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STUDIES ON APHANOMYCES ROOT ROT OF PEAS (*Pisum sativum*) CAUSED BY *Aphanomyces euteiches*

A thesis
submitted in partial fulfilment
of the requirements for the degree
of
Doctor of Philosophy
in the
University of Canterbury

by

Margaret Chan Kit Yok

Lincoln College

1985

Abstract of a thesis
submitted in partial fulfilment
of the requirements for the
Degree of Doctor of Philosophy

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CAUSED BY *Aphanomyces euteiches*

by Margaret Chan Kit Yok

This disease was recorded in 1978 in New Zealand, and found to be a serious disease of peas in the South Island. Disease avoidance is possible, i.e. growing peas only in infested soils with low or moderate amount of inoculum. The literature on the pathogen and factors affecting development of the disease are reviewed, and these aspects studied to determine alternative control methods.

When soil samples from 18 infested fields were tested by the Disease Severity Index method (DSI) and direct counting of oospores, there was a curvi-linear relationship ($r = 0.97^{***}$) between DSI and number of oospores in soil. These results were confirmed when a known number of oospores produced in culture were added to non-infested soil.

On potato dextrose agar, growth of *Aphanomyces euteiches* was inhibited by water potentials between -20 and -30 bars depending on temperature. In glasshouse trials with infested soils, disease increased linearly between -200 and -3 millibars in pea plants grown at 8°, 12°, 16°, 20° and 25°. Yields decreased with increased moisture levels and temperatures.

The disease in peas was related to both the inoculum and growth stage at time of infection. Yield was reduced by 35% when the moisture of an infested soil was raised to field capacity (0 millibars) at 3 weeks after sowing, but yield was not affected in plants in soil raised to field capacity at the

flowering or podding stages.

Aphanomyces euteiches parasitised *Medicago sativa, Trifolium repens, Stellaria media, Viola arvensis* and *Capsella bursa-pastoris* in the glasshouse and field. Infected plants showed no symptoms, but the fungus could be isolated from their roots, and each of the isolates infected peas which showed typical symptoms.

The effect of the fungicides, pyroxyfur, metalaxyl and hymexazol was assessed on *Aphanomyces euteiches in vitro.* Pyroxyfur at 1 ppm inhibited hyphal growth while significant reductions occurred at > 10 ppm with metalaxyl and hymexazol. Zoospore formation was inhibited by pyroxyfur at 10 ppm and by metalaxyl and hymexazol at > 100 ppm, whereas zoospore motility was inhibited by pyroxyfur and hymexazol at 1 ppm and metalaxyl at 5 ppm. Pyroxyfur and metalaxyl at 1 ppm significantly reduced oospore formation with hymexazol effective at 10 ppm.

The same fungicides were tested as seed treatments in glasshouse trials. After 4 weeks of growth all treatments showed similar levels of control. After 12 weeks growth, with soil of DSI 50, the disease was significantly reduced by metalaxyl (-26%) and hymexazol (-45%), but with soil of DSI 100, no differences were observed. In the same trials, fungicide combinations did not improve the efficacy of seed treatments. In a separate trial, pyroxyfur treated seed grown in soil with DSI 100 reduced DSI significantly (-18%) after 6 weeks with root dry matter being increased by 39% and total dry matter by 23%. Significant increases in top dry matter occurred at 2 weeks (+44%) and 8 weeks (+78%). There were no significant interactions between the fungicide treatments, moisture levels (-200, -28 and -3 millibars) and temperatures (20° and 24°).

Growing *Brassica napus* in infested soils for 4 months reduced the DSI; from originally 58 to 27, from 72 to 53,

and from 81 to 56. There was no change in DSI when *Triticum aestivum, Trifolium repens* or no plants were grown in the same soils.

When dried leaves and stems of 5 brassica species were incorporated (0.5% of the soil dry weight) into infested soil, there were significant reductions in DSI and oospore numbers. The trend in reductions with length of time of incorporation was similar with greater effect from 6 weeks incorporation compared to 3 weeks. With soils of DSI 50, 75, and 100, the mean effect of *Brassica napus, Raphano-brassica* and *Sinapis alba* were equally effective, reducing DSI by 32-38% and oospore numbers by 66-72%.

When the same brassica species were grown for 6 months in infested soils and subsequently the roots alone incorporated into the soils for 6 weeks, there was an average reduction of 41% in DSI and 56% in oospore numbers by *Brassica oleraceae, Brassica napus* and *Raphano-brassica*. This cultural method, by reducing inoculum, is of value in the control of aphanomyces root rot.

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PREFACE

The fungus, *Aphanomyces eutèiches*, which causes common root rot or aphanomyces root rot of peas (*Pisum sativum*) has been found in Canterbury and Nelson soils over the summer period of 1978-1979 (Manning and Menzies 1980). This disease occurs in North America, Europe, Australia and parts of Japan (Anon. 1977). In the field, it appears that peas are the only host plant to be severely affected. Infection by the pathogen and disease development are favoured by wet soil conditions and warm temperatures with an optimum temperature of 16° for infection and for symptom development between 20° and 28°.

One of the major problems with this disease is the longevity of the fungus in soil. The fungus has a resistant oospore stage, the survival unit of the life cycle, and thus can persist in field soil for up to 10 years. The fungus appears to infect roots without showing any symptoms on a wide range of plants other than peas.

The lack of pesticide control has added to the problem caused by *Aphanomyces euteiches*. The present control is by disease avoidance using a Disease Severity Index method (Sherwood and Hagedorn 1958) which can give some guidance as to whether or not a field can be used to plant a future crop of peas.

The objectives of the proposed research programme were to investigate in detail certain aspects of the disease, namely:

1. To evaluate methods for assessing inoculum density and potential of *Aphanomyces euteiches* in soil.

2. To determine the effects of water potential, moisture content and temperature of soil as factors in the development of aphanomyces root rot of peas.

3. To study the role of legumes and other weeds as alternate hosts for *Aphanomyces euteiches*.

4. To evaluate potential control methods:
   a) The effectiveness of chemical seed treatments on the pathogen and disease development.
   b) The effects of brassica amendments in aphanomyces infested soil on subsequent disease severity and oospore levels.

CHAPTER 1

THE BIOLOGY, ECOLOGY AND DISEASE POTENTIAL OF *APHANOMYCES EUTEICHES* DRECHSLER - A LITERATURE REVIEW

1.1 BIOLOGY OF *APHANOMYCES EUTEICHES*

1.1.1 Introduction

Common root rot of peas (*Pisum sativum*) caused by *Aphanomyces euteiches* Drechsler was first reported by Jones and Drechsler in 1925. Prior to 1925, there could well have been reports of pea root rots which could have had *Aphanomyces euteiches* as the causal pathogen.

The fact that this important pathogen of peas remained undescribed for so long is due to the difficulty of isolating it in pure culture. One cause of failure has been the brief active vegetative stage the fungus has in host tissues. Thus, Jones and Drechsler (1925) reported that even when the fungus was present in host tissues, it could only be induced to grow out into the culture medium with difficulty. Another problem was the invasion of infected tissues by a large number of vigorous saprophytes which followed the pathogen, and interfered with its isolation.

