BIOLOGY AND CONTROL OF FOOT ROT OF FRENCH BEANS

by

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ABSTRACT

Bean hypocotyls developed resistance to infection and colonization by Fusarium solani f. sp. phaseoli (Fsph) with age when grown in vermiculite but not when grown in soil. Young tissue was more susceptible than old tissue and cotyledons were rarely infected by Fsph. The cotyledon resistance was due to kievitone and an unidentified phytoalexin, both of which did not occur in hypocotyls.

Phytotoxic substances were released when macroconidia germinated and they appeared to facilitate infection of hypocotyls by Fsph.

Fsph was the primary pathogen causing foot rot of beans, but Pythium ultimum and Corticium praticola acted as synergists in mixed inoculations.

Gel filtration and chromatographic separation of soil extracts gave three fractions which induced formation of chlamydospores of Fsph. Depletion of nutrients and sudden withdrawal of carbon sources in restricted feeding experiments also induced formation of chlamydospores in Fsph.

The pathogenicity of Fsph to bean plants was different in different soils; the growing of crops in soil changed the inoculum potential of Fsph in soil. Two types of soil, 'suppressive' and 'conducive' were recognized. Examination showed that the suppressive nature of certain soils is biological in origin and mainly due to soil fungi. In conducive soils more, larger, and thickwalled chlamydospores were formed.

The presence of Fsph in bean rhizosphere soil reduced the number of root nodules probably following interaction between Fsph and soil Rhizobia spp. rathern than through direct effects on bean plants.

Several systemic fungicides were effective in controlling foot rot of beans.

A C K N O W L E D G E M E N T S

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C O N T E N T S

A B B R E V I A T I O N S

a.i.	- active ingredient
ANOVA/ANOVAR	- Analysis of variance
BM	- Basal medium
C	- Carbon
c.	- about
C^{o}	- Centigrade degrees
CE	- Culture Extract
CIF	- Chlamydospore inducing factor/s
cm	- centimeters
C/N	- Carbon:Nitrogen (ratio)
'Col'	- aqueous semicolloidal suspension
Co	- Conidial form
Cp	- Corticium praticola
Cv.	- Cultivar
CSA	- Competitive saprophytic ability
DI	- Disease Index
DNA-Molecule	- Deoxyribonucleic acid
DNA	- Diazotised Nitroaniline
f	- forma
Fig.	- Figure
Fsbt	- Fusarium solani f. sp. batatas
Fsph	- Fusarium solani f. sp. phaseoli
Full	- Inoculated throughout the substrate
f.sp.	- forma(e) specialis (es)
Fspi	- Fusarium solani f.sp. pisi

g	- gram(s)
GSE	- germinated spore extract
h	- Hour(s)
Kc	- Kilocycles
L	- litre
LANS	- Long Ashton nutrient solution
LINREG	- Linear regression analysis
LSD	- Least significant difference
LSR.05	- Least significant range at 0.05 probability level
m	- metre(s)
mg	- milligram(s)
max	- maximum
ml	- millilitre(s)
mm	millimetre(s)
min.	- minutes
M	- Mycelial form
M	- (after numerals, e.g. 0.2M) = Molar
N	- Nitrogen
nm	- nanometre
NH_4-N	- Ammonium nitrogen
NO_3-N	- Nitrate nitrogen
No.	- numbers(s)
p., pp.	- page, pages
PDA	- Potato dextrose agar
ppm	- parts per million
p.s.i.	- Pounds per square inch
Pu	- Pythium ultimum
R	- Rhizobium, Rhizobia spp.
RH	- Relative humidity

RPM	- Revolutions per minute
Rs	- Rhizoctonia solani
R:S ratio	- ratio of number of propagules in the rhizosphere to that in control soil
S	- second(s)
SE	- spore extract
sp./spp.	- species singular and plural
TLC	- Thin layer chromatography
TNV	- Tobacco necrosis virus
TOPLAYER	- Method of substrate inoculation - only the top 1 cm layer was inoculated with fungus
UV	- Ultra violet (light)
v/v	- Volume/volume
V_8/v	- V_8 Juice - Vermiculite fungal inoculum
WP	- water potential
w/v	- weight/volume
YMA	- Yeast mannitol agar
µg	- micrograms
µl	- microlitres
µ	- micron
%	- percent

LITERATURE REVIEW

The soil-borne Fusarium solani f. sp. phaseoli (Burk.) Snyd. & Hans., (Fsph) cause a serious foot rot and root rot disease in Beans (Phaseolus vulgaris L.). In the early stages, affected plants show little or no symptoms. Infected hypocotyls and roots initially develop small reddish brown streaks which eventually coalesce into a larger lesion (Snyder et al., 1959) without a definite margin (Chatterjee, 1958). Later these areas turn brown and longitudinal fissures appear in the cortex. Very often in severely affected plants reddish brown streaks are seen even higher up the hypocotyl near the cotyledons. A few weeks after emerging plants are stunted. At this stage uniform discolouration of the tap root and other secondary roots is evident. Plants are apparently as susceptible a month after they appeared above ground as they were at first (Burkholder, 1919; Weimer & Harter, 1926; Chatterjee, 1958).

Disease development

The pathogen penetrates the host directly through the epidermis of the root and hypocotyl as well as through natural and artificial openings (Burkholder, 1919; Chatterjee, 1958). Christou and Snyder (1962) reported that the broken bases of hypocotyl trichomes are penetrated by the fungus. Hyphae invade the cortex, proceed intercellularly so long as the invaded tissues are alive and subsequently become intracellular (Burkholder, 1919; Chatterjee, 1958; Christour & Snyder, 1962). It seems that infection also commences at natural openings such as root initial ruptures. Very often long fibrous roots appear in diseased plants at soil level. Root

initials are clearly visible on above ground parts of the hypocotyl. These root initials often render the hypocotyl an easy target for the pathogen, infection often starts at the tips of roots. Later there is a gradual drying out of the tissue to the stem bases (Burkholder, 1919).

Histological observations showed that the growth of the fungus in the root is relatively slower than the hypocotyl and that Fsph is primarily a hypocotyl rather than a root pathogen (Christou & Snyder, 1962).

Survival of pathogen in soil

The fungus produces abundant macroconidia in infected tissue where adequate moisture and light were available (Burkholder, 1919; Nash et al., 1961; Christou & Snyder, 1962). Also the formation of chlamydospores within the outer cortex in necrotic lesions were observed by them. At the completion of the vegetative cycle in the necrotic tissue, towards the end of the life of the plant the fungus produces chlamydospores in large numbers. It appears that the exhaustion of the food material in the host tissue by the fungus is a prerequisite for chlamydospore formation. The chlamydospores are of two types:

a. Sub-globular chlamydospores with thick warty walls borne mostly in chains on the surface of infected tissues (a single chlamydospore ranges in size from 9.7 x 8.0 μ to 14.2 x 14.2 μ).

b. Oval or pear shaped with thin and smooth walls mostly found in intercellular spaces (a single chlamydospore ranges in size from 6.2 x 4.4 μ to 8.9 x 7.1 μ) (Nash et al., 1961).

The chlamydospores constitute the primary inoculum in field soil. They are released into soil by disintegration of diseased tissue.

Fsph occurs both in culture and in nature, in conidial (Co.) and mycelial (M) form (Burkholder, 1925; Maloy, 1960). This "dual phenomenon" is a result of heterocaryosis (La Rue, 1925; Hanson, 1938). The Co. type is more pathogenic than the M type which predominates under saprophytic conditions. But it is not a vigorous competitor and its saprophytic activities are greatly restricted. Generally it grow saprophytically under conditions of limited nutrient availability (Maloy, 1960). However Nash et al. (1961) were unable to detect any M type in naturally infested Californian soils.

Nash et al. (1961) found that when macroconidia were seeded into field soil they either germinated to form a short germ tube which eventually produced chlamydospores or else they were converted directly into chlamydospores. Ford et al. (1970) suggested that it is soil fungistasis which converts the fungus to resting structures. The thick walled chlamydospores enable the fungus to survive in the soil between susceptible host crops while the thin walled hyphae and conidia lysed and disappeared from soil (Lockwood, 1960; Nash et al., 1961).

Thus conidia distribute the fungus and replenish the primary inoculum, the chlamydospores, by conversion (Nash et al., 1961). The pathogen therefore survives in soil by saprophytic growth and by resistant resting structures. Most colonies of Fsph arising on dilution plates made from soil, originated from chlamydospores (Warcup, 1955). Using the soil dilution plate technique with peptone-PCNB agar Nash & Snyder (1962) showed that in naturally infested fields the number of propagules per g of soil was as high as 1000-3000. These counts were rather uniform throughout plough depth of 15-20 cm.

Suscept detection

The propagules of many fungi require nutrients for germination (Lockwood, 1964). Toussoun et al. (1960) have demonstrated the necessity for saprophytic growth as a prelude to parasitism. It has been shown that Carbon (C) and Nitrogen (N) sources could maintain the activity of the fungus in soil (Cochrane et al., 1963). Chlamydospores of Fsph in soil depend on exogenous nutrients for germination (Schroth et al., 1963). These observations were confirmed by Cook & Schroth (1965) and established that chlamydospore germination occurred only when C and N sources were available to the fungus. Cochrane et al. (1963) showed that macroconidia required exogenous C and N for germination. The C requirement exceeded the total dry weight of the spore. Similar requirements have been reported for Fusarium roseum conidia (Sisler & Cox, 1954). This could be due to the activity of the biotic environment by immobilization of C and N through competition and by changes in rate of respiration and metabolism of spores by the toxic metabolites of other soil micro-organisms (Cook & Schroth, 1965).

When amino acids such as glutamine and glycine or asparagine were added to soil, germination of chlamydospores was three times greater than when simple sugars only were added. Ammonium form of N was more stimulatory than nitrate N. However inorganic forms of N did not cause chlamydospores to germinate when applied alone, but they did germinate with amino acid alone and with inorganic N and glucose (Cook & Schroth, 1965). Also, Cook and Snyder (1965) showed that higher concentrations of C and N in both organic and inorganic forms are detrimental.

Aspartic acid, glutamic acid, asparagine, glucose and sucrose and to a certain extent fructose and maltose were detected as constituents of bean seed exudates; these constituents individually stimulated germination and growth of chlamydospores. Analysis also indicated the presence of sugars and trace amounts of amino acids in hypocotyl exudates of bean (Schroth & Snyder, 1961). Most of the exudation occurred during the early stages of germination. Root exudation was principally from the growing root tips. Fresh sources of exudates were provided by developing lateral roots and adventitious roots erupting from the hypocotyl (Schroth & Snyder, 1961). The presence of thiamin (Bhuwaneswari & Sulochana, 1955) and auxins in root exudates has been reported (Mitchell et al., 1961)

The importance of exudates from seeds and roots in nullifying fungistasis and in the subsequent germination of resting structures of fungi has been demonstrated by Barton, 1957; Jackson, 1957; Schroth & Snyder, 1961; Buxton, 1962 and Whalley & Taylor, 1973.

Toussoun and Snyder (1961) observed that chlamydospores placed in rewetted air dried soil, did not germinate spontaneously but did germinate /when in contact with bean hypocotyls. The stimulus implicated in chlamydospore germination appeared to be restricted to a peripheral zone about 1 mm deep around the bean seed and growing roots and root tips. Germination was not detected outside this zone. Also mature roots did not stimulate chlamydospores to germinate (Schroth & Snyder, 1961). Chlamydospores of Fsph studied by Schroth and Hendrix (1962) responded to the addition of chopped plant material of various hosts.

Chlamydospores also germinated and produced limited mycelium on which more chlamydospores are formed in close proximity to seed and roots of many non-susceptible plants (Toussoun et al., 1963; Papavizas et al., 1968) such as corn, lettuce, onion and tomato (Schroth & Hendrix, 1962). This transitory saprophytic phase which results in increase in the quantity of inoculum was described as "saprophytic opportunism" by Garrett (1970).

Cook and Snyder (1965) found that due to differences in nutrient content of seed and root exudates the germinating bean seeds were rarely infected whereas hypocotyls are regularly infected. Sugars and amino acids contents are balanced in seed exudates (Schroth et al., 1963), but in hypocotyl exudates there are more sugars and trace amounts of amino acids (Schroth & Snyder, 1961).

Kraft (1974) working with peas reported that exudates of resistant varieties inhibited sporulation of Fusarium solani f. sp. pisi (Fspi), growth of Pythium ultimum (Pu) and germination of conidia of F. solani in soil.

Cook and Snyder (1965) therefore established that chlamydospore will germinate and cause infection in response to nutrient stimuli from host exudates.

Factors affecting pathogenicity

a. pH

Byther (1965) demonstrated that the germination of Fsph declines with increase in pH in a medium containing glucose NH_4-N. The pH of the rhizosphere is an important factor in relation to infection of roots. The form in which N is absorbed determines the rhizosphere pH. NH_4-N reduces rhizosphere pH while NO_3-N increases it (Zentmyer, 1975).

Maurer and Baker (1965) reported that nitrification inhibitors such as 2-chloro-(trichloromethyl)-pyridine, (N-serve) which retard transformation of NH_4-N to NO_3-N by inactivation of nitrifying micro-organisms, increase fusarium root rot of beans by lowering the soil pH.

b. Soil moisture

The importance of soil water to plant pathogenic fungi in soil was clearly elucidated by Griffin (1963). Stem rot of sweet potatoes, stem and root rot of peas caused by Fusarium solani f.sp. batatas (Fsbt) and Fspi respectively were reported to be favoured by dry soil (Harter & Whitney, 1927; Kraft & Roberts, 1969). The minimum water potential (WP) requirements for growth of higher plants range from 0 to -15 bars. But the range that prevents the growth of most root infecting fungi are -30 bars to -50 bars or lower (Cook & Papendick, 1972). In view of this Griffin (1969) suggested that WP per se probably does not directly limit infection by pathogens of growing plants. However Fsph inoculum is usually distributed in the upper soil layer 15-20 cm. (Nash & Snyder, 1962) which is usually at a low WP due to evaporation. Plants absorb water from deeper soil which is at a higher WP (Cook & Papendick, 1972). Schneider (1954) reported that the WP requirements of F. solani for optimal growth was 0 to -32 bars. Growth was reduced by half at -80 to -85 bars and prevented below -120 bars.

When relative humidity (RH) equilibriates internally between 55-75% in chlamydospores DNA molecules undergo a reversible process in which structural integrity is lost by the loss of 4-5 water molecules per nucleotide pair. But

below 55% RH, DNA molecules become irreversibly unstructured (Falk et al., 1963; Cook & Papendick, 1972). Thus very low external WP could be harmful to Fusarium chlamydospores. Also Stanghellini and Hancock (1971) working with bean seeds found that high matric potentials are necessary for higher rate and distance of diffusion of seed exudates which stimulate chlamydospore germination of Fsph.

Compact soil with a bulk density of 1.5 to 1.6 g/cm^3, thus causing host water stress aggravated the root rot of beans. In such cases yield depressing effects were considerably reduced when the hard pan was broken by sub-soiling (Burke et al., 1972a, b). Narrow spacing compared to wider spacing of bean plants favour foot and root rot of beans (Burke, 1965).

Miller and Burke (1974) working with two WPs -200 mb and -800 mb observed that decrease in soil WP favour the foot rot of beans. They concluded that detrimental effects of low WP, increased layer bulk density (equivalent to plough sole of 4 cm which cause host water stress) were additive in infested soil.

c. Soil aeration and CO_2

Increase in CO_2 concentration in soil inhibits chlamydospore production of Fsph but it increases mycelial growth and germination of chlamydospores (Bourret et al., 1965; 1968) leading to a decrease in population level and under such conditions decrease in rate of infection. Louret (1970) confirming these observations reported that aeration favours Fusarium multiplication. He found that after 11 weeks of incubation there were only about one tenth as many propagules

in soil maintained under high CO_2 (17-22% CO_2/air $^{v}/v$) as in soil exposed to a current of air (= 355 propagules/g of soil compared with 3424 propagules/g).

Miller and Burke (1975) observed that excessive wetting by furrow irrigation cause considerable reduction in bean yields, due to temporary O_2 deficit at the root surface. Under such conditions plants become more susceptible to root rot.

d. Soil temperature

Root rot severity is substantially influenced by soil temperatures. An optimum of 24°C in soil was observed by Maier (1961b) for rapid and severe disease development. Working with four temperatures (18, 24, 28, 32°C) he found that 28°C was least favourable for disease development. A relationship between high temperatures and root rot suppression with certain organic amendments such as barley, sorghum and corn without any population reduction was detected.

e. Soil nutrients

In addition to requirements for chlamydospore germination nutrients in the soil influence pathogenesis possibly in all stages. Toussoun et al. (1960) observed rapid and early lesion development at high nutrient levels when conidia are placed in droplets of various nutrient concentrations on excised hypocotyls. They concluded that higher N levels increase whereas glucose decreases disease.

It is evident from the work of Weinke (1962) that thallus development on the hypocotyl before infection is enhanced when N is easily available in surrounding soil. Experiments carried out with KNO_3 and $(NH_4)_2SO_4$ in infested soil indicated that the pathogen is more aggressive when N is available in the NH_4^+ form

in soil (Weinke, 1962). This effect was nullified by the addition of glucose to soil (Maurer & Baker, 1965). Weinke (1962) believed that NH_4-N increases pathogen aggressiveness and also increases secondary infection centres. Maurer and Baker (1965) pointed out that there is a significant reduction in severity of disease when the C:N ratio was altered from 25/1 to 75/1 by soil amendments such as cellulose, glucose alone (4500 ppm.) or in combination with NO_3-N or NH_4-N. This may depend on a group of fungi capable of utilizing either simple C sources of cellulose competitively thus removing available N required for germination of and penetration by the pathogen. In agricultural soil in general there is sufficient N (10-20 ppm.) to support germination and growth of Fsph but excess glucose (C) allows other micro-organisms (including fungi) to grow better causing N starvation for the pathogen resulting in less disease. On the other hand excess N could reduce disease by the lysis of the pathogen (Maurer & Baker, 1965; Toussoun, 1970).

Control

Baker and Nash (1965) indicated that lysis of germinating propagules does not play a significant role in suppression of the bean foot rot. However some control of the disease was obtained, probably through exolysis of hyphae by adding chitin to soil (Mitchel & Alexander, 1961), by adding mature barley residue to immobilize N in competitive action (Maier, 1961a; 1968), and by breaking the hard pan to encourage deep root penetration (Burke et al., 1972).

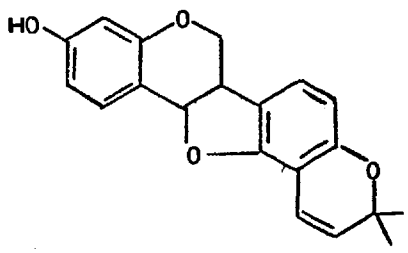
Seed treatments with 75% thiram, benomyl 50w and thiram + dieldrin were not effective in controlling foot rot. But furrow sprays with benomyl + chloroneb gave control. Best control was obtained with 1 oz ai benomyl + 2 oz chloroneb/100 ft. row but yields were low possibly because of phytotoxicity

1. FACTORS AFFECTING PATHOGENICITY

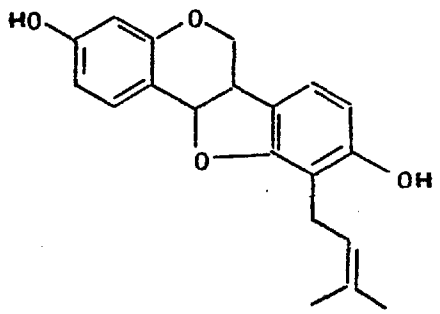
1.1 Introduction

Considerable variation in the severity of infection of beans by Fsph has been reported (Chatterjee, 1958; Bagget et al., 1965). Root lesions were observed to be smaller than those on the hypocotyl in Fsph infected beans (Christou and Snyder, 1962). However, Bateman and Lumsden's (1965) work indicated that bean hypocotyls develop resistance to R. solani infection with age.

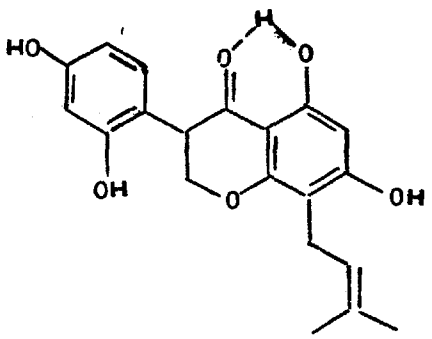
Huber (1963) attributed the resistance of bean to Fsph to a non-specific wound response with enzymatic activity around the infection site. But Müller and Börger's (1940) phytoalexin theory has been the most important working hypothesis for the physiological basis of disease resistance. Since then much research on phytoalexin has been done in several pathogen host, non-host interactions (Cruickshank, 1963) especially in legumes (Cruickshank & Perrin, 1961; 1963; Perrin, 1964; Purkayastha & Deverall, 1965a; b; Deverall et al., 1968; Pierre, 1970; Bailey & Deverall, 1971; Van Etten & Smith, 1975; Smith et al., 1975). This concept of phytoalexin formation in response to fungal infection was later extended because they are formed in response to infection by fungi, a bacterium and a virus (Stolasuta et al., 1971; Bailey & Ingham, 1971). These phytoalexins from beans were identified as phaseollin (Perrin, 1964) kievitone, phaseollinisoflavan (Bailey & Burden, 1973), phaseollidin (Perrin et al., 1972) and 2'-methoxyphaseollinisoflavan (Van Etten, 1973). (Table 1.1, Fig. 1.1).



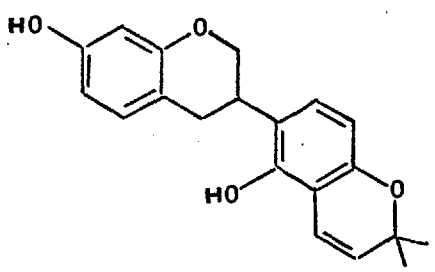
Phaseollin



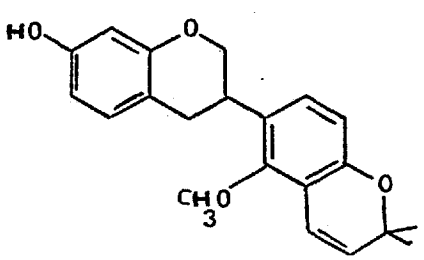
Phaseollidin



Kievitone



Phaseollinisoflavan



2'– Methoxyphaseollinisoflavan

Fig. 1.1 Bean phytoalexins

TABLE 1.1 Chemical and Physical properties of bean phytoalexins and 1a-Hydroxyphaseollone

Properties	PTEROCARPAN			ISOFLAVAN		ISOFLAVANONE
	Phaseollin	Phaseollidin	1a-Hydroxy phaseollone	Phaseolliniso flavan	2'-Methoxyphase ollinisoflavan	Kievietone
Fungitoxicity	+	+	-	+	+	+
UV absorption[1] spectra (Bailey and Burden 1973) λ max EtoH(Logε)	280(4.04); 286sh(3.98); 315(3.42)	281(3.76); 287(3.80)		280(4.01) 310sh(3.27)		293(4.22) 330sh(3.58
λ max KOH	253-281-290	290		290-341		337
nm EtoH[2] λ max (Log ε) as in a,b,c,d,e,f	279(3.96)[a]	286(3.95)[b]	280(3.98)[c]	280(4.01)[d]	280(3.97)[e]	294(4.17)[f]
R_F values in A[3]	0.67	0.48	0.39	0.38	0.67	0.13
R_F values in B[1]	0.50	0.37		0.27		0.05
Reaction with[1]						
DNA	Yellow	Orange		Orange		Orange
Gibbs'	Pale Yellow	Pale Yellow		Blue		Purple

A - Solvent Methanol:Chloroform 4:100 TLC plate Polygram S 1G/UV 254 pre-coated plastic sheets

B - Solvent Ethanol:Chloroform 3:100 - Merck F254 Silica gel pre-coated Al plates

1 - Bailey and Burden (1973)

3 - Van Etten and Smith 1975

2 - a. Cruickshank and Perrin (1963); b. Perrin et al.(1972); c. Heuvel and Van Etten (1973); d. Burden et al.(1972); e. Van Etten (1973); f. Smith et al. (1975)

DNA - Diazotised nitroaniline; Gibbs' - Gibbs' reagent (King et al. 1957)

\+ Fungitoxic

\- Not fungitoxic

Van Etten and Smith (1975) reported that Fsph-infected hypocotyls did not contain kievitone; also Fsph were able to detoxify phaseollin by an inducible oxidase.

Cook and Snyder (1965) observed that seeds were rarely infected by Fsph. Therefore it was decided to investigate the antifungal compounds of cotyledons compared to hypocotyls infected with Fsph, and also the effect of age of hypocotyls on Fsph, and also the effect of age of hypocotyls on Fsph infection.

1.2 Materials and Methods

Pathogen and Host

Virulent isolates of Fusarium solani f. sp. phaseoli (Burk.) Snyder & Hans. Fsph designated S.2d and S2f were obtained from Professor W. C. Snyder, University of California, Berkeley, U.S.A. Cultures were kept on potato dextrose agar (PDA) at 25^{o}C and 16 h daily illumination. Well sporulated cultures grown in PDA in McCartney bottles were preserved under sterile mineral oil. Both strains were inoculated into sterilized agricultural sandy loamy soil in McCartney bottles. After one month from inoculation chlamydospore production in these were confirmed by direct observation of soil using a soil smear technique (Nash, Christou and Snyder, 1961), and they were stored at 18^{o}C. These stock cultures were used to renew the cultures of pathogen used in experiments at least once in six months.

A susceptible cultivar Pinto of French bean, Phaseolus vulgaris (bean) was used throughout the investigation.

A. Seedling Age

i. Substrate for Plant Growth

Horticultural grade vermiculite (Micafil) or sandy loam agricultural soil were used as substrates for plant growth. Soil was collected from "on Hill field and Hill Bottom field" at Silwood Park. Soil was passed through a 7 mm sieve and stored in covered plastic dustbins in the glass-house.

ii. Glass-house Conditions

The glass-house was maintained at 21$\pm$ 2^{o}C through most of the year though during summer periods high temperatures were not infrequent. During the winter a 12 h photoperiod was provided by 400 W mercury vapour discharge lamps (Phillips TYP 571359) suspended about four feet above the bench level. Occasional infestation of white flies were controlled by spraying plants with Bio-Sprayday (Pan Britinnica Ind. Ltd., England). The glass-house was fumigated with Murfume Lindane pellets to control red spider mite and aphids whenever necessary.

Four or more plants were grown in 9 x 9 cm, 510 ml, square, black plastic pots (Rapidex). Pots containing soil were watered with tap water but when grown in vermiculite, Long Aston nutrient solution (Appendix) was used for watering. The pH of vermiculite after soaking with Long Aston nutrient solution was 7.2. Approximate soil moisture levels were maintained at a predetermined level using a "Green-Thumb Plant Communicator" - an electronic soil moisture indicator (Agronomic Corporation of America, Washington).

iii. Substrate inoculation

Inoculum (V8/V) was prepared by mixing 15 ml diluted V8 Juice (1:1 ^{V}/v V8/Water) with 17 g (about 150 ml) horticultural grade vermiculite and sterilizing at 15 p.s.i. for 20 min, in

in 250 ml Erlenmeyer flasks. To the sterilized mixture was added 5 ml containing 1.3 x 10^6 spores/ml. Spores were taken in sterile distilled water from 3 week old PDA plates of Fsph. After mixing the contents, flasks were incubated at 25^{o}C for 7 days before inoculating the pots containing soil or vermiculite. Varney (1961) reported that there was no evidence of any toxic effect of vermiculite on the spores, and Fusarium spp., Pythium spp. and Rhizoctonia spp. grew rapidly in vermiculite medium. Also Fusarium spp. and Pythium spp. produced spores abundantly in this medium (Varney 1961).

iv. Direct inoculation of the hypocotyl

Seedlings were grown in 40.5 x 26.5 x 5 cm polystyrene seed germination trays (Leithan Valley Plastic) containing Long Ashton nutrient solution (LANS) at 25^{o}C and 12 h daily illumination. Seedlings grown for different times were used. Hypocotyls were cut just above the roots and below the cotyledons. Cut ends were sealed with a paraffin/vaseline mixture (1:1). Excised hypocotyls were placed on perspex supporters in boxes containing 10-15 ml water. Spore suspensions were obtained by shaking 3 week old cultures on PDA, with sterile distilled water. They were filtered through 2 layers of muslin and centrifuged at 1000 x g for 10 min. Spores were resuspended in 10 ml sterile distilled water and the procedure repeated twice to eliminate contaminating substances from cultures. Spore concentrations were determined with an haemocytometer (Improved Neubauer BS748, London). Five microlitre drops containing 10^6 spores/ml (5 x 10^3 spores/drop) were placed at each inoculation point on the hypocotyl using an Agla micrometer syringe (Burroughs Welcome & Co., London).

(v) Assessment of damage

Bean plants were harvested 25-35 days from sowing. To remove the root system intact watering was stopped 2 days before harvesting. Roots were carefully washed, and left on Kimwipes tissue to remove surface water on roots. Disease severity was assessed on a scale 0-7 (Fig. 1.2) where 0 = healthy, 7 = dead.

When hypocotyls were inoculated directly with drops of suspensions of macroconidia damage was assessed by a similar scale of lesion rating (Fig. 1.3) where 0 = No lesions or specks, 7 = Highly susceptible lesion.

Percentages of diseased plants are based on total number of emerged plants unless otherwise sated.

Plant height (cm), fresh weight (g) and dry weight (g) per plant were recorded as a measure of effect of the disease on the host.

Each test was replicated 3 to 5 times and each replicate (pot) was seeded with 4 seeds. Fresh and dry weights for plants are given as the weighted average per plant, per replicate. Disease index or plant height values for a treatment are the mean of 12-16 plants. Percentage death or percentage diseased plants was transformed to arcsine percentage by the angular transformation of Fisher and Yates (1963).

vi. Quantitative estimation of Fusarium solani f. sp. phaseoli in soil

Five soil samples were taken from each pot (replicate) using a No. 5 cork borer. Samples from all the pots were bulked together to form a composite sample. These samples were collected in polypropylene wide neck bottles (Gallenkamp)

and were mixed by agitating on a whirlmixer (Fisons) before taking sub-samples for the preparation of soil suspensions. Five sub-samples were taken from each composite sample using No. 1 cork borer. This soil was used to make soil dilution 1:200 (w/v) in sterile distilled water. For the isolation of Fsph a medium recommended by Papavizas (1967) was used.

Davis agar	20.0 g
Mycological peptone (Oxoid)	15.0 g
KH_2PO_4	1.0 g
$MgSO_4.7H_2O$	0.5 g
Oxbile (Oxoid)	0.5 g
Chlorotetracycline HCl	50 mg
Streptomycin sulphate BP (Glaxo)	100 mg
PCNB a.i.	0.5 g
Distilled water	1000 ml

Chlorotetracycline, Streptomycin and PCNB were added at the time of pouring after sterilizing at 15 p.s.i. for 15 minutes. Each plate contained 15 ml medium. Poured plates were allowed to dry in a cool, dark place for 3 days before spreading the soil dilution over agar surface. One ml of the agitated soil suspension was pipetted from a sterile pipette and spread evenly over the solidified agar. Ten plate per soil sample were inoculated and incubated for 5-7 days at $25^{o}C$. Heavy sporulation, large clusters of macroconidia near the edge of the colony, irregular edges, relatively small colony size even after 5 days enabled the easy identification and counting of Fsph (Snyder et al., 1959; Parmeter & Hood, 1961; Nash & Snyder, 1962; Papavizas, 1967).

Colonies of Fsph were counted using a Bactronic colony counter (New Brunswick Scientific Co.). Counts per plate were transformed to propagules/g of soil on the basis of soil dry weight. Results are given as a mean of 10 plates per soil sample.

For the analysis of data statistical methods were taken from Sokal and Rohlf (1969), Steel and Torrie (1969). Also programmed tapes in Wang (700 programmable calculator, Software Group, Wang Europe) scientific routines were used.

a. Analysis of Variance (ANOVAR - Sokal & Rohlf, Wang)
b. Student's t test (Sokal & Rohlf)
c. L.S.D. (Sokal & Rohlf)
d. L.S.R. (Sokal & Rohlf)
e. Linear regression analysis (LINREG - Wang, Sokal & Rohlf, Steel and Torrie).

B. Phytoalexins

i. Production of diseased tissue

Beans were grown in inoculated vermiculite, or healthy excised hypocotyls were directly inoculated with Fsph spore suspension and lesions were excised 2 weeks after germination from the former, or 4 days after inoculation from the latter. Excised tissues were kept on ice during harvesting. After fresh weight had been determined, tissues were stored at $-20^{o}C$ before extraction.

Cotyledons were harvested from seedlings grown for 5 days in polystyrene seed germination trays containing LANS as above (A.iv). Cotyledons were cut into thin slices and dropped into a suspension of Fsph spores (10^{6}spores/ml) in sterile distilled water. The

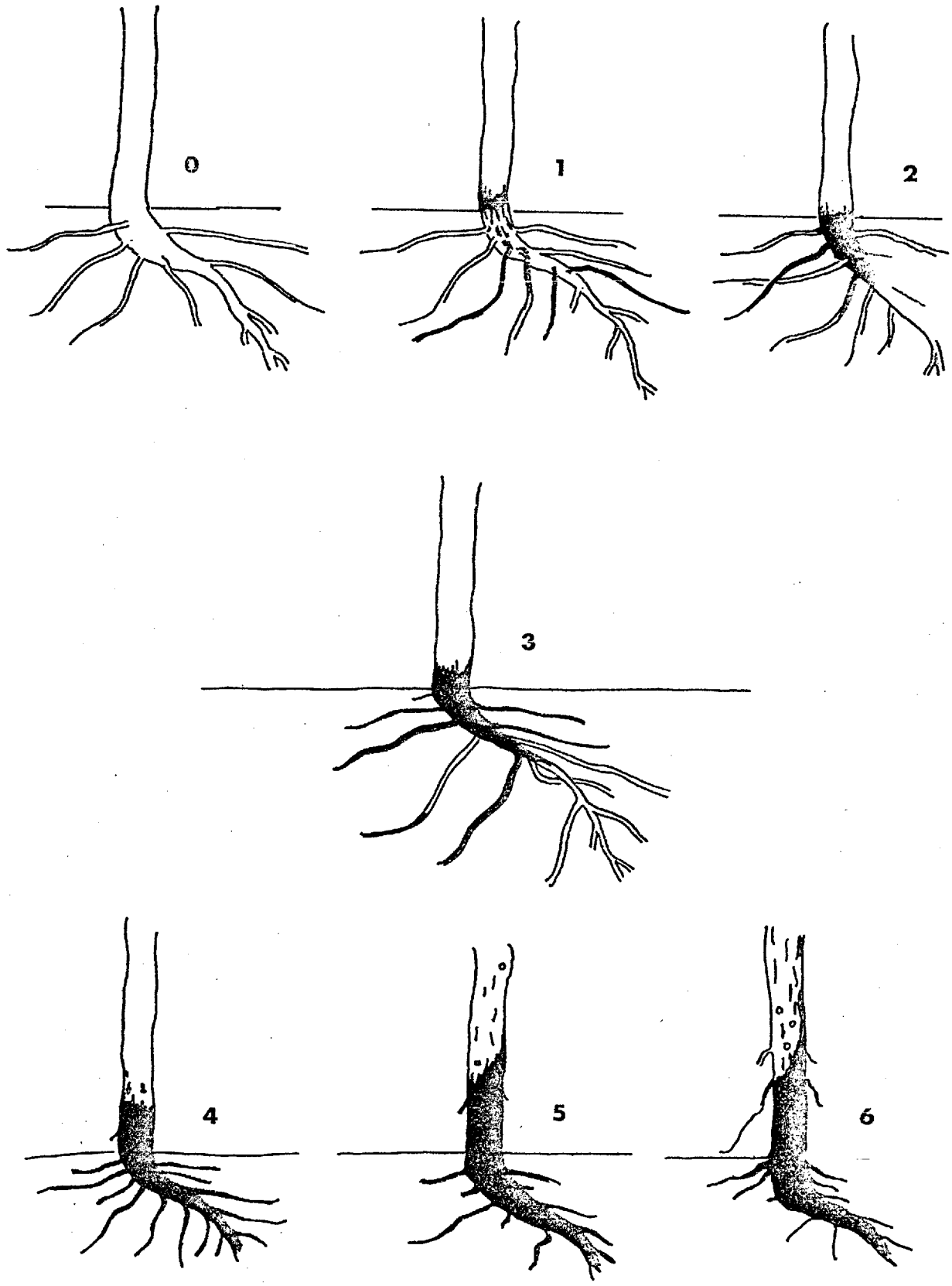


Fig. 1.2 Disease Index: Foot and root rot of French beans.

0 = healthy 7 = dead

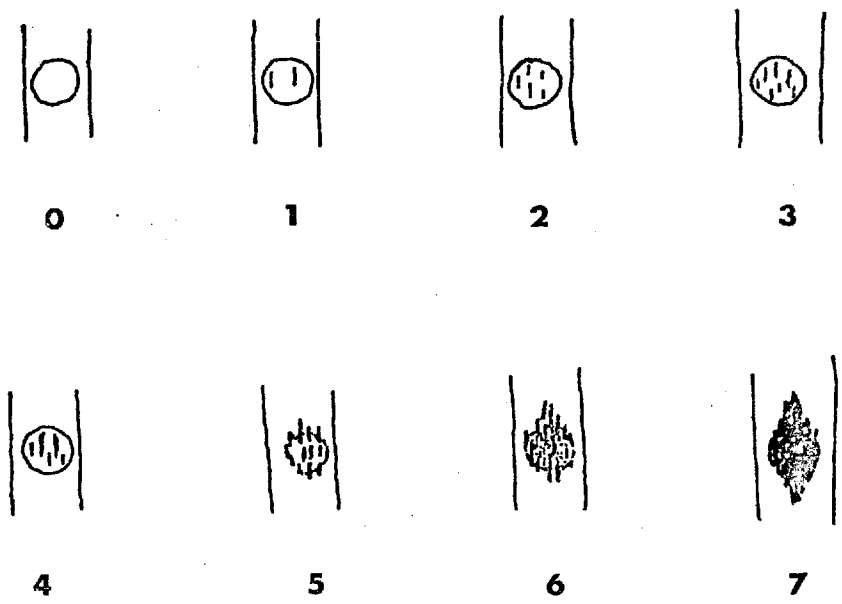


Fig. 1.3 Disease Index to assess damage caused by Fusarium solani f. sp. phaseoli on bean hypocotyls on direct inoculation

suspension was then decanted and the cotyledon slices were left on a wet filter paper in a plastic petri dish under sterile conditions, at 25°C. Three days after inoculation, fresh weights of cotyledon slices were determined and stored at -20°C.

ii. Extraction of antifungal compounds

Hypocotyl lesions (21.7 g), or cotyledon (40.4 g) lesions were comminuted 3 times in 95% ethanol (1 g tissue in 10 ml) in a Sorvall Omni-Mixer homogenizer 3 x 3 minutes at c.7000 RPM. The resulting suspension was vacuum filtered through 2 Whatman No. 1 filter papers, distilled water equivalent to 1/4 of the ethanol volume was added to the extract and the ethanol was removed in vacuo at 40°C. The aqueous extract was then partitioned twice with 1, 2-dichloroethane (1:2 v/v). The combined 1, 2-dichloroethane phase was taken to dryness in vacuo at 40°C and the residue redissolved in 0.2 M Na_2CO_3 equivalent to 1/4 of the original dichloroethane volume. The Na_2CO_3 fraction was partitioned twice with chloroform (1:1 v/v) after taking the pH to c.5.7 with 4 N HCl. The chloroform was evaporated to dryness under reduced pressure at 40°C. The residue was redissolved in c.10 ml of 95% ethanol (hypocotyles 21.7 in 11 ml, cotyledons 40.4 g in 11 ml) and stored at -20°C until assayed. Healthy hypocotyles and cotyledons were used as controls (Smith et al., 1975).

iii. Phytoalexin isolation

a. Chromatography

Chromatography was carried out on 0.25 mm silica gel pre-coated thin layer chromatography (TLC) plates (Polygram 5.1 G /UV 254 20 x 20 cm Camlab, Cambridge or silica gel precoated aluminium plates with fluorescent indicator (Merck F254). The

solvent system used was methanol and chloroform 4:100 (Methanol-Analar; chloroform Analar with c. 2%^{v}/v ethanol). Plates were developed until the solvent front reached 140 mm from the origin in unsaturated tanks.

b. Detection of Compounds

From the ethanol soluble extracts, 0.3 to 0.5 g tissue/cm for observation under ultra violet (UV) light 254 nm, and 1 g tissue/cm for bioassay were streaked on 3 to 5 cm wide origin adjacent to standards on TLC plates.

