated seed

Analysis of variance:

Source	D.F.	S.S.	M.S.	F.
Treatments Ts.	1	0.02	0.020	0.4 n.s.
Time (days) Ti.	5	6.43	1.290	
Interation (Ts X Ti)	5	0.04	0.008	
Error	24	0.14	0.058	
Total	35	6.63		

Table 48: Effect of ceresan-seed treatments on wheat shoot growth in non-infested soil (log transformation of shoot heights).

days	Treatments	Replicates			Mean
		i	ii	iii	
3	T.	0.699	0.477	0.602	0.593
	U.	0.602	0.699	0.602	0.634
4	T.	1.397	1.380	1.342	1.373
	U.	1.568	1.415	1.380	1.454
5	T.	1.591	1.644	1.690	1.642
	U.	1.724	1.663	1.623	1.670
6	T.	1.813	1.771	1.833	1.806
	U.	1.813	1.778	1.833	1.808
7	T.	1.954	1.969	1.935	1.953
	U.	1.785	1.939	1.954	1.993

T. treated seed U untreated seed.

Analysis of variance

Source.	D.F.	S.S.	M.S.	F.
Treatments (Ts)	1	0.01	0.010	0.2 n.s.
Time (days) (Ti)	4	6.56	1.640	
Interaction (TsxTi.)	4	0.02	0.005	
Error	20	0.09	0.0045	
Total	29	6.68		

Table 49: Effect of PP 781 on seedlings stand in infested soil.

Rep	Treatments				
	A	B	C	D	E
i	4	12	13	14	13
ii	7	10	14	13	10
iii	9	13	12	12	13
iv	6	10	12	15	13
v	7	13	12	12	13
Mean	6.6	11.6	12.6	13.2	12.4

Analysis of variance

Source	D.F.	S.S.	M.S.	F.
Replicates	4	2.40	0.6	
Treatments	4	143.44	35.6	15.3***
Error	16	37.20	2.3	
Total	24	183.04		

Table 50: Effect of PP 781 on root growth in infested soil.

Root lengths in (mm.)

Rep.	Treatments				
	A	B	C	D	E
i	238	280	265	273	301
ii	190	262	279	288	255
iii	158	278	292	267	284
1v	209	279	269	300	270
v	241	285	298	269	268
Mean	207	277	281	279	276

Analysis of variance:

Source.	D.F.	S.S.	M.S.	F.
Replicates	4	1,379.0	344.8	
Treatments	4	20,186.6	5,046.7	12.3***
Error	16	6,578.2	411.1	
Total	24	28,143.8		

Table 51: Effect of PP 781 on shoot growth in infested soil.

Rep.	Treatments				
	A	B	C	D	E
i	242	292	325	298	266
ii	225	290	302	300	297
iii	261	279	269	277	254
iv	270	264	305	282	273
v	233	275	295	268	337
Mean	246	280	299	285	285

Shoot heights in (mm.)

Analysis of variance:

Source	D.F.	S.S.	M.S.	F.
Replicates	4	999.4	249.8	
Treatments	4	7,808.6	1,952.1	4.1*
Error	16	7,659.4	478.7	
Total	24	16,457.4		

Table 52: Effect of PP 781 on F. culmorum in vitro

Days	Level of PP 781						
	1	2	3	4	5	6	7
i	0.7	0.8	0.9	1.0	1.4	1.6	1.7
ii	1.4	1.5	1.6	1.9	2.u	3.5	3.3
iii	2.7	2.2	3.0	3.7	4.9	5.7	5.8
iv	3.6	3.8	4.6	5.9	6.7	7.2	7.5
v	4.4	4.9	5 0	6.8	7.8	8.4	8.4

Mean colony diameter in (cm.) of 5 replicate.
1 = 400, 2 = 200, 3 = 100, 4 = 50,
5 = 25, 6 = nil + acetone and 7 = nil
p.p.m.

Table 53:

a - Comparison between the zone of inhibition caused by PP 781 - treated seed and ceresan - treated one.

Treatments	Replicates										Mean
	1	2	3	4	5	6	7	8	9	10	
PP 781	10	8	9	10	11	8	10	10	9	8	9.3
Ceresan	16	17	16	17	17	16	14	16	18	16	16.3

Zone diameter in (mm.) on day 2.