1.1.2 Taxonomy and Nomenclature

In 1860, de Bary (cited by Scott 1961) established a new genus *Aphanomyces*. These fungi have hyphae without cross

walls and produce oospores as resting spores and zoospores or zoosporangia as asexual spores. The taxonomic status of *Aphanomyces* is as outlined in the following (based on Ainsworth *et al.* 1973):

Division	:	Eumycota
Sub-division	:	Mastigomycotina
Class	:	Oomycetes
Order	:	Saprolegniales
Family	:	Saprolegniaceae
Genus	:	*Aphanomyces*
Species	:	*Aphanomyces euteiches*

The generic name *Aphanomyces*, chosen by de Bary, came from the Greek meaning aphanes + myces = obscure fungus. This referred to the macroscopic appearance of the mycelium for when a piece of infected tissue is put in water, the fungus grows forming a very delicate halo of filaments which develop radially from the submerged subtratum and extend outward into the surrounding water. Later, Scott (1961) reported that members of this genus produced two types of zoospores, a phenomenon called diplanetism, fungi possessing this characteristic are said to be diplanetic, or better dimorphic. Within the genus there are 25 species (Ainsworth *et al.* 1973). *Aphanomyces euteiches* Drechs. was reported by Drechsler (1925) as a pathogen causing root rot of peas.

1.1.3 Morphology

The following description is directly from Jones and Drechsler (1925):

"Hyphae hyaline, branching at moderate intervals (20 to 150 μm) at angles approaching a right angle; 4 to 10 μm in diameter, the individual filaments not abruptly varying in width; occurring in nature within cortical cells of the host, in nutrient solutions as extensive nebulous translucent mycelium.

Sporangia in artificial culture arising by conversion of extensive portions of vegetative mycelium delimited by one or more septa; often including many ramifications; discharging through one or several (up to four) tapering branches, the distal portions of which measure usually approximately 8 to 11 μm in diameter, rarely up to 16 μm, diplanetic, the empty spherical wall being distinguished by a protruding evacuation tube 1 μm long by 2.5 to 3 μm in diameter.

Oogonia generally, if not always terminal on a short lateral branch, from which they are delimited by a partition sometimes present as a simple septum, at other times as a columella-like structure protruding into the oogonial cavity; subspherical, measuring usually 25 to 35 μm in diameter, when mature exhibiting a heavy peripheral wall with smooth outer contour and sinous inner contour, hence of irregular thickness varying between 1 to 5 μm (generally between 1 to 2.5 μm).

Antheria typically of diclinous origin, borne on a stalk frequently involved with the oogonial stalk and often branching once or several times measuring 8 to 10 μm in

diameter by 15 to 18 µm in length, or when considerably larger often more conspicuously arched, somewhat lobulate, and becoming compound by the insertion of transverse septa.

Oospores subspherical or more rarely ellipsoidal owing to intruding columella-like septum; 18 to 25 µm (generally 20 to 23 µm) in diameter; provided with a wall of uniform thickness between 1.2 to 1.8 µm (generally 1.5 µm); slightly eccentric in internal structure ('subcentric') germinating without protracted resting period either directly by 1 to 3 germ hyphae or by production of a single unbranched sporangial filament usually 200 to 350 µm in length, in the latter event producing generally 13 to 18 zoospores, approximately half of which are delimited with oospore wall."

1.1.4 Disease Symptoms and Their Development

Peas are susceptible to *Aphanomyces euteiches* root rot at all stages of growth. Infection occurs during the growing season whenever environmental conditions are favourable. The symptoms depend on the stage of development of the host when infection occurs. Zoospores were reported by Scharen (1960) to be the primary infective agent and according to Cunningham and Hagedorn (1962), penetration into the host took place within 2 h after contact.

First symptoms of infection could be apparent 3 to 4 days after penetration of roots and epicotyl as softened, water-soaked and slightly discoloured lesions in the cortical region (Jones and Drechsler 1925). These authors reported

that the water-soaked area was initially firm and gradually became pale yellow to straw colour. Later, the tissue became soft, especially in the epicotyl area, which became darkened with age and eventually collapsed and disintegrated. They maintained that the dark colour in advanced stages of diseases was due partly, if not entirely, to invasion by secondary soil micro-organisms. These fungi were incapable of initiating infection in living intact tissue by themselves, but thrived on the decomposing pea tissues often giving a reddishness to the vascular system of the plant. At this stage, root rot caused by *Aphanomyces euteiches* could not be distinguished from that due to other pathogens. Once the plant roots were destroyed and the epicotyl affected, the plant wilted and collapsed.

According to Haenseler (1926), above-ground symptoms of the disease were not characteristic, except for the few cm of discolouration of the epidermis which extended up the stem especially under humid conditions. However, he reported that if the plants became invaded in the basal stem region below ground as well as in the roots, before they had developed more than 3 to 4 nodes, sudden wilting could result. If infection was delayed until roots became well-developed, the result was a general retardation of growth, with the lower leaves becoming brittle and yellow. Frequently, the plants were observed to persist in a weakened condition until their poorly-filled pods had become mature. If extensive invasion of the roots did not occur until flowering, then under favourable conditions, the plants could mature without any indication of injury, provided there was sufficient soil moisture. A

good crop could be obtained despite rotting of the cortex of the roots.

To distinguish plants infected with *Aphanomyces euteiches* from those attacked by other fungi, Jones and Drechsler (1925) developed two tests that could give a decisive indication of this disease:

1. If an infected plant was pulled by the stem, the vascular cylinder of the taproot would pull out readily from the decayed cortex as a long string, whereas roots of healthy peas would almost always break at the area of seed attachment. However, this test was not satisfactory in early stages of the disease and in dry compact soils.
2. Microscopic examination of the decayed cortex to reveal the presence of oospores which have a very characteristic morphology (Figure 1.1).

1.1.5 Life Cycle

The life cycle of the pathogen is shown in Figure 1.2, after Jones and Drechsler (1925) and Scott (1961).

During the vegetative stage, the pathogen first develops as abundant hyaline, non-septate, moderately branched hyphae within diseased roots. The hyphae vary considerably in diameter, the axial filaments develop short diverticulate spurs, which exhibit only a slight tendency to penetrate host cells (Scott 1961). The mycelium is largely intracellular, the hyphae being orientated longitudinally within the cells.

Figure 1.1: Oospores of *Aphanomyces euteiches* in root tissue from plants grown in naturally infested soil.