Phenolic compounds were located by spraying the developed plates with diazotised nitroaniline (Van Sumere et al., 1965), and Gibbs reagent (King et al. 1957) (Appendix), or by examining the plates under UV and marking the bands corresponding to standards.

c. Bioassay and Spectrophotometry

Developed silica plates were dried in a stream of air and sprayed with a suspension of Cladosporium cucumerinum spores in sterile sucrose/casamino acid solution (Appendix). Plates were then incubated in the dark under high humidity at 25^{o}C for 4 days. Inhibitory areas were clearly visible as white areas on a background of dark hyphae and spores in areas where the fungus grew extensively in the absence of inhibitors.

The bands corresponding to standards or to inhibitory bands from the above were scraped off from similarly developed TLC plates, into 95% ethanol in small specimen bottles and mixed on a Whirl mixer. Specimen bottles were left for c.3 h before suspensions were centrifuged at 1000 x g for 10 minutes and the supernatant was filtered through a sintered filter tube (B & T) containing a Whatman No. 1 Filter paper disc.

After evaporating the ethanol from an aliquot (½ the original volume) of the filtered supernatant in a stream of air, the residue was redissolved in LANS to obtain c.2.5 g tissue/ml for bioassay.

The biological activity of the compounds eluted was estimated by placing three 10 µl drops on each of 5 slides, and overspotting with 5 µl drops of a suspension (10^5 spores/ml) of Fsph spores. Slides were placed on glass frames in a plastic box lined with moist 'Kimwipes' and incubated for 19 h at 20°C, in an illuminated incubator (Gallenkamp). Drops of lacto-fuchsin (Appendix) were added to test drops which were examined under the microscope (1 x 10) and % germination and germ tube length was recorded. The method used to clean slides used in these tests is described in the Appendix.

Eluates in 95% ethanol was used to record the UV absorption spectrum using a Beckman DB spectrophotometer.

d. Preparation of Standard

Phaseollin, phaseollinisoflavan, phaseollidin and kievitone were isolated from tobacco necrosis virus (TNV) infected hypocotyl tissue (Bailey & Burden, 1973). TNV sap (supplied by Dr. I. M. Smith) in 0.1 M phosphate buffer ($Na_2HPO_4 \cdot 12H_2O$) was used to inoculate etiolated hypocotyls. obtained by growing Pinto bean in the dark at 25°C for 5 days. They were inoculated by rubbing the hypocotyls with sap and acid washed Celite (Bailey & Ingham, 1971). Detached hypocotyls were placed in plastic boxes and incubated under high relative humidity at 20°C in the dark. Necrotic lesions (16.5 g fresh weight) were excised and comminuted 3 times in 95% ethanol (1:10 $^{w}/_{v}$). The suspensions

were vacuum filtered and the volume was reduced to $^1/3$ of the original. To the resulting ethanol extract, an equal volume of (c.150 ml) distilled water was added and the ethanol was then evaporated in vacuo at 40°C. The aqueous fraction was partitioned 4 times with an equal volume (150 ml) of ethyl acetate. The ethyl acetate fraction was evaporated to dryness under reduced pressure and the residue redissolved in c.10 ml of 95% ethanol (Bailey and Ingham, 1971). This extract was subject to TLC chromatography on Merck F254 pre-coated aluminium sheets; 0.5 to 1 g tissue per cm was used in 5 cm wide origin. Plates were developed with ethanol/chloroform (3:100), and dried plates were sprayed with Cladosporium cucumerinum as above or with DNA or Gibbs reagent. Bands corresponding to those giving colours with DNA or Gibbs reagents.(Table 1.1) or which inhibited growth of Cladosporium cucumerinum and with R_F the same value as that of phaseollin were eluted in 95% ethanol. Phaseollin was easily identified from its previously described properties (Table 1.1). Other inhibitory bands, phaseollidin and phaseollinisoflavan were purified by successive chromatography in ethanol/chloroform 3:100 (A) and hexane/ethyl acetate 3:1. Kievitone was purified similarly by successive chromatography in A; hexane/Acetone 3:1; and toluene/ethyl formate/formic acid 7:2:1 (Bailey and Burden, 1973).

C. Phytotoxins produced by the fungus

i. Extraction of toxins

Toxins were extracted from cultures (culture extract), spore mucilage (spore extract), by disintegrating macroconidia, or by allowing them to germinate in a liquid medium as follows.

a. Culture extract (CE)

PDA plates were inoculated with Fsph by evenly spreading 0.1 ml of spore suspension (10^6 spores/ml) on the surface of agar and incubated for 6 days at 25°C. To each plate, 10 ml sterile distilled water was added, mixed by shaking and left for 25 minutes before collecting the supernatant liquid. This liquid, containing c. 10^6/ml was then centrifuged at 5000 x g for 1 h at 10°C. The supernatant was sterilized by passing through a 0.25 μ millipore filter and reduced to $^1/_{20}$ of the original volume in vacuo at 40°C. The concentrated extract was stored at -20°C before use.

b. Spore extract (SE)

Fsph was grown in 30 PDA plates as above (C1.a). Spores were collected by shaking each plate with 10 ml of sterile distilled water for 5 seconds. The final suspensions were adjusted to c.10^6 spores/ml, filtered through a sterile muslin, and centrifuged at 5000 x g for 1 h. The supernatant was then passed through a 0.25 μ millipore filter and reduced to $^1/_{20}$ of the original volume (c.15 ml).

c. Sonicated macroconidia extract (SME)

Spores from the above 30 plates (C.i.b) were washed three times by suspending in sterile distilled water, mixing on a Whirl Mixer, followed by centrifuging at 1000 x g for 10 minutes. After recording the fresh weight of the spores (0.4 g) they were sonicated in 14 ml distilled water at an ultrasonic frequency of 24 Kc/s (amplitude 5 microns) for 90 seconds in an MSE-ultrasonic disintegrator. This procedure was repeated 6 times. Throughout the treatment the spore suspension was cooled

in ice. The sonicated suspension was centrifuged at 5000 x g for 1 h, filtered through a 0.25 μ millipore filter and the volume of the extract was reduced to $^{1}/_{10}$ of the original (1.4 ml).

d. Germinated spore extract (GSE)

Wide mouth, 100 ml flasks containing 50 ml of sterilized LANS were inoculated with 3 ml of Fsph spore suspension (10^6 spores/ml) and incubated at 21°C. After 18 h incubation these were bulked (10 flasks) and passed through a 0.25 μ millipore filter. The volume of the filtrate was then reduced to about 5 ml. Sterilized LANS at equivalent concentrations was used as control.

ii. Bioassay

Five microlitre drops of the extracts were placed on 5 day old excised hypocotyls placed on perspex supporters in plastic boxes containing 10-15 ml water. Dilutions 1, 1:2, 1:5, 1:10, 1:50 and 1:100 were tested. Dilutions of the toxin that caused browning or necrosis were considered to be toxic.

1.3 Experimental

A. Seedling Age

i. Inoculum density

Although bean plants develop resistance to R. solani infection with age (Bateman & Lumsden, 1965), Chatterjee (1958) reported that neither susceptibility to infection nor host response to Fsph was materially altered by age of the bean plant. Therefore, in this study an attempt was made to determine the effect of age of the host on Fsph infection and disease expression.

Different inoculum levels were used to study the relationship between disease and amount of pathogen. They were obtained by mixing $^{v}8/v$ inoculum with vermiculite at 0, 3, 6, 12, 25, 50 and 100% $^{v}/v$. Pots were sown with seed at the time of inoculation and kept in the glass-house. Infection was assessed as described above (1.1 A.v)

Plant height was significantly reduced with increase in inoculum density (Table 1.2, Fig. 1.4b). However, increases in inoculum density from 6 to 12 or 12 to 25% did not affect plant height. Therefore, plant height may be a good sensitive indicator of disease in these ranges of inoculum density. Although the Disease Index increased sharply from 0 to 5.2 with increase in inoculum density from 0-25%, further increase in inoculum density (50 to 100%) did not increase the Disease Index significantly. However, it is evident from the Fig. 1.5 that there is a good relationship between Disease Index, dry weight per plant and inoculum density.

Since there was obvious foot rot, severe root damage (Fig. 1.4a) and other symptoms (such as production of root initials on the hypocotyl and differences in dry weight (Fig. 1.5) at Disease Index = 4 (Fig. 1.2) i.e. at 12% inoculum level it was decided to use this density to study disease in relation to age.

In naturally infested soil, populations of Fsph range from 1000 to 3000 propagules/g soil (Nash & Snyder, 1962). Therefore, knowledge of the population of Fsph in inoculated soil

would be useful in the experiments. Accordingly, after incorporating inoculum into soil at 10%, populations of the pathogen were estimated 2 and 10 days after inoculation as described above. During the experiment, soil was kept moist in the glass-house. Soils were collected from the field at two locations; both were sandy loams.

No Fsph was detected in uninoculated soil. Pathogen populations at 10% inoculum density ranged from 7000 to 8000, and 8000 to 10,000 propagules/g dried soil, 2 and 12 days after inoculation respectively. Populations at 10% inoculum density were not significantly different in the two soil samples tested (Table 1.3).

Nash and Snyder (1962) reported that in naturally infested soil, pathogen at 1000 to 3000 propagules/g soil caused considerable damage to plants.

In the current experiments field soil with 10% inoculum, and populations from 7000 to 10,000 propagules/g of soil were associated with a Disease Index of 3-4. To ensure development of disease in experiments on the effects of age of plants on disease, 10% inoculum was used although this gave populations of the pathogen well above those reported for naturally infested soils.

ii. Inoculating seedlings of different ages.

Plants grown in soil or vermiculite were inoculated at 10% level 0, 1, 2 and 3 weeks after emergence. Results are summarized in Table 1.4, 1.5 and Fig. 1.6.

a

b

Fig. **1.4** a – Typical foot rot symptoms caused by Fusarium solani f.sp phaseoli. From left to right in group of 3 plants control, grown in inoculated vermiculite; in soil.

b – Effect of inoculum density on height (1 = 100%, 2 = 50%, 3 = 25%, 4 = 12%, 5 = 6%, 6 = 3%, 7 = control) in vermiculite.

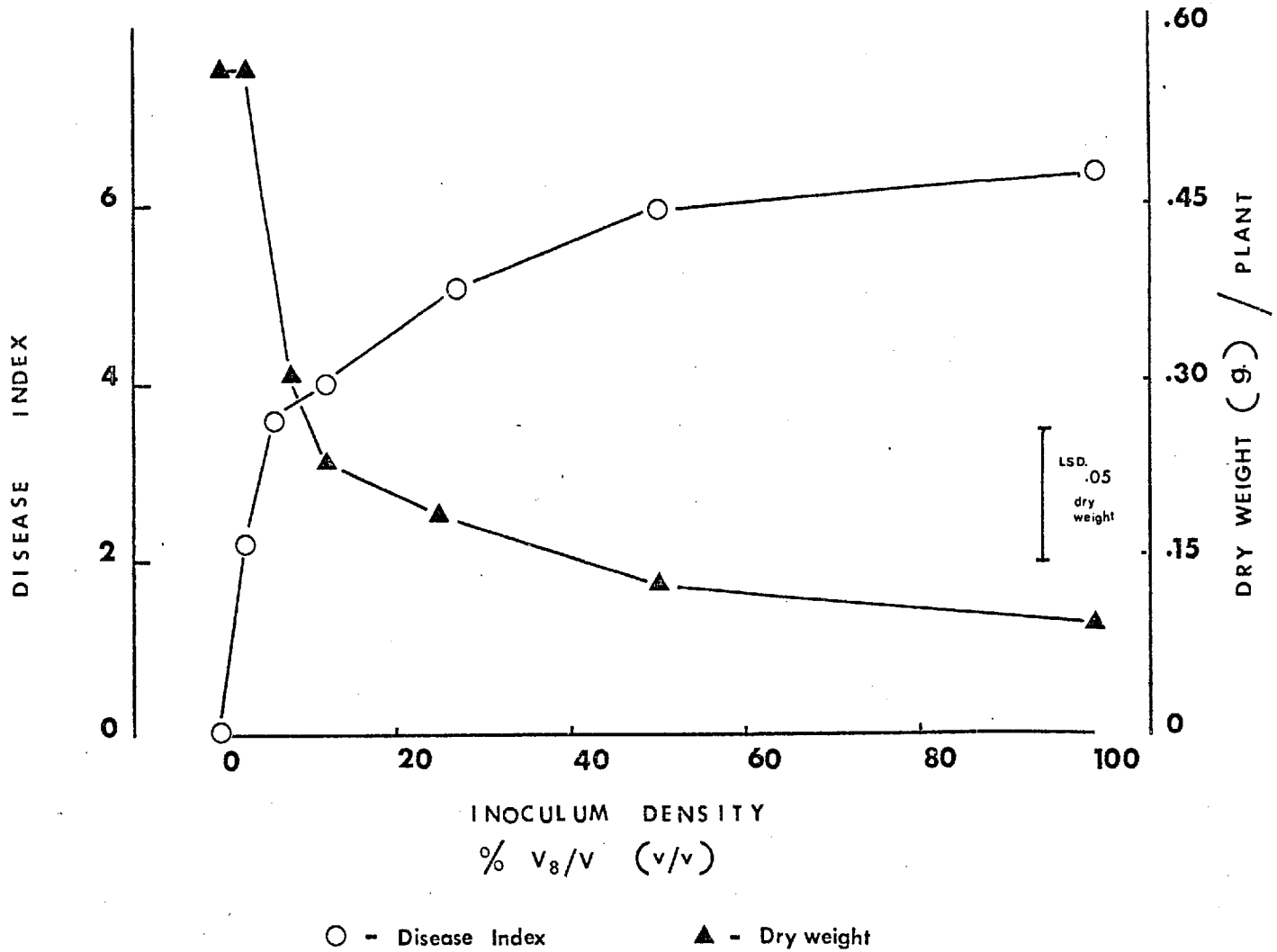


Fig. 1.5 Relationship of inoculum density to Disease Index and dry weight of bean plants grown in vermiculite inoculated with Fusarium solani f. sp. phaseoli at sowing.

TABLE 1.2 Effect of Fusarium solani f. sp. phaseoli on Pinto beans grown in vermiculite inoculated at various inoculum densities

Inoculum density[1] % $^{v}8/v$ ($^{v}/v$)	Disease Index[2]	Plant height[3] (cm)
0	0 w	22.1 z
3	2.2 x	16.5 y
6	3.6 xy	12.8 x
12	4.0 xy	11.8 wx
25	5.1 yz	9.4 w
50	6.0 z	6.9 v
100	6.4 z	6.1 v

Values followed by the same letters in a column are not significantly different at P = 0.05

1 - Inoculated at sowing

2 - Disease Index (Fig. 1.2) 0 = healthy
7 = dead. Means of 11-16 plants

3 - Plant height (cm). Mean of 11-16 plants

TABLE 1.3 *Fusarium solani* f. sp. *phaseoli* population in soil at 10% (^{v}/v) inoculum[a] level in field soil

Days after inoculation	Propagules/g dry weight soil[b]		L.S.D. at 0.01 probability level
	Soil I	Soil II	
2	8127	6816	4599.04
12	9574	7573	3671.60

a - ^{v}8/v inoculum 1:10 ^{v}/v

b - Propagules/g dried soil Mean of 10 replicates

TABLE 1.4 Plant height as a percentage of control of Pinto bean plants inoculated with *Fusarium solani* f. sp. *phaseoli*

Substrate used to grow plants	Age in weeks at inoculation[a]			
	0	1	2	3
	(Plant height as a % of control)			
Vermiculite	36.9 x	34.3 x	72.6 y	81.6 y
Soil	90.2 y	70. 7 x	69.2 x	76.2 xy

a - 10%(^{v}/v) ^{v}8/v inoculum

All values are mean for 13-16 plants. Values followed by the same letter in each horizontal line are not significantly different (P = 0.05)

TABLE 1.5 Dry weight of bean plants grown in soil and in vermiculite inoculated with Fusarium solani f. sp. phaseoli at different age levels of plants

Substrate used to grow plants	Age in weeks at inoculation 0	1	2	3	L.S.D. (P = 0.05)
	(Dry weight/plant as a % of control) in Arcsine transformation				
Vermiculite	51.2	52.8	45.6	57.3	12.52
Soil	65.1	59.9	47.7	61.4	14.51

There is a significant difference in Disease Index at 0.05 probability level in plants grown in vermiculite, between, (0); (1 & 2) and (3) weeks old plants (Fig. 1.6). Also, the fresh weight of infected plants showed a gradual increase with age with a distinct relationship between Disease Index and fresh weight. But no significant change was seen in the disease indices or fresh weight with increase in age in plants grown in soil (Fig. 1.6). Plants in vermiculite showed a significant difference in plant heights in 0 & 1 from 2 & 3 weeks old plants, and there was a general trend of increase in height with age, which was, however, irregular in plants grown in inoculated soil (Table 1.4).

Table 1.5 summarizes the effect of infection on dry weight of plants as arcsine transformation of the dry weight per plant as a % of control. There was no significant increase in dry weight of infected plants with age in comparison to controls in soil or in vermiculite.

iii. Direct inoculation of hypocotyl

Direct inoculation of 8 and 12 day old hypocotyls with washed spores showed that more resistant type lesions (Fig. 1.3) developed towards the root end of the hypocotyl and more susceptible type lesions developed away from the roots (Fig. 1.7). The Disease Index was always lower in 12 day old than in 8 day old hypocotyls for corresponding inoculation points. The bottom half of hypocotyl always produced the more resistant type lesion with the edges of lesions well defined. Younger tissue is, therefore, more susceptible to colonization by the pathogen.

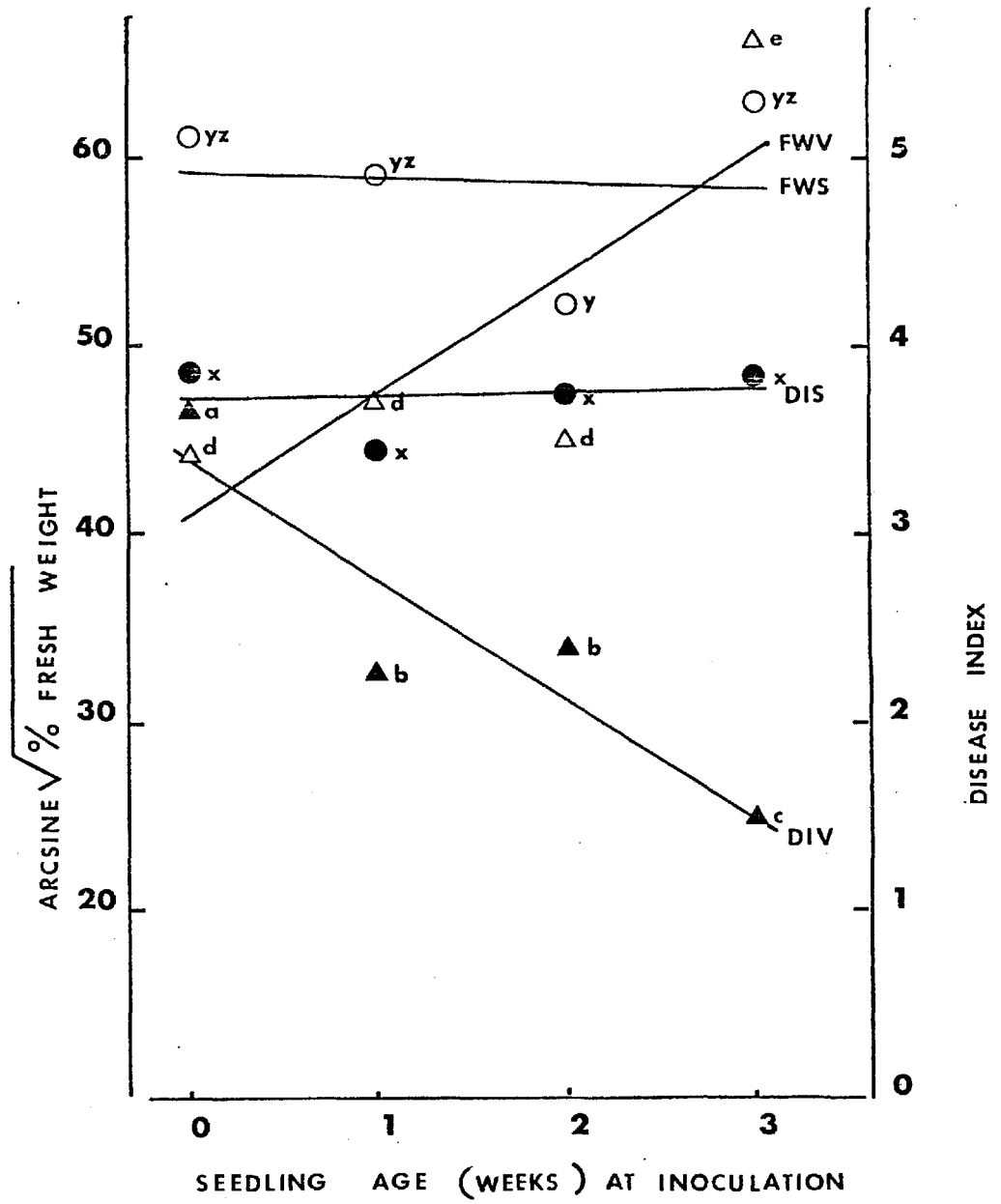


Fig. 1.6 Effect of age at inoculation on foot rot of pinto beans in soil and in vermiculite

Points followed by the same letter/s are not significantly different (P= 0·05)

FWS – ○ – Fresh weight / soil $\bar{y} = 59.32 - 0.23x$
FWV – △ – " vermiculite $\bar{y} = 41.02 + 6.43x$

DIS – ● – Disease Index / soil $\bar{y} = 3.72 + 0.01x$
DIV – ▲ – " " / vermic: $\bar{y} = 3.41 - 0.63x$

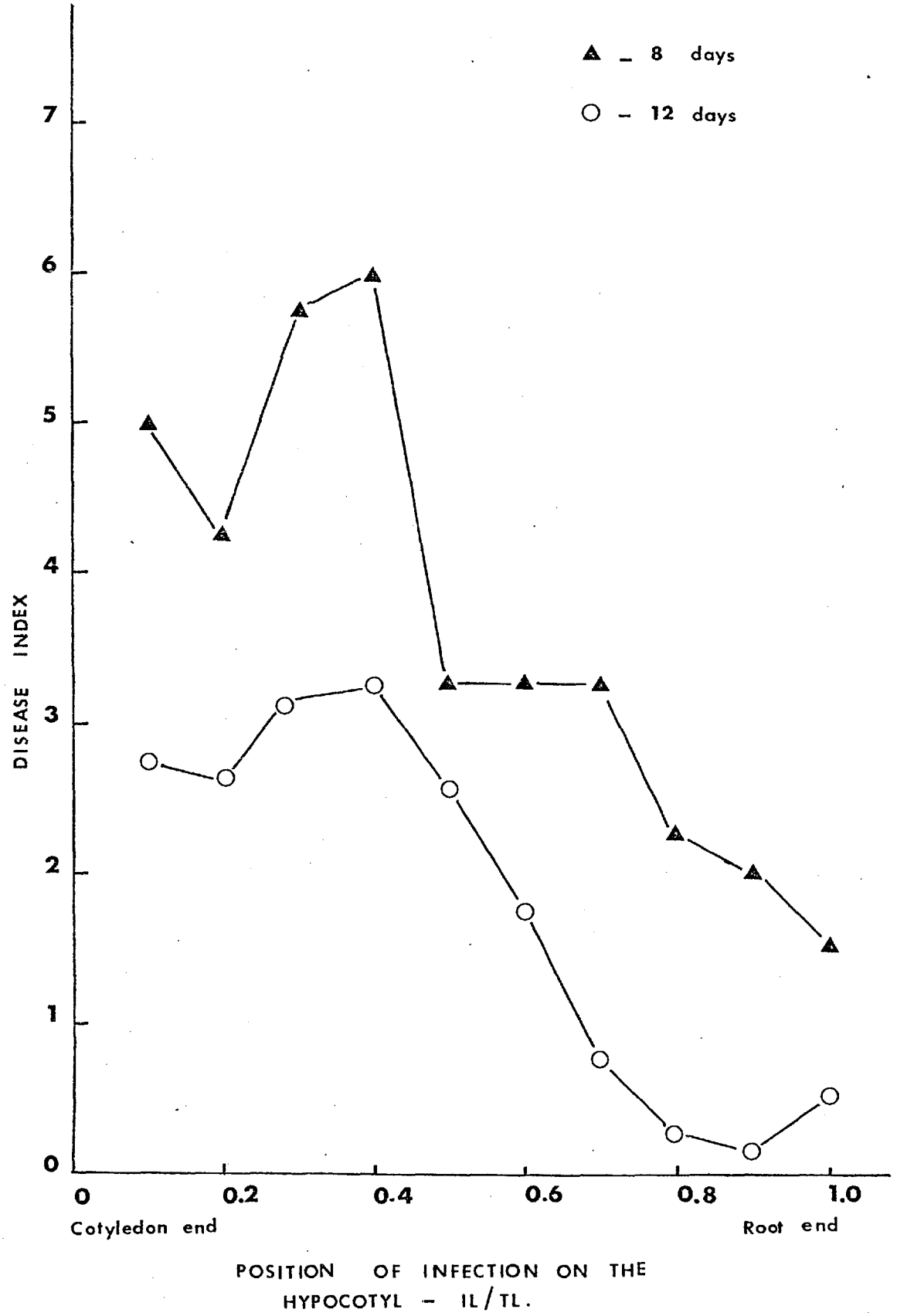


Fig. 1.7 Response to direct inoculation of bean hypocotyls with macroconidia of Fusarium solani f. sp. phaseoli at various positions along the hypocotyl.

IL/TL = Length of the hypocotyl from the cotyledon to the inoculum drop.
Total length of the hypocotyl.

D.I. – 0 = No lesions; 7 = Susceptible lesion; Fig. 1.3

B. Phytoalexins

It was observed in previous work and also by other workers (Cook & Snyder, 1965) that cotyledons were rarely infected even if the seeds germinated in heavily infested soil. The possibility that antifungal compounds were synthesized in cotyledons in response to Fsph infection was investigated.

Cotyledons harvested from plants of various ages were inoculated with 5 µl drops of Fsph spore suspension (10^6 spores/ml) to determine whether the resistance to infection varied with age. Cotyledons from 1, 2, 3, 5, 6 and 8 days old plants were used, and replicated twice with 20 cotyledons per replicate. Inoculated cotyledons were incubated at $25^{o}C$ on moist filter papers in sterile plastic petri dishes. Infection was rated as n - no lesions, S - susceptible spreading lesions and R - resistant non-spreading streaks or spots. Number of cotyledons of various lesion types per replicate (20 cotyledons) were counted and recorded as percentage of cotyledons with N, S or R lesions.

Results are summarized in Table 1.6 as an average for 2 replicates. In subsequent experiments five day old cotyledons were used because they were more resistant to infection than cotyledons at other ages. However, it is important not to overlook the fact that cotyledons were more resistant on the 5th day after emergence and earlier than on the 6th day and later.

Formation of antifungal compound in infected tissue

Aliquots of extract (0.5 g fresh tissue/cm for observation under UV and 1 g fresh tissue/cm for bioassay) of infected tissue from cotyledons and hypocotyls were streaked on to TLC plates on a 5 cm wide origin and developed in chloroform : methanol (4:1). Inspection of developed plates under 250 nm wave length light showed numerous light absorbing bands (Fig. 1.8). When these plates were sprayed with DNA or Gibbs reagent several coloured bands were formed (Fig. 1.8). A band corresponding to phaseollin standard was seen only in hypocotyl extract (Fig. 1.8). In both types of extracts bands corresponding to phaseollidin, phaseollinisoflavan and kievitone standards were visible. However, when developed plates were sprayed with Cladosporium cucumerinum neither phaseollin nor phaseollidin in hypocotyl extracts appeared as inhibitory bands. Phaseollinisoflavan was clearly visible as inhibitory in both cotyledon and hypocotyl extracts (Fig. 1.8 and 1.9).

It was the only inhibitory band visible in the bioassay of hypocotyl extracts. But in cotyledon extracts in addition to phaseollinisoflavan (R_F = 0.41) two other bands, a. at R_F 0.30, and b. at R_F 0.17 corresponding to kievitone were observed. No such inhibitory bands were seen when healthy cotyledon extracts were bioassayed. Therefore the bands further investigated were the 4 UV absorbing bands (Fig. 1.8) up to phaseollinisoflavan (common to hypocotyl and cotyledon) from the origin of the chromatogram. They were eluted (including kievitone) with 95% ethanol and bioassyed with Fsph. Results are given in Table 1.7. Each 10 µl drop of eluate contained compounds from 0.025 g of diseased tissue. Eluate 2 (R_F = 0.17 corresponding to kievitone) was completely inhibitory whereas eluate 4 (R_F = 0.30) inhibited germ tube

growth and germination of macroconidia to a considerable extent. Although eluant 1 inhibited germ tube growth it had no effect on germination of conidia. The UV absorption spectra were recorded in absolute ethanol (Fig. 1.10).

C. Phytotoxins produced by the fungus

It was observed that excised hypocotyls inoculated with suspensions of unwashed spores produced very susceptible type lesions (Type 7 Fig. 1.3) which appeared within 2 to 3 days irrespective of the age of the hypocotyl and position of inoculation. This nullified the effect of age on disease reported above (1.3A). Thus it seemed that suspensions of unwashed spores contain substances, possibly from the mucilage around spores, which stimulate production of lesions.

Fspi causing root and stem rot of peas and other Fusarium spp. from the group Martiella Wr. known to produce several phytotoxic compounds with a naphthazarin structure (Kern & Naef-Roth, 1965; 1967). They observed from in vitro and in vivo studies, that toxin formation and pathogenicity were closely correlated.

The following are the results of experiments which investigated the possibility that spores or spore mucilage of Fsph contain phytotoxins.

The effects of concentrated culture extract (CE) and spore extract (SE) on hypocotyls were similar when applied as 5 µl drops with or without pinprick damage to the surface and are summarized in Table 1.8. Observations were made 72 h after inoculation from 10 hypocotyls, each hypocotyl was inoculated at 5 - 6 inoculation points. Observations presented in Table 1.8 are those that were similar in effect at least in 90-100% of the inoculation points for each treatment.

Since the effects of culture extract and spore extract were similar in causing damage to hypocotyls it was suspected that the toxic compounds are produced by the growing mycelium or germinating spores. It is also possible that toxic materials are carried within the spores and released with germination.

The possibility that dormant spores contain phytotoxins was examined by sonic disintegration of spores. Extracts were diluted with water and assayed on hypocotyls. The maximum dilution that caused visible browning of the hypocotyl cells were recorded (Table 1.11).

To determine whether toxin in spores was released with germination, germinated spore extract GSE was prepared and tested for toxicity. To establish optimum conditions for germination, the concentration of spores, temperature and period of incubation the following tests were done.

a. Spore concentration

Washed spore suspension in distilled water at concentrations from 10^5 to 10^{10} spores/ml were applied in the standard manner as 5 μl drops onto clean glass slides and incubated at 25°C in plastic boxes for 3 days. Results are given in Fig. 1.11. Spores were considered to be germinated when the germ tube length was twice the length of macroconidia.

b. Temperature

Five μl drops of spore suspensions at 10^6 spores/ml in Long Ashton nutrient solution (LANS) was applied onto glass slides and incubated at 6, 5, 17, 21 and 22°C with 6 replicates each for 20 h in the standard manner. Percentage germination and germ tube length was recorded and results are summarized in Table 1.9.

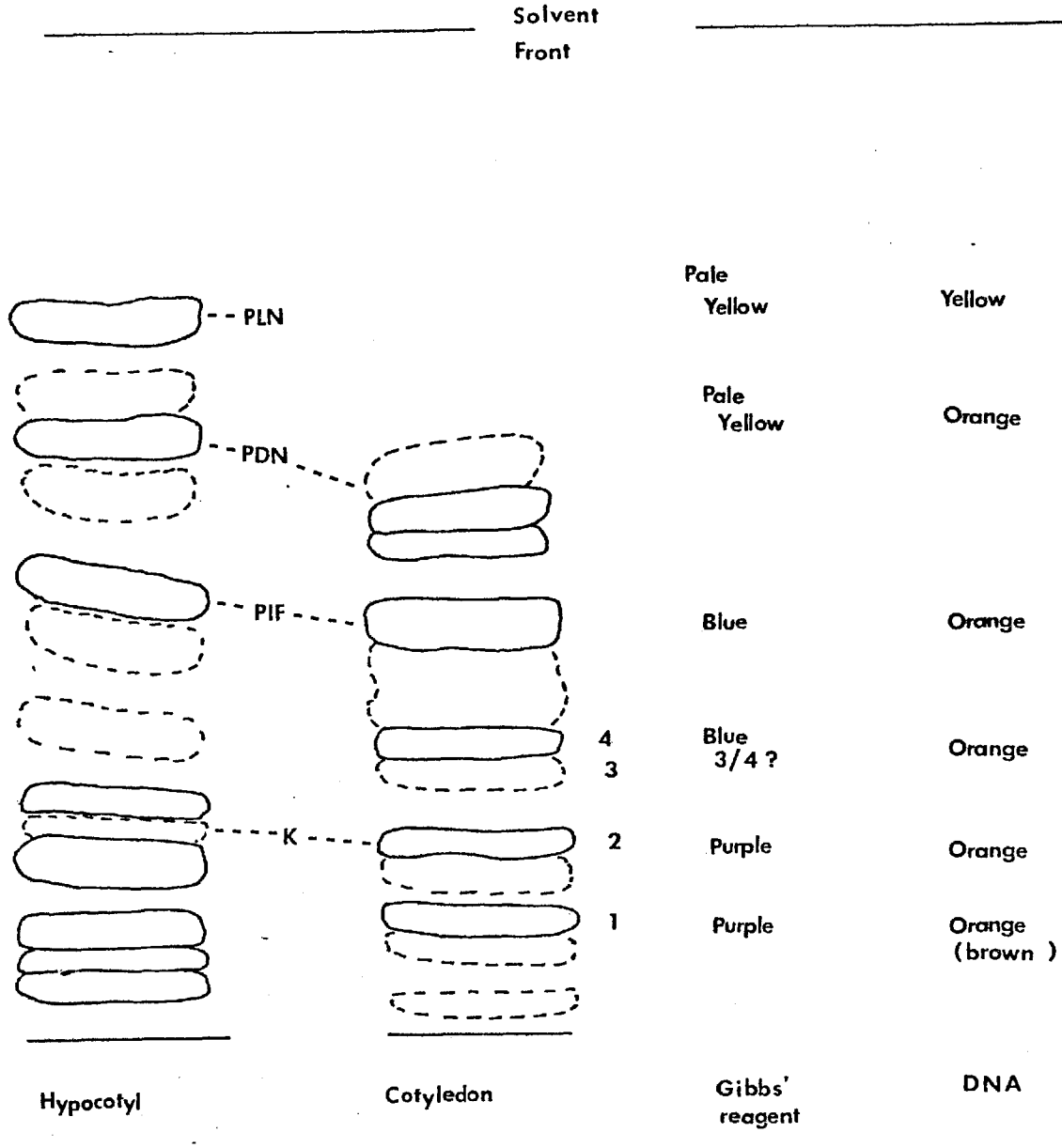


Fig.1.8 Bands visible under UV 254 nm on a TLC plate when Fusarium solani f. sp. phaseoli hypocotyl and cotyledon extract were developed with $CHCl_3$/$CHOH_3$.

PLN – Phaseollin; PDN– Phaseollidin; PIF – Phaseollinisoflavan

K – Kievitone

Dotted lines – faint fluorescent bands

Bold lines – bright " " & dark bands.

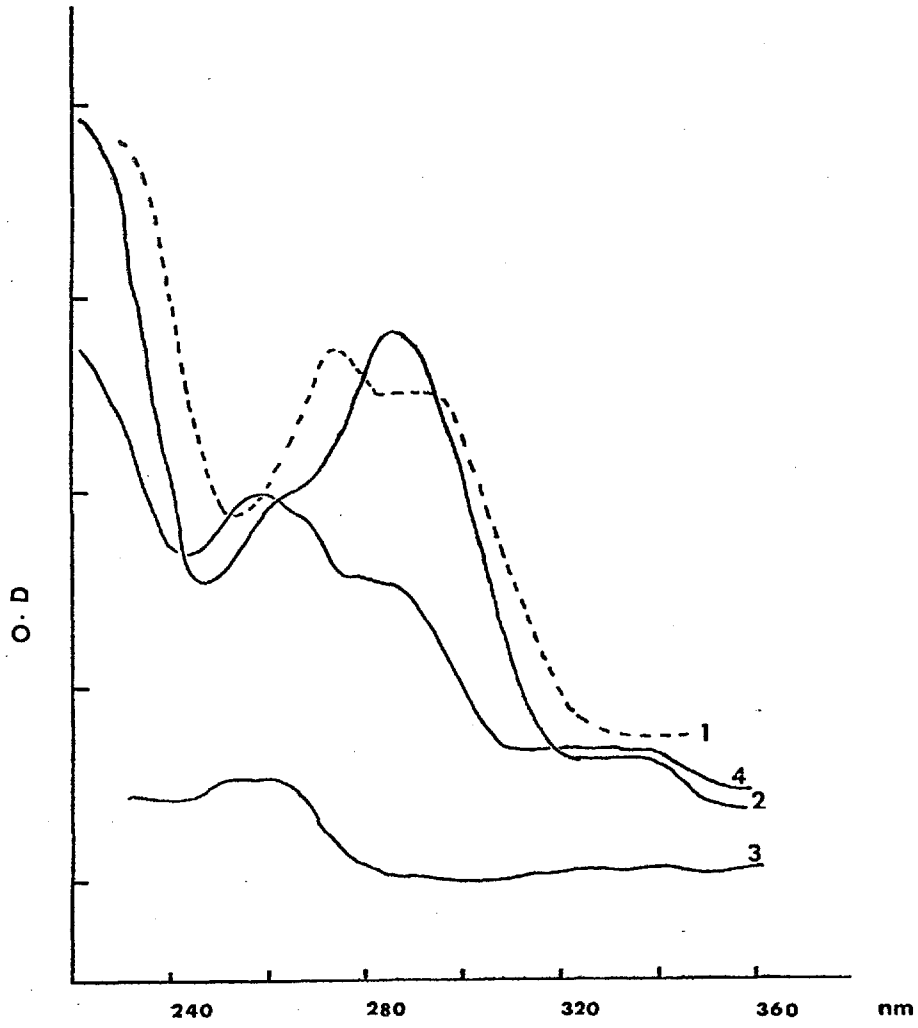


Fig. 1.10 UV Spectra of eluate 1, 2, 3 & 4 in C_2H_5OH (95%) from cotyledon extract. 2 ≡ Kievitone.