Treatments	Mean	Difference
PP 781	9.3	
Ceresan	16.3	7.0 ***
L.S.D. P = 0.001		2.0

(See Table 43 p. 236)

Table 53: b -

Measuremnets of zone of inhibition caused by PP 781 - treated seed (on day 2 10)

Day	Replicates										Mean
	1	2	3	4	5	6	7	8	9	10	
ii	10	8	9	8	10	11	8	10	9	8	9.3
X	7	5	5	7	7	8	5	6	5	5	6.0

Zone diameter in (mm.)

Day	Mean	Difference
ii	9.3	
X	6.0	3.3 ***
L.S.D. P = 0.001		= 2.1

(See Table 43 p.236)

Table 54: Effect of PP 781 - seed treatments on root growth in non - infested soil (Log. transformation of root lengths).

Days	Treatments	Replicates			Mean
		i	ii	iii	
3	T	1.342	0.954	1.176	0.824
	U.	1.230	1.114	0.903	0.749
4	T.	1.690	1.681	1.531	1.634
	U.	1.556	1.663	1.580	1.600
5	T.	2.004	1.978	2.025	2.002
	U.	2.009	2.041	2.053	2.034
6	T.	2.097	2.041	2.107	2.092
	U.	2.009	2.041	2.053	2.052
7	T.	2.152	2.093	2.167	2.137
	U.	2.117	2.134	2.114	2.122

T. treated seed U. untreated seed.

Analysis of variance

Source	D.F.	S.S.	M.S.	F.
Treatments (Ts.)	1	0.02	0.02	2.1 ns.
Time (days) (Ti.)	4	4.19	1.05	
Interaction (Ts. X Ti.)	4	-	-	
Error	20	0.19	0.0095	
Total	29	4.40		

Table 55: Effect of PP 781 - seed treaments on shoot growth in non - infested soil (Log. transformation of shoot heights).

Days	Treatments	Replicates			Mean
		i	ii	iii	
3	T.	0.699	0.301	0.602	0.534
	U.	0.301	0.301	0.602	0.401
4	T.	0.954	0.954	0.778	0.895
	U.	0.845	0.903	0.903	0.884
5	T.	1.568	1.544	1.623	1.578
	U.	1.322	1.544	1.568	1.478
6	T.	1.732	1.699	1.763	1.731
	U.	1.663	1.724	1.740	1.709
7	T.	1.949	1.914	1.964	1.942
	U.	1.914	1.959	1.909	1.927

T. treated seed U. untreated seed.

Analysis of variance

Source	D.F.	S.S.	M.S.	F.
Treatments (Ts.)	1	0.03	0.03	2.7 n.s.
Time (days) (Ti.)	4	9.00	2.25	
Interaction (TsXTi.)	4	0.02	0.005	
Error	20	0.22	0.011	
Total	29	9.27		

Table 56: Effect of soil application of aldrin (5% dust) on seedling stand in infested soil.

Rep.	Treatments				
	A	B	C	D	E
i	2	10	11	11	6
ii	2	6	7	14	6
iii	6	13	5	11	7
iv	7	12	9	11	10
Mean	4.3	10.3	7.8	11.8	7.3

Analysis of variance.

Source.	D.F.	S.S.	M.S.	F.
Replicates	3	17.35	5.8	
Treatments	4	134.00	33.5	5.9**
Error	12	68.40	5.7	
Total	19	219.75		

Table 57: Effect of soil application on aldrin (5% dust) on shoot growth in infested soil.

Rep.	Treatments				
	A	B	C	D	E
i	150	211	185	273	137
ii	94	220	223	298	207
iii	249	230	225	232	260
iv	159	211	178	184	235
Mean	163	218	203	220	209

Shoot heights in (mm.)

Analysis of variance

Source.	D.F.	S.S.	M.S.	F.
Replicates	3	91.87	30.6	
Treatments	4	186.71	46.7	10.2 **
Error	12	54.90	4.6	
Total	19	333.48		

Table 58: Effect of soil application of aldrin (50% dust) on plant dry weight (mg.)