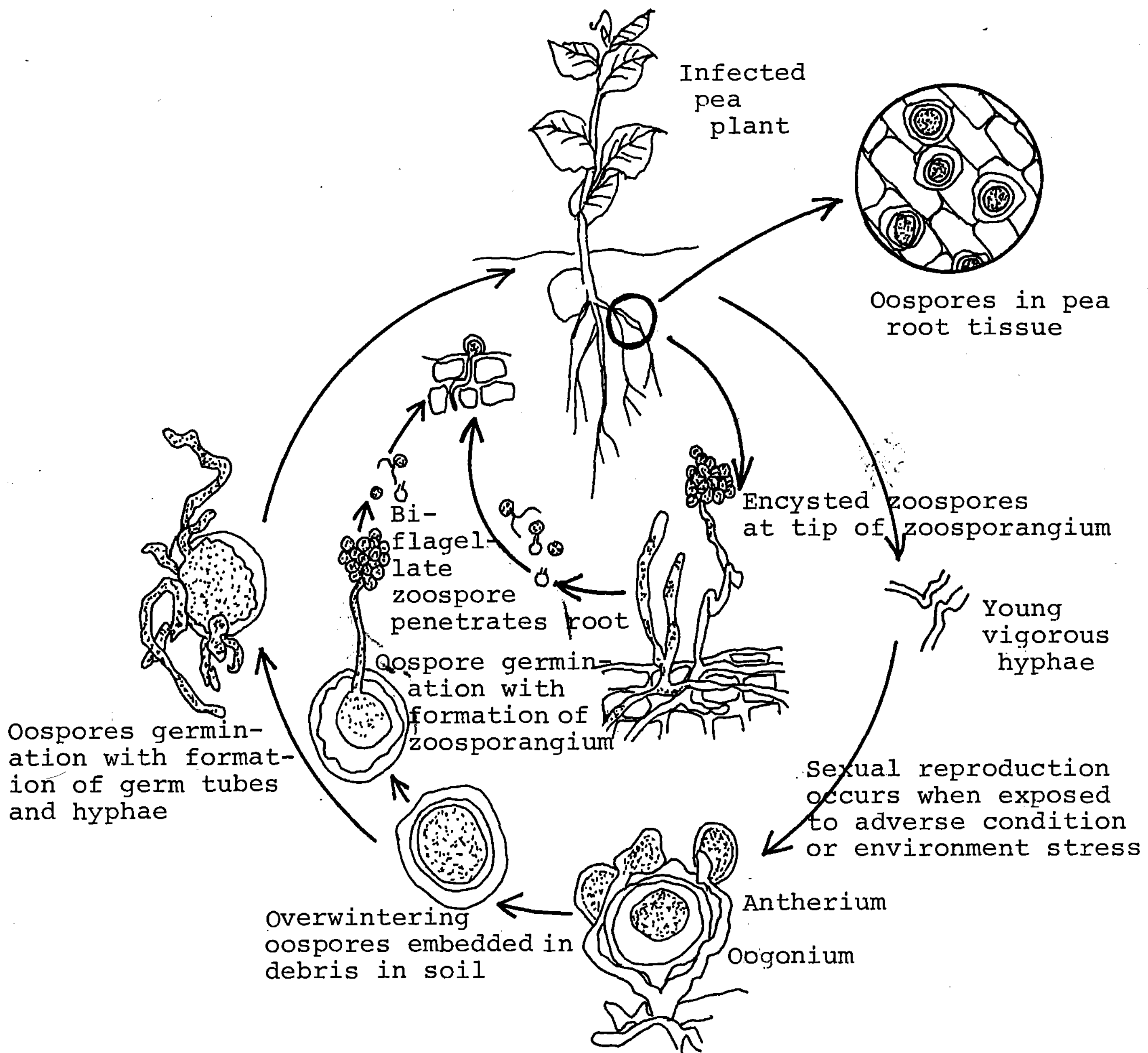


Figure 1.2: The life cycle of *Aphanomyces euteiches* (after Jones and Drechsler 1925, and Scott 1961).

Young, vigorously growing hyphae, and those destined to become zoosporangia, are densely packed with coarsely granular cytoplasm, the hyphal tips sometimes appearing light brown in mass. In older, less vigorous hyphae, the cytoplasmic contents are restricted to a thin peripheral layer with a large, extensive, central vacuole.

According to Scott (1961), the sequence of events occurring in vegetative growth and asexual reproduction may be divided into three distinct phases:

i) vegetative growth of thallus,

ii) differentiation of primary zoospores within the zoosporangium,

iii) discharge of zoospores, and the encystment and aggregation of encysted zoospores at the orifice of the zoosporangium.

Drechsler (1929) reported that primary zoospores, upon discharge, assumed a spherical shape, secreted a cellulose wall and aggregated at the orifice of the sporangium as an irregular mass. The number of zoospores liberated varied from a few to 100. The period of encystment usually lasted from 1 to 3 h when secondary zoospores emerged from encysted primary zoospores.

Haenseler (1925) showed that zoospores were the chief means by which fungus extended its distribution in the soil, but they did not migrate in soil more than 1 - 2 cm. Cunningham and Hagedorn (1962) found that the fungus was

attracted to the host by chemotaxis. The area of maximum attraction was in the region immediately behind the root cap. They also observed that penetration through epidermis of the root occurred after the active zoospores came to rest and encysted on the surface of the root. Each zoospore formed a simple germ tube about 5 μm diameter and up to 10 μm long. After 2 h, many germ tubes penetrated the epidermis of the host plant either between epidermal cells or directly through a cell wall.

Jones and Drechsler (1925) considered oospore formation was induced when mycelium was exposed to adverse conditions such as environmental stress. The purely vegetative condition represented a rather brief stage in the life cycle of the fungus in infected pea tissue and this ended when tissue began to collapse. In their observations, oospores began to form as infected roots disintegrated after a massive invasion by the fungus. The same authors reported that oospores had no dormancy and germinated immediately. Germination was either by zoospores or hyphae depending on the nutrients available. When nutrients were in short supply, germination by means of zoospores predominated.

Sherwood and Hagedorn (1962) concluded that oospores were a major factor in the survival of *Aphanomyces euteiches* between pea crops. However, they doubted whether oospores could survive plant tissue. Boosalis and Scharen (1959) demonstrated that disintegrating root tissue which contained oospores occurred in the soil, while Papavizas and

Ayers (1974) reported that they had obtained evidence which indicated that oospores could survive within root debris for 10 years.

1.1.6 Factors Affecting Disease Development

A. Temperature

Peas can become infected with *Aphanomyces euteiches* throughout the entire temperature range in which the crop is grown. Jones and Drechsler (1925) reported that root infection could occur between 15° and 34° with the optimum between 15° and 30°. Similar results were obtained by Smith and Walker (1941) and Sherwood and Hagedorn (1958). Later studies by Lockwood and Ballard (1959) and Cho and King (1963) narrowed the optimum range for disease development to between 20° and 25°. This temperature range closely approximated that found to be favourable for growth of *Aphanomyces euteiches* in culture (Llanos and Lockwood 1960).

More recently, Burke and Mitchell (1968) and Burke *et al.* (1969) reported that infection of the taproot of peas was greater at 16° than at 24° or 38° even though the latter temperatures were optimal for root rot development. However, infections at the 16° temperature remained latent and symptoms seldom developed unless the plants were subject to higher temperatures. In plants grown at temperatures ranging from 20° to 28°, after an initial incubation at 16°, disease severity was similar after 18 to 21 days. This indicated that in soils where other root pathogens existed,

the optimum temperatures for symptom development ranged from 20 to 28°.