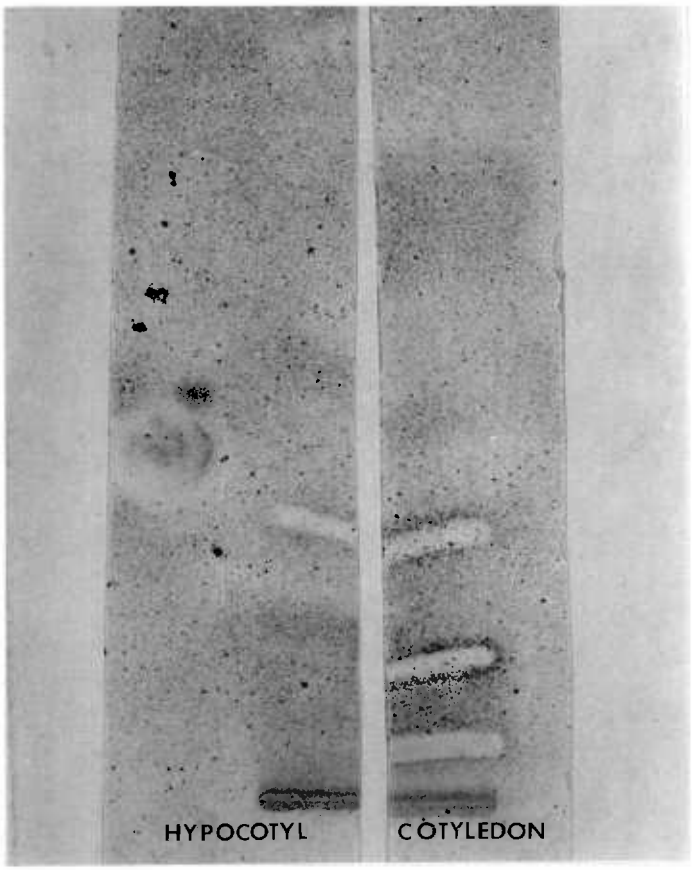


Fig. 1.9 Occurrence of antifungal compounds in extracts of Fusarium solani f. sp. phaseoli infected cotyledons and hypocotyls. Extracts were developed with chloroform/methanol and assayed using cladosporium cucumerinum.

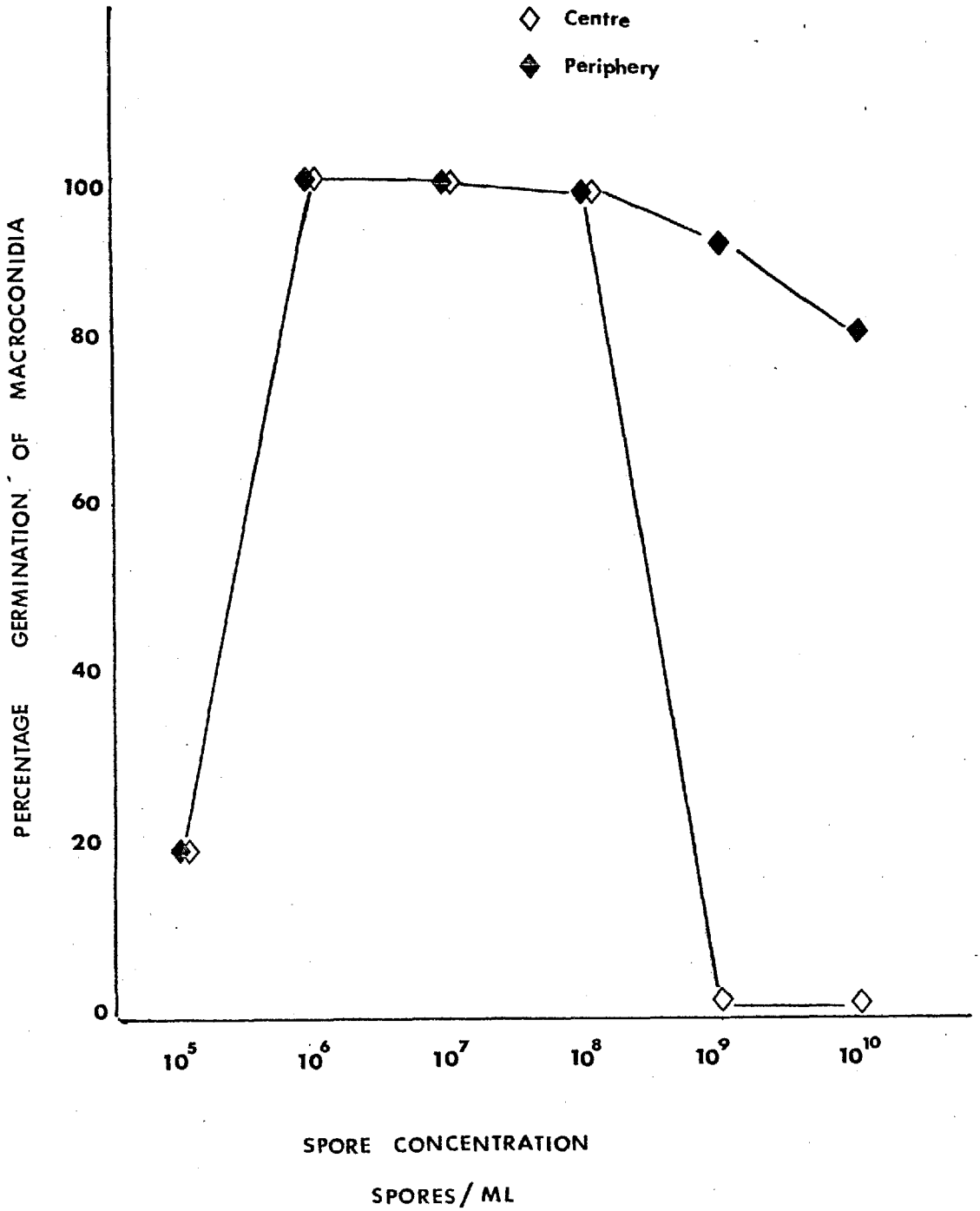


Fig. 1.11 Germination of macroconidia in the centre of inoculum drop when applied on to glass slides at various concentrations.

TABLE 1.6 Effect of direct inoculation of Cotyledons with *Fusarium solani* f. sp. *phaseoli*

Lesion rating	Age of Cotyledons in days					
	1[a]	2	3	5	6	8
	(% of cotyledons with lesions)					
N	37.5	0	2.5	0	0	0
R	40.0	92.5	90.0	100	65.0	5.0
S	22.5	7.5	7.5	0	35.0	95.0

a - Each treatment was replicated twice with 20 cotyledons each. Results are the average of 2 replicates, where the observations were recorded from 20 cotyledons in each replicate.

N - No lesions

R - Resistant non-spreading streaks or spots

S - Susceptible spreading lesion

TABLE 1. 7 Percentage germination and mean germ tube length of macroconidia of Fusarium solani f. sp. phaseoli in four fractions (eluates 1, 2, 3 & 4 Fig. 1.8) from extract of Fsph infected cotyledons

Fraction (eluate) No.	R_F value[a]	Mean percentage germination[b]	Mean germ tube length (μ)[b]
1	0.10	91.0 ± 11.9	94.4 ± 68.1
2 (Kievitone)	0.17	0	0
3	0.26	74.1 ± 14.3	200.2 ± 92.1
4	0.30	9.3 ± 4.8	135.3 ± 68.0
Control[c] LANS	-	95.5 ± 2.5	302.4 ± 92.6

a - R_F value of phaseollinisoflavan on above test = 0.41

b - All figures indicate standard error of mean as Y ± SE

c - LANS = Long Ashton nutrient solution

In 1, 2, 4 and LANS percentage germination was obtained by counting the number of germinated macroconidia per 100 macroconidia and the results are the mean for 10 replicates

Germ tube length was measured in 50 germinated conidia

In fraction 3 percentage germination was obtained as above and given as a mean for 7 replicates.

Germ tube length was measured in 35 germinated conidia

TABLE 1. 8 Influence of culture filtrate or spore filtrate of Fusarium solani f. sp. phaseoli on five day old hypocotyls applied as 5 µl drops

TREATMENT	OBSERVATIONS
CF or SF only	Brown specks.- lesions within the inoculum drop with clearly marked edge of the inoculum drop. The epidermal cells of the hypocotyl under the inoculum drop are faintly discoloured (brown) and interior of cells disorganised. Trichome bases within the inoculum drop are brown.
CF or SF plus pin prick	Dark brown pin prick clearly seen. Two lines spread along the vertical line of the hypocotyls to about twice the diameter of pin prick on either side. The internal walls of the pin prick appear dark brown under the binocular microscope.
Distilled water only	No visible damage
Distilled water plus pin prick	Pin prick easily seen. No browning around them or in internal walls of the pin prick.

TABLE 1.9 Germination of macroconidia in LANS (10^6 spores/ml) on glass slides incubated at various temperatures

Temperature (C^o)	Germination[a] (% ± SE of mean)	Germ tube length[b] (μ ± SE of mean)
5	0	0
6	0	0
17	42.3 ± 7.3	58.8 ± 27.2
21	93.6 ± 5.2	169.0 ±100.0
22	91.5 ± 4.6	109.5 ± 50.2

a - Mean for 6 replicates

b - Mean for 60 readings

TABLE 1.10 Germination of macroconidia in LANS (10^6 spores/ml) on glass slides incubated for various periods of time at 21°C

Period of incubation (h)	germination[a] percentage (% ± SE of mean)
3	9.5 ± 3.4
6	32.7 ± 8.6
9	77.2 ± 10.4
12	92.9 ± 4.3
18	95.2 ± 2.1

a - Mean for 10 replicates

TABLE 1.11 Toxin production by *Fusarium solani* f. sp. *phaseoli*

Source of origin	Dilution end point
LANS	not toxic
spore extract	1:2
culture extract	1:5
germinating spores	1:10
dormant spores sonicated	1:50

LANS - Long Ashton nutrient solution

Final concentration of the original volume (without dilutions) of spore extract, crude extract and germinated spore extract were equivalent to a spore suspension of 10^7 spores/ml. LANS was concentrated to get equal concentrations as germinated spore extract.

Sonicated macroconidia extract = 0.4 g fresh weight spores/1.4 ml, c. 3×10^8 spores.

c. Period of incubation

Experiments were carried out as above (b) and incubated for 3, 6, 9, 12 and 18 h at 21°C. Percentage germination was recorded and given as an average for 10 replicates in Table 1.10.

Maximum germination was obtained at 21°C (Table 1.9; a rather low optimum!), therefore the cultures were incubated for 18 h at 21°C to prepare germinated spore extract (GSE).

It is evident from Table 1.11 that the toxicity of CE and SE is low compared with GSE and sonicated macroconidia extract (SME). The dilution end points of GSE and SME were considerably higher (1:10 and 1:50 respectively) indicating a presence of toxic materials in these extracts. GSE was obtained from a solution equivalent to a spore suspension of 10^7 spores/ml whereas SME had c. 3×10^8 spores/1.4 ml (= 0.4 g of spores in 1.4 ml or c. 2×10^8 spores/ml).

1.4 Discussion

The above (1.3 A) has shown that bean plants develop resistance to infection with age when grown in horticultural vermiculite at pH 7.2 (Disease Index vs. Age graph; slope = - 0.63; Fig. 1.6) and that in more mature tissues, resistant type lesions developed on directly inoculated hypocotyls and more susceptible lesions developed on younger tissue of the hypocotyl (Fig. 1.7). In contrast the fungus behaved differently in field soil (Fig. 1.6 DI vs. Age slope of the graph = + 0.01). It seems that in soil, beans are more susceptible to infection and that susceptibility is independent of age or maturity. Chatterjee (1958) reported

that the shoot is more susceptible than roots. Duque and Muller (1969) suggested that resistance of tolerant and resistant cultivars of beans depends on physiological rather than physical barriers. However, the susceptible Pinto seems to develop barriers when grown in vermiculite.

Toussoun and Patrick (1963) showed that root rot of beans was enhanced when roots and stems were exposed to toxic products associated with plant residue decomposition in soil before inoculating with Fsph. These toxic products could come from soil micro-organism or the plant residue in the bean rhizosphere (Patrick and Koch, 1963). This predisposition to infection of the bean plant in soil apparently breaks down the barriers of plant.

In the present work it was found that in infected cotyledon extract, in addition to kievitone, a previously unidentified antifungal compound was present (Fig. 1.8; 1.9). But it was not possible to demonstrate these compounds in hypocotyl extracts. Van Etten and Smith (1975) reported that there was no kievitone in Fsph infested hypocotyls. But the compound corresponding to kievitone standard (band 2 Fig. 1.8) in cotyledon extract was more inhibitory than the other two bands 1 and 4 (Table 1.7). Although phaseollin was detected in hypocotyl extract it was not detected in cotyledon extracts. Therefore, the resistance of cotyledon could be attributed to their ability to produce kievitone and substance 4 in respons to Fsph infection.

Because the cotyledons and hypocotyl provide the resistant and susceptible site for infection on the same host it would be desirable to investigate further and measure the amounts of antifungal compounds in these parts of bean plants which are particularly liable to challenge by the pathogen.

Results with sonicated spores and germinating spores showed that the toxin came from the spores and were released with germination. This shows that toxin is available for early interaction of Fsph with bean tissue. Although the primary inocula in the field are chlamydospores, macroconidia, may be important as secondary inocula in spreading the disease in the field. Their capacity to germinate rapidly and to produce toxins may be significant in increasing the probability of successful infection.

2. FOOT ROT COMPLEX

2.1 Introduction

Nash (1965) and Chatterjee (1958) observed that 70-90% of the isolates obtained from disease tissue of bean plants affected with foot rot were *Fusarium solani* f. sp. *phaseoli* (*Fsph*). Four per cent of the isolates were pathogenic *Rhizoctonia* spp. (Chatterjee, 1958); 3% of the isolates on the hypocotyl lesions were *Rhizoctonia solani* (*Rs*) and about 2% were *Pythium ultimum* (*Pu*) (Nash, 1965). Species of *Rhizoctonia*, *Pythium*, *Alternaria* and *Thielaviopsis basicola* in addition to *Fsph* have been isolated from foot rot lesions of beans (Maier, 1961; Huber *et al.*, 1966). Also Burke and Kraft (1974) found that bean fields could be heavily infected with *Pu*.

Rs cause pre-emergence and post-emergence damping-off. On emerged seedlings it attacks the underground parts of the hypocotyl causing brown, deeply sunken lesions (Christou, 1962). Houston (1945) observed that *Rs* also infects roots. Hypotocyl tissue of bean was highly susceptible to *Rs* during the first two weeks of plant growth. But with maturity, elongation and concomitant changes in pectic substances and also with increase in calcium content it became resistant (Bateman & Lumsden, 1965).

Pu is widely distributed as a major root pathogen of seedlings. If seedlings survive the pre-emergence damping-off it still causes poor root development, stunting and reduction in yield (Hendrix & Campbell, 1973). It is reported to be commonly associated with root rot of beans (Adegbola & Hagedorn, 1969; Burke & Kraft, 1974).

Although the prime pathogen of foot rot of beans is Fsph it was suspected that Pythium spp. and Rhizoctonia spp. also play a role in inducing foot and root rot in beans. The following experiments were done to study the interactions of Pu, Corticium praticola (Cp) (regarded as a form of Corticium (Rhizoctonia) solani) and Fsph in the induction of foot rot in beans.

2.2 Materials and methods

(i) Host and pathogens

Phaseolus vulgaris cultivar Pinto was used. Fsph was the isolate described in section 1. Pu came from Dr. I. M. Smith and Cp from Dr. B. E. J. Wheeler. All were pathogenic to beans and kept on PDA (Oxoid).

(ii) Substrate for plant growth

Horticultural grade vermiculite or sieved field soil was used.

(iii) Inoculum

The preparation of Fsph $^{v}8/v$ inoculum was as described in section 1. For Pu and Cp the method is essentially similar but each flask was inoculated with comminuted agar from $^{1}/4$ of an 86 mm plate culture on PDA.

(iv) Inoculation

Black square pots (510 ml) were used to grow the plants. Seven different combinations of Fsph, Pu and Cp were used as Fsph, Pu, Cp, Fsph + Pu, Fsph + Cp, Cp + Pu and Fsph + Cp + Pu.

Experiments were done at 0 and 1 week after emergence in soil and 0, 1 and 2 weeks after emergence in vermiculite. Volume of substrate used in a pot was about 500 ml. Each fungal inoculum was incorporated into the substrate at 10% ($^{v}/v$) by mixing 150 ml of inoculated vermiculite with 350 ml soil or

TABLE 2.1 Amount of $^{V}8$/vermiculite inoculum (ml) used per pot to obtain different combinations of Fusarium solani f. sp. phaseoli (Fsph), Pythium ultimum (Pu) and Corticium praticola (Cp)

Pathogen combinations	Volume (ml per pot) (Final 500 ml		
	$^{V}8$/v inoculum	Non inoculated vermiculite	Soil or vermiculite
CONTROLS	NONE	150	350
Fsph	50 Fsph	100	350
Pu	50 Pu	100	350
Cp	50 Cp	100	350
Fsph + Pu	50 Fsph+50 Pu	50	350
Fsph + Cp	50 Fsph + 50 Cp	50	350
Pu + Cp	50 Pu + 50 Cp	50	350
Fsph + Pu + Cp	50 Fsph + 50 Pu + 50 Cp	0	350

fungus free vermiculite. Thus in individual treatments Fsph, Cp, Pu 50 ml of $^{v}8/v$ inoculum of the fungus was mixed with 100 ml non inoculated vermiculite before mixing with 350 ml soil or vemiculite (Table 2.1).

Plants were inoculated 1 and 2 weeks after emergence by removing plants from the pots with minimal root damage. These pots were then emptied and later filled with inoculated soil or vermiculite while plants were kept in their original position in the pots.

Each treatment was replicated 3 times and pots were kept in the glass-house in a randomized design. Glass-house conditions and maintenance of the plants were as described in 1.2.

(v) Disease assessment

Plants were harvested 25 days after inoculation. Death was recorded as a percentage of seeds sown (4 per pot) per pot to reflect effects on both pre-emergence and post-emergence death of seedlings. Length (cm) of the shoots was recorded for surviving plants and is given as a percentage of height of control plants. They were means of 7-12 plants in each treatment. In Cp treatments where numbers of deaths were high means are for 2-5 plants. For controls means were from 12 plants.

Disease severity was assessed on a scale 0-7 where 0 = healthy, 7 = dead (Fig. 1.2) and results are given as means for 12 plants.

(vi) Quantitative estimation of fungal population in soil

a. Methods of isolation

Since three pathogens Fsph, Pu and Cp were used in experiments in single and various combinations only Fsph, Pu,

Cp, Fsph + Pu + Cp and uninoculated control were used for population studies. In single inoculations population counts were restricted to the particular fungus that was used for inoculations. In Fsph + Pu + Cp and control treatments population levels of all three fungi were estimated.

Soil samples for population counts were collected and prepared as described in(1.2).

a.1 Fusarium solani f.sp. phaseoli

The method and medium used was as described in (1.2)

a.2 Pythium ultimum

Kerr's Pythium medium used in estimating population of Pythium spp. in soil was as follows (Kerr, 1962).

$NaNO_3$	2.0 g
KH_2PO_4	1.0 g
KCl	0.5 g
$MgSO_4 \cdot 7H_2O$	0.5 g
$FeSO_4 7H_2O$	0.01g
Sucrose	30.0 g
Yeast extract (Difco)	0.5 g
Davis Agar	15.0 g
Distilled water	1L
*Streptomycin sulphate	50 ppm
*Rose bengal	60 ppm
* Pentachloronitrobenzene	100 ppm
*Mycostatin (Squibb)	100 units/ml

*added just prior to pouring.

Ten to 15 ml of the medium per plate were poured.

Ten to 15 ml of the medium per plate were poured. To facilitate microscopic observation 'Sterilin' plastic petri dishes were used. Poured plates were left in the dark for a maximum of 12 h before use.

Soil dilutions of 1 in 20 (w/v) were used. One ml sample from agitated soil water suspension was poured over the solidified agar and incubated at 25°C in the dark for 2 days before counting colonies. Pythium spp. colonies were easily visible and distinct from sporulating colonies of Penicillium spp. Plates were observed under microscope (x10) to distinguish between occasional Phytophthora colonies and Pythium spp. Colony characters described by Singh and Mitchel (1961). Hendrix and Kuhlman (1965) and Hendrix and Campbell (1973) were used as guidance for the identification of Pythium colonies.

a.3 Corticium praticola

For the isolation of Cp the KO and Hoya (1971) method for Rhizoctonia spp. was used. The medium was essentially similar except that Dexon was replaced with PP 395 "Metazoxolon" [4-(3-chlorophenyl hydrazone)-3 methyl-5 isoxazolono] I.C.I. selected after screening 4 fungicides for specificity to Pythium and Corticium spp. Dexon was replaced because it was unobtainable in the U.K. The modified medium contained the following

$K_2H\ PO_4$	1.0 g
$Mg\ SO_4 \cdot 7H_2O$	0.5 g
KCl	0.5 g
$FeSO_4 \cdot 7H_2O$	0.01 g
$NaNO_2$	0.2 g
*Gallic acid	0.4 g
Agar	20.0 g
Distilled water	1L
*Metazoxolon (ICI)	100 ppm
*Chloramphenicol	50 mg
*Streptomycin sulphate	50 mg

*Added after sterilizing prior to pouring

Poured plates were kept in dark for 12 h before use.

0.5 g of soil was moistened with two drops (c.2x10 ul) of sterile distilled water, compacted with a spatula and then distributed in ten approximately equal clumps on solidified agar. Ten plates per treatment were used i.e. 100 clumps of soil per treatment. Each clump was examined microscopically after 48 h incubation at 25°C. Cp was identified by the morphological characters (Butler & Bracker, 1970). Numbers of soil clumps with growing Corticium spp. mycelium were then counted. The number of soil clumps with growing Corticium mycelium per plate are a measure of the propagules per 0.5 g (fresh weight) soil. Results are given as an average for 10 replicates and as propagules per g of soil on dry weight basis. Although there is a possibility of having more than one propagule/soil clump, modified Ko and Hova medium and techniques were tested and found that the fungus was recovered in a proportionate amount when

different amounts of Rs were inoculated into soil on a $^{v}/_{v}$ basis (personal communication, R. A. Hines).

b. Soil sampling and planting

Fungal populations were estimated in Fsph, Pu, Cp, (Fsph + Pu + Cp) and control 2d, 12d, 42 days after inoculation. Five soil samples were removed from each pot (replicate) on each occasion as described in (1.2). Pots were seeded with Pinto bean (4 seeds per pot) after 12 days from soil inoculation. Soil samples were removed for population estimation immediately before seeding the pots. The final sampling was done after 30 days of cropping that is after 42 d after inoculation as follows. Plants were removed and soils from roots were shaken into the pots. This was necessary because roots ramified profusely within the pot. Soil in the pot as a whole therefore considered to represent a rhizosphere situation. Composite samples were then made from the pots as above.

In all cases Fsph, Cp and Pu populations are expressed as propagules per g of oven dry soil. Results are given as a mean of 10 replicates for each treatment.

Data was analysed by analysis of variance, F test, L.S.D., L.S.R. and student t test.

2.3 Experimental

(i) Pathogenicity

It was shown in section 1 that bean plants when grown in vermiculite became resistant to infection by Fsph with age especially after 2 to 3 weeks growth. However this did not happen in plants grown in soil. Therefore experiments were done at 0,1 and 2 weeks in vermiculite and 0 and 1 week in soil.

a. Fusarium solani f. sp. phaseoli

Infection by Fsph usually begins at the soil level on hypocotyl and on tap root. The fungus gains entry directly through epidermis or through natural openings and wounds. When plants grown in infested soil were examined after 12 days of growth multiple infection points were visible as brown species on the subterranean parts of the hypocotyl and tap root. These brown streaks later coalesce to form larger brown lesions. Brown lesions on the hypocotyl and general browning of the tap root spreads either way from the point of infection.

When inoculated at seeding in vermiculite, Fsph infection significantly reduced the plant height (Table 2.2) but plant became resistant to infection with age (Fig. 2.1).

b. Pythium ultimum

Percentage deaths mainly due to the pre-emergence damping-off were 41.7 in Pu inoculated vermiculite (Fig. 2.2). Pu produced stunting in plants when inoculated at sowing (Table 2.2). In infected plants roots were discoloured and appeared dark brown.

c. Corticium praticola

Percentage deaths of seedlings due to both pre- and post-emergence damping-off were 100% in Cp infested vermiculite (Fig. 2.2). Deaths of seedlings due to post-emergence damping-off in one and 2 week old were 37.5% and 33.3% respectively. However, percentage deaths declined from 100 to 33 with age (Fig. 2.2). In severely infected plants irregular soft brown lesions sometimes covered with mycelium could be seen. Very often seedlings were curved and distorted and cotyledons often carried brown lesions on them. Plants became resistant to Cp attack with age (Figs. 2.1, 2.2).

(ii) Pathogen interaction in vermiculite

a. Fsph and Pu

Results given in Fig. 2.2 show that in Pu and (Fsph + Pu) (pre-emergence mortality) percentage deaths of plant was moderately high. Although they killed only few seedlings when inoculated 1 or 2 weeks after seedling emergence.

Disease Index in general decreased with age in all Fsph, Pu and (Fsph + Pu). It always remained significantly higher in (Fsph + Pu) than Pu or Fsph (Fig. 2.1).

Infected plants were shorter in (Fsph + Pu) than in controls or Pu when inoculated at sowing. However (Fsph + Pu) did not decrease plant height more than Fsph. But these differences in treatment effects on height disappeared with age (Table 2.2).

b. Fsph and Cp

In Cp and (Fsph + Cp) treatments percentage deaths (Pre- plus Post-emergence deaths) were 100 in 0 week inoculations. Although the percentage deaths declined with age they remained significantly higher than controls or Fsph (Fig. 2.2; LSD values in Appendix).

Although the Disease Index in all treatments decline with age it is significantly higher in (Fsph + Cp) and Cp than in Fsph.

All the plants were dead in 0 week treatments. Heights of survived plants were not significantly different in Fsph, (Fsph + Cp) (Table 2.2).

c. Fsph, Pu and Cp

Percentage deaths in (Fsph + Pu + Cp) were significantly higher than in Fsph and follow the same trend as all other treatments (Fig. 2.2).

Disease index of (Fsph + Pu + Cp) was significantly higher than Fsph. Also, there were no significant differences in the

disease index of (Cp + Pu), (Fsph + Cp) with (Fsph + Pu + Cp) at 0, 1 and 2 week old (Fig. 2.1).

Significant differences between the various treatments are summarized in Table 2.3.

(iii) Pathogen interaction in soil

a. Fsph and Pu

Percentage deaths of seedlings caused by (Fsph + Pu) was significantly higher than caused by Fsph alone although it was not different for Pu treatments (Table 2.4), when inoculated at sowing. But when 1 week old plants were inoculated no differences were observed among the treatments Fsph, Pu and (Fsph + Pu).

Disease Index on the otherhand was significantly higher in Fsph than in Pu inoculated plants, but there were no significant differences between (Fsph + Pu) and Fsph in both 0 and 1 week inoculations (Table 2.5).

b. Fsph and Cp

Although there were no significant differences in percentage deaths of seedlings between Fsph and Cp they were much higher in (Fsph + Cp) (50% and 66.7% in 0 and 1 week inoculations respectively) Table 2.4.

Disease Index followed the same pattern as percentage deaths with higher values in (Fsph + Cp) than in Fsph or Cp alone (Table 2.5).

c. Fsph, Pu and Cp

All the seedlings were dead in treatment (Fsph + Pu + Cp) when inoculated at sowing. But percentage deaths declined with age (Table 2.4).

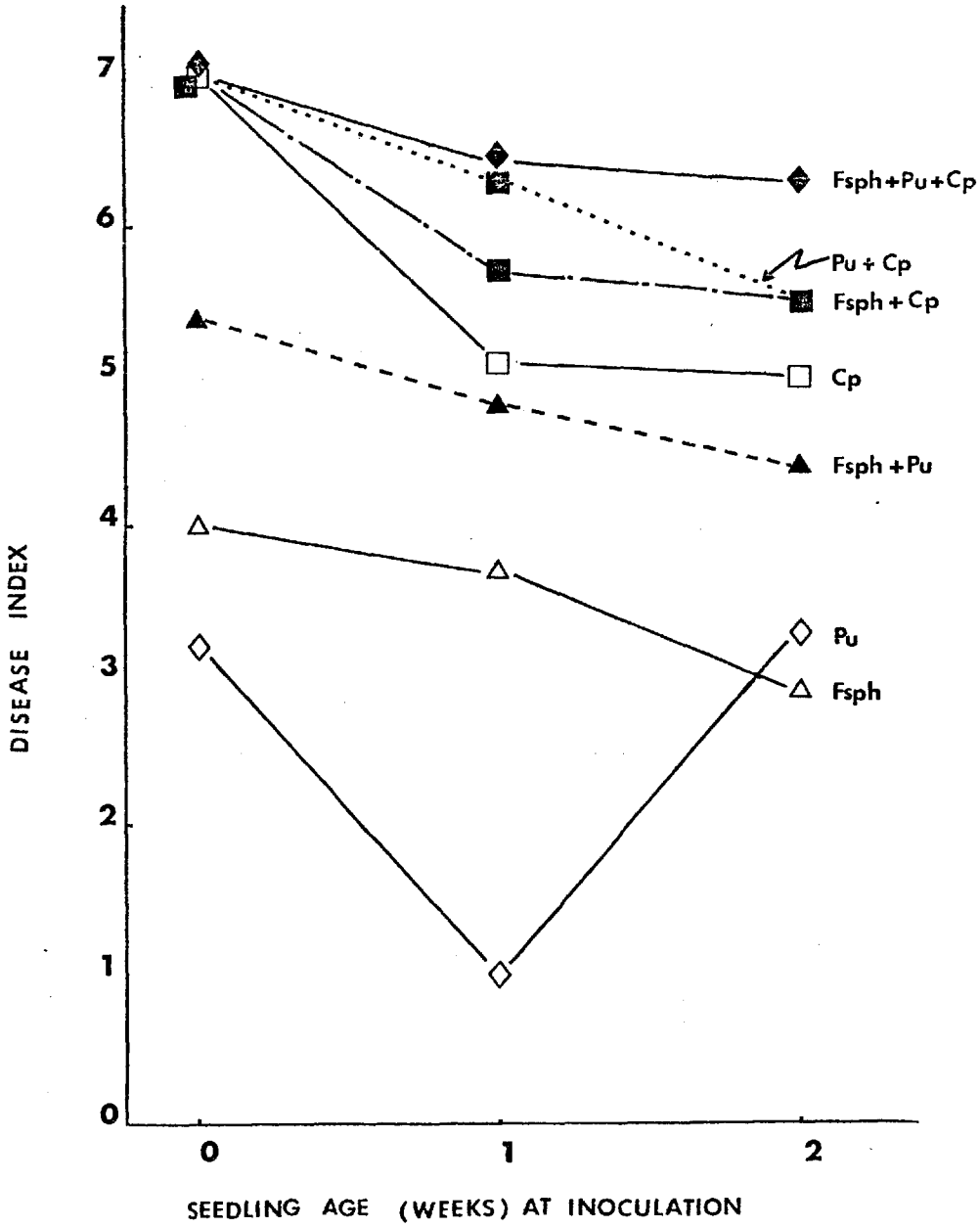


Fig. 2.1 Effect of Pathogen interaction in producing foot-rot in beans inoculated at different ages of the host in Vermiculite.

Fsph – Fusrium solani f.sp. Phaseoli
Pu – Pythium ultimum
Cu – Corticium praticola
Disease index – 0 = Healthy no lesions
7 = Dead

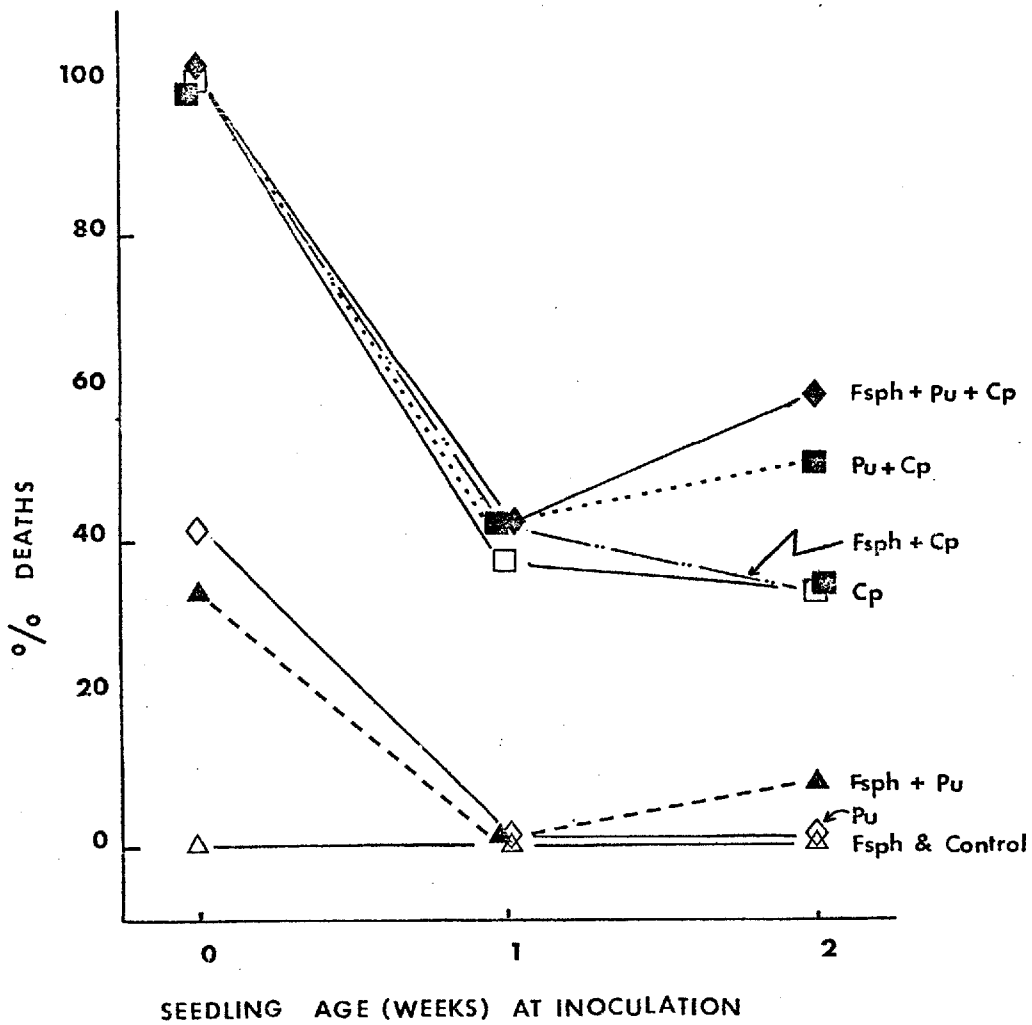


Fig. 2.2 Percentage deaths of seedlings grown in vermiculite inoculated with Fsph, Pu and Cp alone and in various combinations.

Fsph – *Fusarium solani* f. sp. *phaseoli*

Pu – *Pythium ultimum*

Cp – *Corticium Practicola*.

TABLE 2.2 Height of bean plants (as a percentage of control) grown in vermiculite inoculated with *Fusarium solani* f. sp. *phaseoli* (Fsph), *Pythium ultimum* (Pu), *Corticium praticola* (Cp) and with various combinations

Plant age in weeks at inoculation	INOCULATION								(Mean plant weight cm)
	Fsph	Pu	Cp	Fsph+Pu	Fsph+Cp	Pu+Cp	Fsph+ Pu+Cp	CONTROL	
	Plant height % of control								
0	70.4 xy	73.3 y	0	61.7 x	0	0	0	100 z	(20.9)
1	96.8 x	93.2 x	90.8 x	90.7 x	82.6 x	88.1 x	95.3 x	100 x	(20.8)
2	81.8 x	78.5 x	87.3 xy	91.3 xy	101.3 zy	114.4 z	77.4 x	100 zy	(25.0)

Values followed by the same letter in each horizontal row are not significantly different at 0.05 proability level

TABLE 2.3 Summary of statistical analysis of the effects of Fusarium solani f. sp. phaseoli (Fsph), Pythium ultimum (Pu) and Corticium praticola (Cp) and the effects of interactions on bean plants grown in vermiculite

Observation recorded	Plant Age at inoculation (weeks)	INOCULATION							
		CONTROL	Fsph	Pu	Cp	Fsph+Pu	Fsph+Cp	Pu+Cp	Fsph+ Pu+Cp
Percentage deaths	0	x	x	+y	+z	+y	+z	+z	+z
	1	x	x	x	+y	+x	+y	+y	+y
	2	x	x	x	+yz	+xy	+yz	+z	+z
Disease index	0	w	+xy	+x	+y	+z	+y	+y	+y
	1	z	+v	+w	+x	+x	+xy	+y	+y
	2	z	+v	+v	+wx	+w	+xy	+xy	+y
Plant height	0	z	-xy	-y	*	-x	*	*	*
	1	x	-x	-x	-x	-x	-x	-x	-x
	2	yz	-x	-x	-xy	-xy	+yz	+z	-x

Observations indicated by the same letter in each horizontal row are not significantly different at 0.05 probability level

+ values are more than control values - less than control values

* All plants were dead, therefore no records are available

TABLE 2.4 Percentage death of seedlings of beans in soil inoculated with *Fusarium solani* f. sp. *phaseoli* (Fsph), *Pythium ultimum* (Pu) and *Corticium praticola* (Cp) and with various combinations

Seedling age (weeks) at inoculation	INOCULATION							
	Fsph	Pu	Cp	Fsph+Pu	Fsph+Cp	Pu+Cp	Fsph+Pu+Cp	CONTROL
0	0 w	8.3 wx	8.3 wx	16.8 wxy	5.0 y	41.7 xy	100 z	0 w
1	0 y	0 y	25.0 y	0 y	66.7 z	16.7 y	25.0 y	0 y

Results are averages of three replicates

L.S.D. at 0.05 0 week = 41.4 1 week = 29.3

Values followed by the same letter in each horizontal row are not significantly different

TABLE 2.5 Disease severity (Disease Index) of bean plants grown in soil inoculated with *Fusarium solani* f. sp. *phaseoli* (Fsph), *Pythium ultimum* (Pu) and *Corticium praticola* (Cp) and with various combinations

Seedling age (weeks) at inoculation	INOCULATION							
	Fsph	Pu	Cp	Fsph+Pu	Fsph+Cp	Pu+Cp	Fsph+ Pu+Cp	CONTROL
0	5.0 x	1.3 w	1.8 w	5.4 xy	6.3 yz	4.5 x	7.0 z	0 v
1	4.0 y	1.5 x	3.2 y	4.0 y	6.4 z	3.3 y	6.3 z	0 w

Results are means of 12 plants

L.S.D. at 0.05 0 week = 1.178 1 week = 0.979

Values followed by the same letter in each horizontal row are not significantly different

TABLE 2.6 Summary of statistical analysis of the effects of Fusarium solani f. sp. phaseoli (Fsph), Pythium ultimum (Pu) and Corticium praticola (Cp) and the effects of interactions on beans grown in soil

Observation recorded	Plant Age at inoculation (weeks)	CONTROL	Fsph	Pu	Cp	Fsph+Pu	Fsph+Cp	Pu+Cp	Fsph+ Pu+Cp
Percentage	0	w	+w	+wx	+wx	+wxy	+y	+xy	+z
deaths	1	y	y	y	+y	y	+z	+y	+y
Disease	0	v	+x	+w	+w	+xy	+yz	+x	+z
Index	1	w	+y	+x	+y	+y	+z	+y	+z

Observations indicated by the same letter in each horizontal row are not significantly different at 0.05 probability level

+ values indicate more than control

Disease Index was significantly higher in (Fsph + Pu + Cp) than in Fsph or with any one of the pathogens alone (Table 2.5; Fig. 2.3).

Significantly different effects of various treatments are summarized in Table 2.6.

(iv) Influence of interaction between pathogens and cropping on fungal populations

Populations of Fsph, Pu and Cp were estimated after cropping the inoculated soil with beans. Population of pathogens are expressed as propagules per oven dried soil. Results are summarized in Figs. 2.4, 2.5 and 2.6.