Rep.	Treatments				
	A	B	C	D	E
i	30	39	42	39	36
ii	23	44	46	35	34
iii	39	32	38	47	45
iv	32	34	34	36	45
Mean	31	37	40	39	40

Analysis of variance

Source.	D.F.	S.S.	M.S.	F.
Replicates	3	50.33	16.7	
Treatments	4	222.04	55.5	1.6 n.s.
Error	12	419.97	34.9	
Total	19	692.24		

Table 59: Effect of aldrin, ceresan and PP 781 treatments on seedling stand in infested soil.

Rep.	Treatments				
	A	B	C	D	E
i	6	11	14	13	11
ii	8	10	13	11	13
iii	7	8	10	9	10
iv	5	11	12	13	11
v	4	9	11	10	14
Mean	6.0	9.8	12.0	11.2	11.8

Analysis of variance.

Source	D.F.	S.S.	M.S.	F.
Replicates	4	18.16	4.5	
Treatments	4	122.96	30.7	15.3***
Error	16	32.24	2.0	
Total	24	173.36		

Table 60: Effect of aldrin, ceresan and PP 781 treatments on root growth in infested soil.

Rep.	Treatments				
	A	B	C	D	E
i	136	284	266	265	270
ii	196	273	280	247	275
iii	203	246	263	285	271
iv	155	269	286	277	262
v	182	245	279	273	255
Mean	174	263	275	269	266

Root lengths in (mm.)

Analysis of variance.

Source	D.F.	S.S.	M.S.	F.
Replicates	4	370.64	92.7	
Treatments	4	35,807.44	8,951.9	28.7 ***
Error	16	4,990.96	311.9	
Total	24	41,169.04		

Table 61: Effect of aldrin, ceresan and PP 781 treatments on shoot growth in infested soil.

Rep.	Treatments				
	A	B	C	D	E
i	196	258	308	284	310
ii	226	267	296	294	284
iii	159	274	276	256	270
iv	189	266	292	310	290
v	155	289	293	288	307
Mean	185	271	293	286	292

Shoot heights in (mm.)

Analysis of variance.

Source.	D.F.	S.S.	M.S.	F.
Replicates	4	2,265.24	566.5	
Treatments	4	42,071.44	10,517.9	34.8 ***
Error	16	4,842.96	302.7	
Total	24	49,180.24		

Table 62: Effect of soil application of aldrin on the colonization of roots and coleoptiles by F. culmorum.

a. Roots.

Days	Treatments	Replicates								Mean
		1	2	3	4	5	6	7	8	
5	T.	4	3	4	4	9	10	7	5	5.7
	U.	4	7	4	7	8	8	9	9	7.0
7	T.	7	6	6	10	5	9	7	9	7.3
	U.	8	9	8	8	9	8	7	6	7.8
9	T.	5	7	6	8	7	6	8	7	6.8
	U.	7	8	10	8	8	7	7	10	8.0
11	T.	3	3	6	6	4	5	7	8	5.3
	U.	8	7	8	8	9	8	8	9	8.1

T. treated soil

U. untreated soil.

(See Table 43 p.256)

L.S.D.

P. 0.05 = 1.60

b - Coleoptiles.

Days	Treatments	Replicates								Mean
		1	2	3	4	5	6	7	8	
5	T.	4	5	4	3	5	2	4	3	3.8n.s
	U.	4	5	3	3	5	4	4	3	3.9
7	T.	5	5	4	4	4	3	5	4	4.3
	U.	5	4	3	3	5	4	5	5	4.3 "
9	T.	3	3	5	4	4	5	5	5	4.3 "
	U.	5	5	5	4	3	4	4	5	4.4
11	T.	5	5	5	4	4	4	3	4	4.3
	U.	3	3	5	5	5	5	4	4	4.3 "

T. treated soil.

U. untreated soil.

L.S.D.

P. = 0.05 = 1.00

(See Table 43 p.236)

Table 63: Effect of soil application of aldrin on root growth in non - infested soil. (Log transformation of root lengths).

Days	Treatments	Replicates			Mean
		i	ii	iii	
2	T.	1.322	1.279	1.342	1.314
	U.	1.342	1.398	1.122	1.297
3	T.	1.778	1.748	1.716	1.747
	U.	1.732	1.732	1.764	1.742
4	T.	1.914	1.945	1.929	1.929
	U.	1.924	1.799	1.887	1.870
5	T.	2.017	2.000	2.025	2.140
	U.	2.017	2.000	2.013	2.010
6	T.	2.093	2.065	2.033	2.064
	U.	2.017	2.025	2.049	2.030
7	T.	2.161	2.097	2.090	2.116
	U.	2.068	2.086	2.134	2.096

T. treated soil, U. untreated soil.

Analysis of Variance.