B. Soil Moisture

Aphanomyces euteiches is dependent on a high level of moisture for infection, root rot development and rapid spread. The favourable effect of high soil moisture has been observed many times and serious outbreaks are exclusively associated with wet seasons. Smith and Walker (1941) reported that when soil moistures reached 30 to 35 per cent saturation, severe root rot could occur. Jones and Linford (1925) observed in their survey that frequent rains that maintained a high soil moisture increased root rot severity. Haenseler(1926) reported that root rot was favoured by high soil moisture with 30 per cent of waterholding capacity being close to the minimum for the disease. Burke *et al.* (1969) were able to show that only brief periods of soil saturation were necessary for seedling infection and these may occur after periods of only a few hours of rain. Reinking and Newhall (1950) observed in their surveys that pea rot was not important during extreme dry growing seasons, even though the soil might be infested with *Aphanomyces euteiches*.

Pea losses, due to root rot, may not be proportional to the amount of infection but may depend on the time of infection in relation to the stage of pea growth and the amount of soil moisture. Haenseler (1926) found that if infection occurred early or if infected plants were subjected to extreme drought at pod development, injury could be so great that the entire crop could be lost. In wet soils and especially if

infection occurred at late stages of plant growth, damage to the plants was not severe as long as the water conduction system was intact and provided other parasites or saprophytes did not invade the affected tissues.

C. Soil Type

There is lack of agreement about the effect of soil type on development of root rot. Drechsler (1925) concluded that any soil that retained water or in which water was held because of its relation to impervious subsoil could provide favourable conditions for development of the disease. Jones and Linford (1925) reported that no soil type provided an environment in which root rot could develop more readily than any others. However, in soils with similar cropping histories, more severely infested fields were found on clays or clay loams than on loams, silt loams or lighter soils.

D. Plant Age

The older the pea plant is at the time of infection, the lower will be the infection rate and the amount of root rot. Lockwood and Ballard (1959) obtained more pea root rot when 4 and 6 day old seedlings were inoculated than with 8 day old seedlings. Lockwood (1960) inoculated plants at 0, 8 and 21 days after planting and concluded that disease development decreased with increasing plant age.

E. Interaction with Other Pathogens

Root rots of peas in the field are complex diseases which may be caused by several fungal pathogens (Alconero and Hagedorn 1967) and possibly by nematodes (Hag-

lund and King 1959). It is very difficult to ascertain what is the role of each pathogen and the nature of the interrelationships between pathogens. Pivaral (1967) reported no interaction between *Pythium* and *Aphanomyces euteiches* in the pea root rot syndrome. Nematodes associated with pea root rot and their relationship to *Aphanomyces euteiches* have been studied (Haglund and King 1961; Taylor 1960; Davis 1963; Temp and Hagedorn 1968), but no interactions were found.

Viruses appear to have a significant role in the development of aphanomyces root rot of peas. Farley and Lockwood (1964) reported that virus-infected plants showed increased susceptibility and this observation was supported by Beute and Lockwood (1968) who presented evidence which indicated that virus-infected plants exhibited an increased exudation of nutrients, including amino acids, carbohydrates, organic acids and nucleotides.

1.1.7 Host Range

The papers listing plants reported to be parasitised by *Aphanomyces euteiches* have been reviewed by Papavizas and Ayers (1974). Most inoculation studies on plants other than peas have been performed using pure culture techniques and many species shown to be susceptible under these conditions may not be attacked by the fungus in the field. Haenseler (1926) tested the susceptibility of many hosts in naturally infested soil and showed that most suscept-

ible species were legumes. Later investigators had also reported that legumes were the main hosts while other species were also susceptible (Linford 1927; Geach 1936; Geard 1961). The later host range studies were based on inoculations of plants with pure cultures under aseptic conditions. Table 1.1 shows only hosts reported to be susceptible from tests on infested soil collected from the field.

Table 1.1: Plant species reported as hosts of *Aphanomyces euteiches*.

Host	Common name	Reference
GRAMINEAE		
Avena sativa	oat	Geach (1936)
Zea mays	corn	Haenseler (1926)
LEGUMINOSAE		
Glycine max	soybean	Haenseler (1926)
Lathyrus latifolius	perennial pea	Linford (1927)
Medicago sativa	lucerne	Haenseler (1926)/ Linford (1927)
Melilotus alba	white sweet clover	Linford (1927)
Phaseolus vulgaris	bean	Geach (1936)
Trifolium hybridum	alsike clover	Haenseler (1926)
Trifolium subterranean	subterranean clover	Geach (1936)
Vicia angustifolia	narrow leaf vetch	Geach (1936)
Vicia benghalensis	purple vetch	Geach (1936)
Vicia dasycarpa	woollypod vetch	Linford (1927)
Vicia ervillia	bitter vetch	Geach (1936)
Vicia fulgens	scarlet vetch	Linford (1927)
Vicia pannonica	Hungarian vetch	Linford (1927)
Vicia sativa	common vetch	Linford (1927)
Vicia angustifolia	cowpea	Haenseler (1926)
Vicia villosa	hairy vetch	Haenseler (1926)

1.2 GEOGRAPHIC DISTRIBUTION

Aphanomyces euteiches has been reported from Asia, Australasia, Europe and North America (CMI 1977). In Asia, the only report has been from Japan (Yokasawa *et al.* 1974).

In Australia, Geach (1936) reported that the severity of the disease had forced abandonment of pea growing in some areas of Tasmania. In New Zealand, the fungus was found in parts of the Nelson and Canterbury districts in the late 1970s (Manning and Menzies 1980). In Northern Europe, the fungus has been recorded from France (CMI 1977); Britain (Dennis and Foister 1942; Moore 1943) and in Norway (CMI 1977). In Southern Sweden, the fungus regularly causes considerable economic losses (Linford and Hetmberg 1941). It has also been reported to occur in Denmark (Solberg 1926) and in the non-chemozem zone of the U.S.S.R. (Kotova 1969).

In the United States, it is found in practically every pea growing district, occurring frequently and often destructively (Papavizas and Ayers 1974), except in Northern New England and North Pacific coast where it seldom appears to be severe except in irrigated areas. The disease is concentrated in those States near to the Great Lakes where soil moistures may be high during spring and summer.