No Fsph or Cp was found in the uninoculated field soil used in the experiment.

a. Fusarium solani f. sp. phaseoli

In Fsph treatments no increase in number of propagules was observed with cropping. However, Fsph in (Fsph + Pu + Cp) treatments increased with cropping from 5 400 to 8 000 propagules/g of soil. Although equal amounts were added at the start the population of Fsph 2 days after inoculation in Fsph and (Fsph + Pu + Cp) were significantly different, 6 800 propagules/g in Fsph, 4 300 propagules/g dried soil in (Fsph + Pu + Cp). No significant difference was found between the population of Fsph and (Fsph + Pu + Cp) treatments on the 12th day Fig. 2.4 (also appendix).

b. Corticium praticola

Cp counts declined sharply from 9 to 2 or 3 propagules/g dry weight soil in both Cp and (Fsph + Pu + Cp) inoculations after 30 days of cropping. Cp followed the same pattern of population change in both treatments (Fig. 2.5, also appendix).

c. Pythium ultimum

In the controls Pythium spp. population showed a gradual increase from 140 to 700 propagules/g dry weight soil. Numbers of propagules estimated in soil after cropping is significantly higher than that at seeding in control soil. But the increase in population levels in Pu inoculated treatments was not significant although it follows same pattern of population increase as in the controls. On the other hand Pythium spp. population in (Fsph + Pu + Cp) declines significantly with cropping (Fig. 2.6, also appendix).

2.4 Discussion

Data resulting from the experiments must be interpreted bearing in mind the high level of inoculum that was used. Since the susceptibility of bean to Fsph infection differs in vermiculite from that in soil the effects of pathogen interactions differ accordingly.

Bean plants appear to develop resistance to infection by Fsph, Cp and Pu with age when grown in vermiculite (Fig. 2.1, 2.2) clear synergistic effects were reflected in (Fsph + Pu) over and above Fsph or Pu separately in disease severity and in reducing plant height (Fig. 2.1, 2.3; Table 2.2). Whenever Cp was present in treatments, except in (Fsph + Cp + Pu), the effects on the host were similar to those of the Cp treatment.

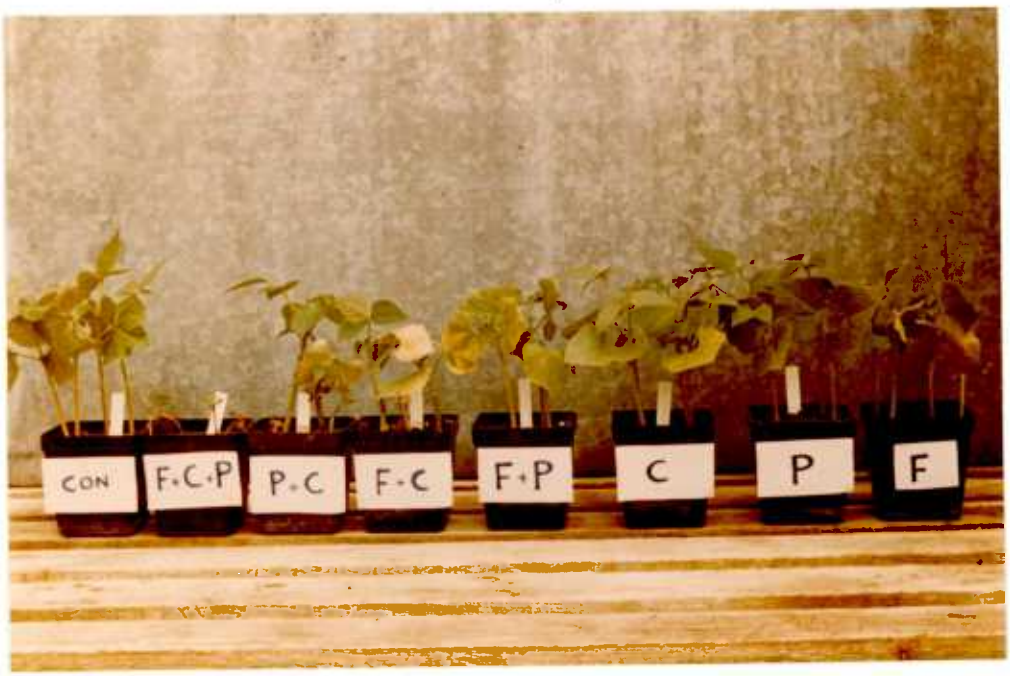


Fig. 2.3 Interaction of Fusarium solani f.sp. phaseoli, (F), Pythium ultimum, (P), & Corticium praticola, (C), on Pinto bean.

CON = Control.

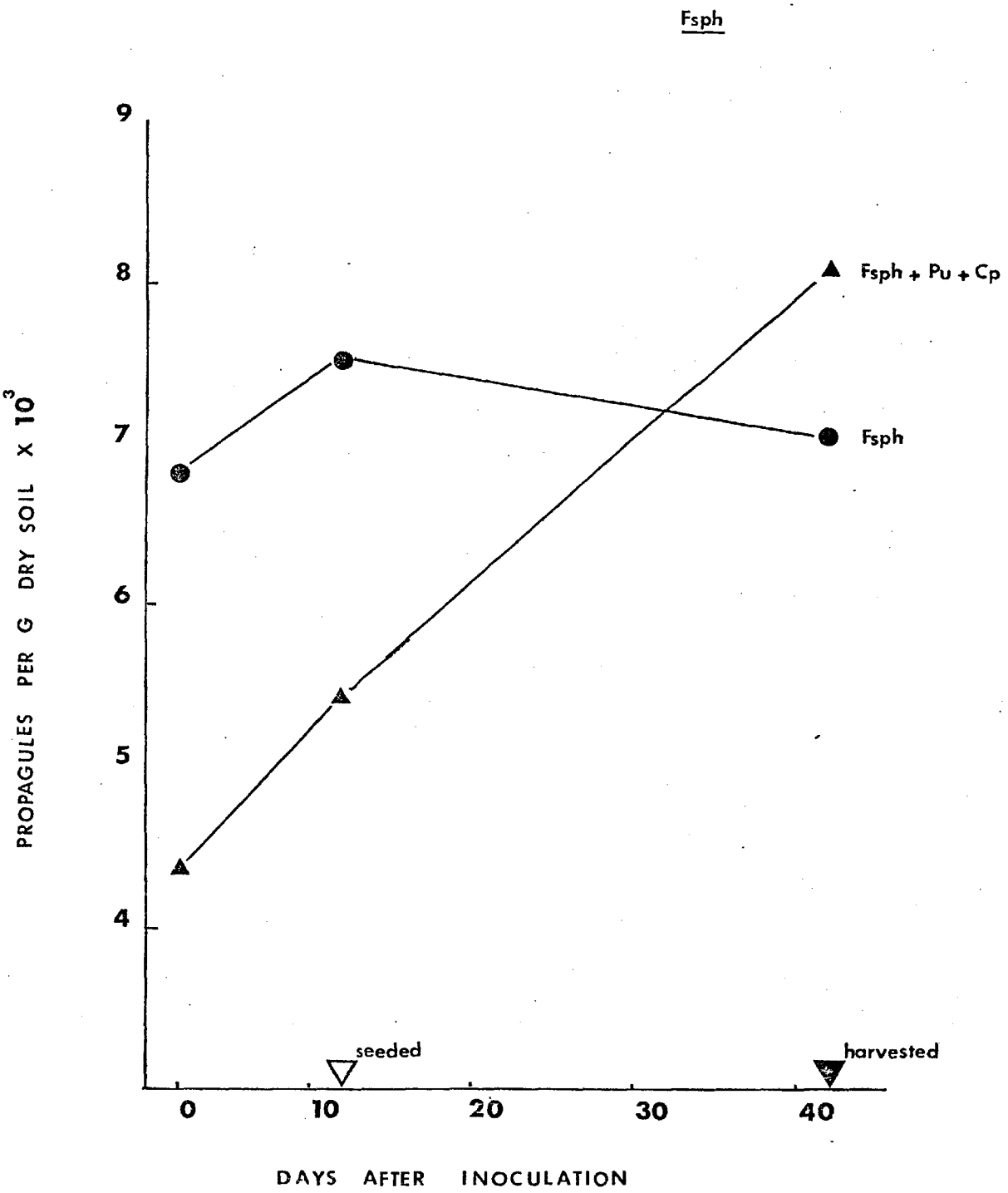


Fig. 2.4 Populations of Fusarium solani f. sp. phaseoli in soil inoculated with Fsph and in soil inoculated with Fsph + Pu + Cp.

No Fs ph was detected in uninoculated field soil

Fsph - Fusarium solani f. sp. phaseoli

Pu - Pythium ultimum

Cp - Corticium praticola

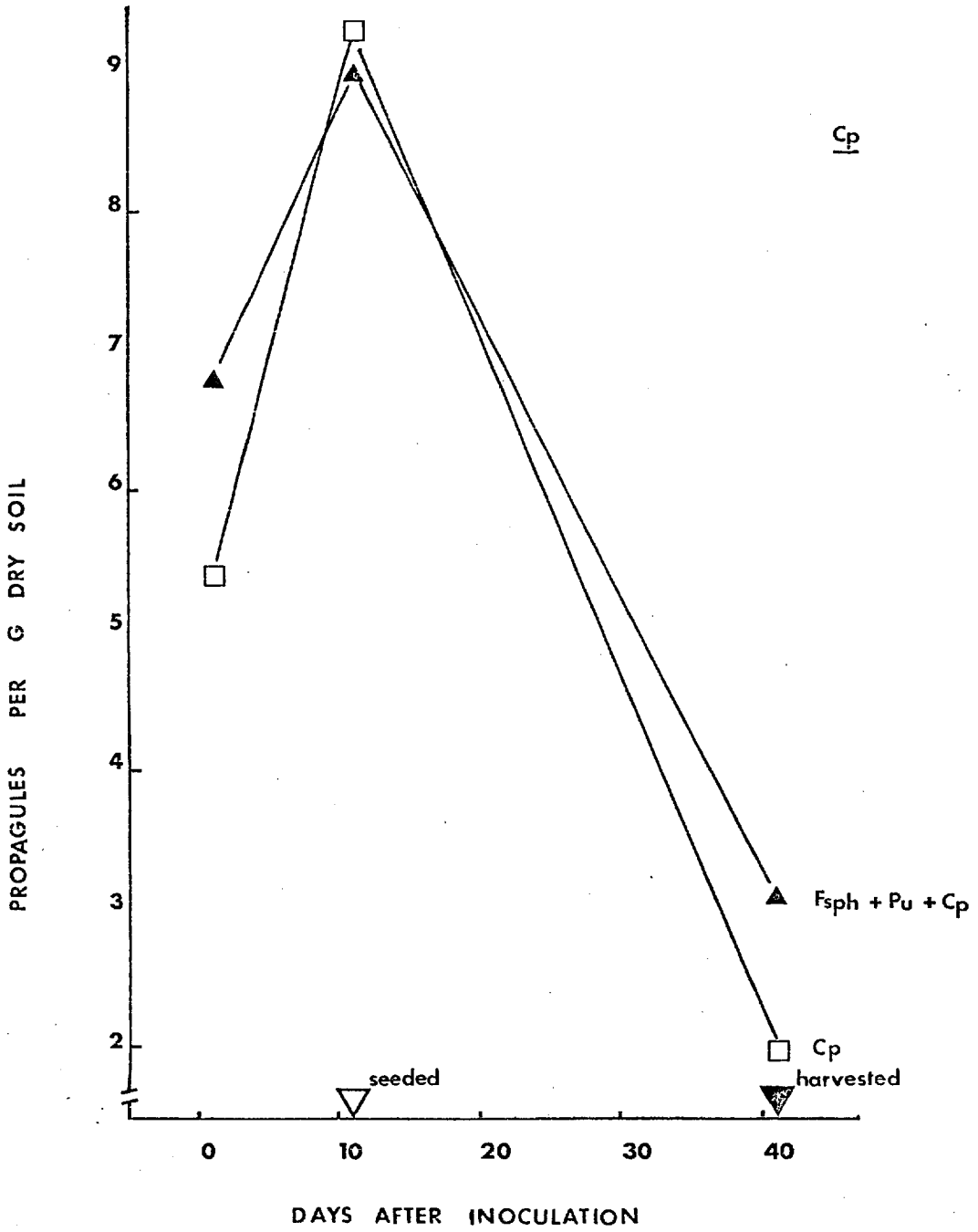


Fig. 2.5 Population of Corticium praticola in soil inoculated with Cp and in soil inoculated with Fsph + Pu + Cp.

No Corticium spp. was detected in uninoculated field soil.

Fsph = Fusarium solani f. sp. phaseoli

Pu = Pythium ultimum

Cp = Corticium praticola

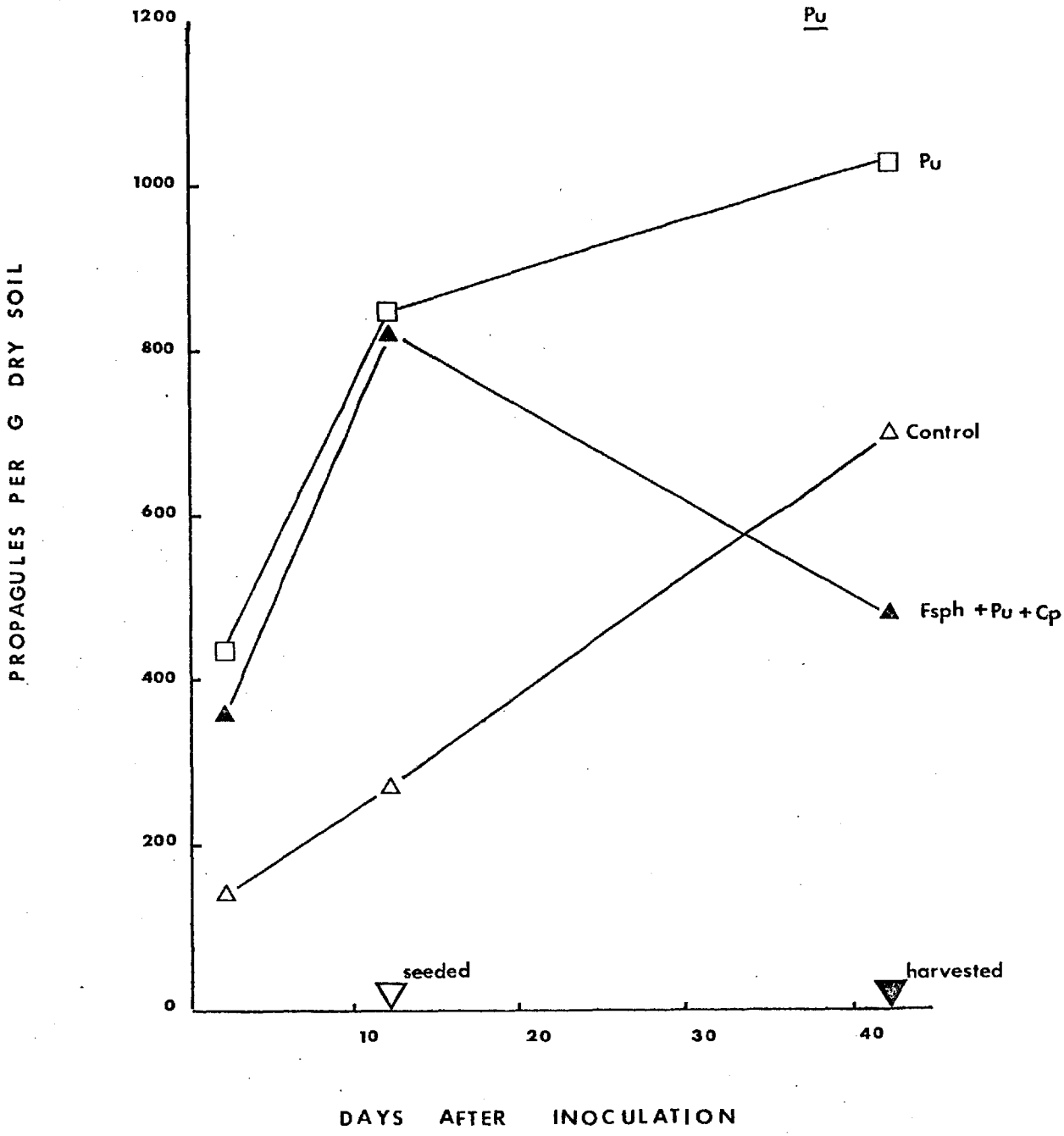


Fig. 2.6 Populations of Pythium ultimum in soil inoculated with Pu and in soil inoculated with Fsph + Pu + Cp.

Fsph – Fusarium solani f. sp. phaseoli
Pu – Pythium ultimum
Cp – Corticium praticola

This prominent effect of Cp could be due to the larger inocula used. However the (Fsph + Pu + Cp) combination produced more disease than any single pathogen treatment.

In soil the susceptibility of the host appeared to be different from that in vermiculite. Fsph in the presence of Cp damaged the plants to a greater extent than did either pathogen alone (Table 2.5). This interaction effect of Fsph and Cp was more prominent than Fsph and Pu or Pu and Cp interactions, but was similar to the effects of (Fsph + Pu + Cp).

These interaction effects are summarized in Table 2.6.

Lindsey (1965) reported that linear growth of Rhizoctonia spp. is faster than that of Pythium spp. and thus Rhizoctonia spp. have a higher competitive saprophytic ability (CSA) sensu Garrett. Fungi with low CSA cannot establish a high inoculum potential sensu Garrett as quickly as the fungi with high CSA. Also, Cp being more aggressive it could predispose bean plant to Fsph infection resulting in quicker build up of foot rot. This effect could also be due to the high inoculum level of Cp (9 propagules/g dried soil) that was used compared to 0.9 propagules per g dried soil reported from naturally infested soil (Ko & Hora, 1971).

In the (Fsph + Pu) interactions more root damage was observed than in Fsph, Pu or Cp. This was also true for general damage to the plant (Table 2.5). Similarly, Kerr (1962) reported that Fusarium oxysporum was more damaging to pea plants in the Pu.

However, it is clear from the results that unless Fsph is prevalent, damage to plants could be very low. These results are similar to those found by Burke and Kraft (1974) which suggested that the Fsph is the primary pathogen involved

in bean foot rot complex. Therefore it may be concluded that Fsph is essential for significant damage to plants in the foot rot complex. But necrosis caused by other pathogens such as Pu, Cp may predispose plants to foot rot and thus be important factors influencing the development of disease.

Cropping did not change the population significantly in Fsph or (Fsph + Pu + Cp) treatments. The faster build up of Fsph population in Fsph alone treatments compared to the slow and gradual increase in (Fsph + Pu + Cp) could be due to the higher CSA gained by Fsph in the absence of Cp and low levels of Pythium spp. in soil and in the bean rhizosphere (Fig. 2.4).

Pythium spp. population in Pu inoculated treatments were significantly higher than in the controls. Within the first 12 days after inoculation Pythium population increased in both Pu and (Fsph + Pu + Cp). This sudden increase in population may have been due to germination and subsequent rapid growth of resting spores after inoculation (Stanghallini & Hancock, 1971) in Pu and (Fsph + Pu + Cp) compared to the more stable populations in uninoculated field soil (Fig. 2.6). On the 12th day after inoculation i.e. at sowing Pythium spp. populations were significantly higher in Pu and (Fsph + Pu + Cp) than in controls. In the rhizosphere of a susceptible host, populations of Pythium spp. showed a significant increase in number from 270 to 700 propagules/g dry weight soil in uninoculated control (Fig. 2.6 also in appendix). Populations after cropping in each treatment were significantly different from each other. Surprisingly the Pu population in (Fsph + Pu + Cp) declines

significantly with cropping. High populations, about 730 propagules/g dried soil in infested soil were reported by Singh and Mitchell (1960) and 100 to 1000/g dried soil by Kraft and Roberts, 1970. Pu on peas increased from 237 to 870 propagules/g dried soil after cropping (Kerr, 1962).

Pythium spp. population in (Fsph + Pu + Cp) declined with cropping from 821 to 480 propagules per g dried soil. Lindsey (1965) working with soil microbiological sampling tubes reported that Pythium spp. were unable to colonize tubes in the presence of Rhizoctonia spp. but Pythium spp. had no influence on the Rhizoctonia spp. in his experiments. As Lindsey (1965) suggested, Pythium spp. are unable to compete for root exudates of bean plants in the presence of Cp. However, Butler (1957) reported that Rs parasitizes Pythium spp.

Ko and Hora (1971) reported that in infested soil, Rhizoctonia spp. populations are as low as 1 - 9 / 10 g dried soil (= 0.1 to 0.9 propagules/g dried soil). Therefore, severe decline in populations of Cp after cropping in Cp inoculated soil, or in (Fsph + Pu + Cp) may be due to the introduction of unusually high (5.4 to 6.8 propagules/g dried soil) inoculum (Fig. 2.5). On the other hand it was found that field soil used in the experiment did not carry any detectable amount of Cp and this could be because field soil was not contaminated or the field soil may be highly antagonistic or suppressive to Cp. If the latter is correct a drastic decline in Cp even after cropping with bean would be expected.

3. FACTORS INFLUENCING CHLAMYDOSPORE FORMATION AND DISEASE EXPRESSION IN SOIL

3.1 Introduction

3.1 A Chlamydospore formation

Pathogenic Fusaria exist in soil as chlamydospores (Nash et al., 1961; French & Nielson, 1966; Cook, 1968). The survival of Fsph depends largely on the production of these resting structures which could survive in soil for long periods of time in the absence of the host.

Several factors are known to induce chlamydospore formation, among them, nutrient starvation (Ko & Lockwood, 1970; Hsu & Lockwood, 1973), C/N ratio of growing medium (Wilson, 1960; Qureshi & Page, 1970), accumulation of staling products (Park, 1961; Griffin, 1965; Ford, 1969), unfavourable growing conditions (Park, 1954), stimulatory substances occurring in natural soil (Alexander et al., 1966; Ford et al., 1970), soil bacteria (Venkata Ram, 1952; Ford et al., 1970b), and by lowering pH (Cochrane & Cochrane 1971).

These could be broadly categorised into two hypotheses that chlamydospore formation is an endogenous response to nutrient starvation (biotic or abiotic) or that chlamydospore formation is an aspect of the general phenomena of soil fungistasis operating through a balance of inhibitory and stimulatory substances that occur in soil (Watson & Ford, 1972).

Alexander et al. (1966) working with sterile soil extract suggested that more than one substance in soil extracts may be responsible for the induction of chlamydospores.

Ford et al. (1970) confirmed their findings. In the present study an attempt was made to understand the effects of different fractions of aqueous soil extract on chlamydospore formation.

3.1 B Disease expression in soil

The expression of disease symptoms of foot rot of bean seems to vary in different soils (Burke, 1965). Such differences in host susceptibility in various host-pathogen/soil combinations have been attributed to effects of previous cropping and crop residue in soil (Williams & Schmitthenner, 1960; Maier, 1961; Schroth & Hendrix, 1962), continuous monoculture (Williams & Kaufman, 1962), mycolytic bacteria in soil (Mitchell & Alexander, 1961) and in general to rhizosphere microflora (Venkata Ram, 1960). Therefore the soils could be broadly grouped into two groups, namely conducive soil, that make the plant susceptible to disease, and suppressive soil, that suppress the disease even under conditions favourable for disease development. In these experiments the nature of suppressive and conducive soils was investigated.

3.2 Materials and methods

Two pathogenic strains of Fsph S.2d and S.2f and a susceptible cultivar Pinto beans were used (1.2).

3.2 A Chlamydospore formation

i. Preparation of soil extract

Soil collected from "on hill and hill bottom field" from a plot where field beans (Vicia faba) were grown in the previous season, was kept moist in covered plastic dustbins

in the glass-house. Soil when required was passed through a 1680 micron, mesh 10 (Gallenkamp) sieve and 380 ml distilled water was added to 1140 ml loosely packed soil. The final soil/water extraction ratio was 3:1 ($^{v}/v$) (Ford et al., 1970). The suspension was then distributed equally between 8 flasks (250 ml) and shaken for 20 minutes at a medium speed on a Griffin flask shaker. Soil extract was recovered after passing the agitated soil suspension through glass wool. The extract was then sterilized by passing through an 0.20 μ millipore filter (Alexander et al., 1966) and reduced in vacuo at 37°C to $^{1}/10$ of its original volume.

ii. Gel filtration

Polymerized dextran gel 'Sephadex' G25 (Pharmacia Fine Chemicals, Sweden) was used as the gel matrix in a Sephadex column K 15/30. The K 15/30 chromatographic tube is made of borosilicate glass with an internal diameter 15 mm. The bottom of the tube is fitted with an endpiece with a 10 μ nylon bed supporting net. G25 coarse (with water gain value 2.5 ml/g dry gel; dry particle diamter 100 to 300 μ and with a fractionation range 1000 to 5000 molecular weight) was swelled in distilled water for 12 h and poured carefully into the column down a glass rod. The gel was allowed to settle to a column of height 25.8 cm. To obtain even sedimentation eluant (distilled water) flow was started soon after filling the column (Sephadex, 1973).

700 μl of concentrated soil extract was applied to the surface of the bed using a micropipette and eluted with sterile

distilled water. Fractions were collected manually under aseptic conditions and UV absorption of the fractions were recorded at 254 nm by UV cord before collecting the fractions.

Experimental conditions used were as follows:

Gel	- G25 coarse
Column	- Sephadex K 15/30 length 30 cm diameter 1.5 cm
Bed height	- 25.8 cm
Amount of soil extract applied	- 700 µl
Eluant	- Distilled water (sterile)
Flow rate	- 3.18 ml/min
UV absorption recorded at	- 254, 280 nm

iii. Thin layer chromatography (TLC)

Soil extract was streaked onto TLC plates (20 x 20 cm Kieselgel, Camag pre-coated glass and aluminium plates with UV indicator) and developed with 64% n-propanol (w/w) (or 7:3 n-propanol:water $^{v}/v$) in an unsaturated tank.

The developed plates were inspected under (UV) ultra violet light 254 nm and all absorbing bands were marked. Location of chlamydospore inducing factors (CIF) was done by eluting different bands in 6 ml steriled distilled water and subsequent bioassay for CIF. The bands were separated for bioassay as follows:

a. Chromatogram divided into 3 equal portions

b. 1 cm broad bands parallel to origin

c. UV absorbing and non-absorbing bands separately.

iv. Chlamydospore induction by abrupt removal of C source in culture

To study the induction of chlamydospores by abrupt removal of Carbon (C) source macroconidia were allowed to germinate in a basal medium (BM) [$NaNO_3$ 2.0g, KH_2PO_4 1.0 g, $MgSO_4.7H_2O$ 0.5g, KCl 0.5 g and $FeSO_4.7H_2O$ 0.01 g in a litre of distilled water] by shake flask technique. Cultures were supplied with a restricted supply of monosaccharides and disaccharides from diffusion capsules (L. H. Engineering Co. Ltd., England). The capsule is a nylon cylindrical container (total volume 1.2 ml) which may be filled with concentrated substrate and sealed at one end with a semi-permeable membrane. Semi-permeable membranes were cut from visking cellulose dialysis tubing $^{8}/32"$. Rate of release of sugars from capsules were linear and gave slow diffusion up to concentration of 30% w/v with 3 or 2 membranes over 20-24 h with monosaccharides and disaccharides (Ph.D. Thesis, R.M.Cooper). The filled and sealed capsules were autoclaved at 10 p.s.i. for 10 min and aseptically introduced into flasks containing macroconidia in BM. All capsules were removed. After 24 h incubation and start of chlamydospore production was recorded in each case as time (h) after withdrawal of C source from the culture.

v. Assay of chlamydospore formation

Eluates collected by gel filtration and TLC were bioassayed for chlamydospore inducing factors (CIF) by adding 0.2 ml of spore suspension (10^6 spores/ml) of S.2d and S.2f in sterile distilled water to 2 ml of the fraction in 10 ml sterile specimen bottles with caps. Following inoculation each container was shaken on a whirlmixer 2-3 minutes and incubated for 5-6 days at 25°C. Vials were then shaken until a homogenous suspension of chlamydospores were obtained. Aliquot of the suspension was removed onto a slide and stained with a drop of lacto-fuchsin. Numbers of chlamydospores produced on macroconidia or on hyphae for 100 macroconidia were counted.

3.2B Soil factors affecting expression of disease

i. Soils

Soils from "on hill and hill bottom field" were collected on the basis of the previous season's crop (1974 summer) namely barley, field beans (Vicia faba), cabbage and potatoes designated 1, 2, 3 and 4 respectively (Fig. 3.1). Soil designated 6 was collected from virgin land under a thick cover of grass near Silwood Park golf links. Soils were passed through a 7 mm sieve and stored in large plastic dustbins in the glass-house. The soils are described in Table 3.1.

ii. Soil sterilization

Moist soils were sterilized at 100°C in an oven within an aluminium foil (aluminium cooking foil) casing as follows. A galvanized iron wire basket (32 x 26 x 8 cm)

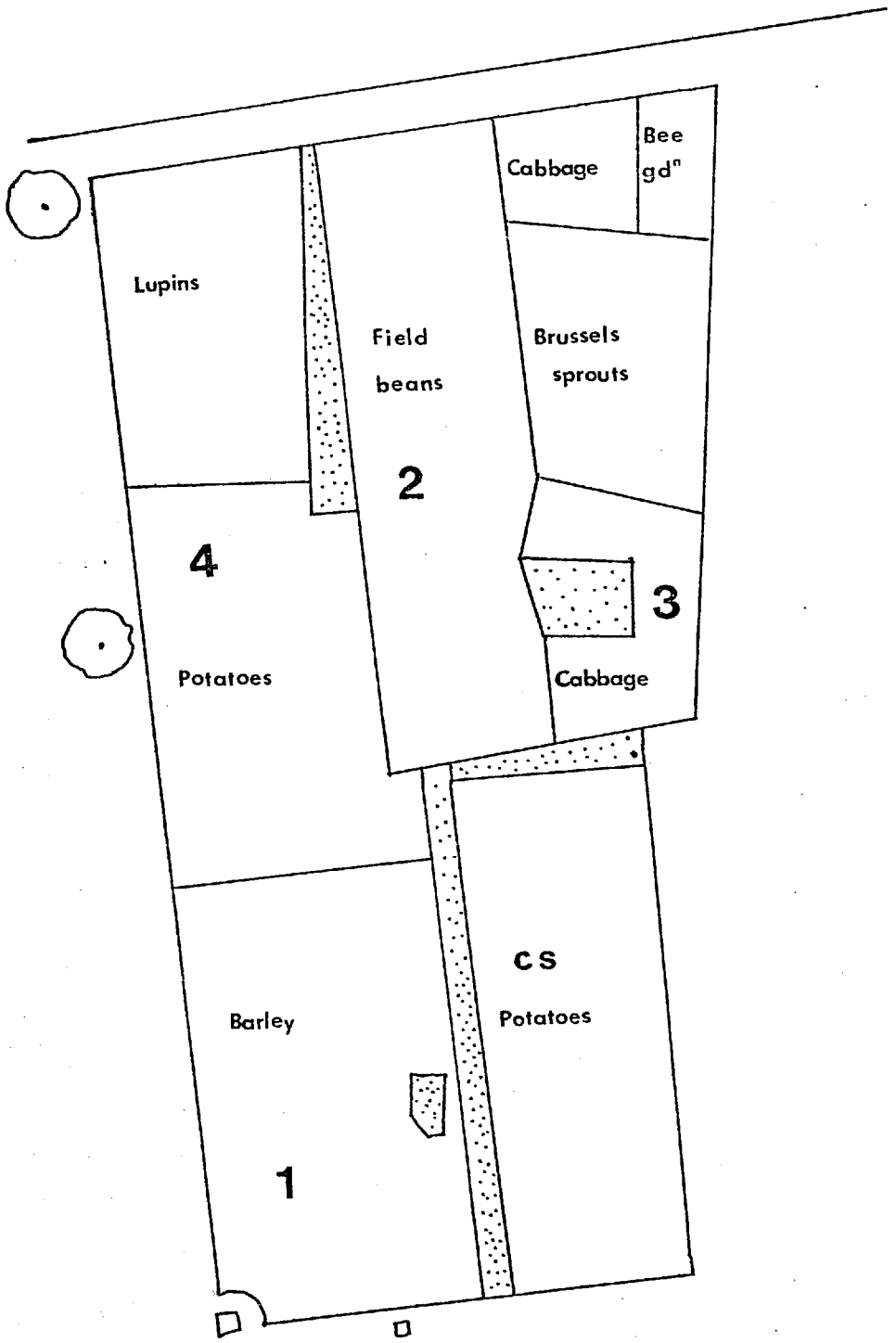


Fig. 3.1 Crop rotation (1974 season) "on hill and hill bottom" field, Silwood Park. Top soil was removed for experiments from the plots where indicated by numbers (1 – 4) & CS.

TABLE 3.1 Soils sampled[ab]

Soil (Number)	Previous Crop	pH	C%	N%
1	Barley	7.25	1.4	0.12
2	Field bean	7.1	1.5	0.13
3	Cabbage	7.0	1.2	0.12
4	Potato	6.4	1.3	0.12
6	Virgin grass	5.7	1.7	0.13
CS	Potato	4.75	0.9	0.08

a. See Fig. 3.1 for Location of sampled plots
All soils were sandy loams
C and N in soils were analysed by the
Soil Scientist, Soil Analysis Department
Ministry of Agriculture, Coley Park, Reading

b. No Fusarium solani f. sp. phaseoli was detected in these soils when tested with Papavizas (1967) medium.

lined with 2 layers of aluminium foil was filled with 2 cm layer by layer. Each layer was moistened (c. to field capacity) before adding the subsequent layer of soil. Once the tray was filled the top surface was completely covered with aluminium foil to prevent the escape of moisture (steam). Then a thermometer was introduced at an angle until its bulb reached the centre of the soil tray and the surface was sealed with Sellotape and aluminium foil. The soil basket was then placed in an oven and increased the temperature gradually to 100°C. Soil trays were left in the oven for 30-40 min. after the temperature at the centre of the soil bed reached 100°C. Sterilized soil was then aerated for 2-3 days before using in experiments.

iii. Inoculation

Inoculation of soils with Fsph was done at 7.5 or 10% v/v with v8/v inoculum as described above (1.2).

iv. Quantitative estimation of fungi, bacteria and actiomycetes in soil

After inoculating the soils with Fsph they were left in the glass-house in closed containers for 3 to 13 weeks for the conversion of macroconidia to chlamydospores.

Soil samples were taken from pots using a No. 5 cork borer before and after cropping. Five core samples were taken from each pot and samples from all the pots were bulked to form a composite sample. When sampling was done after cropping, rhizosphere soil was obtained by removing plants with intact roots and shaking the roots into a polythene bag.

The soil that adhered to roots as a thin layer around them was considered as rhizosphere soil. Care was taken to avoid any larger clumps of soil that adhered to roots. From the rhizosphere soil 4 to 5 sub samples were removed with a No. 1 cork borer to prepare soil dilutions.

a. Fusarium solani f. sp. phaseoli

Populations were estimated by the dilution plate method (1:200) using Papavizas' (1967) medium as in 1.2.

b. Fungal spectrum

Populations of Aspergillus spp., Trichoderma spp., Penicillium spp., Fusarium spp., Zygorrhynchus spp. and other fungi were estimated by the diluation plate technique using Martin's medium (1950). The composition of this medium is given below:

Agar	20.0 g
KH_2PO_4	1.0 g
$MgSO_4.7H_2O$	0.5 g
Peptone	5.0 g
Dextrose	10.0 g
Rose bengal	0.033 g
*Streptomycin	30 mg
*Chlortetracycline	30 mg
Distilled water	1000 ml

One ml aliquots from a soil dilution ($1:10^4$) were pipetted into sterile plastic petridishes followed by 10 ml of autoclaved Martin's medium to each plate and the soil suspension was mixed thoroughly with melted agar by

swirling the freshly poured plates. Plates were incubated at 25°C in the dark for 5-7 days and numbers of colonies of Aspergillus spp., Trichoderma spp., Penicillum spp., Fusarium spp., Zygorrhynchus spp. and other fungi were counted. Different species were identified by the characters described by Barnett (1969) and Gilman (1957).

c. Actinomycetes

Soil samples collected were air dried for 4-5 days to eliminate vegetative bacterial cells (Williams & Cross, 1974) before making the soil dilution (1 in 10^6). One ml aliquots were mixed with 10 ml of glycerol-casein medium in petri dishes. The glycerol-casein medium had the following ingredients:

Glycerol	10.0 g
Casein (vitamin free)	0.3 g
KNO_3	2.0 g
NaCl	2.0 g
K_2HPO_4	2.0 g
$MgSO_4.7H_2O$	0.05 g
$CaCO_3$	0.02 g
$FeSO_4.7H_2O$	0.01 g
Agar	20.0 g
Distilled water	1000 ml
*Cycloheximide	50 mg
*Nystatin	20 mg

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

Glycerol was substituted for soluble starch in starch-casein medium used by Küster and Williams (1964).

Inoculated medium was incubated for 8-10 days at 25°C. before counting the number of actinomycetal colonies per plate.

d. Spore-forming bacteria - Bacillus spp.

About 25 ml of soil suspension (1 in 10^3) was immersed in a water bath maintained at 80-85°C for 10 min. and during the procedure suspension was rotated gently to ensure even heating of the soil suspension. The heated suspension was then diluted to obtain a soil suspension of 1 in 10^5 in sterile distilled water. One ml portions from the resultant suspension were mixed with 10 ml of Yeast-mannitol agar containing congo red (36 mg congo red/L), in petridishes and incubated for 5 days at 25°C. The composition of Yeast-mannitol agar is given in 4.2.

Results of the population counts are recorded as propagules per g dried soil. Disease in plants was assessed on a scale 0-7 where 0 = healthy 7 = dead (Fig. 1.2).

3.3 Experimental

3.3A Chlamydospore formation

Three ml of washed macroconidia suspension (10^6 spores/m) was added to 15 ml of unsterile or sterile field soil in McCartney bottles (25 ml) and incubated for 5 to 21 days at 25°C. Observations after various periods of incubation were made by a soil smear technique (Nash et al., 1961) where a small amount of soil was mixed with water to make a slurry and poured onto a glass slide. This was then stained with lacto-fuchsin and covered with a large coverslip. Observation

under the microscope revealed that chlamydospores were formed in field soil 6 days after inoculation but in sterilized soil only extensive mycelial growth with micro- and macroconidia were seen. Chlamydospores were seen 21 days after inoculation in sterilized soil.

i. Chlamydospore formation in soil extract

When sterile soil extract was bioassayed initiation of chlamydospore production was noted 5 days after inoculation. Thick walled chlamydospores were formed either directly on macroconidia or on hyphae as intercalary chlamydospores; or on tips of hyphae.

a. Gel filtration

Since soil extract produced abundant chlamydospores it was decided to investigate whether any particular substance in the extract is responsible for induction of chlamydospores. Therefore the soil extract was fractionated by gel filtration and the fractions from the column were collected as follows:

(1) 3 fractions on the basis of absorption peaks -(Fig. 3.2 - x, y, z)

(2) 4 fractions i. ii, iii and iv (9.6 ml each) Fig. 3.3

(3) 7 fractions 1 to 7 (4.8 ml each) Fig. 3.3

Each fraction was bioassayed for chlamydospore inducing substance as described above and the results are summarized in Tables 3.2, 3.3, 3.4 and 3.5.

Peaks x and z (Fig. 3.2) did appear to contain chlamydospore inducing factor (CIF) (Table 3.2). When 9.6 ml fractions (i. to iv.) were tested for CIF fraction i was

chlamydospore inducing (Fraction i (9.6 ml) = peak x, Fig. 3.2 and 3.3). But fraction iii (Fig. 3.3) which includes peak z and a portion of peak y was no chlamydospore inducing (Table 3.3).

This apparent mixture of chlamydospore inducing and chlamydospore inhibitory substances were fractionated by collecting small volumes (4.8 ml, Fig. 3.3, Table 3.4).