Source	D.F.	S.S.	M.S.	F.
Treatments (Ts)	1	0.01	0.01	4.0 n.s.
Time (days) (Ti.)	5	2.68	0.54	216.0 ***
Interaction (TiXTs)	5	0	-	-
Error	24	0.06	0.0025	
Total	35	2.75		

Table 64: Effect of soil application of aldrin on shoot growth in non - infested soil

Days	Treatments	Replicates			Mean
		i	ii	iii	
2	T.	0.477	0.477	0.477	0.477
	U.	0.477	0.477	0.477	0.477
3	T.	1.079	1.041	0.041	1.053
	U.	0.903	1.00	0.954	0.952
4	T.	1.505	1.505	1.491	1.500
	U.	1.462	1.415	1.447	1.411
5	T.	1.716	1.756	1.732	1.735
	U.	1.748	1.724	1.699	1.441
6	T.	1.875	1.875	1.833	1.855
	U.	1.857	1.806	1.748	1.804
7	T.	1.969	1.964	1.935	1.956
	U.	1.863	1.929	1.945	1.912

T. treated soil & U. untreated soil

Analysis of variance.

Source.	D.F.	S.S.	M.S.	F.
Treatments (Ts)	1	0.02	0.02	25.0***
Time (days) (Ti)	5	9.55	1.91	
Interaction (TsXTi)	5	0.01	0.002	
Error	24	0.02	0.0008	
Total	35	9.60		

Table 65: Effect of aldrin on F. culmorum in vitro:

a.

Days	Level of aldrin					
	1	2	3	4	5	6
i	1.7	1.6	1.6	1.5	1.7	1.7
ii	3.1	3.4	2.9	3.3	3.6	3.2
iii	6.0	6.2	6.3	6.4	6.2	6.0
iv	7.4	7.3	7,7	7.8	7.3	7.6
v	8.3	8.4	8.4	8.4	8.3	8.4

Mean colony diameter in (cm.) of 5 replicates.

Level of aldrin viz: -

1 = 10,000 , 2 = 5,000 , 3 = 2,500 ,
4 = 1,250 , 5 = 650 , 6 = nil , p.p.m.

b.

Days	Level of aldrin					
	1	2	3	4	5	6
i	1.4	1.6	1.6	1.7	1.8	1.6
ii	3.3	3.4	3.2	3.2	3.1	3.3
iii	6.3	6.8	6.5	6.1	6.0	6.6
1v	7.2	7.7	7.6	7.6	7.5	7.9
v	8.2	8.3	8.3	8.4	8.4	8.4

Mena colony diameter in (cm.) of 5 replicates

Level of aldrin viz:

as in a.

Table 66: Effect of aldrin on F. culmorum in liquid medium.

Rep	level of aldrin						
	1	2	3	4	5	6	7
i	96	103	112	102	95	111	103
ii	107	97	98	100	108	101	107
iii	98	105	96	109	99	98	97
iv	110	109	107	98	106	103	108
Mean	103	104	103	102	102	103	104

Mean dry weight of mycelium in (mg.)

Levels of aldrin viz:-

as in Table 65a except for level 7 = basic medium + an appropriate amount of acetone.

Analysis of variance:

Source	D.F.	S.S.	M.S.	F.
Replicates	3	110.11	36.7	
Treatments	6	10.22	1.7	0.05 n.s.
Error	18	620.64	34.5	
Total	27	740.97		

Table 67: Effect of aldrin on the spore germination of F. culmorum.

Rep	Levels of aldrin						
	1	2	3	4	5	6	7
i	98	98	96	98	95	97	96
ii	95	94	98	99	97	98	98
iii	95	98	97	96	99	95	97
iv	98	96	94	98	95	97	94
v	97	96	97	95	96	95	97
v1	97	98	96	95	94	95	97
Mean	96.5	96.7	96.3	96.8	96.0	96.2	96.5

Mean % Germination.