1.3 ECONOMIC IMPORTANCE

Estimation of yield losses caused by *Aphanomyces euteiches* is extremely difficult since numerous factors can be responsible for variation in yield. Furthermore, since this fungus is almost always accompanied by other parasitic and quasi-parasitic fungi, it is difficult, if not impossible, to apportion the part played by each one. Zaumeyer (1962) considered the fungus to be one of the most important pathogens in the pea root rot complex, while Carley (1969) considered common root rot of peas incited by *Aphanomyces euteiches* to be one of the limiting factors in pea production in the United States. In Minnesota in the 1950s, Johnson (1953) reported that it was conservatively estimated that *Aphanomyces euteiches* accounted for 80 per cent of the root rot of peas. During 1951-60 in the United States, an average annual loss of ten per cent was attributed to *Aphanomyces euteiches* (US Dept of Agri. 1965). The pathogen is also of major economic importance in other areas of the world. The abandonment of pea-growing in Tasmania in the 1920s and 1930s was due to heavy infestation with *Aphanomyces euteiches* (Geach 1936) which caused increasing economic losses (Stubbs 1971). In Sweden, when temperature and moisture have been favourable for disease development, damage has been so severe that the crop has not been worth harvesting (Olofsson 1967).

Manning and Menzies (1980) were the first to report that *Aphanomyces euteiches* was responsible for severe losses

in pea crops in New Zealand. Symptoms shown by diseased plants from affected crops in the 1977-78 and 1978-79 seasons in Nelson districts were similar to those described overseas as being caused by the pathogen. Later, a survey showed that the fungus was also responsible for severe losses throughout Mid-Canterbury (Manning and Menzies 1980).

1.4 CONTROL

The literature on control methods has been reviewed by Papavizas and Ayers (1974). No reliable economic methods have yet been found that will control the disease in peas sown in heavily infested soil.

1.4.1 Resistant Cultivars

Breeding for resistance has been attempted without success. In the 1920s, some pea cultivars were shown to be more tolerant than others to *Aphanomyces euteiches* (Haenseler 1925; Jones 1926), but no cultivars were reported to be highly resistant. Lockwood (1960) reported that problems associated with breeding of resistant cultivars were due to:

i) low levels of resistance in breeding material,

ii) inability of this type of resistance to express itself in the field,

iii) the presence of races of the fungus,

iv) the synergistic action of several pathogens.

1.4.2 Chemical Control

There have been no reports on economic and effective chemical control of *Aphanomyces euteiches* (Papavizas and Ayers 1974). There is little information on the effect of seed treatment. In the 1960s and early 1970s, considerable research was on control of the disease by soil fungicides. Some chemicals showed beneficial effects in greenhouse trials and limited effects in field trials; however, none could be recommended for widespread field application. The cost of soil sterilisation by chemical treatments does not permit its use in the field.

Field and glasshouse studies by Katan and Eshel (1973), Harvey *et al.* (1975), Jacobsen and Hopen (1975), Grau and Reiling (1977) and Tesdale *et al.* (1978) with dinitroaniline herbicides showed that they suppressed root rot.

1.4.3 Cultural Control

Jones and Linford (1925) reported that one of the major problems with aphanomyces root rot was the longevity of the pathogen in soil where it could persist for up to 10 years as oospores. Walker and Hare (1943) showed evidence that persistence for 5 to 6 years was common. Thus, once a field has become heavily infested with the pathogen, it is many years before it is safe to grow peas in it again.

The value of crop rotation as a control method was questioned by Olofsson (1967) who considered that once a heavy infestation occurred, it could take 10 to 15 years for the inoculum density to reduce. Temp and Hagedorn (1967) made the first systemic study of the effect of cropping patterns on the disease. They found crop rotations of even 10 years were not always effective enough to eradicate root rot, but could reduce inoculum density to such an extent that a profitable pea crop could be grown. Jones and Linford (1925) stated that there was a direct relationship between the number of pea crops and root rot severity and thus rotation of crops may help keep root rot in check.

The best method of disease control appears to be disease avoidance. A method which involved indexing of soil to show its disease potential, was devised by Sherwood and Hagedorn (1958) and Reiling *et al.* (1960). Soils were collected at random from the fields and in a glasshouse, peas were sown in each soil as bait plants. The inoculum potential of the pathogen and infectivity of field samples were determined by the severity of infection under glasshouse conditions. Hazardous fields were identified and distinguished from non-infested or slightly infested areas. This method of land selection, if performed routinely and accurately, can be valuable in avoiding severe crop losses from root rots caused by *Aphanomyces euteiches* (Papavisas and Ayers 1974).

CHAPTER 2

EVALUATION OF METHODS FOR ASSESSING INOCULUM DENSITY OF *APHANOMYCES EUTEICHES* IN SOIL

2.1 INTRODUCTION

If more is to be learned about the survival and spread of *Aphanomyces euteiches* in the soil, it will be necessary to have available a reliable and accurate method for quantifying the inoculum present in soil samples. Although the glasshouse technique for assessing root rot potential of field soil, devised by Reiling *et al.* (1957) and improved by Sherwood and Hagedorn (1958), is a valuable tool for grower advisory services, the method is not sufficiently accurate for use in research into the population biology of the pathogen (Pfender *et al.* 1981).

Inoculum density can be defined as the abundance of the pathogen both in the saprophytic and pathogenic phases in the biological cycle in the soil (Bouhot 1979). Several methods for precise measurement of inoculum density of *Aphanomyces euteiches* in soil have been developed. One method, to determine inoculum density, devised by Boosalis and Scharen (1959) was to count the number of oospores of *Aphanomyces*, embedded in plant debris, per unit volume of soil by microscopically examining organic debris separated from soil by wet sieving. However, they reported the method

to be tedious, and furthermore, it did not indicate the viability of the propagules. Mitchell *et al.* (1969) also separated organic debris from soil but used a host bioassay technique to quantify infectivity of the material obtained. Burke *et al.* (1969) did not separate infested soil into organic and mineral fractions, but estimated the inoculum level by counting the number of infected plants when the roots of test pea plants were allowed to grow through a layer of infested soil placed over vermiculite. However, a 'most probable number' (MPN) approach failed because the roots of pea plants were not infected when infested soil was diluted with non-infested soil. Pfender *et al.* (1981) developed a MPN bioassay in field soil and gave results that were consistent with those from the root rot potential test of Sherwood and Hagedorn (1958) and correlated well with epidemiology and yield measurements from fields in which the disease was present. The disadvantage of this technique was that the resultant MPN was a statistical estimate of the most probable number of infective inoculum in a given amount of soil (McGrady 1915; Geldreich *et al.* 1967) and thus the result could not be regarded as an actual count of the inoculum population in the soil sample.

As the methods reported in the literature did not appear to be suitable for precise measurement of *Aphanomyces euteiches* inoculum density, it was decided to evaluate the techniques presently in use and to ascertain whether they are reliable and if they were sufficiently simple and rapid to be used in this project on large numbers of samples.

In the first part of this chapter, the Literature Review provides information on the facts and concepts required for ecological studies of soil fungi including the three techniques chosen to test. In the second part, the results obtained using the three techniques were compared and discussed. The techniques tested were:

1. Disease severity index method (DSI) of Reiling *et al.* (1957) and Sherwood and Hagedorn (1958).
2. Direct microscopic examination method and counting of oospores devised by Boosalis and Scharen (1959).
3. Dilution-end point technique (most probable number) of Pfender *et al.* (1981).