Fractions 1 and 2 (at 0-9.6 ml elution vol) and fraction 6 (at 24.1 to 28.8 ml el. vol) were chlamydospore inducing (Table 3.4). Fraction 1 was as inducive as crude extract. Since fraction 1 and 2 were chlamydospore inducive and belong to the same absoprtion peak x (Fig. 3.2), transmission of eluents was scanned at 280 nm (instead of 254 nm) and slightly larger volume (5.2 ml) fractions were collected in another experiment (Fig. 3.4, Table 3.5). Fraction a and b (el.vol 0-10.5 ml) and fraction e (el. vol 21.1 to 26.2 ml) were found to be chlamydospore inducing. It seems that fraction e when less contaminated with fraction d, is more active in chlamydospore induction than fraction a (Table 3.5).

Table 3.6 describes the behaviour of macroconidia in the fractions (1-7) collected as in Fig. 3.3. Chlamydospores were mainly intercalary in fraction 6 or f.

b. Thin layer chromatography

Concentrated soil extract was subjected to chromatography using different solvent phases on silica gel plates. Several solvent systems such as ethanol:chloroform (1:4),

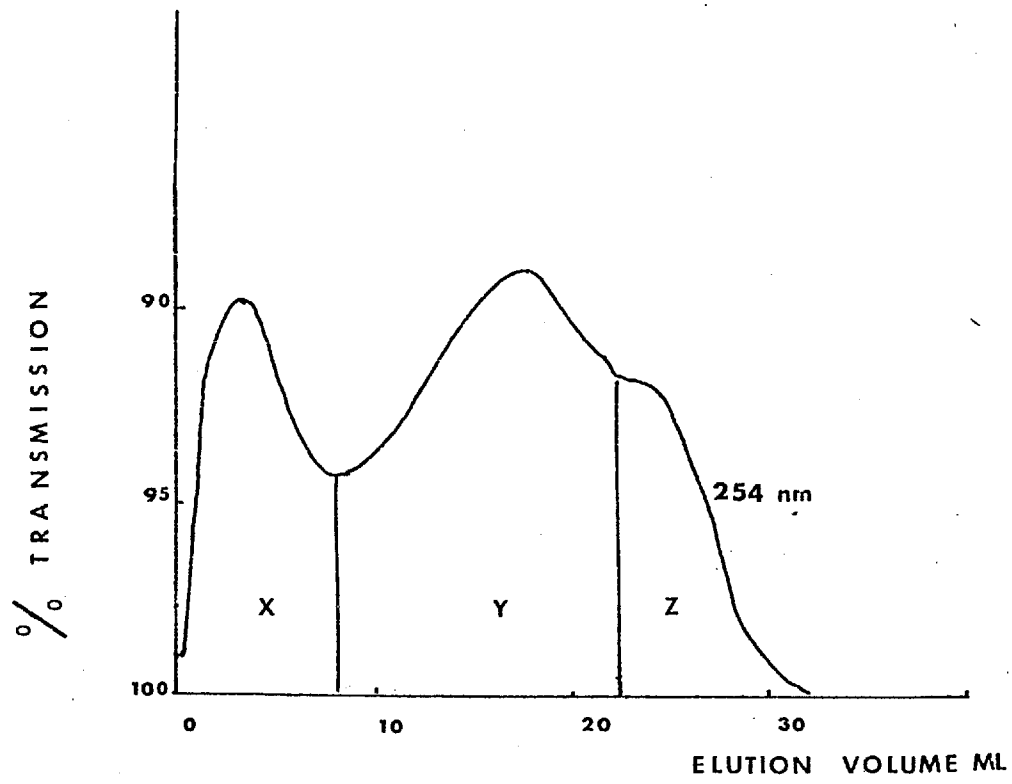


Fig. 3.2

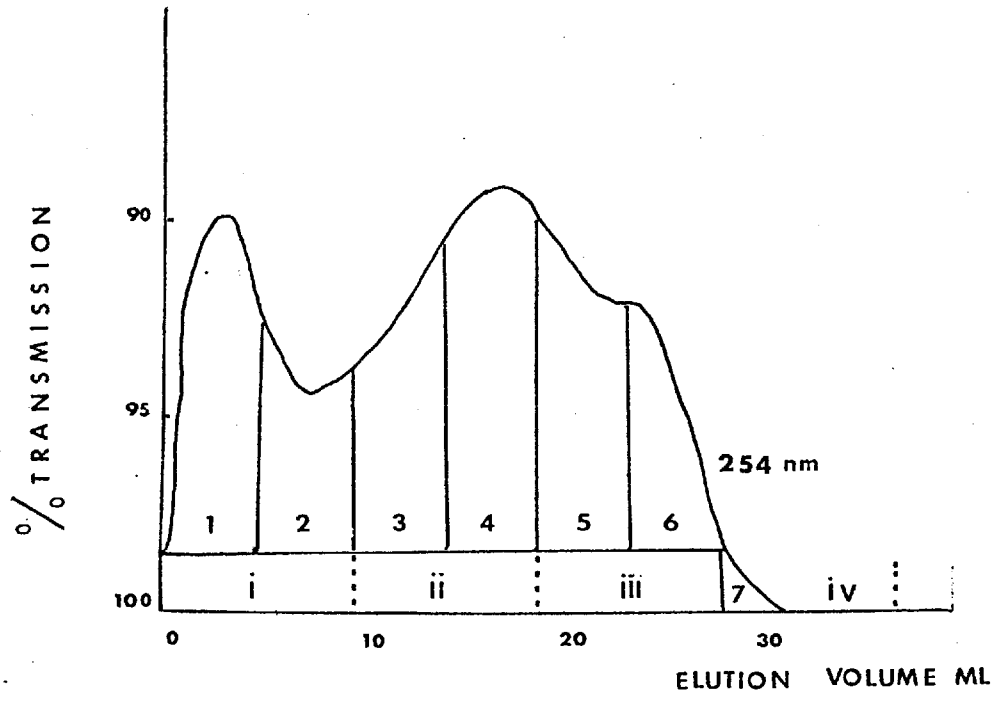


Fig. 3.3

Fig. 3.2 & 3.3 Gel filtration of soil extract on Sephadex G-25, Eluant - distilled water.

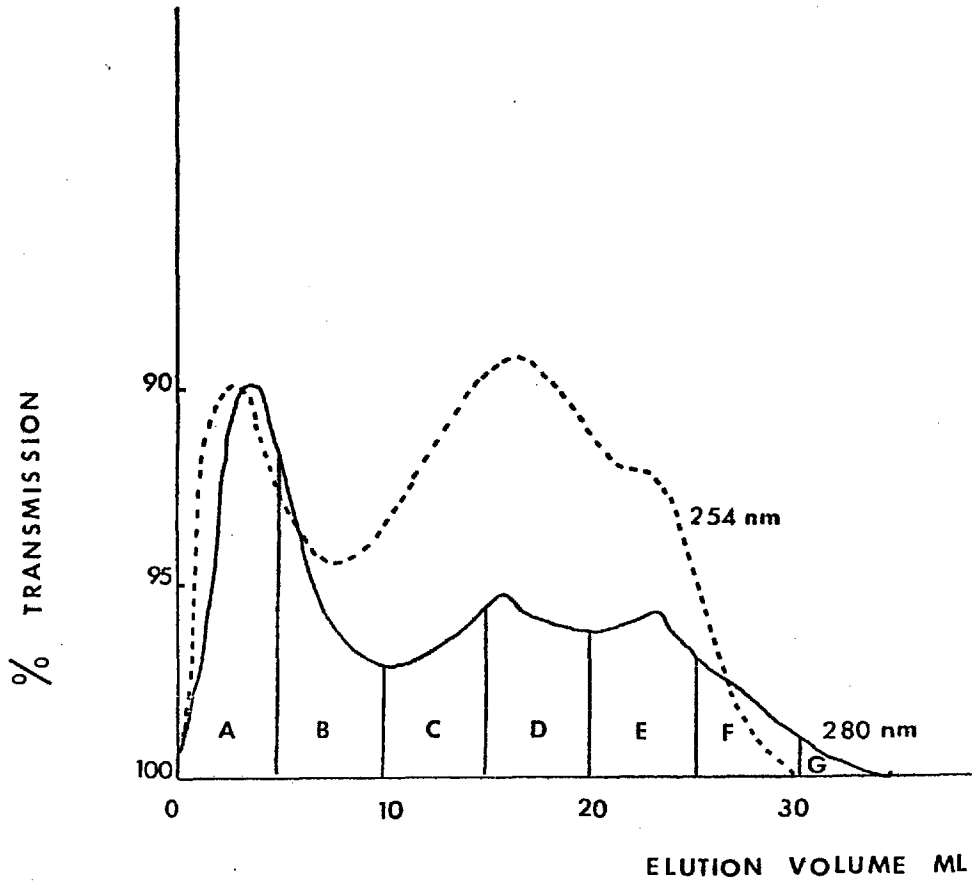


Fig. 3.4 Gel filtration of soil extract on Sephadex G-25, Eluant distilled water.

TABLE 3.2 Induction of chlamydospores of *Fusarium solani* f. sp. *phaseoli* by different fractions of soil extract fractionated on Sephadex G25 with water as an eluant

Fsph Strain	fractions of soil extract[a] (based on 254 nm absorption peak)			Crude Soil Extract
	x	y	z	
	(Chlamydospores per 100 macroconidia)[b]			
S.2d	37.3±8.1	8.0±3.8	14.7±5.5	80.5±15.1
S.2f	47.2±3.3	8.0±3.3	8.2±2.1	94.7±15.0

a. See Fig. 3.2

b. Mean ± Standard Error of mean. Mean number of chlamydospores formed from 100 macroconidia. Results are average of 6 observations

TABLE 3.3 Chlamydospore induction in Fusarium solani f. sp. phaseoli by various fractions (9.6 ml) obtained by fractionation of soil extract with water on a Sephadex G25 column

Soil extract[a] fractions (9.6 ml)	Elution volume ml	Mean number of[b] chlamydospores per 100 Macroconidia	Standard error of Mean
i	0 - 9.6	26.8	6.1
ii	9.7-19.2	5.0	3.0
iii	19.3-28.8	0.6	1.2
iv	28.9-38.4	5.0	4.1
Crude Soil Extract		80.5	15.1

a - see Fig. 3.3

b - Mean value for 6 observations

TABLE 3.4 Induction of chlamydospores in Fusarium solani f. sp. phaseoli by different fractions (4.8 ml) of soil extract fractionated on Sephadex G25 with water as eluant

Soil extract fractions[a] (4.8 ml) No.	elution volume ml	Chlamydospores for 100 macroconidia[b] S.2d	S.2f
1	0 - 4.8	75.3±13.7	80.2±12.4
2	4.9-9.6	50.3± 4.3	35.7±13.8
3	9.7-14.4	7.0± 3.0	5.5± 2.9
4	14.5-19.2	1.0± 1.0	1.7± 2.7
5	19.3-24.0	0	0.3± 0.8
6	24.1-28.8	26.7± 3.9	32.3± 5.1
7	28.9-33.6	15.7± 4.4	12.8± 3.5
Crude Soil Extract	-	80.5±15.1	94.7±15.0

a. See Fig. 3.3

b. Means for 6 observations Mean ± Standard error of Mean

TABLE 3.5 Chlamydospore induction in Fusarium solani f. sp. phaseoli by fractions (5.2 ml) obtained by fractionation of soil extract with water on a Sephadex G25 column

Soil extract fractions[a]		Chlamydospores for 100 macroconidia[b]	
		S.2d	S.2f
	elution volume ml		
a	0 - 5.2	25.5±11.3	82.7±16.7
b	5.3-10.5	46.7± 0.9	30.2± 3.3
c	10.6-15.7	4.0± 0.8	29.0± 9.2
d	15.8-21.0	0	0
e	21.1-26.2	85.0± 9.5	107.5±10.5
f	26.3-31.5	0	4.7± 1.9
g	31.6-36.7	1.0± 0.8	0
Crude Soil Extract	-	98.7±10.9	148.7 ± 8.5

a. See Fig. 3.4

b. Mean for 6 observations, Mean ± Standard error of mean. Fraction e, mainly induced intercalary chlamydospores often 2 to 3 together on macroconidia with less mycelial growth, whereas in crude extract fewer intercalary chlamydospores were formed. Therefore less chlamydospores in crude extract than total of a-g.

TABLE 3.6 Behaviour of Fusarium solani f. sp. phaseoli macroconidia in different fractions (4.8 ml) of soil extract fractionated on Sephadex G25 with water as eluant, see Fig. 3.3

Soil extract Fractions	Inception of chlamydospores	Behaviour of macroconidia 6 days after inoculation
1	++	All conidia with germ tubes most germ tubes ending in chlamydospores which are single and club shaped. Cytoplasm of the conidial cells are separated from the cell walls.
2	++	Similar to fraction 1.
3	+	Large number of fat globules in macroconidia, with long germ tubes chlamydospores when present similar to fraction 1 and 2.
4	-	Macroconidia faintly stained as a whole mass. Cells of macroconidia are barrel shaped long germ tubes, extensive mycelial growth, no plasmolysis of conidia.
5	-	Similar to fraction 4.
6	+	Chlamydospores are intercalary on macroconidia, rarity on hyphae 2 to 3 chlamydospores together, they are thick walled and circular. Mycelial growth prominent.
7	+	Similar to fraction 1.

methanol:chloroform (1:3), n-butanol:acetic acid glacial:water (12: 3: 5) and 64% (w/w) n-propanol were used. Since 64% n-propanol gave the best separation of compounds it was used to develop the chromatograms. Concentrated soil extract (0.3ml) was applied as a streak and six UV absorbing bands were detected in developed plates (Fig. 3.5).

Different fractions from the chromatogram were eluted for bioassay as (1) dividing into 3 equal bands (4.5 cm bands) A, B and C (2) UV absorbing bands and other non-absorbing bands separately (1-9) (Fig. 3.5).

Each fraction was bioassayed in 4 separate experiments. Although Fraction A, B and C did not induce any chlamydospores but extensive mycelial growth even after 14 days of incubation. Crude extract equivalent to A, B and C together induced chlamydospores. However, when fraction 1-9 was tested for CIF it was found that initiation of chlamydospore production in certain fractions begins 6-7 days after inoculation. The bioassay revealed that UV absorbing fractions 2, 5, 7 were more active in inducing chlamydospores than were others (Table 3.7). The R_F values of bands 2, 5 and 7 were 0.10, 0.44 and 0.74.

UV absorption spectra of these fractions in 10 ml distilled water are illustrated in Fig. 3.6. Fraction 4 and 5 had a minor peak at 260, probably similar to ribonucleic acid. Fraction 7 had a maximum absorption at 230 nm.

ii. Chlamydospore induction by abrupt withdrawal of carbon source

Depletion of exogenous C source is known to stimulate chlamydospore formation in Fusarium spp. in pure culture. To simulate such depletion of C source in a short period Fsph S.2d was grown in a nutrient medium containing (Peptone5 g,

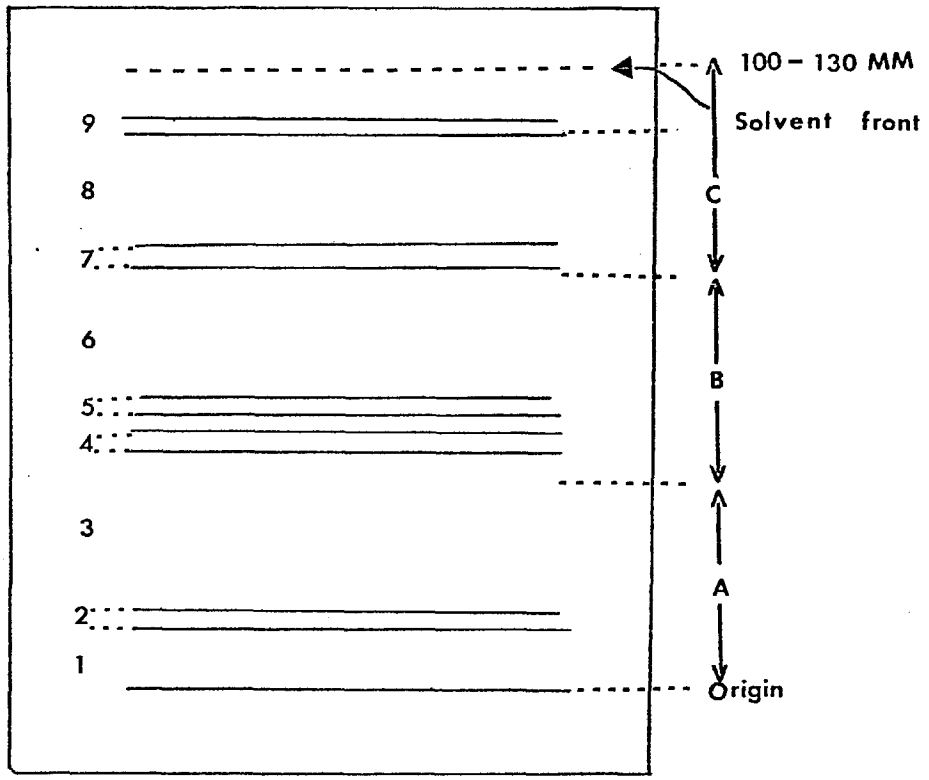


Fig. **3.5** A sketch of the chromatogram seen under UV (254 nm) light, when soil extract was developed with 64% n-propanol (w/w).

A, B, C & 1-9 are the fractions eluted for biossay.

Bands 2, 4, 5, 7 & 9 are UV absorbing (brown) bands.

R_F values are band 2 = 0.10,
4 = 0.41,
5 = 0.44,
7 = 0.74,
9 = 0.96.

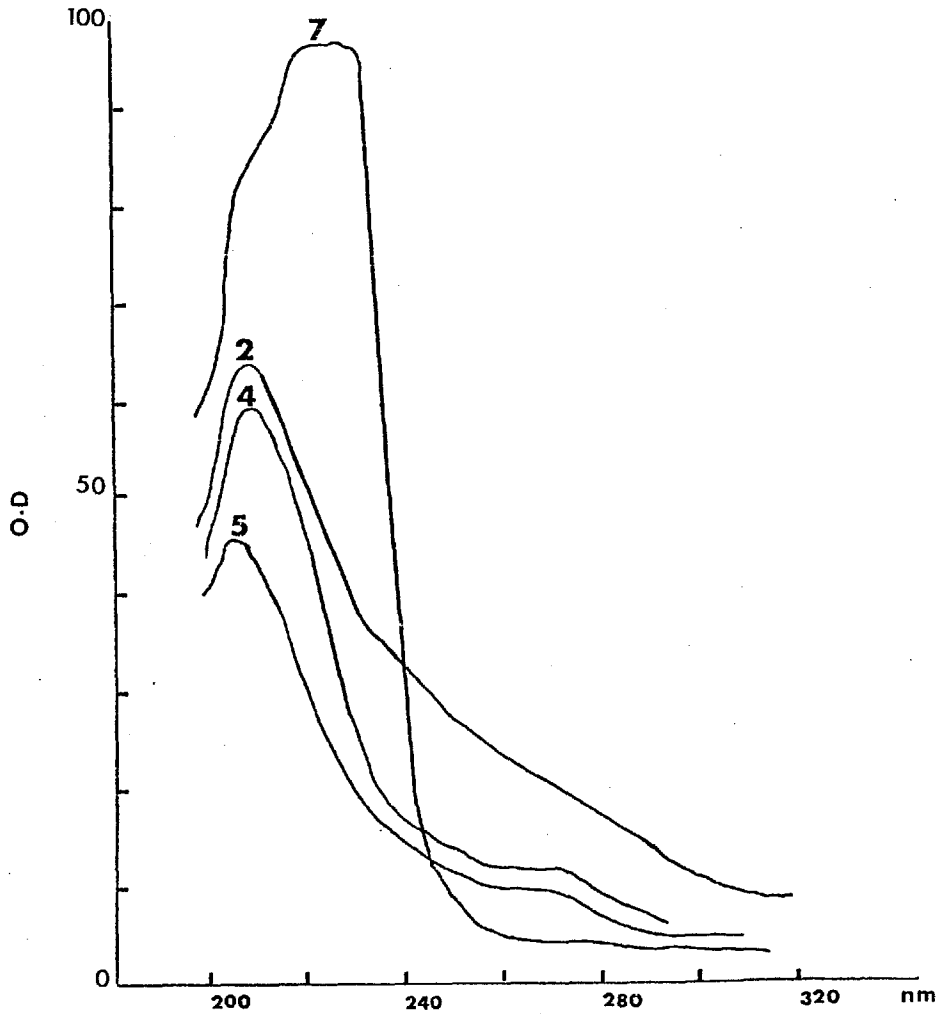


Fig.3.6 Absorption spectra of UV absorbing, (2,4,5 & 7 Fig.3.5) chlamydospore inducing bands eluted from a chromatogram obtained by developing soil extract with 64 % n-propanol

TABLE 3.7 The chlamydospore induction in Fusarium solani f. sp. phaseoli by water eluants of different regions of a silica gel thin layer chromatogram developed with 64% (w/w)n-propanol to effect separation of the components of soil extract in water

Soil extract (Component) bands No. (Fig. 3.5)	Chlamydospore induction in Fsph (Chlamydospores/100 Macroconidia) S.2d	S.2f	UV absorption 254 nm
1	39±27	3± 3	-
2	88± 16	304± 45	+
3	8± 8	4± 5	-
4	58± 23	3± 2	+
5	196± 76	114± 10	+
6	55± 29	9± 4	-
7	156±112	125± 17	+
8	0	3± 3	-
9	3±6	12± 9	+
Crude Soil Extract	89± 25	128± 29	

Fraction 7 mainly induced intercalary chlamydospores often 2 to 3 together on macroconidia, but fewer intercalary chlamydospores were formed in crude extract, therefore less chlamydospores in crude extract than total of 1-7.

TABLE 3. 8 Chlamydospore induction in Fusarium solani f. sp. phaseoli after removal of the carbon source from shake cultures. Carbon in the form of sugars were supplied as a restricted supply through diffusion capsules into basal medium for 24 hours

Carbon Source	Time (days) of chlamydospore inception after removal of C source
5% Glucose	11
5% Sucrose	10
1% Glucose	9
1% Sucrose	6
0.1% Glucose	9
0.1% Sucrose	6
Basal Medium without C	14

NaCl 5 g, yeast extract 2 g, Agar 15 g in 1 litre of distilled water) concentrations of 1 (as above), $\frac{1}{2}$, $\frac{1}{4}$ and $\frac{1}{8}$ of the constituents, for 6 days at 25°C. Then the plates were leached with sterile distilled water by first washing with 15 ml sterile water and subsequent leaching with another 15 ml of sterile distilled water for 3 hrs. Leached plates were incubated for 3-4 days at 25°C with 12 replications and observed for onset of chlamydospore production.

For all concentrations of constituents inception of chlamydospores were observed 3 days after leaching with sterile distilled water. Controls without leaching fail to produce chlamydospores even after 14 days of further incubation.

Since depletion of exogenous nutrients had a marked effect on chlamydospore induction Fsph was grown in shake flask culture with a restricted C source (each 1% and 0.1% of sucrose and glucose fed through diffusion capsules) for 24 h. Approximate time for the onset of chlamydospores after the removal of C source was observed and the results are given in Table 3.8.

3.3 B Disease expression in soil

i. Pathogenicity of Fsph in different soils

Pathogenicity of Fsph was tested in various soils 1, 2, 3, 4 and 6 after inoculating with Fsph in $^{v}8/v$ inoculum at 10% (u/v). Results are given in Table 3.9. Conducive indices $\bar{/}$(Percentage diseased plants x Disease Index)/100$\bar{/}$ were used to obtain a weighted damage to plants in a given soil. Table 3.9 shows that potato soil with a C:N ratio 10.8 has the highest conducive index of 2.56 compared to soil 6 (virgin soil} with C:N ratio 13.0) with conducive index of 0.19.

The conducive or suppressive nature of soils tested was not related to C:N ratio (Table 3.9). Therefore it was assumed that previous crops had some effect on their suppressive nature. The effect of growing different crops in soil on the pathogenicity of Fsph on beans were tested by growing various crops in soil CS collected from another potato field (Table 3.1). Soil CS was inoculated with Fsph 7.5% ($^{v}/v$) and kept moist in closed plastic dustbins in the glass-house for 90 days for the conversion of macroconidia to chlamydospores. Populations of Fsph were estimated 6d and 90d after inoculation. There was an initial drop in the Fsph population from 5349$\pm$ 1090 propagules per g dry soil to 1408$\pm$575 (Appendix) during the 3 months storage in the glass-house. This represents the survival during chlamydospore formation (Nash & Alexander, 1965). There was no Fsph detected in any of the soils tested above.

Plastic pots (diameter 13 cm) were filled with these soils and thickly sown with beans(Phaseolus vulgaris cv. Pinto) cabbage (Brassica oleracea var. capitata cv. Savoy cabbage January King), Capsicum (Capsicum annuum var. grossum, cv. world beater), Onions (Alium cepa cv. Ailsa craig) Rice (Oryza sativa cv. Ballila Gruesco), Sorghum (Sorghum vulgare African variety) or left fallow. Each treatment was replicated 4 times, and crops were grown for 95 days (beans were not harvested even after fruiting) before taking soil samples for population estimations. Since the pots were heavily seeded the whole pot was regarded as rhizosphere soil when sampling was done. When plants were harvested, soil in roots were shaken into their pots and 9 samples per pot were removed for population estimation. These were then seeded with beans for pathogenicity tests.

Disease in beans was assessed 25 days from inoculations. Results are summarized in Table 3.10.

Although the population of Fsph apparently increased in soil where bean, rice and sorghum had been grown it was significantly higher only in sorghum soil. Similarly there were reductions in population in cabbage, onion and capsicum soils and populations in cabbage soil were significantly lower than in fallow (treatments) controls. In cabbage and onion soil disease indices were significantly higher even with such low population of Fsph compared to controls and sorghum soil (Table 3.10).

The conducive and suppressive nature of soils were studied further with respect to the biological spectra of soils. Two soils (4 and 6) were selected from 3.2 i where soil 4 (C:N 10.8) for conducive and 6 (C:N = 13.0) for suppressive soil.

A concept introduced by mathematical model (Baker et al., 1967) to describe the relationship between non-motile inocula about a fixed infection court describes the conditions encountered by Fsph in infections of beans i.e. a. in the presence of a host the propagules germinate in the rhizosphere under a directional stimulus towards the infection court. Therefore additional inoculum results in a proportional increase in infection.

b. For a rhizoplane influence only propagules immediately adjacent to the surface of the infection court can germinate, penetrate and infect. Under such conditions the relationship of inoculum and infection $Y = {}^{2}/3X + C$ where Y = log number of successful infection, X = log inoculum density C = log K (constant).

Since the disease suppression appears to operate in rhizosphere the above concept was used to study the properties of two soils 4 and 6. Thus to determine whether the interference to the rhizosphere influence is biological in suppressive soil, conditions a and b above were simulated in soil 4 and 6. This was done by testing pathogencity at various inoculum densities (1, 3, 10, 25 and 50% inoculum) using sterilized and non-sterile soil.

The correlation between the inoculum density as % $^{v}8/v$ inoculum $^{v}/v$) and number of propagules/g dry soil in soil 4 and 6 were found to be 0.94 and 0.96 (Fig. 3.7). Thus in all experiments inoculum density is given an % inoculum ($^{v}/v$) incorporated into soil.

Fig. 3.8 illustrates the linear relationships between Disease Index and inoculum density in soil 6, sterile soil 6, soil 4 and sterile soil 4. The slopes of the graph in soil 4, Sterile 4 and sterile 6 are the same (0.09) indicated by 3 parallel lines whereas in soil 6 the slope was much lower (0.06). Thus sterilizing has eliminated microbiological effects operating in the suppression of disease in soil 6. In other words the factors interfere with the rhizosphere influence on germination of chlamydospores and penetration. The graph with 0.09 slope represents disease in the presence of a rhizosphere influence and the graph with slope 0.06 represents the disease when only a rhizoplane influence is operating. The fact that the soil 4, sterilized soil 4 and sterilized soil 6 all have the same slope indicates that soil 4 was conducive to foot rot because of the absence of a suitable microflora. Therefore, the biological spectra of soils 4 and 6 were examined in order to investigate the biological agents that suppress disease in soil 6.

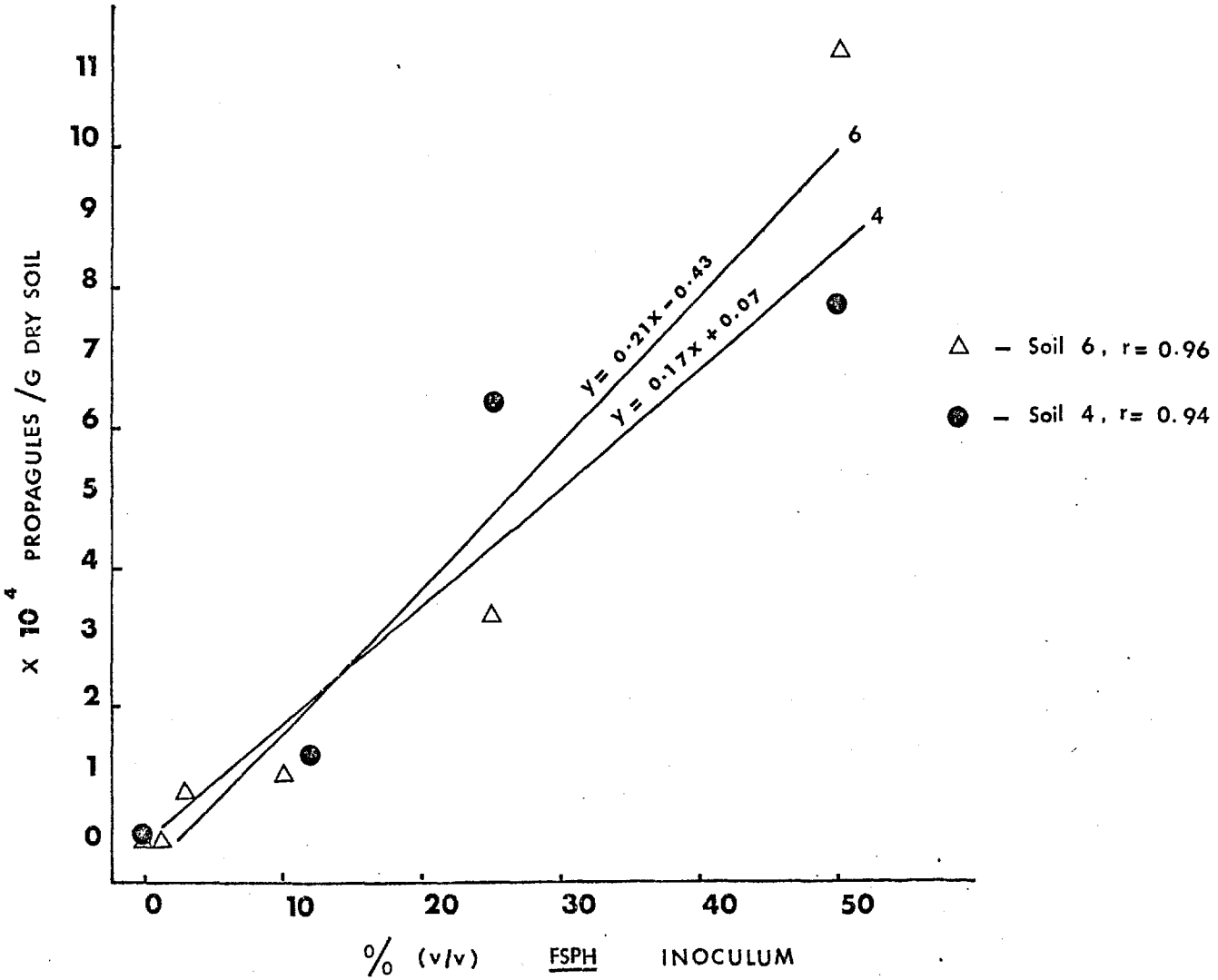


Fig. 3.7 Relationship between % inoculum incorporated and resultant population of Fusarium solani in soil. All points are means for ten replicates. Regression based on raw data.

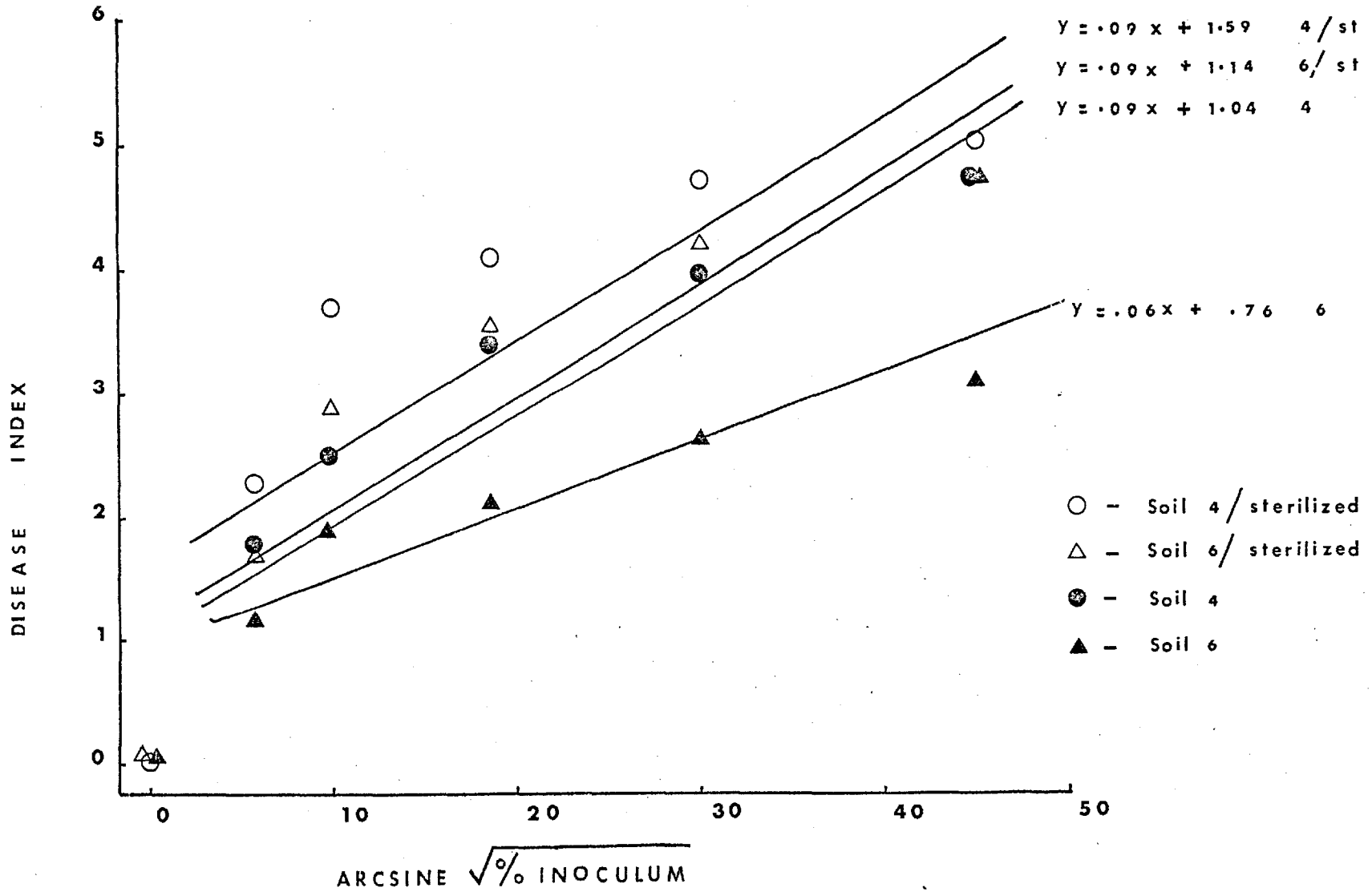


Fig. 3.8 Relationships of inoculum density to Disease Index of Foot rot in soil 6 and 4

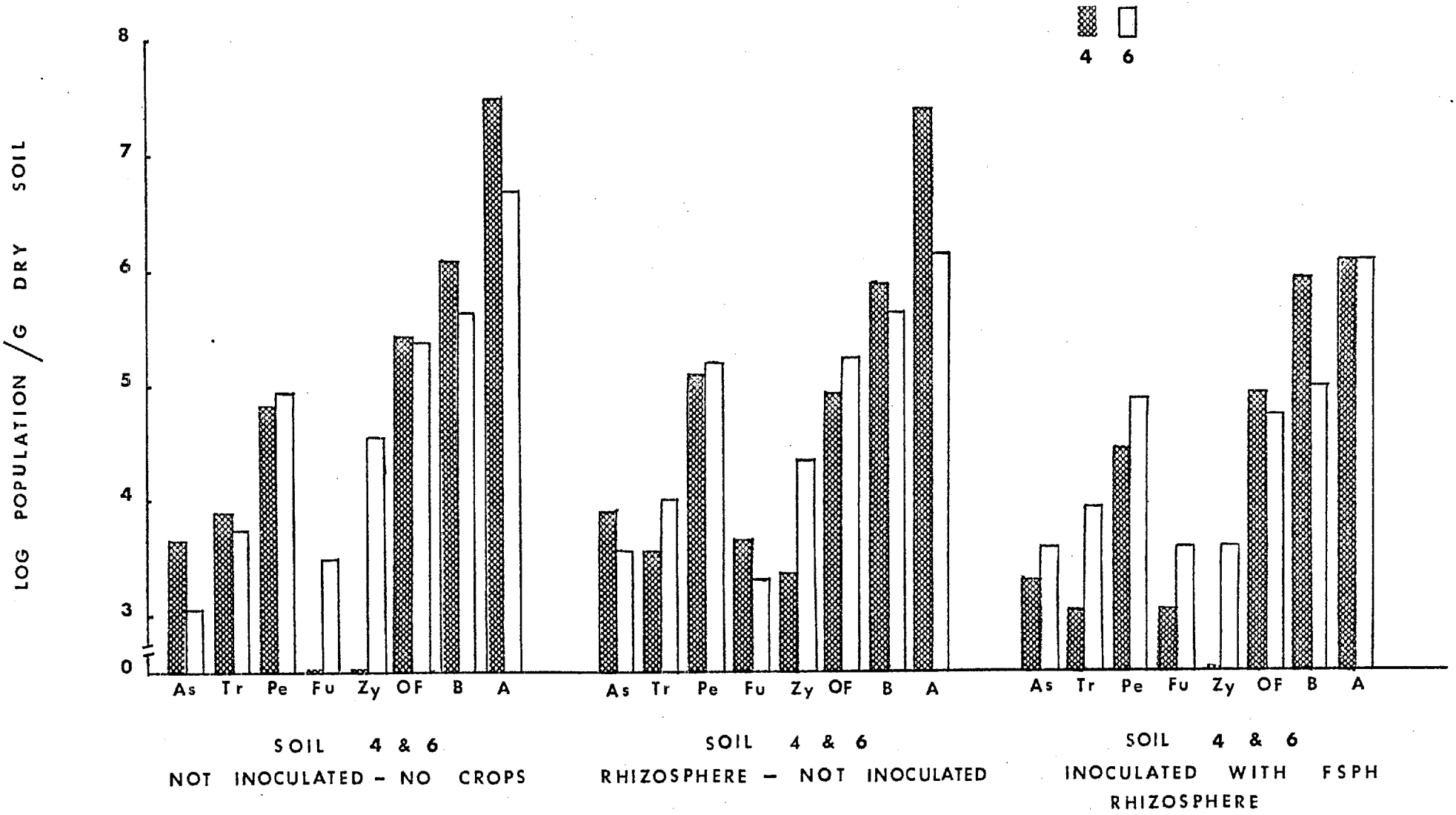


Fig. 3·9 Distribution of fungi, Actinomycetes and spore formers in non rhizosphere- and rhizosphere of conducive (4) and suppressive soil (6).

As – Aspergillus spp., Tr – Trichoderma spp., Pe – Penicillium spp., Fu – Fusarium spp., Zy – Zygorrhynchus spp., OF – Other fungi, B – Bacillus spp., A – Actinomycetes.