Analysis of variance:

Source	D.F.	S.S.	M.S.	F.
Replicates	5	8.3	1.66	
Treatments	6	2.96	0.49	0.02 n.s.
Error	30	73.04	2.11	
Total	41	84.03		

Table 68: Effect of soil extract of soil treated with aldrin on F. culmorum

a - Spore germination

Treatments	Replicates												Mean
	1	2	3	4	5	6	7	8	9	10	11	12	
T.	76	80	86	74	77	76	88	68	83	86	90	75	79.9
U.	93	96	95	95	94	93	96	91	97	90	97	94	94.3

T. treated soil & U. untreated soil
Mean % germination

Treatments	Mean	Difference
T	79.9	
U	94.3	14.4 ***

L.S.D. P. = 0,001 7.6

(See Table 43 p. 236)

b - Lengths of germ-tube

Treatments	Replicates												Mean
	1	2	3	4	5	6	7	8	9	10	11	12	
T.	40	66	91	113	101	98	117	58	47	77	89	116	84.4
U.	158	146	123	137	152	118	143	121	139	145	121	129	136.0

T. treated soil & U. untreated soil
Mean germ-tublengths in (U.)

Treatments	Mean	Difference
T.	84.4	
U.	136.0	51.6***

L.S.D. P. = 0.001 = 32.5

(See Table 43 p. 236)

Table 69: Effect of soil extracts from soil treated with aldrin (planted with wheat) on growth of F. culmorum.

a. In liquid medium

Rep.	Level of aldrin					
	A	B	C	D	E	F
i	100	97	93	86	84	90
ii	90	96	95	89	86	103
iii	101	101	89	90	85	98
1v	98	89	94	91	92	88
Mean	97.3	95.6	92.8	89.0	86.8	94.8

Mean mycelium dry weight in (mg.)

Analysis of variance:

Source	D.F.	S.S.	M.S.	F.
Replicates	3	19.96	6.6	
Treatments	5	333.16	66.6	2.9 *
Error	15	349.84	23.3	
Total	23	702.96		

b. On spore germination

Rep.	Level of aldrin					
	A	B	C	D	E	F
i	94	97	92	96	89	91
ii	99	100	43	92	94	97
iii	100	99	96	92	79	94
iv	94	98	94	94	92	98
v	88	96	97	97	88	99
v1	98	95	95	95	94	94
Mean	95.5	97.5	94.5	94.3	87.5	95.5

Mean % Germination

Analysis of variance:

Source	D.F.	SS.	M.S.	F.
Replicates	5	33.66	6.7	
Treatments	5	342.33	68.5	5.4**
Error	25	317.01	12.7	
Total	35	693.00		

c - On germ - tube lengths

Rep.	Level of aldrin					
	A	B	C	D	E	F
i	149	150	134	132	127	140
ii	151	155	155	137	137	154
iii	145	148	146	128	130	146
1v	158	139	123	139	136	147
v	144	153	146	141	123	155
v1	151	148	141	136	119	152
Mean	150	149	141	136	129	149

Mean germ - tube lengths in (μ).

Analysis of variance:

Source	D.F.	S.S.	M.S.	F.
Replicates	5	345.88	69.1	
Treatments	5	2,254.91	451.0	10.4 **
Error	25	1,086.26	43.5	
Total	35	3,386.75		

Table 70: Effect of soil extracts from soil treated with aldrin (only) on growth of F. culmorum.

a - In liquid medium.

Rep.	Level of aldrin					
	A	B	C	D	E	F
i	95	88	91	84	82	90
ii	102	101	88	90	77	103
iii	90	97	86	85	84	98
iv	89	87	97	93	77	88
Mean	94.0	93.3	90.5	88.0	80.0	94.8

Mean mycelium dry weight in (mg.)

Analysis of variance:

Source	D.F.	S.S.	M.S.	F.
Replicates	3	103.50	34.5	
Treatments	5	613.34	122.7	4.1 *
Error	15	451.00	30.1	
Total	23	1167.84		

b - On spore Germination

Rep.	Level of aldrin					
	A	B	C	D	E	F
i	92	98	96	86	80	97
ii	94	96	95	89	74	92
iii	97	93	91	94	82	97
iv	97	92	100	89	91	93
v	95	89	86	92	90	91
vi	96	97	91	91	74	94
Meam	95.2	94.2	93.2	90.2	81.8	94.0

Mean % germination

Analysis of variance.