2.2 REVIEW OF LITERATURE

Prediction of crop losses required a considerable amount of fundamental epidemiological information, which is often difficult to obtain and to interpret (James 1971). Information on inoculum density and an understanding of the relationship between inoculum density and disease intensity is of basic importance for this type of study on a soil-borne problem (Ashworth *et al.* 1981).

The difficulty of studying the biology of plant pathogens in soil is mainly due to the soil's opacity, this together with the presence of an established population of micro-organisms with their own biology and not necessarily having any connection with host plant (Park 1963), further

complicates matters. The study of such pathogens has been hindered because there are no suitable methods for direct observation or measurement of their occurrence. Menzies (1963), in his review, stated that techniques and sampling methods developed for above-ground plant pathogens, where the inoculum almost always consisted of discrete propagules could not be applied easily to studies of the soil's environment. Gilligan (1983) supported Menzies' (1963) statement and added that the role of disposal was frequently reversed in the soil, for ironically it was usually the host that "advertently seeks out" the pathogenic propagule, by growing close to it.

According to Menzies (1963), the occurrence of disease in the plant root system was dependent upon the presence of a certain minimum amount of inoculum of the pathogen in the vicinity of the roots. Geypen (1973) defined inoculum as "viable material which could infect a host." Many factors in the soil environment may intervene in one way or another to influence the effectiveness of inoculum.

The severity of soil-borne plant pathogens has been expressed in terms of the amount of the disease produced in the host plant. According to Baker (1968), the two factors, most important in determining disease severity, were inoculum potential and disease potential. Dimond and Horsfall (1960) in their discussion of inoculum and inoculum potential, defined the latter as "the resultant of the action of the environment, the vigour of the pathogen to establish an infection, the susceptibility of the host and the amount of

inoculum present." These authors drew an analogy between inoculum potential and potential energy, the magnitude of which could be expressed as the product of the intensity factor and the capacity factor. Environmental influences comprises the capacity factor and the inoculum expressed in suitable units was the intensity factor. Garett (1960) also viewed inoculum potential as a combination of the population of the pathogen and the interacting environmental factors that affected the 'nutritional status' of the inoculum at the surface of the host. Disease potential, according to Baker (1978), was "the susceptibility of the host over the period of its life cycle as influenced by disease proneness." Menzies (1963) pointed out that whatever definition is used for describing the disease-producing power of a soil, the inoculum component must be measured before the environmental effects can be analysed. He showed that it was also important to know whether these effects operated on the inoculum directly by changing its ability to survive and infect or on the host plant by changing its susceptibility.

Thus, it can be concluded that for any detailed study of a soil-borne disease, a means of quantitative evaluation of the inoculum potential of the pathogen is a prerequisite.

Methods for quantitative studies

1. Plant infection tests for determining the disease severity index - Menzies (1963) stated that the presence of disease in a plant infection test was an integration of many factors. An important one was the population of the

pathogen and there could be significant changes in the population of the pathogen from the time of sampling to the time of assessment as a significant period was required to grow a host plant and obtain disease symptoms. As a result the use of indicator plants was generally not suitable as an assay method for measuring pathogen. According to Bouhot (1979), the only valid measure of inoculum potential of soil-borne fungi using this bioassay was the number of successful infections that were obtained under optimum environmental conditions on a standard susceptible host. He concluded the following requirements should be fulfilled when measuring the inoculum potential of a soil:

a) the test plants should be susceptible to the parasite,
b) the test plants should be at their most sensitive period of growth,
c) naturally infested soil samples should be applied to the most sensitive part of the test plants to obtain a rapid response to the inoculum,
d) environmental conditions should be standardised so that inoculum potential induced to maximum disease,
e) quantitative studies should be made using a dilution-end point technique,
f) optimum conditions for the highest selectivity and sensitivity of the technique employed should be determined.

2. <u>Direct examination methods</u> - Many pathogens produce macroscopic sclerotia, sporophores or rhizomorphs that can be easily separated from soil and seen either with the naked eye or with a stereomicroscope. Using appropriate

sampling and screening methods, a quantitative determination of the inoculum can be obtained. However, Menzies (1963) reported that one of the problems encountered was that large fruiting bodies or rhizomorphs often only constitute part of the thallus of most pathogens. According to Boyle (1961) and Garren (1961), a direct quantitative relationship between observed structures and the total mass of the pathogen was unlikely to be obtained.

Warcup (1959) reported that a pathogenic fungus that produced spores or mycelium, sufficiently characteristic for positive microscopic identification, it was possible to count these structures in preparations from soil samples. Unfortunately, he found that sufficiently distinctive morphology was rare and the number of pathogens able to be identified in this way were so few that the method was not used very often. Bouhot (1979) commented that this technique detected inoculum but did not allow the separation of the pathogenic strains from saprophytic ones. Thus the measure of the inoculum density needed to be confirmed by inoculation of susceptible hosts with each colony counted. Such techniques are time-consuming and could not be used routinely.

3. 'Most probable number' technique - Gilligan (1983) concluded that difficulties with 'most probable number' (MPN) techniques were mainly related to statistical analysis, "one of estimating a population parameter, the mean μ, from a dilution series." According to Finney (1964), the technique was based on the theory of the maximum likelihood, estimated iteratively as that value of the mean density that gave the

highest probability to the observed results. Data was required only on the proportion of replicates giving a negative response at each dilution. As pointed out by Gilligan (1983), estimation of the mean inoculum density by a quantal response obscured the natural variation in the host-pathogen system because no allowance was made for variation in disease expression due to the interaction between inoculum potential of propagules (Garett 1960) and disease potential of the host (Baker 1978). As a result, the mean inoculum density was therefore a crude biological statistic that masked a large amount of variation.

According to Cochran (1950), there were two fundamental assumptions based on an ingenious application of the theory of probability in the MPN procedure. The first was that organisms were distributed randomly throughout the test medium so that the numbers of propagules in aliquots followed a Poisson distribution. The second assumption was that every propagule was capable of evoking a quantal response, e.g. growth or infection. A bias result, i.e. deviation of the mean of the sampling distribution from μ, would be obtained if any of the inoculum remained clumped after mixing (Taylor *et al.* 1981) or if infection counts of an assay plant did not explore the entire volume (Pfender *et al.*, 1981). Cochran (1950) suggested that by adjusting the range of dilutions in an assay, the dilution ratio and the numbers of samples per dilution, precision of the MPN procedure, i.e. closeness of the estimate to μ, could be maximised.

Numerous refinements to the MPN procedure have been suggested of which a most promising example is the inclusion of a randomisation test, after De Man (1975), by Pfender *et al.*, (1981).

Conclusion on methods

Gilligan (1983) reported the use of MPN procedures in combination with plate counts or bioassays involving a host was to be preferred to direct observation and counting of propagules of inoculum which was seldom practical or appropriate. However, direct examination procedures often measured the total amount of propagules which were important in the survival of the pathogen, as there was rarely information on virulency. Thus the use of host indexing methods was of limited value because of lack of information on the number of propagules required for infection (Weinhold 1977). However, for some purposes, e.g. quantitative studies of soil-borne pathogens, one approach may be more valuable than another. Therefore, it is tempting to utilise existing methods not only to elucidate the mechanisms involved but also as it was to be worthwhile to compare the potentialities and limits of techniques of soil-borne pathogen population studies to judge their value when forecasting the severity of the disease.