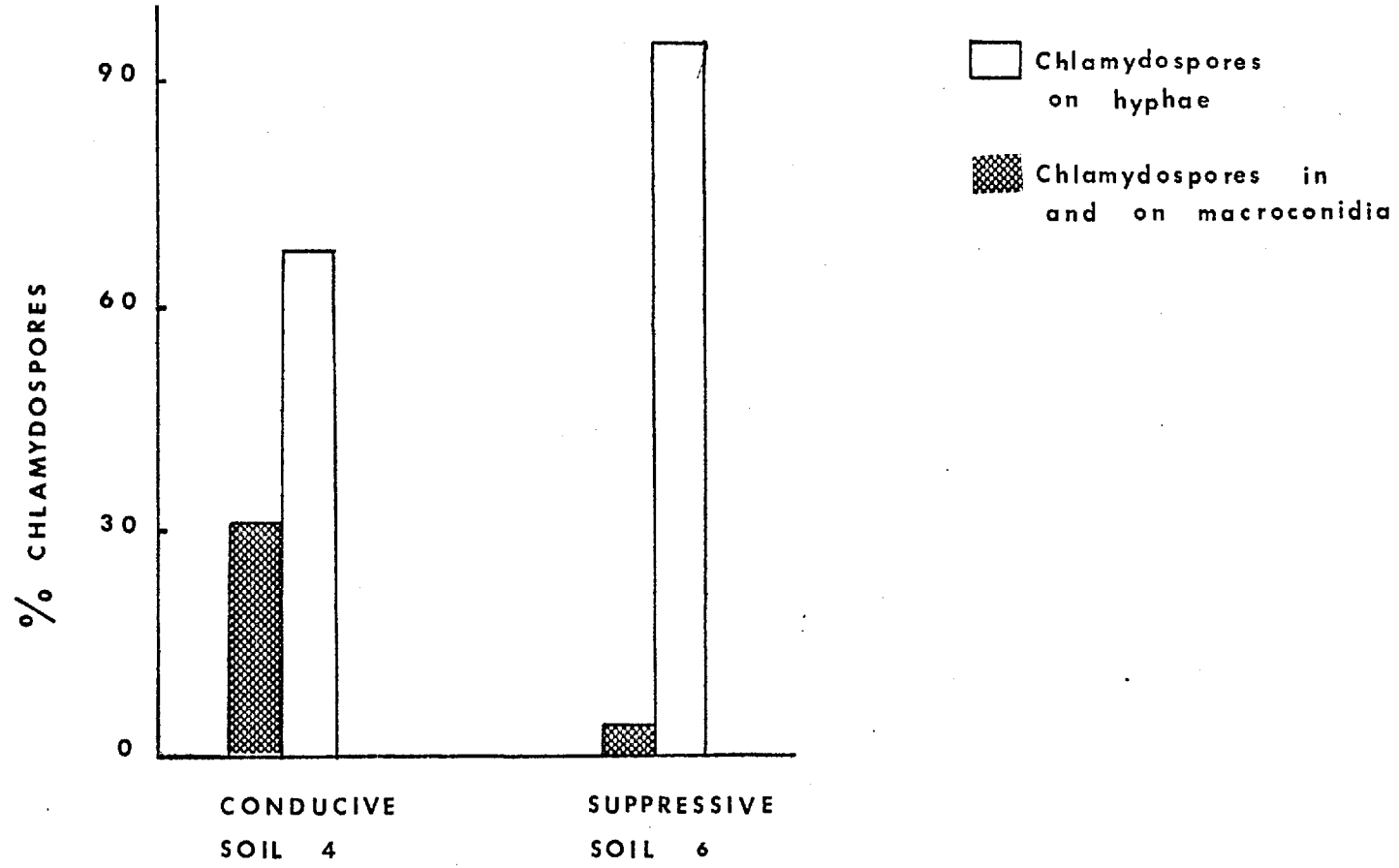


Fig. **3.10** Proportion of the two types of chlamydospores formed in soil extract of conducive (4) and suppressive soil (6).

TABLE 3.9 Pathogenicity of *Fusarium solani* f. sp. *phaseoli* to Pinto bean in various soils

	SOILS TESTED				
Nature	Cultivated				Virgin
Soil No.	1	2	3	4	6
Previous crop or vegetation	Barley	Beans	Cabbage	Potatoes	under grass cover
C/N ratio	11.7	11.5	10.0	10.8	13.0
% Disease plants	68.8 x	83.3 x	100 y	100 y	40 x
Disease[1] Index	0.9 a	1.93 b	2.12 b	2.56 b	0.49 a
Conducive[2] Index	0.62	1.61	2.12	2.56	0.19

Values followed by the same letter in each horizontal line are not significantly different at 0.05 probability level.

[1] Disease Index = 0 = healthy 7 = dead (Fig.1.2)

[2] Conducive Index = $\frac{\text{\% disease plants x Disease Index}}{100}$

TABLE 3.10 Effect of host and various non host crops on soil population of Fsph and subsequent pathogenicity on beans

Treatment	Rhizosphere population after cropping (prop./g)	Disease Index in subsequent bean crop
Control (no crops)	1548 bc	2.4 a
Bean (host)	2404 cd	2.8 ab
Cabbage	572 a	3.4 c
Capsicum	1494 ab	3.1 bc
Onion	755 ab	3.9 d
Rice	2372 cd	3.4 c
Sorghum	2553 d	2.5 a

Values followed by the same letter/letters in the same column are not significantly different at 0.05 probability level

Control: No plants were grown but the soil was kept moist throughout the experiment. The original population at the time of sowing in soil was 1408 ± 575 propagules/g dry soil

TABLE 3.11 Ratios of numbers of different types and groups of organisms in rhizosphere soil to numbers in control soil or non rhizosphere soil (R:S ratio)

Group	S O I L			
	Not inoculated		Inoculated (*Fsph*)	
	4	6	4	6
Aspergillus spp.	1.50	1.02	2.0	1.66
Trichoderma spp.	0.14	2.83	1.0	1.33
Penicillium spp.	1.90	1.77	0.68	1.80
Fusarium spp.	45×10^4/0	1.01	0.11	49×10^4/0
Zygorrhynchus spp.	22×10^4/0	0.61	0	0.34
Other fungi	0.31	0.64	0.94	2.96
Bacillus spp.	0.64	0.94	1.01	0.72
Actinomycetes	0.76	0.28	0.23	0.99

Data on soil microflora before cropping and microflora in rhizosphere soil in inoculated and non-inoculated soils 4 and 6 are illustrated in Fig. 3.9. It is evident from Fig. 3.9 that more Trichoderma spp. Zygorrhynchus spp. and Fusarium spp. are present in the rhizosphere soil in soil 6. Also the presence of more Zygorrhynchus spp. in both non-rhizosphere and rhizosphere soil 6 are evident although the Bacillus spp. and Actinomyatus are higher in soil 4.

The ratios of numbers of different types and groups of organism in rhizosphere soil to numbers in control soil (R:S ratio) for soil 6, 4 and Fsph inoculated 6 and 4 are given in Table 3.11.

The rhizosphere influence, that is the increase in number of organisms in the rhizosphere over that in the control soil is indicated by R:S ratio greater than 1. R:S ratio for Aspergillus, Trichoderma, Penicillium, Fusarium, other fungi and actinomycetes in soil 6 was greater than 1 compared to soil 4.

When chlamydospore formation was studied in the two soils using soil extracts it was found that in conducive soil (soil 4) a larger proportion of ($31.2\pm17.1\%$) chlamydospores were formed directly on macroconidia compared to $4.82\pm12.89\%$ in soil 6 (Fig 3.10). These chlamydospores had the following size ranges:

soil 6 - on macroconidia

$10.75 \pm 2.37\ \mu \times 6.5 \pm 1.64\ \mu$

soil 6 - on hyphae

$8.25 \pm 2.73\ \mu \times 5.37 \pm 0.91\ \mu$

soil 4 - on macroconidia

$10.83 \pm 2.43\ \mu \times 6.17 \pm 1.20\ \mu$

soil 4 - on hyphae

$9.87 \pm 2.06\ \mu \times 6.56 \pm 1.20\ \mu$

Chlamydospores formed on hyphae on short germ tubes in soil 4 were larger than chlamydospores of similar origin in soil 6.

3.4 Discussion

A. Factors affecting chlamydospore formation

There are two types of chlamydospores, the intercalary produced directly on macroconidia or in hyphae, and those produced on hyphal tips (Table 3.6). Usually, intercalary chlamydospores have thick walls. When soil extract was fractionated by molecular weight, a fraction with a higher molecular weight (el. vol. 0-9.6 ml Table 3.4 and 0.-10.5 ml Table 3.5) and a fraction with a low molecular weight (el. vol. 24-28.8 ml Table 3.4 and 21-26 ml Table 3.5) were found to be chlamydospore inducing. The higher molecular weight fraction induced chlamydospores on short germ tubes or on hyphal tips; they were club shaped and comparatively thin walled. The low molecular weight fraction induced thick walled, more circular, intercalary chlamydospores in and on macroconidia and hyphae. Therefore, it could be assumed that two chlamydospore inducing factors are present in soil extract. Ford et al. (1970) reported that at least 3-4 substances in soil could induce chlamydospore formation.

It was also found that depletion of exogenous nutrients and abrupt withdrawal of carbon source from a restricted shake culture could induce chlamydospore formation (Table 3.8). Similar results have been reported by Venkata Ram (1952, Ford et al.(1970) and Meyers and Cook (1972).

In the gel filtration experiments, whenever the two chlamydospore inducing fractions are contaminated by chlamydospore inhibitory fractions, the numbers of chlamydospore formed were reduced and their formation was delayed (Table 3.3, 3.4). These chlamydospore inhibitory fractions stimulate mycelial growth (Table 3.6) and provide exogenous nutrients.

However, with depletion of nutrients (with time) the effects of chlamydospore inducing substances appeared as delayed inception of chlamydospores. Watson and Ford (1972) suggested that "soil fungistasis is caused by the presence in soil microenvironments of complex inhibitors of biotic or abiotic origin effective at low concentrations of stimulators (mostly nutrients) present in the soil. The control of fungistasis is effected by specific balances of inhibitor and stimulator concentrations." Thus the production of more chlamydospores in crude soil extract could be due to the presence of growth stimulators and subsequent chlamydospore formation may be caused by nutrient depletion.

The chlamydospore inducing factors were again obtained in 3 UV absorbing bands (R_F 0.13, 0.46 and 0.70) in TLC experiments. When these bands were mixed with other bands, as before, they were not chlamydospore inducing (Fig.3.5). The possibility of the occurrence of chlamydospore inducing non-UV absorbing bands was eliminated by elution and bioassay of 1 cm bands of developed TLC plates.

The absorption spectra (Fig. 3.6) showed that some UV absorbing bands had λmax at 260 nm in distilled water. Meyers and Cook (1972) suggested thatbbiological compounds such as adenosine 5' monophosphric acid (AMP) or 3':5' cyclic adenosine monophosphoric acid could act as chlamydospore stimulants. Therefore, the possible relationship of chlamydospore inducing substances in soil to nucleotides, also present in soil, cannot be ignored.

The above results are consistent with the concept that the induction of the formation of Fusarium chlamydospores is a response to particular substances and not merely due to low concentrations of nutrients or other unfavourable conditions. Macroconidia of Fsph generally are not affected by fungistasis until the nutrients (within or outside spores) are depleted. When these are exhausted they soon respond to chlamydospore inducing substances in soil. This formation of survival structures sensitive to soil fungistasis could also occur in response to endogenous chlamydospore inducing substances. These chlamydospore inducing substances are, therefore, indentified as compounds which are biotic in origin (Ford et al. 1970 a, b; Meyers & Cook, 1972).

B. Factors affecting disease expression in soil

Pathogenicity of Fsph in various soils was different when observed under controlled conditions (Table 3.9). Two types of soils were recognised, those that are conducive and suppressive to foot rot. These differences did not depend on the previous crop in the soil.

The population of the Fsph in inoculated soil (7.5% ^{v}/v) 6 days after inoculation was found to be 5349 propagules/g dry weight soil but 90 days after inoculation the population had declined to 1408 propagules/g dry soil. Nash and Alexander (1965) showed that only 30-50% of the conidia are converted to chlamydospores and this initial decline represents the conversion of macroconidia to chlamydospores. Since the original inoculum was mainly macroconidia, a marked decline in the population during the first 1-2 months could be expected.

When various crops were grown in this soil (Soil CS) increases in population occurred with host and non-host crops (Table 3.10). Also there were significant decreases in populations with certain non-host crops, e.g. cabbage and onion. These results are similar to those reported by Schroth and Hendrix (1962). However, the amount of disease in a subsequent bean crop was consistently lower after sorghum and higher after cabbage and onion. This meant that there was a poor correlation between populations of the pathogen and disease indices (Table 3.10). But certain evidence from experiments in which inoculum was produced and incorporated under similar and controlled conditions (Fig. 3.8, Baker et al., 1967) gave good correlations (soil PS = 0.81, 6 = 0.80) between Disease Index and inoculum density. Therefore, the inoculum associated with different crops changes in quantity and in quality. The inoculum potential (sensu Garrett) is, therefore, affected by the crop. A higher Disease Index with a low inoculum density and a low Disease Index with a high inoculum density (Table 3.10) reflected, therefore, differences in inoculum potential.

Chlamydospores that formed on macroconidia are larger in size in both conducive and suppressive soil (soil extract) than were those formed on hyphae (10.75 x 6.5 μ cf. 8.25 x 5.37 μ). In soil 4 (conducive soil) a larger proportion of chlamydospores were formed on macroconidia. It seems likely that the larger chlamydospores have a higher inoculum potential than smaller chlamydospores formed on hyphae. Thus the substances of biotic origin in soil may modify the inoculum potential of chlamydospores of Fsph.

Soil treatments (with different crops) inducing changes in population of Fsph may stimulate selectively certain components of the microflora which in turn may modify incidence and severity of disease. This was seen when conducive (4) and a suppressive (6) soils were studied for their ability to suppress disease at different inoculum densities (Fig. 3.8). The similar slopes of curves (disease x inoculum density) for sterilized soils and unsterile conducive soil 4 indicate that in the absence of a particular soil microflora (of soil 6) disease increases proportionally with increase in inoculum density. The less steep slope of suppressive soil 6 indicates a contraction of the rhizosphere influence (which stimulates chlamydospore germination and penetration of host) to a rhizoplane influence which results in a lower Disease Index (Baker et al., 1967, 1970). This decrease in the influence of the rhizosphere has also been obtained by incorporating crop residues (Menson et al., 1957; Maier, 1959; Snyder et al., 1959; William & Schmitthenner, 1960; Maier, 1961).

The presence of broader range of competitive saprophytes in suppressive soil 6 and their preferential stimulation in the rhizosphere (Fig. 3.9, Table 3.11) of beans probably effectively interfered with and immobilized nutrients necessary for the germination of chlamydospores. Also in this context it seems that the presence of a wider range of fungi is more important in decreasing disease than the presence of actinomycetes or spore forming bacteria.

4. EFFECT OF *FUSARIUM SOLANI* F.SP. *PHASEOLI* ON ROOT NODULE FORMATION

4.1 Introduction

Earlier experiments have shown that there were fewer root nodules on roots of infected bean plants than on roots of control plants. Normally *Rhizobium* spp. multiply readily in the rhizosphere of legumes, and it is also known that legumes stimulate growth of *Rhizobium* spp. more than other soil micro-organisms. Brown (1961) showed that the ratio of number of *Rhizobium* spp. in the legume rhizosphere to ordinary soil (R : S ratio), is rarely less than 10^2 and often more than 10^6, but for other organisms it is 10^1-10^2. Nutman (1965) reported that a factor necessary for root nodule formation comes from the cotyledon or plumule, and that root exudates are also important in root nodule initiation. Therefore the following experiments investigated the effect of the pathogen on the formation of nodules.

4.2 Materials and Methods

(i) Pathogen and Host

These were as above (Section I). Bean plants were grown (one plant per pot) in 8 cm diameter plastic pots in soil or vermiculite, both inoculated with the pathogen.

(ii) Nodule Bacteria

A normal virulent *Rhizobium* sp. (R) isolated from *Phaseolus vulgaris*, designated Cambridge isolate 1975, R.C.No. 3644 obtained from the Curator, Rothamsted Rhizobium Collection, Soil Microbiology Department, Rothamsted Experimental Station, Harpenden, Herts., was used. It was kept on yeast-mannitol agar at 25°C.

(iii) Inoculation

a. Fusarium solani f.sp. phaseoli (Fsph)

Fsph inoculum (Section I, materials and methods $^{v}8/v$ inoculum) at 10% $^{v}/v$ was added either as a 1 cm layer (TOP LAYER) above the seeds or throughout the soil or vermiculite (FULL).

b. Rhizobium sp.

Bacterial cultures were multiplied on YMA (Yeast mannitol Agar, Fred and Waksman, 1928):

Yeast extract (Oxoid)	10.0 g
Mannitol	10.0 g
K_2HPO_4	0.5 g
$MgSO_4$ $7H_2O$	0.2 g
NaCl	0.1 g
$CaCO_3$	3.0 g
Agar	15.0 g
Distilled Water	1000 ml

Sterilized at (120°C) 15 p.si for 20 minutes. Bacteria from five day old cultures grown on YMA were washed off into nitrogen-free Long Aston nutrient solution (Appendix), filtered through sterile muslin, centrifuged at 1000 x g for 15 minutes and resuspended in fresh mineral solution. This washing procedure was repeated twice to avoid carrying over nutrients from YMA with the bacterial cells into the substrate. Procedure was adopted from the method used by Lim (Ph.D. Thesis 1961).

The bacterial suspension was diluted to 10^7 cells/ml, and sprayed on to soil while mixing, at 10 ml per pot of soil (about 200 cm^3 of soil 190 g oven dry soil). The final bacterial density in soil was $\sim 5.2 \times 10^4$ / g. dry soil.

(iv) Assessment of Nodulation and Disease

Pots were watered with tap water when required and kept at $21\pm3^{0}C$ in the glasshouse as described in Section I. Plants were harvested when they began to flower. Two days before harvesting watering was stopped to facilitate removal of roots which were carefully washed and the number of root nodules per plant were counted. Damage to the plants by the pathogen was assessed on scale 0-7 using the disease index (Fig. 4.2). Dry weight of shoot and roots per plant was also recorded.

4. 3 Experimental

(i) Nodulation in Vermiculite

Nodulation was studied in Fsph inoculated vermiculite as a preliminary experiment. Pots were inoculated with the pathogen as TOP LAYER or FULL. Each treatment (method of pathogen inoculation) was replicated 5 times, and results are given as an average of 5 replicates.

Results from the above experiment are summarized in Table 4.2 and Table 4.3.

(ii) Nodulation in Soil

The formation of root nodules of Fsph / infected plants was studied in Rhizobium sp. inoculated soil and ordinary field soil (not inoculated with Rhizobium sp.). Soil used in the experiment was collected from Silwood Hill bottom field, and is described in Table 4.1.

TABLE 4.1 Description of the soil used in nodulation experiment

Description	- Sandy Loam
Previous crop (summer 1974)	- Potato
pH	- 6.4
Total Carbon	- 1.3
Total Nitrogen	- 0.12

Fsph inoculated soil was left in plastic bins for 15 days in the glass-house before inoculating with Rhizobium sp. 3644. Nodulation was studied in the following different treatments:

1. CONTROL - Not inoculated with either Rhizobium sp. or Fusarium solani f. sp. phaseoli (Fsph)
2. R - Soil inoculated with Rhizobium sp.
3. R/F - Inoculated throughout with Rhizobium sp. and Fsph
4. R/T - Inoculated throughout with Rhizobium sp. and with Fsph as a 1 cm layer above the seed.
5. F - Soil inoculated throughout with Fsph
6. T - Soil inoculated with Fsph as a 1 cm layer above the seed.

Pots were seeded just after Rhizobium sp. inoculation. Each treatment was replicated 10 times.

Results from this experiment are summarized in Tables 4.4 and 4.5.

TABLE 4.2 Nodulation in bean plants grown in vermiculite inoculated with *Fusarium solani* f. sp. *phaseoli* (10% v/v), as a 1 cm layer above the seed (TOP LAYER) or added throughout vermiculite (FULL)

REPLICATES	INOCULATION OF PATHOGEN		
	FULL	TOP LAYER	NOT INOCULATED
	No. of nodules/ plant		
1	11	20	67
2	9	37	22
3	13	32	33
4	7	22	26
5	4	45	26
MEAN	8.8	31.2	34.8

L.S.D. at 0.05 = 17.08 L.S.D. at 0.01 = 23.94

TABLE 4. 3 Effect of Fusarium solani f. sp. phaseoli[a] on nodulation, dry weight of shoot and roots of bean plants grown in vermiculite.

METHOD OF INOCULATION	DRY WEIGHT OF SHOOT (g) per plant	DRY WEIGHT OF ROOTS (g) per plant	DISEASE INDEX[b] (Fig.)	NO. ROOT NODULES
FULL[c]	0.58 x	0.08 x	3.6 x	9 x
TOP LAYER[d]	0.66 x	0.13 x	2.2 y	31 y
CONTROL	0.82 x	0.13 x	0 z	35 y

a - Vermiculite was inoculated with the pathogen as in c and d at 10% (v/v)

b - Damage caused by the pathogen was assessed using the Disease Index (Fig. 1.2)

c - Pots were inoculated with pathogen throughout vermiculite

d - Inoculated as 1 cm layer above the seeds

Values followed by the same letter in each column are not significantly different at 0.05 probability level

TABLE 4.4 Nodulation in bean plants grown in soil inoculated with Rhizobium sp. R.C. No. 3644 and Foot rot pathogen Fusarium solani f. sp. phaseoli

REPLICATES	METHOD OF INOCULATION					
	CONTROL	R	R/F	R/T	F	T
1	51	53	1	2	23	76
2	85	66	25	96	14	69
3	72	53	35	56	30	32
4	51	43	15	57	38	107
5	57	66	11	38	27	40
6	140	39	41	4	8	44
7	140	66	8	83	73	42
8	85	45	9	94	45	118
9	110	86	3	68	36	60
10	50	13	0	61	50	87
MEAN	84.9	53.0	14.8	56.1	34.4	67.5

L.S.D. at 0.05 = 23.68

TABLE 4.5 Effect of Fusarium solani f. sp. phaseoli on nodulation, dry weight of roots of bean plants grown in soil

OBSERVATION PER PLANT	METHOD OF INOCULATION					
	CONTROL	R	R/F	R/T	F	T
No. of root nodules	85[a] z	53 xy	15 w	56 xy	34 wx	68 yz
Dry weight of roots (g)	0.52 x	0.57 x	0.49 x	0.55 x	0.69 x	0.68 x
Disease[b] Index	0.2 z	1.22 y	4.1 w	3.2 x	3.0 x	3.2 x

a - All values are means of ten replicates

b - See Fig. 1.2

Values followed by the same letter in the same horizontal line are not significantly different at 0.05 probability level

4.4 Discussion

Nodulation in beans grown in vermiculite were significantly lower when vermiculite was inoculated throughout (FULL) with Fsph compared to TOP LAYER inoculation or the non-inoculated control (Table 4.2). Although treatments caused no significant difference in shoot or root dry weight, the disease index was significantly higher in fully inoculated than in other treatments (Table 4.3).

When plants were grown in soil inoculated with Fsph, nodulation followed the same pattern as above (Table 4.4), with or without Rhizobium inoculum. Although the nodulation in the control was higher than in other treatments (including Rhizobium inocuated soil) it was not significantly different from the TOP LAYER inoculated treatment. However, the variability between plants in number of nodules formed was very high in both experiments (Table 4.2 and 4.4).

The dry weight of roots was not significantly different between any treatments although F had higher dry weight than control (Table 4.5). It is interesting to note the higher disease index in R/F treatments than in similar treatments that includes Fsph without Rhizobium inoculation (Table 4.5).

It is clear from Tables 4.2 and 4.4 that the pathogen markedly inhibits root nodule formation, presumably in the rhizosphere of the bean plants. This may be due to root damage, direct inhibition of Rhizobium spp. growth or by interfering with factors controlling formation of root nodules (Nutman, 1965) as a result of hypocotyl and taproot damage. However it is also evident from the results (Tables 4.3 and 4.5) that the root weight (a measure of root damage) is little different in full, top-layer, or non-inoculated control plants.

Therefore, it is possible that the inhibition of root nodule formation is due to direct interaction between the pathogen and Rhizobium rather than on effects operating through the host plant.

Soybean cyst nematode (Heterodera glycines)-infected plants usually have fewer bacterial nodules (Agrios, 1969). Lim (1961) showed that certain rhizoplane fungi (Verticillium spp.) consistently decreased Rhizobium infection without affecting the numbers of Rhizobium in the rhizosphere, whereas other fungi such as Paecilomyces spp. were either stimulatory or had no effect.

Hely et al. (1957) reported that failure of inoculation of clover seed was due to microbial antagonism in the rhizosphere which prevented normal colonization of the rhizosphere by Rhizobium. They could not however establish the identity of the antagonists after a survey of bacteria and actinomycetes.

Lim (1963) showed that fewer than 100 bacteria in the whole rhizosphere are sufficient to start infection. In the above experiments about 5.2×10^4 bacterial cells/g dry soil was used. Therefore the lower number of root nodules in bean plants grown in Rhizobium spp. 3644 inoculated soil (Table 4.4) could be due to interaction between inoculated and native Rhizobia (Means et al., 1961).

5. CHEMICAL CONTROL OF FOOT ROT

5.1 Introduction

Fsph persists in soil as chlamydospores. Therefore it is difficult to eradicate compared to other pathogens involved in foot rot namely Pu and Cp. Thus it is desirable to evaluate certain chemicals recently introduced as fungicides, mostly with systemic activity, for the ability to control Fsph.

5.2 Materials and Methods

(i) Pathogens and Host - Fsph, Pu and Cp used in section 2 and Pinto beans were used in experiments.

(ii) Fungicides

The following fungicides were used in the experiments:

1. PP 395 formulation JF 3937:- 4-(3-chlorophenyl hydrazon:)-3 methyl-5 isoxazolone. "Metazoxolon" as an aqueous semi-colloidal suspension ('col') containing 400 g PP 395/L supplied by ICI Plant Protection Ltd., Jealott's Hill Research Station, Bracknell, U.K. Recommended for - Pythium, Phytophthora, Fusarium and Rhizoctonia spp.

2. DAM 18654 - Formulation 6233a:- 1-(5 cyanopentylcarbamoyl)-2-(methoxy corbomyl amino)-benzimidazole.
"Folcidin" or "Cypendazol" - systemic 50% WP supplied by Dr. W. Linke, Bayer Agrochem Ltd., Eastern Way, Bury St. Edmunds, Suffolk. Product of Chemagro - Bayer, Germany, Recommended for - Fusarium, Septoria etc.

3. F 319:
3 hydroxy-5methylisoxazole
"Hymexazol" or "Tachigaren" - 30% liquid systemic supplied by Dr. Mitsuo Ishida, Agricultural Research Laboratory, Tokyo, Japan. Product of Sankyo Co., Ltd., No. 7-12 Ginza 2-chome Chuo-Ku, Tokyo, Japan. Recommended for - Fusarium, Aphanomyces Pythium Corticium and Rhizoctonia spp.

4. 4322:

5 ethoxy-trichloromethyl 1, 2, 4, thiodiazole 15% and

Dimethyl 4, 4-O-phenylembis (3-thioallophanate) 25%

"Banrot" - systemic

Produced and supplied by Mallinkrodt Chemical Works, Second and Mallinkrodt Streets, St. Louis, Montreal 63160, U.S.A.

Recommended for - Pythium, Rhizoctonia, Fusarium and Botrytis spp.

5. Quintozene:

Pentachloronitrobenzene (PCNB) 20% DUST

"Botrilex" - Commercial product of Plant Protection Ltd., ICI Agricultural Division, Yalding, Kent, U.K.

Recommended for - Rhizoctonia spp.

(iii) Specificity test on agar

Fungicides were incorporated in 15 ml PDA (Oxoid) after sterilizing at 15 p.s.i. for 20 minutes just before pouring plates; 0, 100, 500 and 1000 ppm of the a.i. were used. Three to four plates of each were inoculated separately at the centre of the plate with disks obtained from one week old PDA cultures of Fsph, Pu and Cp. Inoculum was removed with a sterile No. 1 cork borer (diameter = 4mm). Plates were incubated in the dark at 25°C.

(iv) Pot experiments

a. Inoculation

Sieved field soil in 9 cm, 510 ml black square plastic pots were inoculated with Fsph $^{v}8/v$ inoculum at 10% $^{v}/v$. Inoculated pots were left in the glass-house for two weeks before seeding and treating with fungicides. Treatments were replicated 5 times; each pot was seeded with 4 bean seeds.

b. Method of fungicide application

b.1 Soil drench

All fungicides were applied just after seeding except Botrilex which as a dust was incorporated to soil 4 days before seeding. The following dosages were used in the experiments. All are the rates recommended by manufacturers.

JF 3937 - (Metazoxolon)	250 ppm 2.5 g a.i. of PP395 in 10 L of water @ of 300-400 ml/22 x 35 x 5 cm^3 or to 10 ml soil added 1 ml i.e. 50 ml/pot.
6233a - (Folcidin)	100 ppm 1g/5L/M^2 (of product not a.i.) i.e. 0.5 g a.i./5 L @ 50 ml/pot
F 319 - (Hymexazol)	600 ppm a.i. @ 3L/M^2 i.e. 25 ml/pot
4322 - (Banrot)	170 ppm 200 g/455 L to 400 ft^2 of product. i.e. 147 ml/pot
Quintozene - (Botrilex)	1 g of Botrilex dust/1122 cm^3 of soil i.e. 0.4542 g/pot

b.2 Seed dressing

Liquid formulations

Seeds were dipped in 'Col' and liquid formulations for 3-4 minutes. The following concentrations were used as recommended by the manufacturers.

JF 3937 - 0.1 - 1g PP395/kg of seed

F319 - 600 ppm a.i., 0.2 g of F319/100 ml

Dust

Seeds were treated with fungicides after making a paste with water so that seeds could carry sufficient of the fungicides. To provide the bulk required for coating seeds active ingredients of fungicides were thoroughly mixed with talc before making the

paste. The following concentrations were used as recommended by the manufacturers.

6233 a - 0.02 g a.i./10 g seeds

4322 - 0.02 g a.i./10 g seeds

Quintozene - 0.05 g (Botrilex)/10 g seeds

(v) Assessment of fungicide activity and disease in plants

In specificity tests on agar, colony diameters (mm) in treated plates were recorded when the fungus in untreated control PDA plates reached the periphery of the petriplate (85mm). Thus colony diameters were recorded for Pu, Cp and Fsph 2, 3 and 23 days after inoculation respectively. Results are recorded as a percentage of control and given as an average for 3-4 replicates.

In glass-house experiments percentage emergence was recorded 10 days after sowing based on number of seeds (4 seeds) sown. Number of diseased plants were recorded as a percentage of total number of emerged plants per replicate. Fsph infection was assessed on a scale 0-7 and given as a mean for 4-20 plants (Fig. 1.2).

Fresh and dry weights per plant were recorded (per plant weights are given as weighted average for number of survived plants in each replicate) 25 days after sowing.

5.3 Experimental

(i) Specificity test on agar

Because fungicide 4322 ("Banrot") is not a single compound but a mixture of two fungicides it was not used in this test. Results are summarized in Table 5.1.

Plates containing JF 3937 were bright yellow in colour. In plates inoculated with Pu white haloes were observed around the growing mycelium in plates containing 100 and 500 ppm a.i. But no fungal growth was detected within these white haloes. Mycelial growth of Fsph was markedly reduced at low concentrations (100 ppm) and it tends to grow better at higher concentrations of 500 and 1000 ppm. Pu was effectively inhibited at 100 ppm. Although in general Cp was inhibited by JF3937 mycelial growth was better than that of Pu at all concentrations (Table 5.1).

Folcidin, 18654 decreased the mycelial growth of Cp and Fsph at all concentrations used. But it had little effect on the growth of Pu at 100 or 500 ppm (Table 5.1).

Hymexazol, F319 completely inhibited the growth of all three fungi at 1000 ppm and of Pu and Cp even at 500 ppm. It was less effective at 100 ppm against Fsph and Cp although the colony diameter of Pu was only 15% of that of control at 100 ppm.

(ii) Soil drench experiment

The effects of fungicides applied as soil drenches at sowing on Fusarium root rot are summarized in Table 5.2.

Although the percentage of diseased plants were generally lower in fungicide treated soil than in untreated, Fsph inoculated soil, it was significantly lower only in 4322 treated soil. The Disease Index of plants grown in 4322, F319 and 6233a treated soil were significantly lower than those grown in JF 3937 or Botrilex treated or Fsph inoculated soil. However Disease Index of plants grown in Botrilex treated soil did not differ from that of inoculated, untreated soil.

The percentage emergence of seedlings in all inoculated treatments except Botrilex treated soil were significantly lower than that in non-inoculated untreated control.

(iii) Seed dressing experiments

Results of this experiment are given in Table 5.3 and 5.4. Percentage diseased plants were significantly lower in 6233 a than in other treatments although there were no significant differences in percentage emergence between treatments (Table 5.3).

Similar results were reflected in Disease Indices where 6233 a had significantly lower values (Table 5.3). However, these differences were not clear in fresh and dry weight observations (Table 5.4).

5.4 Discussion

It was observed that Folcidin at 100 ppm Fsph on agar (Table 5.1). Folcidin gave the best control in seed dressing experiments although it was not as effective as Banrot in soil drench experiments. Folcidin and Banrot in soil drench experiments were used at 100 and 170 ppm respectively. But the quantity per pot at manufacturer's recommended rate were three times more for Banrot than Folcidin (5.2, IV-2). The prominent effect of Banrot in controlling foot rot could be due to the presence of Truban (= 5 ethoxy-trichloromethyl 1, 2, 2, 4, thiodiazole) which controls any effect due to Pu.

TABLE 5.1 Colony diameters of Fsph, Pu and Cp grown on PDA containing various concentrations of Metazoxolon, Folcidin and Hymexazol

Fungicide	Pathogen	Concentrations of fungicide (ppm)			
		0	100	500	1000
		(colony diameter as a percentage of control)			
Metazoxolon	Fsph	100	36.2 (1.21)	51.4 (6.58)	56.2 (7.03)
JF 3937	Pu	100	9.0 (0.79)	0	0
	Cp	100	18.0 (0.39)	16.1 (0.39)	10.0 (0.34)
Folcidin	Fsph	100	33.7 (2.18)	32.2 (1.41)	28.6 (1.71)
6233 a	Pu	100	99.6 (0.4)	62.7 (0.77)	39.2 (1.41)
	Cp	100	4.3 (1.47)	0	0
Hymexazol	Fsph	100	66.7 (2.40)	22.2 (1.39)	0
F 319	Pu	100	15.3 (0.83)	0	0
	Cp	100	40.4 (0.39)	0	0

Figures in brackets are standard errors of means. Colony diameters were recorded when the control reached 85 mm.

Fsph - Fusarium solani f. sp. phaseoli. Pu - Pythium ultimum. Cp - Corticium praticola.

PDA - Potato dextrose agar (Oxoid).

TABLE 5.2 Effect of fungicides applied as a soil drench at sowing on Fusarium foot rot of bean

Fungicide/ Treatments	Percentage Emergence[1]	Percentage diseased plants[2]	Disease index	Disease index as a % of untreated
Uninoculated	95 yz	0 w	0 x	0
Banrot	50 x	58.3 y	0.90 x	29.0
Hymexazol	20 x	75.0 yz	1.25 x	40.3
Folcidin	35 x	75.0 yz	1.57 x	50.6
Metazoxolon	30 x	91.6 yz	2.67 yz	74.1
Quintozene	100 z	100 z	2.30 y	74.1
Inoculated untreated	55 xy	100 z	3.09 z	100

1. Means for five replicates - 4 plants per pot

2. Percentage diseased plants out of total emerged

Percentage emergence L.S.D. at 0.05 = 43.44

Disease Index and Percentage diseased L.S.R. was calculated using multiple range test see appendix

Values followed by the same letter in each column are not significantly different at 0.05 probability level

TABLE 5.3 Effect of seed treatments with various fungicides on foot rot bean

Fungicide Treatments	Percentage[1] emergence	Percentage[1] diseased plants	Disease[2] index	Disease index as a % of untreated
Uninoculated	95 x	0 x	0 u	0
Banrot	100 x	100 z	2. x	85.3
Hymexazol	100 x	95 z	2.7 xy	88.9
Folcidin	95 x	63 y	0.9 w	31.3
Metazoxolon	95 x	100 z	3.11 z	103.6
Quintozene	100 x	100 z	3.1 z	101.9
Inoculated untreated	100 x	100 z	3.0 yz	100

Values followed by the same letter in each column are not significantly different at 0.05 probability level

1. Mean for 5 replicates
2. Mean for 18 plants

TABLE 5.4 Fresh and dry weight of bean plants grown in Fsph[1] inoculated soil from treated seeds with various fungicides

Fungicide/ Treatment	Fresh weight[2] per plant (g)	Dry weight[2] per plant (g)
Uninoculated	8.37 z	1.22 z
Banrot	6.84 xy	0.87 x
Hymexazol	7.05 xy	0.95 xy
Folcidin	6.43 xy	0.88 x
Metazoxolon	7.79 yz	1.10 yz
Quintozene	7.30 xy	0.97 xy
Inoculated untreated	6.97 xy	0.92 x

Values followed by the same letter in each column are not significantly different at 0.05 probability level

Fresh weight $L.S.D._{.05}$ = 1.08 Dry weight $L.S.D._{.05}$ = 0.18

1. Fsph - Fusarium solani f.sp. phaseoli
2. Means for five replicates. Weight per plant for each replicate was a weighted average for 3-4 plants

The effect of Folcidin in seed dressing experiments was prominent in percentage diseased plants and disease index (Table 5.3). This could also be due to its inhibitory effect on Cp which decreases any cumulative effect on foot rot. Because it reduced the incidence of disease in both soil drench and seed dressing treatments at low conc. 100 ppm it would be a desirable fungicide for further experiments on foot rot control.

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A.1

1. Chromatographic spray reagents.

   a. Diazotised 4-nitroaniline DNA

   10 ml 0.1% aqueous 4-nitroaniline solution are mixed with 10 ml 0.2% aqueous $NaNO_2$ solution and 20 ml 10% K_2CO_3 solution in water are added.

   b. Gibbs' reagent - 2, 6 - Dibromoquinonechloroimide

   Spray reagent: Freshly prepared 0.4% methanolic solution of 2, 6 - dibromoquinonechloroimide.

   Treatment after spraying: Sprayed with 10% aqueous Na_2CO_3 solution or placed in a chamber containing 25% NH_4OH

2. Sucrose-Casamino acid medium

Casamino acid (vitamin free)	4.6 g
KH_2PO_4	1.0 g
$MgSO_4.7H_2O$	0.5 g
Sucrose	15.0 g
Glucose	0.5 g
Long Ashton nutrient solution	10.0 ml
Distilled water	990.0 ml

Sterilized at 15 p.s.i. for 15 minutes

3. Long Ashton nutrient solution

Preparation of stock solution

After Hewitt E. J. (1966). sand and water culture methods used in the study of plant nutrition. 2nd Ed. Commonwealth Agric. Bureaux, Tech. Comm. No. 22.

	Compound	g/2 litres
1.	KNO_3	404
2.	$Ca(NO_3)_2.4H_2O$	944
3.	$NaH_2PO_4.2H_2O$	208
4.	$MgSO_4.7H_2O$	368
5.	Fe EDTA Na Salt	77.02
6.	$Mn\ SO_4.4H_2O$	4.46
7.	$CuSO_4.5H_2O$	0.50
8.	$ZnSO_4.7H_2O$	0.58
9.	H_3BO_3	3.72
10.	$(NH_4)_6Mo_7O_{24}.4H_2O$	0.176

To prepare 1 litre of standard nutrient solution (LANS) 1 ml of

each of solution 5-10, and 2 ml of each of solutions 1-4 are added to about 900 ml distilled water and then made up to one litre. The phosphate stock (3) is added last to avoid the risk of precipitation.