Source	D.F.	S.S.	M.S.	F.
Replicates	5	57.58	11.5	
Treatments	5	775.58	155.1	8.4**
Error	25	459.59	18.4	
Total	35	1292.75		

c - On germ - tube lengths.

Rep.	Level of aldrin					
	A	B	C	D	E	F
i	168	155	134	123	113	160
ii	138	146	155	130	121	151
iii	129	134	146	126	110	152
iv	165	146	124	129	101	136
v	149	156	148	137	115	158
	135	133	131	129	123	190
Mean	148	145	140	128	113	150

Mean Germ - tube lengths in (µ,)

Analysis of variance

Source	D.F.	S.S.	M.S.	F.
Replicates	5	804.00	160.8	
Treatments	5	5,685.33	1,137.1	10.6 **
Error	25	2,743.67	109.8	
Total	35	9,233.00		

Table 71: Effect of dieldrin on F. culmorum in vitro

Days	Level of dieldrin						
	1	2	3	4	5	6	7
i	0.4	0.6	0.8	1.0	1.3	1.7	1.6
ii	0.9	1.2	1.8	2.2	2.5	3.2	3.2
iii	1.8	2.0	3.0	3.9	4.4	5.6	5.9
iv	2.8	3.2	4.1	4.9	5.8	7.2	7.4
v	3.6	4.3	5.7	5.9	7.0	8.4	8.4

Mean colony diameter in (cm.) of 5 replicates

1 = 100, 2 = 50, 3 = 25, 4 = 12, 5 = 6.25

6 = nil + acetone and 7 = nil p.p.m.

Table 72: Effect of dieldrin on growth of F. culmorum.

a - In liquid medium.

Rep.	Level of dieldrin						
	1	2	3	4	5	nil+	nil
i	55	59	62	64	73	106	103
ii	56	60	57	69	80	101	96
iii	54	55	64	66	82	98	99
iv	50	57	61	75	79	102	107
Mean	53.8	57.8	61.0	68.5	78.5	101.8	101.3

* (nil + acetone & nil = basic medium only)

Mean mycelium dry weight in (m g.)

Analysis of variance:

Source	D.F.	S.S.	M.S.	F.
Replicates	3	15.00	5.0	
Treatments	6	9,613.43	1,602.2	110.0***
Error	18	262.00	14.6	
Total	27	9,890.43		

b - On spore germination.

Rep.	Level of dieldrin						
	1	2	3	4	5	nil+	nil
i	72	79	78	87	87	96	98
ii	70	78	77	88	86	97	98
iii	64	76	82	86	87	98	95
iv	63	73	90	83	87	97	96
v	55	75	84	84	88	96	97
vi	60	74	86	87	87	96	97
Mean	64.0	75.8	81.2	85.8	87.0	96.7	96.8

Mean % germination.

Analysis of variance:

Source.	D.F.	S.S.	M.S.	F.
Replicates	5	39.62	7.9	
Treatments	6	4,872.95	812.2	8.8 ***
Error	30	277.05	92.4	
Total	42	5,189.62		

c - On germ - tube lengths.

Rep.	Level of dieldrin						
	1	2	3	4	5	nil+	nil.
i	40	59	87	91	113	146	143
ii	46	58	88	98	115	143	148
iii	42	72	86	101	105	150	166
iv	35	73	90	106	102	139	152
v	38	68	93	98	110	144	139
vi	41	65	92	103	107	162	146
Mean	40.0	65.8	89.3	99.5	108.7	147.3	149.0

Mean germ - tube lengths in (µ.)

Analysis of variance:

Source:	D.F.	S.S.	M.S.	F.
Replicates	5	186.57	37.3	
Treatments	6	57,348.33	9,558.1	24.9 ***
Error	30	1,147.10	382.4	
Total	41	58,682.00		

Table 73: Long - term effect of ceresan and aldrin treatments on the following characters:

a - Seedling stand (no.)

Rep.	Treatments					
	A	B	C	D	E	F
i,	9	9	7	5	10	8
ii	8	10	6	6	10	9
iii	10	9	7	4	10	10
iv	8	10	8	4	9	9
v	9	8	8	6	8	9
Mean	8.8	9.2	7.2	5.0	9.4	9.0

Mean no. of seedling stand of 5 replicates

Analysis of variance:

Source:	D.F.	S.S.	M.S.	F.
Replicates	4	0.53	0.13	
Treatments	5	73.10	14.62	17.2***
Error	20	17.07	0.85	
Total	29	90.70		

b - Tillering (no.)