2.3 MATERIALS AND METHOD

2.3.1 Collection of Soil Samples

From the results of a survey for *Aphanomyces euteiches* in Canterbury by Plant Health Diagnostic Station, Ministry of Agriculture and Fisheries, Lincoln in 1979-80, 18 fields were selected and soil samples were obtained from each. Information on the characteristics of the soils is provided in Table 2.1. Soil from each field was collected by taking samples at a minimum of 20 pace intervals along a zig-zag course diagonally across the field. One trowelful of soil (approximately one kg of soil) was taken to a depth of 15 cm at every stop and placed in a plastic bag. Later these samples were thoroughly mixed and all lumps were broken so that the soil would pass through a screen with 1.70 mm mesh. After sieving, the samples were stored at +/- 5° until required.

2.3.2 Determination of Disease Severity Index (DSI)

The method, developed by Reiling *et al.* (1957) and Sherwood and Hagedorn (1958) was used. Three plastic pots (15 cm diameter x 20 cm deep) were filled to within 2 cm of the top with soil from each sample from the field. Eleven captan-treated seeds of the pea cultivar "Canterbury 39" were placed on the soil surface and covered with an additional 2 cm of the soil. The pots were labelled and randomised on a glasshouse bench. Two controls were used:

1. a soil known to be highly infested, and

Table 2.1: Soil type, topography, natural drainage class and pH of soil samples from Mid-Canterbury (all information derived from NZ Soil Bureau Bull. 1968)

Soil type	Topography	Natural drainage class	pH	Soil symbol	No. of samples
Barrhill fine sandy loam	Flat to undulating	Well-drained	5.1	B	7
Highbank silt loam	" "	" "	5.3	H	2
Lyndhurst silt loam	" "	Somewhat excessively drained	4.7	L	4
Mayfield stony sandy loam	Flat to gently undulating	Well-drained	4.9	M	2
Templeton silt loam	Flat to undulating	" "	5.4	T	1
Templeton shallow silt loam	Flat	Somewhat excessively drained	5.3	T_S	1
Waterton silt loam	Flat, low lying land	Poorly drained	5.7	W	1

2. a soil known to be free from *Aphanomyces euteiches*. All treatments were replicated three times. The glasshouse temperature was kept between 24-28° throughout the test. The soil was kept at a 65% of waterholding capacity, based on gravimetric measurement (g of water per g of soil oven-dried for 72 h at 105°) during seed germination and emergence. Nine to 11 days after planting, when two leaves were fully expanded, the waterholding capacity of the soil was raised to 80% for 12 to 15 days. Subsequently, it was maintained at 65% waterholding capacity until final assessment.

The pea plants in all test pots were assessed for aphanomyces root rot when all the plants in the heavily infested check soil had died, usually at 28 to 33 days after planting. The appearance of the check plants at this stage is illustrated in Figure 2.1. The plants and soil were removed from the pots and the roots were carefully separated and freed from soil by washing under running water. According to the symptoms present, each root was assigned to one of the following arbitrary disease classes (after Sherwood and Hagedorn 1958) as shown in Figure 2.2:

0 - no disease, root free of symptoms,

1 - about 5 water soaked, light brown areas on the roots,

2 - water soaked, light brown areas confluent and more extensive, but not involving the entire root system, tissue firm,

3 - water soaking and browning involved all root and epicotyl (stem above seed), tissue soft but not collapsed, epicotyl not markedly shrivelled,

Figure 2.1: Check plants in *Aphanomyces euteiches*-free soils (Pot A) and infested soils (Pot B) at time of assessment for glasshouse common root rot disease index.

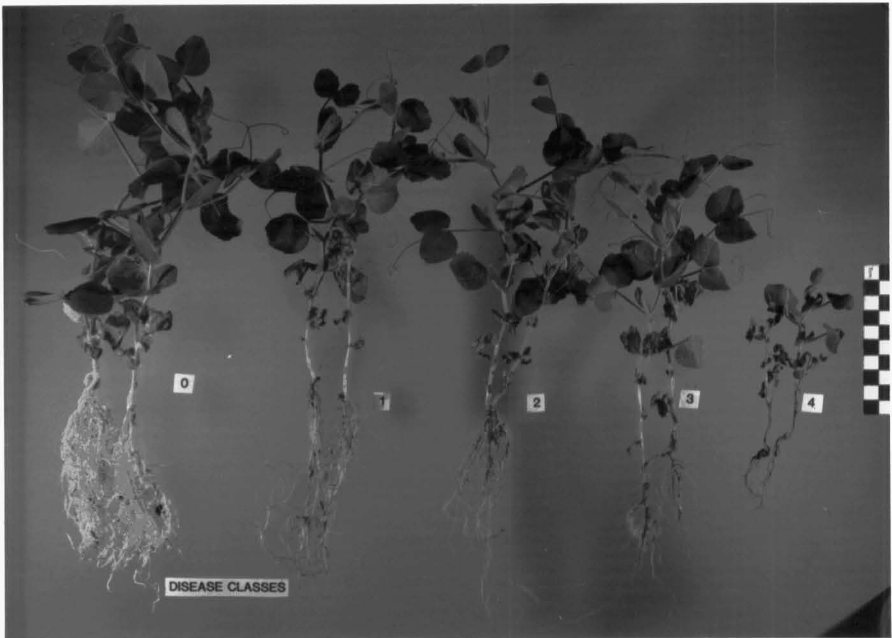


Figure 2.2: Pea plants from the disease severity index method for root rot potential showing from left to right, appearance of roots in disease classes 0, 1, 2, 3 and 4 respectively.

4 - water soaking, browning and decay involving total root system; cortex easily sloughed off and epitocyl shrivelled or rotted; dead plants included.

The disease severity index (DSI) was calculated for each pot using the formula:

$$DSI = \frac{\text{Sum of (disease class x No. of plants in that class) x 100}}{\text{Total number of plants x 4}}$$

According to the authors, three categories of fields can be distinguished on the basis of the DSI; fields with DSI of 0-50 can be safely planted with peas; 51-69 of questionable safety and 70-100, definitely dangerous and should not be planted with peas.