3. Method of cleaning slides used in spore germination tests

New glass slides were rubbed with soap water and rinsed in hot water followed by distilled water. Glass slides were then soaked in 5% acetic acid for c. 12 h and rinsed with glass distilled water; these slides were then left overnight in absolute ethanol and dried at 70°C in closed containers.

4. Lacto-fuchsin (Carmichael, 1955)

Acid fuchsin	0.1 g
Lactic acid	100 ml

Carmichael, J. W. 1955, Lacto-fuchsin: a new medium for mounting fungi. Mycologia 47, 611.

TABLE A 1.1 (Reference Fig. 1.5)

Relationship between inoculum density and Disease Index

Replicates	Inoculum density (% inoculum)						
	100	50	25	12	6	3	0
1	7	7	5	4	3	1	0
2	7	6	5	4	3	2	0
3	7	6	5	4	3	1	0
4	7	6	-	4	4	2	0
5	6	6	5	4	5	3	0
6	6	7	4	4	3	2	0
7	6	6	-	3	4	-	0
8	6	6	-	4	3	-	-
9	6	5	6	4	3	3	0
10	6	5	6	4	3	2	0
11	6	6	5	4	3	2	0
12	6	5	6	-	-	-	0
13	6	6	5	4	3	3	0
14	6	6	5	4	6	3	0
15	6	6	5	4	3	-	0
16	7	7	4	5	6	-	0
Mean	6.3	6.0	5.1	4.0	3.6	2.2	0

ANOVA

Source	df	SS	Ms	F
Between groups	6	442.6	73.76	20.43
within groups	94	339.4	3.61	
Total	100	782.0		

$$LSR = Q_{.05\ [\bar{K}.94]} \sqrt{\text{Ms with}} \times \sqrt{\frac{n_1 + n_2}{2n_1n_2}}$$

Rank	1	2	3	4	5	6	7
Means	0	2.2	3.6	4.0	5.1	6.0	6.3

LSR for rank 1 and 2 ($LSR_{1\ \ 2}$) = 1.51

2 — 7 = 2.204
2 — 3 = 1.51
2 — 4 = 1.83
2 — 5 = 1.99
3 — 7 = 1.96
3 — 5 = 1.71
3 — 6 = 1.72

4 — 7 = 1.72
4 — 5 = 1.41
4 — 6 = 1.58
5 — 7 = 1.71
6 — 7 = 1.30

TABLE A. 1.2 (reference Fig. 1.5)

Relationship between inoculum density and dry weight g per plant (weighted average per plant per replicate)

Replicates	Inoculum density (% inoculum)						
	100	50	25	12	6	3	0
1	0.125	0.075	0.167	0.200	0.350	0.450	0.600
2	0.075	0.125	0.200	0.250	0.350	0.450	0.600
3	0.075	0.150	0.175	0.250	0.300	0.600	0.650
4	0.100	0.175	0.200	0.250	0.225	0.750	0.400
Mean	0.094	0.131	0.186	0.238	0.306	0.563	0.563

ANOVA

Source	df	SS	MS	F
between groups	6	3.073	0.512	85.3
within group	21	0.120	0.006	
Total	27			

$$LSD = t_{.05\ (21)} \sqrt{\frac{2}{n}} \cdot MS\ within = 0.114$$

TABLE A 1.3 (reference Fig. 1.6)

Effect of Age at inoculation on Fusarium foot rot of beans in soil and in vermiculite

Seedling age and Disease Index in vermiculite

Replicate	Age (weeks) at inoculation			
	0	1	2	3
		(Disease Index)		
1	4	4	3	3
2	4	2	3	2
3	4	2	2	2
4	5	2	2	1
5	4	1	3	2
6	4	2	1	1
7	4	2	1	2
8	3	4	-	2
9	3	2	3	1
10	3	3	3	2
11	3	2	3	-
12	3	1	2	1
13	4	3	3	1
14	4	2	3	1
15	3	2	2	-
16	4	2	2	-
Mean	3.687	2.250	2.40	1.538

ANOVA

Source of variation	df	SS	MS	F
among groups	3	35.715	11.905	24.495
within groups	56	27.269	0.486	
Total	59	62.984		

LINREG

regression coefficient = -0.632
y intercept = 3.4126

regression line, y = 3.413 - 0.632 x

Rank	1	2	3	4
Means	1.54	2.25	2.40	3.69

LSR 1-4 = 0.57 LSR 1-3 = 0.56 LSR 3-4 = 0.50

LSR 1-2 = 0.52 LSR 2-3 = 0.50

TABLE A 1.4 (reference Fig. 1.6)

Seedling age and Fresh weight per plant (weighted average per pot) as a percentage of control (arcsine transformation) in vermiculite

	age (weeks) at inoculation			
Replicate	0	1	2	3
	(arcsine $\sqrt{\text{Fresh weight as \% of control}}$)			
1	44.5	44.7	40.5	60.6
2	48.3	37.2	47.9	70.9
3	46.5	52.8	45.9	62.0
4	37.2	54.2	45.6	71.8
Mean	44.2	47.2	44.9	66.3

ANOVA

Source of variation	df	SS	MS	F
among groups	3	1328.87	442.95	13.63
within groups	12	389.99	32.49	
Total	15	1718.86		

regression coefficient = 6.435

y intercept = 41.005

regression line y = 41.0 + 6.43 x

LSD at .01 = 12.30

LSD at .05 = 8.78

TABLE A 1.5 (reference Fig. 1.6)

Seedling Age and Disease Index in soil

Replicate	Age (weeks) at inoculation			
	0	1	2	3
		(Disease Index)		
1	3	3	3	3
2	4	3	3	4
3	4	3	6	4
4	3	3	4	4
5	4	4	3	4
6	4	4	3	4
7	3	2	3	4
8	4	3	-	-
9	4	4	3	4
10	4	5	6	3
11	4	3	3	-
12	4	-	-	-
13	4	4	3	4
14	5	4	3	4
15	4	4	6	3
16	4	3	-	5
Mean	3.87	3.47	3.77	3.85

ANOVA

Source of variation	df	SS	MS	F	
Among groups	3	1.570	0.523	0.781	ns
Within groups	53	35.480	0.669		
Total	56	37.050			

regression coefficient = 0.014
y intercept = 3.717

regression line y = 3.717 + 0.014x

Rank	1	2	3	4
Means	3.47	3.77	3.85	3.87

LSR 1-4 =.639 ns

TABLE A 1.6 (reference Fig. 1.6)

Seedling age and Fresh weight per plant (weighted average per pot) as a percentage of control (arcsine transformation) in soil

Replicate	Age (weeks) at inoculation			
	0	1	2	3
	(arcsine	fresh weight as % of control)		
1	65.6	63.6	51.5	53.0
2	55.9	60.5	61.4	71.5
3	68.0	54.8	54.0	64.0
4	55.9	58.9	42.0	63.4
Mean	61.35	59.37	52.22	62.97

ANOVA

Source of variation	df	SS	MS	F
among groups	3	269.45	89.82	2.533 ns
within groups	12	425.52	35.46	
Total	15	694.97		

Regression coefficient = -0.2275

y intercept = 59.322

regression line y = 59.32 - 0.227 X

LSD at 0.01 = 12.86

LSD at 0.05 = 9.17

TABLE A. 1.7 (reference Fig. 1.7)

Response to direct inoculation of bean hypocotyls with macroconidia of Fusarium solani f. sp. phaseoli at various positions along the hypocotyls

(Disease Index from Fig. 1.3)

8 days old hypocotyls

Replicate	Position of infections on the hypocotyl									
	Cotyledon end									Root end
	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
	(Disease Index)									
1	4	2	5	4	3	1	1	0	1	-
2	5	6	6	7	4	5	2	1	2	1
3	5	4	5	7	4	5	6	5	3	2
4	6	5	7	-	2	2	4	3	-	-
Mean	5.0	4.25	5.75	6.0	3.25	3.25	3.25	2.25	2.0	1.50
95% confidence limit 1	3.70	1.53	4.23	1.69	1.73	-0.03	-0.28	-1.28	0.48	-4.85
2	6.29	6.97	7.27	10.30	4.71	6.53	6.78	5.78	4.48	7.85

12 days old hypocotyls

Replicate	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
1	6	5	3	7	-	2	2	1	0	-
2	4	3	4	0	3	1	1	-	-	-
3	4	3	5	4	4	2	2	1	1	1
4	3	3	3	2	3	4	0	0	0	-
5	2	3	4	4	2	1	0	0	0	-
6	1	2	-	4	2	1	0	0	0	-
7	0	0	2	3	2	2	1	0	0	-
8	8	2	1	2	2	1	0	0	0	0
Mean	2.75	2.63	3.14	3.25	2.57	1.75	0.75	0.29	0.14	0.5
95% confidence limit 1	1.16	1.45	1.89	1.53	1.84	0.88	0.01	-0.16	-0.2	
2	4.35	3.80	4.39	4.97	3.29	2.61	1.49	0.74	0.49	

TABLE A 1.8 (reference Table 1.2)

Effect of Fusarium solani f. sp. phaseoli on beans grown in vermiculite at various inoculum densities

Relationship between inoculum density and Disease Index see Table A 1.1.

Relationship between Plant height and Inoculum density

Replicates	Inoculum density (%(^{v}/v) inoculum)						
	100	50	25	12	6	3	0
	(Plant height cm)						
1	5.0	4.5	11.0	14.0	12.0	18.0	24.0
2	5.5	8.0	8.0	16.0	17.0	15.0	23.0
3	3.5	8.0	11.0	14.5	16.0	18.0	20.0
4	6.0	8.5	-	10.0	10.0	14.0	21.0
5	5.0	7.0	8.5	16.0	8.0	13.0	21.0
6	6.0	6.0	10.0	13.5	12.0	11.0	18.0
7	7.0	7.0	-	15.0	9.0	-	23.0
8	4.0	7.0	-	10.0	13.5	-	-
9	8.0	9.0	8.5	7.0	18.0	22.5	20.0
10	6.0	9.0	8.5	14.0	18.0	27.0	18.0
11	7.0	8.0	10.5	10.0	19.0	22.0	18.0
12	7.0	9.5	8.0	-	-	-	17.0
13	9.0	4.5	6.0	12.0	15.0	10.5	33.0
14	6.5	4.0	10.0	9.0	6.0	10.0	27.0
15	8.0	8.0	10.0	10.5	12.0	-	26.0
16	4.0	2.0	12.0	6.0	7.0	-	22.0
Mean	6.1	6.9	9.4	11.8	12.8	16.5	22.1

TABLE A 1.9 (reference TABLE 1.3)

Population of Fusarium solani f. sp. phaseoli in soil at 10% inoculum level

Replicate	Days after inoculation			
	2		12	
	Soil I (Propagules/g	Soil II dry soil)	Soil I (Propagules/g	Soil II dry soil)
1	13830	3061	14042	12761
2	17872	6735	7659	6067
3	5319	7143	7872	4393
4	3404	7347	11276	9623
5	5319	6122	7659	7950
6	4894	10612	10851	7322
7	7234	4490	6170	7322
8	9362	8775	11702	7950
9	9149	7751	4468	7113
10	4894	6122	14042	5230
Mean	8127	6816	9574	7573
$LSD_{.05}$	4599.04		3671.6	

Inoculum = $^{V}8$/vermiculite Fsph inoculum

TABLE A 1.10 (reference TABLE 1.4)

Plant height as a percentage of control of Pinto bean plants inoculated with Fusarium solani f. sp. phaseoli

In Vermiculite

Replicate	Age in weeks at inoculation			
	0	1	2	3
	(Plant height as a % of control)			
1	34.2	27.4	71.3	71.0
2	45.9	33.8	38.4	62.1
3	34.2	32.2	64.0	46.1
4	11.0	42.6	60.3	62.9
5	64.0	25.7	47.5	66.4
6	39.3	27.7	133.6	85.2
7	36.4	37.0	82.3	103.4
8	42.0	10.4	62.2	98.5
9	47.5	41.5	72.2	102.0
10	22.5	43.4	56.7	78.2
11	55.2	35.4	85.9	83.8
12	32.0	40.2	113.3	82.4
13	32.0	33.8	64.0	118.7
14	30.0	41.8	57.6	81.0
15	36.4	47.5	79.5	82.0
16	29.1	29.0	72.5	81.5
Mean	36.9	34.3	72.6	81.6

ANOVA

Source of variation	df	SS	MS	F
among groups	3	28151.2	9383.73	33.34
within groups	60	16886.0	281.43	
Total	63			

LSD at 0.05 = 11.86 LSD at 0.01 = 15.77

TABLE A 1.10 contd. (reference Table 1.4)

In Soil

Replicate	Age in weeks at inoculation			
	0	1	2	3
	(Plant height as a % of control)			
1	95.0	108.4	61.0	61.5
2	98.5	58.6	74.6	86.5
3	79.2	70.0	50.9	83.7
4	112.6	78.5	65.1	82.0
5	66.9	53.3	74.9	88.9
6	80.6	54.2	82.1	80.3
7	121.4	43.5	88.2	85.4
8	70.4	78.2	85.1	52.6
9	140.0	46.2	44.1	77.9
10	80.9	49.8	74.6	83.7
11	82.3	103.2	54.3	83.7
12	128.2	68.4	93.2	89.9
13	72.2	76.4	50.9	34.5
14	58.0	67.9	-	-
15	72.1	94.2	-	-
Mean	90.2	70.7	69.2	76.2

ANOVA

Source of variation	df	SS	MS	FS
among groups	3	4020.48	1340.16	3.44 ns
within groups	52	20209.07	388.63	
Total	55			

Rank	1	2	3	4
Means	69.2	70.7	76.2	90.2

LSR at 0.05 probability level

LSR_{1-2} = 14.95 ns $\qquad$ LSR_{2-3} = 14.95 ns

1 — 3 = 18.57 ns $\qquad$ 2 — 4 = 1729

1 — 4 = 19.76 $\qquad$ 3 — 4 = 14.95 ns

TABLE A 1.11 (reference TABLE 1.5)

Dry weight per plant (weighted average per plant) in vermiculite

Replicate	Age in weeks at inoculation			
	0	1	2	3
	(Dry weight/plant as a % of control in arcsine transformation)			
1	55.8	47.2	39.9	52.7
2	50.0	40.3	47.8	64.6
3	62.1	61.8	48.5	52.7
4	38.6	61.8	46.3	59.1
Mean	51.2	52.8	45.6	57.3

LSD at 0.05 probability level = 12.52

Dry weight per plant (weighted average) in soil

Replicate	Age in weeks at inoculation			
	0	1	2	3
	(Dry weight/plant as a % of control in arcsine transformation)			
1	64.1	60.3	53.2	52.0
2	72.6	60.3	30.5	71.9
3	72.6	63.7	61.4	62.7
4	51.2	55.5	45.8	58.9
Mean	65.1	59.9	47.7	61.4

LSD at 0.05 probability level = 14.51

TABLE A1.12 (reference TABLE 1.6)

Effect of direct inoculation of cotyledons with Fusarium solani f. sp. phaseoli

Replicate	Age of cotyledons (days)					
	1	2	3	5	6	8
	(Number of cotyledons with different lesions)					
1	9 N	ON	1N	ON	ON	ON
	7 R	18R	18R	20R	11R	1R
	4 S	2S	1S	OS	9S	19S
2	6 N	ON	ON	ON	ON	ON
	9 R	19R	18R	20R	15R	1R
	5 S	1S	2S	OS	5S	19S

TABLE A 1.13 (reference TABLE 1.7)

Bioassay of eluates 1, 2, 3 and 4 (Fig. 1.8) with Fusarium solani f. sp. phaseoli macroconidia

Germination of spores were estimated by counting number of germinated macroconidia for 100 macroconidia in LANS

Percentage germination 98, 94, 96, 96, 94, 96, 95, 90, 99, 97 Mean 95.5

Germ tube length μ
330, 200, 450, 420, 550 -260, 360, 300,210, 470-
500, 320, 380, 420, 200 -400, 200, 240,240, 320
320, 380, 270, 280, 310 -210, 340, 240,260, 370
310, 300, 340, 230, 330 -270, 120, 400,240, 300
220, 150, 230, 320, 210 -330, 300, 410,220, 140

Mean germ tube length 30.24 μ s = 92.6

Fraction 1

Percentage germination 100, 98, 97, 96, 98, 99, 84, 70, 98, 70 Mean 91.

Germ tube length μ
120, 120, 130, 170, 130 -120, 110, 90, 180, 80
220, 350, 220, 50, 90 - 50, 20, 90, 100,180
100, 30, 20, 220, 60 - 90, 100,100, 200,100
20, 40, 50, 50, 50 - 20, 20, 40, 50, 40
50, 180, 120, 60, 40 - 60, 30, 60, 50, 50

Mean germ tube length 94.4 μ s = 68.1

Fraction 2

Percentage germination 0, 1, 0, 2, 1, 0, 0, 0, 0, 0, 0 Mean 0.2
0, 0, 0, 0,

Fraction 3

Percentage germination 67, 75, 50, 80, 67, 88, 92 Mean 74.14

germ tube length μ
220, 70, 270, 70, 110 -320, 150, 200, 120, 300
120, 300, 300, 300, 230 -400, 110, 220, 250, 340
150, 70, 200, 300, 80 - 250, 250, 200,100, 260
250, 100, 220, 110, 70 - 60

Mean germ tube length = 200.2 μ s = 92.1

Fraction 4

Percentage germination 16, 6, 15, 14, 8, 9, 3, 2, 11 Mean 9.3

germ tube length μ
210, 150, 200, 130, 160 -200, 220, 70, 240, 80
80, 120, 50, 40, 30 - 70, 310, 50, 250, 180
120, 70, 80, 130, 130 -130, 90, 100, 100, 210
150, 150, 210, 30, 210 -210, 120, 170, 100, 40
200, 70, 100, 220, 110

Mean germ tube length 135.3 μ s = 68.0

A.2

TABLE A 2.1 (reference Fig. 2.1)

Effect of pathogen interaction in producing foot rot in beans inoculated at different ages of the host in vermiculite

The seedling age at inoculation = 0 week (at sowing)

Pathogen Combination	Disease Index	Mean
Fsph	4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4	4.0
Pu	0, 7, 7, 7, 1, 0, 1, 7, 0, 0, 1, 7	3.2
Cp	7, 7, 7, 7, 7, 7, 7, 7, 7, 7, 7, 7	7.0
Fsph+Pu	4, 7, 5, 5, 7, 5, 4, 5, 5, 4, 7, 7	5.4
Fsph+Cp	7, 7, 7, 7, 7, 7, 7, 7, 7, 7, 7, 7	7.0
Cp+ Pu	7, 7, 7, 7, 7, 7, 7, 7, 7, 7, 7, 7	7.0
Fsph+Cp+Pu	7, 7, 7, 7, 7, 7, 7, 7, 7, 7, 7, 7	7.0
Control	0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0	0
1 week		
Fsph	3, 4, 4, 3, 4, 4, 3, 4, 4, 4, 4	3.7
Pu	1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1	1.0
Cp	5, 5, 5, 5, 0, 7, 7, 7	5.1
Fsph+Pu	5, 4, 5, 4, 5, 5, 5, 5, 5, 5, 5, 5	4.8
Fsph+Cp	6, 7, 6, 7, 5, 5, 5, 7, 2, 7, 7, 4	5.6
Cp+Pu	6, 6, 7, 7, 6, 6, 6, 7, 6, 5, 7, 7	6.3
Fsph+Cp+Pu	6, 6, 7, 7, 6, 6, 7, 7, 6, 6, 6, 7	6.4
Control	0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0	0
2 week		
Fsph	3, 3, 3, 3, 3, 4, 4, 3, 3, 2, 3, 1	2.6
Pu	4, 4, 4, 4, 1, 2, 2, 3, 4, 4, 4, 4	3.3
Cp	4, 4, 7, 7, 4, 4, 4, 7, 7, 4, 4, 4	5.0
Fsph+Pu	4, 4, 4, 4, 7, 4, 4, 4, 4, 4	4.3
Fsph+Cp	5, 5, 7, 7, 4, 4, 4, 4, 6, 6, 7, 7	5.6
Cp+Pu	4, 4, 7, 7, 4, 4, 7, 7, 4, 4, 7, 7	5.5
Fsph+Cp+Pu	6, 7, 7, 7, 5, 7, 7, 7, 5, 6, 5, 7	6.3
Control	0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0	0

TABLE A 2.2 (reference Fig. 2.2)

Percentage death of seedlings in vermiculite inoculated with Fsph, Pu and Cp at various ages

0 week - at sowing

Fsph - 0, 0, 0, Mean $\bar{Y}$ = 0; Pu - 75, 25, 25, $\bar{Y}$ = 41.7;
Cp - 100, 100, 100, $\bar{Y}$ =100; Fsph+Pu - 25, 25, 50, $\bar{Y}$ = 33.3
Fsph+Cp - 100, 100, 100, $\bar{Y}$ =100; Cp+Pu -100,100,100, $\bar{Y}$ =100
Fsph+Cp+Pu - 100, 100, 100, $\bar{Y}$ =100 ; Control - 0, 0, 0 $\bar{Y}$ =100

LSD at 0.05 = 27.21

1 week

Fsph - 0, 0, 0, $\bar{Y}$ = 0; Pu - 0, 0, 0, $\bar{Y}$ = 0
Cp - -, 0,75, $\bar{Y}$ =37.5; Fsph +Pu- 0, 0, 0, $\bar{Y}$ = 0
Fsph+Cp - 50,25,50, $\bar{Y}$ =41.7; Cp+ Pu - 50, 25, 50, $\bar{Y}$ = 41.7
Fsph+Cp+Pu - 25,50,50, $\bar{Y}$ =41.7; Control - 0, 0, 0, $\bar{Y}$ = 0

$LSR_{.05}$ 0-37.5 = 31.6; $LSR_{.05}$ 0-41.67 = 38.98; $LSR_{.05}$ 37.5-41.67 = 28.58 ns

2 week

Fsph - 0, 0, 0, $\bar{Y}$ = 0 ; Pu - 0, 0, 0, $\bar{Y}$ = 0
Cp - 50, 25, 25, $\bar{Y}$ =33.3; Fsph+Pu- 0, 25, 0, $\bar{Y}$ = 8.3
Fsph+Cp - 50, 0, 50, $\bar{Y}$ =33.3; Cp+Pu - 50, 50, 50, $\bar{Y}$ = 50
Fsph+Cp+Pu - 75, 75, 25 $\bar{Y}$ = 58.3 Control - 0, 0, 0. $\bar{Y}$ = 0

LSD at 0.05 = 27.93

TABLE A 2.3 (reference Fig. 2.4)

Population of Fusarium solani f. sp. phaseoli in soil inoculated with Fsph and Fsph+Pu+Cp

2 days after inoculation - Propagules/g dry soil

Fsph - 3061, 6735, 7142, 7347, 6122, 10612, 4490, 8775, 7755, 6122
Mean = 6816

Fsph+Pu+Cp -

5102, 4490, 3265, 5306, 3469, 4898, 2449, 4082, 4898, 5510
Mean = 4347

non-inoculated control - no Fsph was detected

$LSD_{.05} = t_{.05}\sqrt{\frac{2}{n}\ MS_{within}}$

MS_{within} = weighted average variance $= \frac{\sum^{a}(n_i - 1)S_i^2}{\sum^{a}(n_i - 1)}$

LSD = 1551.55

12 days after inoculation at sowing Propagules of Fsph/g dry soil

Fsph -

12761, 6066, 4393, 9623, 7949, 7322, 7322, 7949, 7112, 5230
Mean = 7573

Fsph+Pu+Cp -

7726, 6181, 4635, 7064, 1324, 2869, 2649, 4415, 7726, 9492
Mean = 5408

No Fsph in controls was detected

LSD = 2350.96

42 days after inoculation - after cropping - Fsph propagules/g dry soil

Fsph -

6250, 9722, 7407, 4629, 10185, 2546, 5093, 7407, 8333, 8796
Mean = 7037

Fsph+Pu+Cp -

9685, 9927, 11138, 8474, 4116 6779, 2905, 8474, 4358, 15012
Mean 3087

Controls - No Fsph was detected

LSD = 2923.48

TABLE A 2.4 (reference Fig. 2.5)

Population of *Corticium praticola* in soil inoculated with *Cp* and *Fsph+Pu+Cp*

No *Corticium* spp. was detected in controls

2 d after inoculation - Propagules/g dry soil

Cp	- 1, 7, 6, 7, 8, 10, 2, 5, 2, 5	Mean = 5.3
Fsph+Pu+Cp	- 9, 7, 10, 2. 6, 1, 9, 9, 7, 7	Mean = 6.8

LSD at 0.05 = 2.79

12 d after inoculation - at sowing - Propagules/g dry soil

Cp	- 9, 4, 4, 11, 4, 11, 13, 13, 13, 9	Mean = 9.3
Fsph+Pu+Cp	- 9, 7, 4, 7, 4, 9, 9, 13, 13, 15	Mean = 9.0

LSD at 0.05 = 3.54

42 d after inoculation - after cropping - Propagules/g dry soil

Cp	- 2, 0, 4, 2, 0, 4, 2, 0, 2, 2	Mean =.1.8
Fsph+Pu+Cp	- 2, 0, 2, 0, 7, 5, 5, 2, 2, 5	Mean = 3.1

LSD at 0.05 = 1.85

TABLE A 2.5 (reference Fig. 2.6)

Population of Pythium ultimum in soil inoculated with Pu and Fsph+Pu+Cp

2 days after inoculation - Propagules/g dry soil

Pu -

531, 490, 265, 408, 428, 286, 469, 531, 449, 510 Mean 436.73

Fsph+Pu+Cp -

408, 326, 265, 428, 367, 347, 306, 387, 265, 490 Mean 359.08

Control -

82, 306, 204, 82, 184, 143, 61, 204, 102, 41 Mean 140.82

LSD at 0.05 = 76.74

12 days after inoculation, at sowing - Propagules/g dry soil

Pu -

943, 1006, 1216, 985, 755, 713, 797, 734, 650, 901 Mean 870.02

Fsph+Pu+Cp -

817, 706, 684, 993, 949, 1037, 1059, 751, 596, 618 Mean 821.19

Control -

421, 189, 232, 232, 358, 253, 189, 379, 147, 295 Mean 269.47

LSD at 0.05 = 139.23

42 days after inoculation, after cropping - Propagules/g dry soil

Pu -

1225, 897, 1247, 765, 1072, 1182, 1072, 1182, 788, 875 Mean 1030.6

Fsph+Pu+Cp -

557, 412, 339, 460, 605, 533, 412, 557, 412, 508 Mean 479.4

Control -

889, 622, 844, 778, 556, 778, 644, 667, 600, 578 Mean 695.6

LSD at 0.05 = 105.17

TABLE A 2.6 (reference Table 2.2)

Height of bean plants as a % of control grown in vermiculite inoculated with Fsph, Pu, Cp and with various combinations

Vermiculite was inoculated at 3 stages of plant growth at sowing 0 week, 1 week and 2 week from emergence

0 week - at sowing Plant height as a % of control
Mean height of control = 20.95 cm

Fsph -
76.4, 71.6, 59.7, 59.7, 66.8, 66.8, 71.6, 85.9, 62.0, 64.4, 93.5, 66.8
= 70.4
Pu - 66.8, 78.7, 85.9, 71.6, 81.1, 62, 66.8 = 73.3
Cp -
Fsph+Pu - 66.8, 57.2, 57.2, 62.0, 66.8, 64.4, 57.2, 62.0
= 61.7
Fsph+Cp -
Fsph+Pu+Cp-

1 week Plant height as a % of control
Mean height of control = 20.75 cm

Fsph -
86.7, 89.1, 67.4, 86.7, 96.4, 108.4, 106, 96.4, 96.4, 106, 125.2
= 96.79
Pu -
96.4, 110.8, 115.6, 110.8, 96.4, 96.4, 89.1, 93.9, 67.5, 86.7
67.5, 86.7 = 93.15
Cp - 81.9, 91.6, 82.9, 96.4, 101.1 = 90.78
Fsph+Pu -
98.7, 84.3, 96.4, 77.1, 91.6, 86.9, 81.9, 72.3, 101.1, 106.0,
106, 91.6 = 90.74
Fsph+Cp - 81.9, 62.6, 67.5, 96.4, 81.9, 101.1, 86.7 = 82.59
Pu+Cp - 72.3, 86.7, 110.8, 81.9, 101.1, 77.1, 86.7 = 88.09
Fsph+Pu+Cp - 81.9, 96.4, 101.1, 101.1, 110.8, 91.6, 84.3
= 95.31

2 weeks Plant height as a % of control
Mean control height = 25.04 cm

Fsph -
77.8, 71.9, 75.8, 83.8, 99.8, 79.8, 87.8, 87.8, 91.8, 67.9,
75.8, 81.8 = 81.82
Pu -
75.8, 79.8, 71.9, 83.8, 71.9, 75.8, 75.8 83.8, 83.8, 67.9
= 78.49
Cp - 99.8, 91.8, 95.8, 99.8, 95.8, 95.8, 55.9, 63.9 = 87.33
Fsph+Pu
75,8, 91.8, 95.8, 87.8, 75.8, 123.7, 79.8, 89.8, 95.8,
95.8, 91.8 = 91.25
Fsph+Cp - 99.8, 119.7, 111.8, 91.8, 99.8, 95.8, 107.8, 83.8
= 101.29
Cp+Pu- 123.7, 139.7, 111.8, 103.8, 103.8. 103.8 = 114.40
Fsph+Pu+Cp - 71.8, 83.7, 95.8, 59.9, 75.8 = 77.42

TABLE A 2.6 - contd.

ANOVA 0 week

SV	df	SS	MS	F
among groups	2	573.97	286.99	3.89
within groups	24	1771.22	73.8	
Total	26			

Rank	1	2	3
Mean	61.7	70.4	73.3

LSR .05 1−3 = 9.76 LSR .05 2−3 = 8.43 ns

1−2 = 9.18 ns

1 week

ANOVA

SV	df	SS	MS	F
among groups	7	1797.88	256.84	1.33 ns
within groups	65	12590.11	193.69	
Total	72	14387.99		

2 week

ANOVA

SV	df	SS	MS	F
among groups	7	9027.76	1289.68	8.4
within groups	66	10134.69	153.56	
Total	73	19162.45		

Rank	1	2	3	4	5	6	7	8
Mean	77.4	78.5	81.8	87.3	91.2	100	101.3	114.4

$LSR_{.05}$

1 - 8 = 23.55
1 - 7 = 21.53
1 - 6 = 19.40
1 - 5 = 18.80 ns
1 - 2 = 13.12 ns

2 - 8 = 18.87
2 - 7 = 16.64
2 - 6 = 14.23
2 - 5 = 19.33 ns

3 - 6 = 13.37
4 - 8 = 18.82
4 - 7 = 16.30 ns
4 - 6 = 13.59 ns

5 - 8 = 16.62
5 - 7 = 13.84 ns
5 - 6 = 10.52 ns
6 - 8 = 14.89 ns

TABLE A 2.7 (reference TABLE 2.4)

Percentage death of seedling in soil - Pathogen combinations

	I N O C U L A T I O N							
	Fsph	Pu	Cp	Fsph+ Pu	Fsph+ Cp	Pu+Cp	Fsph+ Pu+Cp	control
	(% death of seedlings)							
0 week	0	0	0	25	50	0	100	0
	0	0	0	25	100	50	100	0
	0	25	25	0	0	75	100	0
	0	8.33	8.33	16.67	50	41.67	100	0
1 week	0	0	25	0	50	25	0	0
	0	0	0	0	50	0	25	0
	0	0	50	0	100	25	50	0
	0	0	25	0	66.67	16.67	25	0

0 week - ANOVA

SV	df	SS	MS	F	LSD at 0.05
among groups	7	24973.96	3567.71	6.227	41.43
within groups	16	9166.67	572.92		
Total	23				

1 week - ANOVA

SV	df	SS	MS	F	LSD at 0.05
among groups	7	11250.0	1607.14	5.61	29.297
within groups	16	4583.33	286.46		
Total	23				

TABLE A 2.8 (reference TABLE 2.5)

Disease Index of bean plants grown in soil inoculated with various pathogen combinations

	Fsph	Pu	Cp	Fsph+ Pu	Fsph+ Cp	Pu+Cp	Fsph+ Cp+Pu	control
0 week								
1	6	0	1	6	6	2	7	0
2	6	2	1	6	5	2	7	0
3	6	0	1	6	7	2	7	0
4	6	0	1	7	7	1	7	0
5	6	0	2	6	5	4	7	0
6	5	0	2	6	6	4	7	0
7	5	0	3	6	6	7	7	0
8	5	0	2	7	6	7	7	0
9	4	2	0	5	7	4	7	0
10	3	2	1	4	7	7	7	0
11	4	3	1	3	7	7	7	0
12	5	7	7	3	7	7	7	0
	5.08	1.33	1.83	5.42	6.33	4.5	7.0	0
1 week								
1	4	1	7	4	5	4	6	0
2	5	1	2	4	5	4	6	0
3	5	2	2	4	7	3	6	0
4	4	3	2	4	7	7	6	0
5	4	2	1	4	5	3	6	0
6	4	3	2	4	6	2	6	0
7	4	1	2	4	7	2	6	0
8	4	2	2	4	7	2	7	0
9	4	0	2	4	7	1	6	0
10	2	1	2	4	7	2	6	0
11	4	1	7	4	7	2	7	0
12	4	1	7	4	7	7	7	0
	4.0	1.5	3.17	4.0	6.42	3.25	6.25	0

ANOVA - 0 week Disease Index

SV	df	SS	MS	F
Among groups	7	547.792	78.256	37.05
within groups	88	185.833	2.112	
Total	95			

LSD at 0.05 = 1.178

ANOVA - 1 week Disease Index

SV	df	SS	MS	F
Among groups	7	395.40	56.49	38.69
within groups	88	128.08	1.46	
Total	95	523.49		

LSD at 0.05 = 0.9797

TABLE A 3.1 (reference Fig. 3.7)

Relationship between % inoculum and population of Fsph

Soil 6

Replicate	Percentage inoculum v/v (x)					
	0	1	3	10	25	50
	(Propagules/g dry soil) (y)					
1	0	889	6889	9627	34560	103304
2	0	889	4667	10746	35280	112174
3	0	889	9111	5821	25680	120261
4	0	1111	6000	9403	38160	124174
5	0	889	10889	13880	40080	113739
6	0	667	10667	11193	29040	122869
7	0	667	8444	7612	32160	85826
8	0	444	8222	11418	33360	121826
9	0	444	8000	11866	26640	114522
10	0	889	6000	11418	35760	116869
Mean	0	777.8	7889	10298	33072	113556

ANOVA

SV	df	SS	MS
Regression	1	0.854689556092 ex 11	
Residuals	58	0.618535526580 ex 10	0.106644056306 ex 9
Total	59	0.916543108750 ex 11	

Parameter	value	SE	confidence limits lower	upper
Y intercept	-4356.896	1732,863	-7753.309	-960.484
Regression coefficient	2112.704	74.628	1966.433	2258.975

Correlation = 0.9657

Regression line $y = 2112.7 x - 4356.89$

OR

$y = 0.21 x - 0.43$

as used in Fig. 3.7

Potato Soil

Percentage inoculum vs population of Fsph

Replicate	Percentage inoculum (v/v) (x)				
	0	3	12	25	50
	(Propagules/g dry soil) (y)				
1	0	2320	14184	68675	67734
2	0	928	14184	71566	78818
3	0	1624	13712	68675	70690
4	0	4640	11820	72289	79557
5	0	696	8274	48916	82759
6	0	2088	15366	59759	76601
7	0	1160	14657	50843	82759
8	0	696	18440	78554	82266
9	0	1856	13712	51807	82020
10	0	3480	14657	63373	83005
Mean	0	1949	13901	63446	78621

TABLE A 3.1 - contd.

ANOVA

SV	df	SS	MS
Regression	1	0.488988639018 ex 11	
Residual	48	0.654819633620 ex 10	0.136420757004 ex 9
Total	49	0.554470602380 ex 11	

Parameter	Value	SE	Confidence limits lower	upper
y intercept	671.080	2322.562	-3881.142	5223.303
Regression coefficient	1717.343	90.708	1539.554	1895.131

Correlation = 0.9391

Regression line $y = 1717.34 x + 671.08$

OR

as used in Fig. 37 $y = 0.17 x + 0.07$

TABLE A 3.2 (reference Fig. 3.8)

Relationship of inoculum density (Arcsine$\sqrt{\%\ \text{inoculum}}$) to Disease Index in soil 6 and 4

Soil 4 - Disease Index

Percentage Inoculum (v/v)	Replicate Pot No.	Disease Index (recorded from the plants grown in each pot)	Replicate Mean
0	1-5	-o-	0
1	6	2, 2, 1, 1	1.5
	7	2, 3	2.5
	8	2, 2, 2, 1	1.8
	9	3, 2, 2, 2	2.3
	10	1, 1, 1	1.0
3	11	3, 2, 2, 2, 2	2.2
	12	2, 2, 2, 3, 3	2.4
	13	2, 2, 2, 2, 2	2.0
	14	3, 5, 2, 3	3.3
	15	2, 3, 2, 2	2.3
10	16	4, 4, 4, 4	4.0
	17	3, 3, 4	3.3
	18	2, 5, 5, 4	4.0
	19	3, 1, 2, 1	1.75
	20	4, 5, 3	4.0
25	21	5, 4, 5, 5	4.7
	22	2, 4, 4, 2	3.0
	23	5, 3, 4	4.0
	24	2, 4, 4, 3, 4	3.4
	25	4, 5, 5	4.7
50	26	5, 5, 5, 5	5.0
	27	4, 5, 5, 5	4.8
	28	5, 4, 5, 4	4.5
	29	4, 5, 4	4.3
	30	5, 5, 5, 5	5.0
Soil 4 (Sterilized)- Disease Index			
0	31-35	-o-	0
1	36	3, 3, 3	3.0
	37	1, 2, 2	1.7
	38	3, 3, 3, 4, 4	3.4
	39	2, 1, 2	1.7
	40	1, 1, 2, 2, 2	1.6
3	41	5, 5, 3	4.3
	42	3, 4, 4	3.6
	43	4, 2, 2	2.7
	44	5, 4, 4	4.3
	45	4, 3, 4, 4, 3	3.6
10	46	4, 4, 4, 4, 3	3.8
	47	4, 5, 5, 5, 4	4.6
	48	2, 4, 5	3.7
	49	5, 5, 4, 4, 4	4.4
	50	4, 4	4.0
25	51	5, 4, 4, 5, 5	4.6
	52	5, 5, 5, 5, 5	5.0
	53	3, 4, 4, 5, 4	4.0
	54	5, 5, 5	5.0
	55	5, 5, 5, 5	5.0

TABLE A 3.2 - contd.