Rep.	Treatments					
	A	B	C	D	E	F
i	42	63	89	30	66	83
ii	75	56	63	47	72	77
iii	62	60	56	41	58	86
iv	64	58	69	34	73	84
v	54	78	72	12	55	78
Mean	59.4	63.0	67.8	32.8	64.8	81.6

Analysis of variance:

Source:	D.F.	S.S.	M.S.	F.
Replicates	4	123.54	30.9	
Treatments	5	6,424.57	1,284.9	9.7 **
Error	20	2,649.26	132.5	
Total	29	9,197.37		

c - Ear weights in (g.)

Rep.	Treatments					
	A	B	C	D	E	F
i	71	80	87	18	146	124
ii	141	115	106	42	96	146
iii	93	123	91	40	76	93
iv	103	113	103	35	112	116
v	61	67	91	8	133	94
Mean	93.8	99.6	95.6	28.6	112.6	114.6

Analysis of variance:

Source:	D.F.	S.S.	M.S.	F.
Replicates	4	3,508.8	877.2	
Treatments	5	25,100.0	5,020.0	11.0 ***
Error	20	9,092.0	454.6	
Total	29	37,700.8		

d - Grain weights in (g.)

Rep.	Treatments					
	A	B	C	D	E	F
i	47	58	63	10	56	86
ii	101	78	70	28	69	105
iii	66	62	74	25	88	60
iv	75	75	75	26	80	76
v	40	60	92	3	42	62
Mean	65.8	66.6	74.8	18.4	67.0	77.8

Analysis of variance:

Source.	D.F.	S.S.	M.S.	F.
Replicates	4	2,582.54	645.6	
Treatments	5	11,873.07	2,374.6	12.5 ***
Error	20	3,796.26	189.8	
Total	29	18,251.87		

e. - Straw weights in (g.)

Rep.	Treatments					
	A	B	C	D	E	F
i	98	79	111	31	130	103
ii	123	105	90	53	97	106
iii	98	142	87	42	94	140
iv	118	123	130	50	114	129
v	86	116	124	37	140	117
Mean	104.6	113.0	108.4	42.6	115.0	119.0

Analysis of variance:

Source	D.F.	S.S.	M.S.	F.
Replicates	4	1,241.87	313.7	
Treatments	5	20,731.90	4,146.3	13.2 ***
Error	20	6,277.60	313.9	
Total	25	28,251.37		

f - Plant heights in (cm.)

Rep.	Treatments					
	A	B	C	D	E	F
i	88	95	91	56	98	101
ii	104	102	103	65	101	96
iii	98	100	105	58	105	105
iv	92	97	87	83	104	103
v	96	101	104	57	91	100
Mean	95.6	99.0	98.0	63.8	99.8	101.0

Analysis of variance:

Source:	D.F.	S.S.	M.S.	F.
Replicates	4	220.13	55.0	
Treatments	5	5,152.67	1,030.5	22.3***
Error	20	924.67	46.2	
Total	29	6,297.47		

g - Ear Lengths in (cm.)

Rep	Treatments					
	A	B	C	D	E	F
i	10	11	10	9	11	11
ii	10	11	11	9	11	12
iii	12	10	12	9	11	12
iv	11	12	11	8	11	12
v	9	10	11	7	12	11
Mean	10.4	10.8	11.0	8.2	11.2	11.6

Analysis of variance:

Source.	D.F.	S.S.	M.S.	F.
Replicates	4	3.80	0.95	
Treatments	5	36.67	7.33	13.3***
Error	20	11.00	0.55	
Total	29	51.47		

Table 74: Colonization of root surfaces by F. culmorum in sterilized and non - sterilized soil.

Days	Treatments	Replicates								Mean
		1	2	3	4	5	6	7	8	
5	S.	8	10	10	7	10	9	10	10	9.3
	U.	8	7	6	8	10	8	10	7	8.0
7	S.	9	8	10	10	10	9	10	10	9.5
	U.	9	9	9	8	7	6	6	10	7.8
9	S.	10	10	10	10	10	10	9	9	9.8
	U.	6	9	8	9	8	9	10	8	8.4
11	S.	10	10	10	10	10	10	10	10	10.0
	U.	6	8	10	8	9	8	8	6	7.9

S. sterilized soil

U. unsterilized soil.

(see Table 43 p.236)

L.S.D.

P. = 0.05 = 1.3

P. = 0.01 = 1.4

Table 75: Decline of F. culmorum inoculum with time in non - sterilized soil.

Rep.	Time in (weeks)							
	0	1	2	3	4	5	6	7
i	8	6	3	6	8	9	5	6
ii	4	9	7	7	9	5	8	9
iii	6	4	7	9	6	7	8	7
iv	7	5	9	6	7	8	9	9
Total	25	24	26	28	30	29	30	31

Total out of 60 seeds.

Table 76: Effect of seed treatments with an isolate of Penicillium on seedling stand and growth in sterlie and infested sand.

a - Seedlings stand (no.)

Rep.	Treatments			
	A	B	C	D
i	2	5	8	8
ii	4	6	9	9
iii	1	7	10	9
iv	3	7	10	10
Mean	2.5	6.3	9.3	9.0

Analysis of variance:

Source:	D.F.	S.S.	M.S.	F.
Replicates	3	6.50	2.17	
Treatments	3	118.50	39.50	59.0***
Error	9	6.00	0.67	
Total	15			

b - Root lengths in (mm.)

Rep.	Treatments			
	A	B	C	D
i	101	120	166	147
ii	112	110	144	153
iii	96	113	132	168
iv	123	98	156	129
Mean	108	110	150	149

Analysis of variance:

Source;	D.F.	S.S.	M.S.	F.
Replicates	3	119.5	39.8	
Treatments	3	6,490.5	2,163.5	9.7 **
Error	9	1,99.0	222.1	
Total	15	8,609.0		

c - Shoot heights in (mm.)

Rep.	Treatments			
	A	B	C	D
i	153	198	220	228
ii	148	196	234	227
iii	141	193	231	214
iv	139	208	197	221
Mean	145	199	221	223

Analysis of variance:

Source:	D.F.	S.S.	M.S.	F.
Replicates	3	245.0	84.7	
Treatments	3	15,533.5	5,177.8	48.2 ***
Error	9	967.5	107.5	
Total	15	16,755.0		

Table 77: Effect of seed treatments with antagonistic micro - organisims on seedling stand and growth in non - sterile and infested soil.

a - Seedling stand.

Rep	Treatments						
	A	B	C	D	E	F	G
i	7	12	8	6	11	10	11
ii	8	13	7	7	8	12	12
iii	6	11	9	9	9	9	14
iv	5	14	6	7	11	10	12
v	8	12	9	6	8	11	11
Mean	6.8	12.4	7.8	7.0	9.4	10.4	12.0

Analysis of variance:

Source.	D.F.	S.S.	M.S.	F.
Replicates	4	0.69	0.2	
Treatments	6	159.20	26.5	14.3***
Error	24	44.51	1.9	
Total	34	104.40		

b - Root lengths in (mm.)

Rep	Treatments						
	A	B	C	D	E	F	G
i	182	286	214	158	200	225	287
ii	187	261	201	209	196	210	261
iii	208	266	191	190	191	181	267
iv	159	264	198	172	209	177	247
v	210	255	165	187	162	198	289
Mean	189	266	194	183	192	198	270

Analysis of variance:

Source:	D.F.	S.S.	M.S.	F.
Replicates	4	1,388.98	347.3	
Treatments	6	43,115.78	7,185.9	21.9 ***
Error	24	7,857.42	327.4	
Total	34	52,362.18		

c. Shoot heights in (mm.)

Rep.	Treatments						
	A	B	C	D	E	F	G
i	237	313	243	203	265	254	274
ii	210	284	231	209	220	266	311
iii	198	285	220	243	225	260	300
iv	181	260	241	230	262	258	262
v	213	277	235	184	218	285	279
Mean	208	284	234	214	238	265	285

Analysis of variance

Source	D.F.	S.S.	M.S.	F.
Replicates	4	894.39	223.6	
Treatments	6	30,058.29	5,058.3	13.2^{xxx}
Error	24	9,130.01	380.4	
Total	34	40,082.69		

Corrections and Additions.