Percentage Inoculum (v/v)	Replicate Pot No.	Disease Index (recorded from the plants grown in each pot)	Replicate Mean
Soil 4 (Sterilized) - Disease Index			
50	56	5, 5, 5, 5	5.0
	57	5, 5, 5, 5, 5	5.0
	58	5, 5, 5	5.0
	59	5, 5, 5, 5	5.0
	60	6, 5, 5, 5, 5	5.2
Soil 6 (Sterilized) Disease Index			
0	65-69	0 - 0	0
1	70	1, 2, 3, 3, 4	2.6
	71	0, 1, 2, 3	1.5
	72	1, 0, 0, 1	0.5
	73	0, 0, 2, 2, 1	1.0
	74	3, 3, 3, 3	3.0
3	75	0, 4, 4, 3, 3	2.8
	76	3, 3, 3, 4, 1	2.8
	77	2, 2, 3, 3, 3	2.6
	78	3, 3, 3, 3, 4	3.2
	79	3, 2, 3, 4, 3	3.0
10	80	4, 4, 4	4.0
	81	4, 3, 3, 4, 3	3.4
	82	4, 3.5, 3, 4	3.6
	83	3, 2	2.5
	84	4, 5, 4, 4, 4	4.2
25	85	7, 3, 5, 4, 5	4.8
	86	4, 4, 5, 5	4.5
	87	5, 3, 3, 4	3.8
	88	4, 3, 4, 4, 4	3.8
	89	5, 3, 4, 4.5, 4.5	4.2
50	90	4.5, 4, 5	4.5
	91	5, 5, 4	4.7
	92	4, 4, 5, 5, 5	4.6
	93	5, 5, 5, 5	5.0
	94	5, 4, 5, 5, 5	4.8
Soil 6 - Disease Index			
0	95-99	- 0 -	0
1	100	2, 0, 1, 2	1.3
	101	1, 2, 3, 2, 2	2.0
	102	1, 0, 1, 1	0.75
	103	1.5, 1, 2, 2, 1	1.5
	104	0, 1, 2, 0	0.75
3	105	3, 2, 3, 2, 2	2.4
	106	0, 1, 2	1.0
	107	1, 2, 2	1.7
	108	2, 1, 3, 2, 1	1.8
	109	3, 3, 3, 1, 3	2.6
10	110	3, 3, 3, 3	3.0
	111	2, 1.5, 2.5	2.0
	112	2, 1, 2	1.7
	113	1, 1.5, 2, 1	1.4
	114	2, 3, 2.5, 1.5, 3	2.4
25	115	3, 1, 1, 3	2.0
	116	2, 3, 3, 3, 3	2.8
	117	3, 3, 2, 3	2.8
	118	3, 2, 3	2.7
	119	3, 3, 2, 3, 3	2.8
50	120	4, 4, 3.5, 4	3.9
	121	4, 4, 4, 4	4.0
	122	1.5, 2, 3, 1	1.9
	123	3, 3, 4, 4, 2	3.2

TABLE A 3.3 (reference Fig. 3.8)

Relationship of inoculum density (Arcsine$\sqrt{\%\ \text{inoculum}}$) to Disease Index in soil 6 and 4.

Data are the mean Disease Index (Replicate mean) from Table 3.2 and % inoculum in Arcsine$\sqrt{\%}$ values

Soil 4 (field soil)

Replicate	Inoculum density (Arcsine$\sqrt{\%}$ inoculum) (x)					
	0	5.74	9.97	18.43	30.0	45.0
	(Disease Index - Replicate mean) (y)					
1	0	1.5	2.2	4.0	4.7	5.0
2	0	2.5	2.4	3.3	3.0	4.8
3	0	1.8	2.2	4.0	4.0	4.5
4	0	2.3	3.3	1.75	3.4	4.3
5	0	1.0	2.3	4.0	4.7	5.0
Mean	0	1.82	2.48	3.4	3.96	4.72

ANOVA

SV	df	SS	MS
Regression	1	60.857	
Residuals	28	19.405	0.6930
Total	29	80.262	

Parameter	Value	SE	Confidence limits lower	upper
y intercept	1.043	0.236	0.580	1.505
Regression Coefficient	0.093	0.009	0.073	0.112

Correlation = 0.871
Regression line - y = 0.09x + 1.04

Soil 4 (Sterilized Soil

Replicate	Inoculum density (Arcsine$\sqrt{\%}$ inoculum) (x)					
	0	5.74	9.97	18.43	30. 0	45.0
	(Disease Index - Replicate mean) (y)					
1	0	3.0	4.3	3.8	4.6	5.0
2	0	1.7	3.6	4.6	5.0	5.0
3	0	3.4	2.7	3.7	4.0	5.0
4	0	1.7	4.3	4.4	5.0	5.0
5	0	1.6	3.6	4.0	5.0	5.2
Mean	0	2.28	3.7	4.1	4.72	5.04

ANOVA

SV	df	SS	MS
Regression	1	62.696	
Residuals	28	32.223	1.151
Total	29	94.919	

Parameter	Value	SE	Confidence limits lower	upper
y intercept	1.592	0.304	0.997	2.188
Regression Coefficient	0.094	0.012	0.069	0.119

Correlation = 0.813
Regression line - y = 0.09x + 1.59

TABLE A 3.3 - contd.

Soil 6 (field soil)

Replicate	Inoculum density (Arcsine $\sqrt{\%\ \text{inoculum}}$) (x)				
	0	5.74	9.97	18.43	30.0	45.0
	(Disease Index - Replicate mean) (y)				
1	0	1.3	2.4	3.0	2.0	3.9
2	0	2.0	1.0	2.0	2.8	4.0
3	0	0.75	1.7	1.7	2.8	1.9
4	0	1.5	1.8	1.4	2.7	3.2
5	0	0.75	2.6	2.4	2.8	2.5
Mean	0	1.26	1.90	2.10	2.62	3.10

ANOVA

SV	df	SS	MS
Regression	1	24.547	
Residuals	28	13.431	0.479
Total	29	37.978	

Parameter	Value	SE	Confidence limits lower	upper
y intercept	0.757	0.196	0.373	1.141
Regression Coefficient	0.059	0.008	0.043	0.075

Correlation = 0.804

Regression line y = 0.06x + 0.76

Soil 6 (Sterilized)

Replicate	Inoculum density (Arcsine $\sqrt{\%\ \text{inoculum}}$) (x)				
	0	5.74	9.97	18.43	30.0	45.0
	(Disease Index - Replicate mean) (y)				
1	0	2.6	2.8	4.0	4.8	4.5
2	0	1.5	2.8	3.4	4.5	4.7
3	0	0.5	2.6	3.6	3.8	4.6
4	0	1.0	3.2	2.5	3.8	5.0
5	0	3.0	3.0	4.2	4.2	4.8
Mean	0	1.72	2.88	3.54	4.22	4.72

ANOVA

SV	df	SS	MS
Regression	1	61.844	
Residuals	28	21.750	0.777
Total	29	83.595	

Parameter	Value	SE	Confidence limits lower	upper
y intercept	1.144	0.249	0.655	1.633
Regression Coefficient	0.094	0.010	0.073	0.114

Correlation = 0.860

Regression line y = 0.09 x + 1.14

TABLE A 3.4 (reference Fig. 3.9)

Distribution of fungi, Actinomycetes and spore formers in non rhizosphere and bean rhizosphere of Soil 4 and 6

As - Aspergillus spp. Tr - Trichoderma spp. Pe - Penicillium spp.
Fu - Fusarium spp. Zy - Zygorrhynchus spp. OF - other fungi
B - Bacillus spp. A. - Actinomycetes

Soil 4 - Not inoculated without crops

	Propagules/g dry soil							
Replicates	As	Tr	Pe	Fu	Zy	OF	B	A
1	0	0	67039	0	0	324022	111732	327×10^5
2	11173	11173	33519	0	0	312849	1340782	412×10^5
3	0	11173	89385	0	0	279329	893855	316×10^5
4	11173	0	55865	0	0	346368	1117318	262×10^5
5	0	0	67039	0	0	234636	1564245	314×10^5
6	0	11173	89385	0	0	279329	1229050	412×10^5
7	0	11173	33519	0	0	301675	2234636	303×10^5
8	0	11173	78212	0	0	279329	1452513	399×10^5
9	22346	11173	111731	0	0	312849	1675977	233×10^5
10	0	11173	78212	0	0	301675	1452514	363×10^5
Mean	4469	7821	70391	0	0	297206	1307262	334.1×10^5

Soil 6 - Not inoculated with Fsph - no crops

	As	Tr	Pe	Fu	Zy	OF	B	A
1	0	0	89888	11236	56180	157303	337079	78×10^5
2	11236	0	134831	0	22472	382022	561798	52×10^5
3	0	22471	89888	0	44944	202247	224719	39×10^5
4	0	0	89888	0	33708	325843	1123595	23×10^5
5	0	0	101123	11236	33708	325843	786517	47×10^5
6	0	11236	123595	0	11236	224719	224719	53×10^5
7	0	0	78652	0	22472	348315	337079	34×10^5
8	0	11236	89888	0	33708	202247	112359	26×10^5
9	0	11236	67416	0	22472	337079	561798	139×10^5
10	0	0	44944	0	89888	235955	449438	40×10^5
Mean	1123.6	5618	91011.3	2247.2	37078.8	274157.3	471910.1	53.1×10^5

Soil 4 - Rhizosphere soil (not inoculated with Fsph)

	As	Tr	Pe	Fu	Zy	OF	B	A
1	0	0	123134	22388	11194	89552	671642	25×10^6
2	0	0	89552	0	0	134328	783582	32×10^6
3	0	11194	78358	11194	0	89552	335821	26×10^6
4	0	0	167910	0	0	78358	671642	30×10^6
5	0	0	89552	0	11194	55970	1007463	22×10^6
6	0	0	190298	0	0	33582	783582	17×10^6
7	11194	0	179104	0	0	89552	1679104	21×10^6
8	44776	0	179104	0	0	134328	1007463	27×10^6
9	0	0	55970	11194	0	156716	559701	26×10^6
10	11194	0	167910	0	0	67164	-	28×10^6
Mean	6716.4	1119.4	132089.2	4477.6	2238.8	92910.2	833333	25.4×10^6

TABLE A 3.4 - contd.

Soil 6 - Rhizosphere soil (not inoculated with Fsph)

Replicate	Propagules/g dry soil							
	As	Tr	Pe	Fu	Zy	OF	B	A
1	11407	22814	102662	22814	0	353612	228137	0
2	0	22814	171103	0	11407	193916	456274	$2x10^6$
3	0	11407	171103	0	11407	182509	342205	$3x10^6$
4	0	45627	296578	0	11407	148289	1026616	0
5	0	0	136882	0	22814	250951	228137	$4x10^6$
6	0	11407	68441	0	34220	79848	456274	$1x10^6$
7	0	11407	148289	0	68441	102661	456274	0
8	0	11407	228137	0	11407	193916	228137	$1x10^6$
9	0	11407	159696	0	22814	182509	570342	$1x10^6$
10	0	11407	125475	0	34220	57034	-	$3x10^6$
Mean	1140.7	15969.7	160836.6	2281.4	22813.7	174524.5	443599.56	$1.5x10^6$

Soil 4 - Rhizosphere soil - Inoculated with Fsph

Replicate	As	Tr	Pe	Fu	Zy	OF	B	A
1	0	0	33333	11111	0	100000	777778	0
2	0	0	44444	0	0	88889	1111111	$2x10^6$
3	0	11111	33333	0	0	66667	1111111	$2x10^6$
4	0	0	33333	0	0	88889	1333333	$1x10^6$
5	0	0	22222	0	0	44444	1000000	$1x10^6$
6	11111	0	22222	0	0	88889	1000000	0
7	0	0	22222	0	0	144444	444444	$1x10^6$
8	0	0	22222	0	0	122222	888889	$1x10^6$
9	0	0	22222	0	0	111111	1333333	$2x10^6$
10	11111	0	33333	0	0	55555	555555	$3x10^6$
Mean	2222.2	1111.1	28888.6	11111	0	91111.0	955555.4	$1.3x10^6$

Soil 6 - Rhizosphere soil - Inoculated with Fsph

Replicate	As	Tr	Pe	Fu	Zy	OF	B	A
1	0	0	142857	10989	10989	21978	219780	$1x10^6$
2	0	0	21978	0	0	54945	0	$2x10^6$
3	0	21978	43956	0	0	120879	219780	0
4	0	10989	65934	0	0	43956	329670	$1x10^6$
5	0	10989	65934	10989	0	65934	109840	$3x10^6$
6	10989	10989	43956	0	21978	0	0	$1x10^6$
7	10989	0	164835	21978	0	65934	219780	0
8	10989	0	98901	0	10989	10989	109890	$3x10^6$
9	0	10989	120879	0	0	142857	219780	$1x10^6$
10	-	-	-	-	-	-	0	$1x10^6$
Mean	3663	7326.0	85470.0	4884.0	4884.0	58608	142857	$1.3x10^6$

TABLE A 3.5 (reference Fig. 3.10)

Proportion of the two types of chlamydospores formed in soil extract of soil 4 and 6

Soil 4		Soil 6	
Proportion of chlamydospores		Proportion of chlamydospores	
On Macroconidia	On hyphae	On Macroconidia	On hyphae
14.09	85.91	0	100
27.91	72.09	0	100
33.08	66.92	50	50
22.84	77.16	0	100
40.66	59.34	9.6	90.4
38.76	61.24	5.08	94.92
50.0	50.0	7.64	92.36
29.94	70.06	0	100
37.23	62.77	0	100
63.69	36.31	0	100
13.79	86.21	0	100
0	100	0	100
37.78	62.22	0	100
49.32	50.68	0	100
8.82	91.18	0	100
Mean 31.194	68.806	4.82	95.18
Sd. 17.15	17.15	12.89	12.89

TABLE A 3.6

Chlamydospore sizes of *Fusarium solani* f. sp. *phaseoli* formed on macroconidia and on hyphae in soil extract of soil 6 and 4

(Length x breadth u)

Soil 6		Soil 4	
On hyphae (u)	On macroconidia (u)	On hyphae (u)	On macroconidia (u)
12.5 x 5.0	10.0 x 5.0	7.5 x 7.5	10.0 x 5.0
12.5 x 5.0	12.5 x 5.0	15.0 x 7.5	15.0 x 6.25
10.0 x 5.0	12.5 x 5.0	8.75 x 7.5	7.5 x 7.5
5.0 x 5.0	7.5 x 10.0	12.5 x 5.0	12.5 x 5.0
5.0 x 5.0	10.0 x 5.0	10.0 x 6.25	10.0 x 5.0
15.0 x 7.5	12.5 x 7.5	7.5 x 5.0	7.5 x 7.5
7.5 x 7.5	10.0 x 7.5	8.75 x 7.5	12.5 x 7.5
5.0 x 5.0	7.5 x 6.25	11.25 x 5.0	15.0 x 7.5
6.25 x 5.0	10.0 x 6.25	10.0 x 5.0	10.0 x 7.5
10.0 x 5.0	15.0 x 7.4	8.75 x 8.75	12.5 x 6.25
7.5 x 5.0		10.0 x 5.0	10.0 x 5.0
7.5 x 7.5	Mean 10.75 x 6.5	7.5 x 6.25	7.5 x 5.0
8.75 x 5.0	Sd 2.37 1.64	8.75 x 7.5	10.0 x 5.0
5.0 x 5.0		12.5 x 7.5	12.5 x 5.0
7.5 x 5.0		8.75 x 7.5	10.0 x 7.5
10.0 x 5.0		12.5 x 5.0	
7.5 x 5.0		8.75 x 7.5	Mean 10.83 x 6.17
7.5 x 5.0		11.25 x 7.5	Sd 2.43 1.20
7.5 x 5.0		7.5 x 6.25	
7.5 x 5.0		10.0 x 6.25	
Mean 8.25 x 5.37		Mean 9.87 x 6.56	
Sd 2.73 0.915		Sd 2.06 1.20	

TABLE A 3.7

Population of Fusarium solani f. sp. phaseoli in soil (used for crop type experiment) 6 days and 90 days after inoculation. Inoculated soil was kept in plastic dustbins

	Propagules/g dry soil	
Replicate	6d	90d
1	5895	1363
2	7423	1136
3	4148	1363
4	4585	1591
5	4585	1591
6	4367	2727
7	6332	1591
8	4367	455
9	5895	1136
10	5895	1136
Mean	5349.2	1408.9
Sd.	1090.3	574.8

TABLE A 3.8 (reference TABLE 3.2)

Chlamydospore induction in Fusarium solani f. sp. phaseoli by soil extract fractions x, y and z (Fig.3.2)

Fsph S.2d - chlamydospores/100 Macroconidia

Fraction	1	2	3	4	5	6	Mean ± SE
x	43	37	25	41	31	47	37.3 ± 8.1
y	8	4	15	5	8	8	8.0 ± 3.8
z	9	12	25	16	13	13	14.7 ± 5.5
Crude extract	91	84	79	54	98	77	80.5 ± 15.1

Fsph S.2f - chlamydospores/100 Macroconidia

Fraction	1	2	3	4	5	6	Mean ± SE
x	51	47	44	43	50	48	47.2 ± 3.2
y	8	11	5	5	13	6	8.0 ± 3.3
z	6	10	9	11	7	6	8.2 ± 2.1
Crude extract	105	120	80	85	91	87	94.7 ± 15.0

TABLE A 3.9 (reference TABLE 3.3)

Chlamydospore induction in Fusarium solani f.sp. phaseoli 9.6 ml fractions of soil extract (Fig. 3.3 fraction i, ii, iii and iv)

Fsph - S.2d - Chlamydospores/100 macroconidia

Fraction	Elution volume	1	2	3	4	5	6	Mean ± SE
i	0 - 9.6	31	36	25	20	21	28	26.8± 6.1
ii	9.7-19.2	9	8	3	5	1	4	4.0 ± 3.0
iii	19.3-28.8	1	3	0	0	0	0	0.66± 15.1
iv	28.9-38.4	12	1	4	5	1	7	5.0 ± 15.1
Crude soil extract		91	84	79	54	98	77	80.5 ± 15.1

TABLE A 3.10 (reference TABLE 3.11)

Chlamydospore induction in Fusarium solani f. sp. phaseoli in 4.8 ml fraction (1-7 Fig. 3.3) of soil extract

Fsph - S.2d

Replicate	Fractions of soil extract							Crude Soil extract
	1	2	3	4	5	6	7	
	(chlamydospores/100 macroconidia)							
1	78	48	4	2	0	26	16	91
2	62	52	4	0	0	32	14	84
3	80	50	6	2	0	28	12	79
4	56	46	8	0	0	20	12	54
5	92	48	8	0	0	26	16	98
6	84	58	12	2	0	28	24	77
Mean	75.3	50.3	7.0	1.0	0	26.7	15.7	80.5
Standard error	13.7	4.3	3.0	1.0		3.9	4.4	15.1

Fsph - 2.2f

	Fractions of soil extract							Crude soil extract
	1	2	3	4	5	6	7	
	(chlamydospores/100 macroconidia)							
1	97	34	2	2	2	31	10	105
2	75	18	3	0	0	23	18	120
3	60	39	9	1	0	33	16	80
4	79	25	4	7	0	35	11	85
5	86	40	8	0	0	34	13	91
6	84	58	7	0	0	38	9	87
Mean	80.2	35.7	5.5	1.7	0.3	32.3	12.8	94.7
Standard error	12.4	13.8	2.9	2.7	0.8	5.1	3.5	15.0

TABLE A 3.11 (reference TABLE 3)

Chlamydospore induction in Fusarium solani f. sp. phaseoli in soil extract fractions a - g (Fig. 3.4)

Fsph - S.2d

	Fractions of soil extract							Crude soil extract
	a	b	c	d	e	f	g	
	(chlamydospores/100 macroconidia)							
1	17	46	4	0	96	0	2	94
2	20	47	5	0	73	0	1	111
3	23	48	3	0	84	0	0	86
4	42	46	4	0	87	0	1	104
Mean	25.5	46.7	4.0	0	85.0	0	1.0	98.7
Standard error	11.3	0.9	0.8	-	9.5	-	0.8	10.9

Fsph - S.2f

	Fractions of soil extract							Crude soil extract
	a	b	c	d	e	f	g	
	(chlamydospores/100 macroconidia)							
1	92	30	20	0	102	6	0	160
2	128	35	23	0	100	2	0	150
3	44	28	33	0	123	6	0	145
4	60	28	40	0	105	5	0	140
Mean	82.7	30.2	29.0	0	107.5	4.7	0	148.7
Standard error	16.7	3.3	9.2	-	10.5	1.9	-	8.5

TABLE A 3.12 (reference TABLE 3.)

Chlamydospore induction in Fusarium solani f. sp. phaseoli by various bands eluted from a soil extract chromatogram (Fig. 3.5)

Fsph - S.2d

	Fractions eluted (soil extract fractions)									Crude soil extract
	1	2	3	4	5	6	7	8	9	
	(chlamydospores/100 macroconidia)									
	8	112	0	28	176	48	360	0	4	128
	32	80	4	56	184	40	104	0	16	104
	48	72	16	88	328	32	56	0	0	64
	64	72	12	56	120	40	64	0	0	64
	72	96	0	40	136	56	168	0	0	96
	8	96	16	80	232	112	184	0	0	76
Mean	39	88	8	58	196	55	156	0	3	89
Standard error	26	15	7	23	65	29	112	-	6	25

Fsph - S.2f

	Fractions eluted									Crude soil extract
	1	2	3	4	5	6	7	8	9	
	(chlamydospores/100 macroconidia)									
	0	360	14	6	112	7	128	2	24	178
	6	256	3	4	128	13	110	3	8	118
	6	280	3	3	110	14	134	8	7	144
	2	363	0	0	124	14	132	4	5	106
	2	284	2	2	109	10	146	2	6	100
	0	283	0	3	102	6	100	0	22	120
Mean	3	304	4	3	114	9	125	3	12	128
Standard error	3	45	5	2	10	4	17	3	9	29

TABLE A.3.13 (reference TABLE 3.)

Pathogenicity of Fusarium solani f. sp. phaseoli to beans in various soils

Percentage diseased plants per pot

	Soils				
	Barley	Beans	Cabbage	Potato	Grass Virgin
	1	2	3	4	6
	100	33	100	100	0
	100	100	100	100	0
	75	100	100	100	60
	0	100	100	100	100
Mean	68.75	83.25	100	100	40

ANOVA

SV	df	SS	MS	F
among groups	4	10097.6	2524.4	2.19
within groups	15	17285.2	1152.3	
Total	19	27382.8		

LSD at 0.05 = 51.14

Disease Index of plants grown in inoculated soil 1-4 and 6

	Soil				
	Barley	Beans	Cabbage	Potato	Grass Virgin
	1	2	3	4	6
			(Disease Index)		
1	2	0.25	2	1	0
2	3	0	2	1	0
3	2	0	1	2	0
4	1	2	2	2	0
5	0.25	3	3	3	0
6	1	4	2	4	0
7	1	3	4	3	0
8	2	3	3	3	0
9	2	2	3	2	1
10	0.25	2	2	3	0.75
11	0.25	4	1	2	0.75
12	0.50	3	2	3	0
13	0	1	1	3	0
14	0	2	1	2	1
15	0	0.75	1	2	2
16	0	1	3	3	1
17	0		3	3	2
18				4	
Mean	0.9	1.93	2.12	2.56	0.49

TABLE A 3.13 - contd.

ANOVA

SV	df	SS	MS	F
among groups	4	52.34	13.09	13.93
within groups	80	75.38	0.94	
Total	84	127.68		

Rank	1	2	3	4	5
Means	0.49	0.90	1.93	2.12	2.56

$LSR_{.05}$ 1-5 = 0.903
1-2 = 0.656 ns
1-3 = 0.809

$LSR_{.05}$ 2-3 = 0.667
3-5 = 0.796 ns
3-4 = 0.667 ns
4-5 = 0.648 ns

TABLE A 3.14 (reference TABLE 3.10)

Effect of various non host and host crop on soil population of Fusarium solani f. sp. phaseoli- and subsequent pathogenicity on beans

Fsph - Population in soil after cropping

	CROPS						
	Beans	Cabbage	Capsicum	Fallow	Onions	Rice	Sorghum
1	4270	458	0	1505	444	2791	1171
2	2697	229	1149	2796	667	2558	2108
3	4494	0	4598	2366	2222	2326	2810
4	3146	459	1609	860	1111	930	1873
5	674	0	919	1075	444	4651	1405
6	2247	686	1609	1720	222	2326	3981
7	2022	686	1839	645	444	2326	3747
8	899	458	460	645	667	930	1639
9	1573	1601	2299	1935	0	2326	3279
10	2022	1144	460	1935	1333	2558	3513
Mean	2404	572.1	1494	1548	755	2372	2553

ANOVA

SV	df	SS	MS	F
Among groups	6	38990175.80	6498362.63	6.8017
Replicates	9	14556816.13	1617424.01	
Interactions	54	45632882.77	845053.38	
Error			955392.05	
Total	69	99179874.699		

LSD at 0.05 = 874.25 LSD at 0.01 = 1162.75

Disease Index

Beans - 4, 4, 4, 4, 3, 2, 2, 2, 2, 3, 4, 3, 4, 2, 4, 2, 2, 1, 3, 2, 3, 2, 4, 2, 2; Mean 2.8

Cabbage - 3,3,3,3,3,4,4,4,4,3,3,3,2,2,3,4,4,4,4,4,4,3,3,4,4; Mean 3.4

Capsicum - 4,3,2,3,3,2,2,2,2,2,2,3,3,2,3,4,4,4,4,4,4,3,4,4,4,3; Mean 3.1

Fallow - 3,4,2,2,2,2,3,2,2,3,2,2,2,3,3,4,2,2,2,2,2,2,3,2; Mean 2.4

Onion - 5,4,3,4,3,4,3,5,5,4,4,5,5,3,3,4,4,3,4,4,4,3,4,4,4, 4,4,4; Mean 3.9

Rice - 4,2,4,3,3,3,2,5,4,4,4,4,5,3,4,3,4,2,3,2,3,4,3,5,2; Mean 3.4

Sorghum - 2,4,4,4,4,4,4,0,1,1,1,2,2,2,3,3,3,3,3,3,2,3,2,2,3,2,2,2; Mean 2.5

Means	2.4	2.5	2.8	3.1	3.4	3.4	3.9
Rank	1	2	3	4	5	6	7

ANOVA

SV	df	SS	MS	F
Among groups	6	46.1	7.683	13.57
within groups	174	98.5	0.566	
Total	180	144.6		

$LSR_{.05}$ 1-7 = 0.621 2-7 = 0.582 3-7 = 0.570 4-7 =0.521
1-2 = 0.415 ns 2-3 = 0.410 ns 3-4 = 0.418 ns 4-5 =0.418 ns
1-3 = 0.501 ns 2-4 = 0.477 3-5 = 0.496 5-7 =0.482
1-4 = 0.542

A 4

TABLE A 4.1 (reference TABLE 4.2)

ANOVA of TABLE 4.2 - Nodulation in bean plants grown in vermiculite inoculated with Fsph

ANOVA

SV	df	SS	MS	F
Among groups	2	1984.53	992.26	6.462
Within groups	12	1842.40	153.53	
Total	14	3826.93		

LSD at 0.01 = 23.94 LSD at 0.05 = 17.08

TABLE A 4.2 (reference TABLE 4.4)

ANOVA OF TABLE 4.4 - Nodulation in bean plants grown in soil inoculated with Rhizobium spp. 3644 and Fsph

ANOVA

SV	df	SS	MS	F
Among groups	5	30337.9	6067.58	8.69
Within groups	54	37712.3	698.38	
Total	59	68050.2		

LSD at 0.05 = 23.68

TABLE A 4.3 (reference TABLE 4.3)

Effect of Fsph on root and shoot dry weight (g)

Dry weight of shoot (g)

Replicate	Method of inoculation: Full	Top Layer	Control
		(Dry weight/plant g)	
1	0.8	0.9	0.6
2	0.5	0.5	0.9
3	0.7	0.9	0.8
4	0.2	0.6	0.6
5	0.7	0.4	0.82
Mean	0.58	0.66	0.82

ANOVA

SV	df	SS	MS	F
Among groups	2	0.150	0.0750	1.3089 ns
Within groups	12	0.688	0.0573	
Total	14	0.838		

LSD at 0.05 = 0.329

Dry weight of roots (g)

Replicate	Method of inoculation: Full	Top layer	Control
1	0.10	0.10	0.10
2	0.10	0.15	0.20
3	0.10	0.15	0.15
4	0.05	0.15	0.05
5	0.05	0.10	0.15
Mean	0.08	0.13	0.13

ANOVA

SV	df	SS	MS	F
Among groups	2	0.008	.004	2.5
Within groups	12	0.019	.0016	
Total	14			

LSD at 0.05 = 0.053

Disease Index of Fsph inoculated bean plants (reference TABLE 4.3)

Replicate	Method of inoculation: Full	Top layer	Control
		(Disease Index)	
1	3	2	0
2	3	2	0
3	4	2	0
4	4	3	0
5	4	2	0
Mean	3.6	2.2	0

ANOVA

SV	df	SS	MS	F
Among groups	2	34.94	17.47	104.79
within groups	12	2.0	0.1667	
Total	14			

LSD at 0.05 = 0.5626

TABLE A 4.4 (reference TABLE 4.5)

Dry weight of roots (g) of bean plants grown in soil inoculated with Rhizobium sp. 3644 and Fsph

Replicate	Control	R	R/F	R/T	F	T
	(dry weight (g) roots per plant)					
1	0.30	0.58	0.15	0.10	0.50	0.95
2	0.40	0.50	0.65	0.90	0.70	0.50
3	0.60	0.55	0.80	0.55	0.70	0.80
4	0.10	0.80	0.60	0.45	0.60	0.55
5	0.60	0.50	0.20	0.60	0.65	0.60
6	0.50	0.50	0.60	0.35	0.45	0.85
7	0.65	0.50	0.45	0.50	0.70	0.65
8	0.60	0.50	0.70	0.65	0.60	0.60
9	0.90	0.85	0.15	0.90	1.0	0.60
10	0.55	0.50	0.55	0.50	1.0	0.70
Mean	0.52	0.57	0.49	0.55	0.69	0.68

ANOVA

SV	df	SS	MS	F	
Among groups	5	0.36	0.072	1.846	ns
Within groups	54	2.1	0.039		
Total	59				

LSD at 0.05 = 0.18

Disease Index of bean plants grown in soil inoculated with Rhizobium sp. 3644 and Fsph

Replicate	Control	R	R/F	R/T	F	T
1	0	4	5	5	4	3
2	0	0	4	2	2	4
3	1	0	2	3	2	3
4	0	0	4	2	4	3
5	1	1	4	3	3	4
6	0	1	4	4	4	2
7	0	1	4	2	1	3.5
8	0	4	4	3	4	3
9	0	0	5	4	2	2
10	0	1	5	4	4	4
Mean	0.2	1.2	4.1	3.2	3.0	3.15

ANOVA

SV	df	SS	MS	F
Among groups	5	106.98	21.39	20.357
within groups	54	56.73	1.05	
Total	59	163.71		

LSD at 0.05 = 0.918

A.5

TABLE A 5.1 (reference TABLE 5.1)

Colony diameters of Fsph, Pu and Cp grown on PDA containing Metazoxolon, Folcidin and Hymexazol

Metazoxolon JF 3937

Pathogen	Concentrations of fungicides (ppm)			
	Control 0	100	500	1000
	(Colony diameter as a % of control)			
Fsph	100	37.35	60.24	63.86
	100	37.35	55.42	42.17
	100	33.73	38.55	62.65
$\bar{Y}$	100	36.14	51.40	56.23
SE	0	1.21	6.58	7.03
Pu	100	8.24	0	0
	100	10.59	0	0
	100	8.24	0	0
$\bar{Y}$	100	9.02	0	0
SE	0	0.79	0	0
Cp	100	18.82	16.47	9.41
	100	17.65	16.47	10.59
	100	17.65	15.29	9.41
	100	-	-	10.59
$\bar{Y}$	100	18.04	16.08	10.0
SE	0	0.39	0.39	0.34

Folcidin 6233a

Pathogen	Concentrations of fungicides (ppm)			
	Control 0	100	500	1000
	(Colony diameter as a% of control)			
Fsph	100	36.47	29.41	25.88
	100	35.29	34.12	31.76
	100	29.41	32.94	28.24
$\bar{Y}$	100	33.72	32.16	28.63
SE	0	2.18	1.41	1.71
Pu	100	98.8	63.5	36.5
	100	100.0	63.5	40.0
	100	100.0	61.2	41.2
$\bar{Y}$	100	99.6	62.7	39.2
SE	0	0.40	0.77	1.41
Cp	100	0	0	0
	100	5.88	0	0
	100	5.88	0	0
	100	5.88	0	0
$\bar{Y}$	100	4.4	0	0
SE	0	1.47	0	0

TABLE A 5.1 - contd.

Hymexazol - F 319

Pathogen		Concentrations of fungicides (ppm)			
		Control 0	100	500	1000
		(Colony diameter as a % of control)			
Fsph		100	70.83	20.83	0
		100	66.67	20.83	0
		100	62.50	25.0	0
	$\bar{Y}$	100	66.67	22.22	0
	SE	0	2.4	1.39	-
Pu		100	15.29	0	0
		100	17.65	0	0
		100	14.12	0	0
		100	14.12	0	0
	$\bar{Y}$	100	15.29	0	0
	SE	0	0.83	-	-
Cp		100	41.18	0	0
		100	40.0	0	0
		100	40.0	0	0
	$\bar{Y}$	100	40.39	0	0
	SE	0	0.68	-	-

TABLE A 5.2 (reference TABLE 5.2)

Fungicide applied as a soil drench to Fsph inoculated soil

Percentage emergence

	Fungicide/treatment						
	Uninoculated	Banrot	Hymexazol	Folcidin	Metazoxolon	Quintozene	Inoculated Untreated
1	100	50	0	50	25	100	25
2	100	0	0	0	0	100	50
3	100	0	0	50	0	100	100
4	100	0	100	25	25	100	75
5	75	0	0	50	100	100	25
Mean	95	50	20	35	30	100	55

ANOVA

SV	df	SS	MS	F
Among groups	6	29500.0	4916.67	4.370
Replicates	4	1357.14	339.29	
Interactions	24	30142.86	1255.95	
Error			1124.99	
Total	34	60999.99		

LSD at 0.05 = 43.44

Percentage diseased plants out of total emerged

	Fungicide/treatment						
	Uninoculated	Banrot	Hymexazol	Folcidin	Metazoxolon	Quintozene	Inoculated Untreated
1	0	100	-	100	100	100	100
2	0	50	-	-	-	100	100
3	0	-	-	50	-	100	100
4	0	-	75	100	100	100	100
5	0	25	-	50	75	100	100
Mean	0	58.3	75	75	91.6	100	100

ANOVA

SV	df	SS	MS	F
Among groups	6	35440.04	5906.67	19.24
Within groups	19	5834.0	307.05	
Total	25			

Rank	1	2	3	4	5	6	7
Mean	0	58.3	75	75	91.6	100	100

LSR $_{.05}^{1-2}$ = 26.97 LSR $_{.05}^{2-6}$ = 38.82 LSR $_{.05}^{2-5}$ = 40.03 ns

LSR $_{.05}^{3-6}$ = 33.47 ns

TABLE A 5.2 - contd.

Disease Index

Treatment Fungicide		Mean
Uninoculated	- 0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0;	0
Banrot	- 2,2,2,0,0,1,0,0,0,2	0.9
Hymexazol	- 3,1,0,1	1.25
Folcidin	- 2,3,0,2,2,0,2	1.57
Metazoxolon	- 2,2,3,3,3,3	2.67
Quintozene	- 3,2,3,2,3,3,2,3,2,1,3,2,2,2,3,2,2,2,2,2	2.3
Inoculated untreated	- 3,3,3,3,3,3,3,3,3,3,4	3.09

ANOVA

SV	df	SS	MS	F
Among groups	6	97.49	16.25	38.69
Within groups	71	29.8	0.42	
Total	77	127.29		

Rank	1	2	3	4	5	6	7
Mean	0	0.9	1.25	1.57	2.3	2.67	3.09

$LSR_{.05}$ 1-7 = 0.74 $LSR_{.05}$ 2-7 = 0.83 $LSR_{.05}$ 3-7 = 1.04

1-2 = 0.51 2-5 = 0.68 3-5 = 0.85

2-4 = 0.76 ns 3-4 = 0.80 ns

$LSR_{.05}$ 3-6 = 0.85 $LSR_{.05}$ 5-7 = 0.58 $LSR_{.05}$ 6-7 = 0.63 ns

4-5 = 0.56 5-6 = 0.60 ns

TABLE A 5.3 (reference TABLE 5.3)

Seed treatment with various fungicides

Percentage emergence

	Uninoculated	Banrot	Hymexazol	Folcidin	Metazoxolon	Quintozene	Inoculated Untreated
1	100	100	100	75	100	100	100
2	75	100	100	100	100	100	100
3	100	100	100	100	75	100	100
4	100	100	100	100	100	100	100
5	100	100	75	100	100	100	100
Mean	95	100	100	90	95	100	100

ANOVA

SV	df	SS	MS	F
Among groups	6	464.2857	77.380	1.238
Replicates	4	71.4286	17.857	
Interactions	24	1678.5714	69.940	
Error			62.499	
Total		2214.2857		

LSD at 0.01 = 13.815 LSD at 0.05 = 10.240

Percentage diseased plants

	Uninoculated	Banrot	Hymexazol	Folcidin	Metazoxolon	Quintozene	Inoculated Untreated
1	0	100	100	67	100	100	100
2	0	100	75	25	100	100	100
3	0	100	100	100	100	100	100
4	0	100	100	25	100	100	100
5	0	100	100	100	100	100	100
Mean	0	100	95	63.4	100	100	100

ANOVA

SV	df	SS	MS	F
Among groups	6	42500.971	7083.495	32.296
Replicates	4	1151.600	287.900	
Interactions	24	4989.600	207.900	
Error			219.328	
Total		48642.171		

LSD at 0.01 = 25.879 LSD at 0.05 = 19.182

TABLE A 5.3 - contd.

Disease Index

	Uninoculated	Banrot	Hymexazol	Folcidin	Metazoxolon	Quintozene	Unoculated Untreated
1	0	3	3	0	4	3	3
2	0	3	3	1	4	3	4
3	0	2	3	1	2	4	3
4	0	3	3	0	3	3	3
5	0	3	2	0	3	3	3
6	0	3	1	0	3	3	2
7	0	2	3	1	3	3	3
8	0	2	2	2	3	3	3
9	0	2	3	2	3	3	3
10	0	3	3	1	3	3	3
11	0	2	3	3	3	3	3
12	0	3	3	0	3	3	3
13	0	3	3	0	2	3	3
14	0	2	3	0	2	3	3
15	0	2	3	1	4	3	3
16	0	3	2	2	5	3	3
17	0	3	2	1	3	3	3
18	0	2	3	2	3	3	3
Mean	0	2.56	2.67	0.94	3.11	3.06	3.0

ANOVA

SV	df	SS	MS	F
Among groups	6	156.857	26.142	82.35
Replicates	17	5.492	0.323	
Interactions	102	32.285	0.316	
Error			0.317	
Total		194.635		

LSD at 0.01 = 0.491 LSD at 0.05 = 0.371

TABLE A 5.4 (reference TABLE 5.4)

Fresh and dry weight(g) of bean plants grown from fungicide treated seeds

Fresh weight per plant (g) - seed treatment with fungicides (Weighted average)

	Uninoculated	Banrot	Hymexazol	Folcidin	Metazoxolon	Quintozene	Inoculated Untreated
1	9.57	5.85	6.43	6.03	8.38	7.50	7.38
2	7.80	8.00	7.88	5.4	6.90	8.45	6.10
3	7.75	6.80	6.98	5.68	8.25	7.25	6.85
4	8.08	6.75	6.15	7.83	8.38	5.60	7.65
5	8.63	6.80	7.83	7.20	7.03	7.68	6.85
Mean	8.37	6.84	7.05	6.43	7.79	7.30	6.97

ANOVA

SV	df	SS	MS	F
Among groups	6	12.50	2.080	2.976
Within groups	28	19.56	0.699	
Total	34	32.06		

LSD at 0.05 = 1.083

Dry weight per plant (g) seed treatment with fungicides (weighted average)

	Uninoculated	Banrot	Hymexazol	Folcidin	Metazoxolon	Quintozene	Inoculated Untreated
1	1.53	0.73	0.75	0.72	0.98	0.95	0.94
2	1.15	0.83	1.13	0.93	0.93	1.08	0.85
3	1.09	0.89	0.93	0.85	1.23	1.14	1.00
4	1.09	0.94	1.00	1.10	1.33	0.78	0.85
5	1.23	0.98	0.95	0.80	1.03	0.91	0.95
Mean	1.22	0.87	0.95	0.88	1.10	0.97	0.92

ANOVA

SV	df	SS	MS	F
Among groups	6	0.48	0.08	4.0
within groups	28	0.56	0.02	
Total	34	1.04		

LSD at 0.05 = 0.18 LSD at 0.01 = 0.25