2.3.3 Estimation of Inoculum Density by a Wet-Sieving Flotation Method

A microscopic method for detection of oospores in plant debris, developed by Boosalis and Scharen (1959), was modified to increase the efficacy of the procedure. Three replicate soil samples, each of 100 g were assessed from each field. Each replicate was placed in 500 ml of water and homogenised in a Waring Blender at a low speed for 3 min. This step was added into the original method and served to disperse aggregates of soil particles and comminute the larger particles of plant debris, so that the infected fragments of plant debris were more evenly distributed in the suspension. The resulting soil suspension was transferred to a 2-litre glass measuring cylinder and made

up to 2 l with tap water. The suspension was allowed to stand for one min and then the supernatant liquid was slowly decanted on to a stainless steel screen of 75 μm mesh (Gallenhamp Test Sieve BS410). Soil and organic debris that had settled was resuspended in 2 l of tap water and allowed to settle again for one min and the supernatant was also passed through the screen. This procedure was repeated 5 - 8 times until the supernatant liquid was free from plant debris. Most of the soil deposited on the screen was removed by holding the screen under running tap water and sharply tapping the frame of the screen against the sink. The debris retained was washed to the edge of the screen and transferred to a 100 ml measuring cylinder through a funnel. It was resuspended in about 50-70 ml of tap water, and this suspension used in the membrane filter method. If the sample was not examined immediately, it was stored at 4°.

For microscopic examination, the original procedure of Boosalis and Scharen (1959) of examining 7 aliquots of each of 5 ml on water agar was replaced by the membrane filter method developed by Adams (1967). This method reduced the time required to examine each sample. In this method, a 25 mm Type GA-1 (5 μm filter) membrane filter (Gelman, Part No. 60002 Metricel) was mounted in a filter holder. Two ml of the suspension was passed through the filter by vacuum filtration. The membrane filter was gently removed and placed on a microscopic slide and covered with lactophenol cotton blue. The slide was steamed for 3 min

over a waterbath at 70°. The stained filter was placed back on the filter holder and washed with fresh lactophenol by vacuum filtration and then with glycerin. The membranes were mounted on glycerin on a microscopic slide for oospore counts. Five slides were prepared from each replicate of the three soil samples.

Each slide was examined at 100x magnification with a compound microscope to determine the gross morphology of the oospores embedded in plant debris fragments. The whole area of the membrane filter was examined as the oospores were counted. The numbers of oospores on each of the five slides from one replicate were added together and the total number of oospores per 100 g of soil was calculated using the following formula:

$$\text{No. of oospores per 100 g of soil} = \frac{x}{10} \times y$$

where x = total number of oospores counted on 5 slides per replicate

y = volume of final suspension in ml.

To assess the validity of this method of determining inoculum density, a known number of oospores from a culture of *Aphanomyces euteiches* Isolate 919 (obtained from Department of Scientific and Industrial Research, Auckland) were added to non-infested soil at 11 inoculum levels ranging from 0 to 700 oospores per 100 g of soil. The soil used was Templeton silt loam collected from Lincoln College Mixed Cropping Farm and sieved through a 1.70 mm mesh. The number of oospores in each of the 11 inoculum levels was determined by the methods previously described.

The oospores were produced in cultures grown in oatmeal broth, using a method developed by Schneider and Yoder (1973) and Schneider (1978). Oatmeal broth was prepared by comminuting 5 gm of rolled oats in 500 ml distilled water in a Waring Blender for 5 min. The mixture was strained through a muslin cloth and sufficient water was added to the total volume one litre. The broth was heated to 50° for a min, adjusted to pH 6.6 with N/20 HCl and autoclaved in wide mouthed Erlenmeyer flasks (250 ml) containing 100 ml per flask for 15 mins. When cold, the broth in each flask was inoculated with an agar plug from a culture of *Aphanomyces euteiches* Isolate 919 and incubated at 25° for approximately three weeks. To obtain a mycelium-free suspension of oospores, the method developed by Jimenez and Lockwood (1981) was used. The mycelium in oatmeal broth culture was killed by freezing at -10° for 12 to 15 h. Thawed mycelial mats were rinsed thrice with sterile distilled water and ground for 10 mins at 6000 rpm in a Sorvall Omni-mixer (172000 Ivan Sorvall Inc., Newtown, Connecticut 06470, USA) with the container surrounded by an ice waterbath. The homogenate was diluted and sieved through a screen (74 μm mesh). The filtrate containing the oospores was layered over 1 M sucrose and centrifuged for one min at 1500 g in a centrifuge (Wifug XI Sweden Model) and this was repeated four times to obtain oospores free from hyphae. These oospores were suspended in distilled water and the concentration in the resulting suspension was determined with a Bright Line Metallized Counting Chamber (Clay Adams, Bacton, Dickenson and Company, Parsippany, NJ 07054). The suspension was diluted with distilled water to obtain the desired oospore

density, added to the soil and mixed thoroughly in a Griffin Flask Shaker (Griffin and George Ltd, Britain). The soil samples, inoculated with oospores also were assayed for DSI as described in Section 2.3.2. The DSI results obtained from the assay at varying inoculum levels were compared to results obtained from the soil samples from the field.

2.3.4 'Most Probable Number' Method of Assessing Inoculum Potential

The 'Most Probable Number' (MPN) method used to estimate infective inoculum density in field soil samples was based on the procedure described by Pfender *et al.* (1981). However, a 10-fold dilution series was used and there was no computer programme. In a preliminary trial, three diluent media, non-infested soil, non-infested soil steamed soil (Barrhill silt loam and Templeton silt loam) and sterilised vermiculite were tested. Non-infested soil was steam-treated at 80° for 30 min in an autoclave. To ensure the desired temperature was achieved, the centre of the soil bulk was constantly checked with a thermometer. Vermiculite was autoclaved for 30 min. Since sterilised vermiculite, when mixed with inoculum, gave the highest infectivity, it was selected for subsequent use.

Soil dilutions were prepared by mixing a weighted quantity of infested soil with sterilised vermiculite, made up to 150 ml which was the volume of 100 g of soil. The range of dilutions was undiluted soil (100 g), 10 g of soil + vermiculite, and 1 g of soil + vermiculite. Each dilution

was thoroughly mixed by vigorously shaking the mixture with the Griffin Flask Shaker.

The apparatus consisted of Plix Rootrainers (Tinus - 352 ml volume book style; Spencer-Lemaire Industries Ltd, Edmonton, Canada; and Winstone Merchants Ltd, New Zealand). In this equipment, 100 books, each consisted of 4 cavities measuring 39 cm wide x 51 cm deep x 184 cm high (Figure 2.3). Cotton wool was placed at the bottom of the cavities to prevent vermiculite from sifting through. In each cavity, the infested soil or soil/vermiculite was placed between two layers of sterilised vermiculite with a thin layer of sand (5 mm) being placed below the inoculum layer to prevent soil or the mixture from being washed through the vermiculite. Two captan-treated seeds of pea cultivar "Canterbury 39" were sown in each cavity in the middle of the top layer of vermiculite. Each tray of books of cavities was placed partly immersed in water in a plastic container (50 cm x 35 cm x 15 cm), and the water level was kept constant and checked daily. The soil moisture in each inoculum layer was held constant at approximately -15 millibars because the lower surface of each inoculum layer was always 15 mm above the water. The trays were kept in a temperature light humidity controlled cabinet, maintained at $24^{o} \pm 1^{o}$ with a relative humidity of 90% and a 12 h light was used to obtain equal day and night length.

After 14 days, plants were removed from the containers and washed free from soil and vermiculite. Those plants which showed the characteristic water-soaked

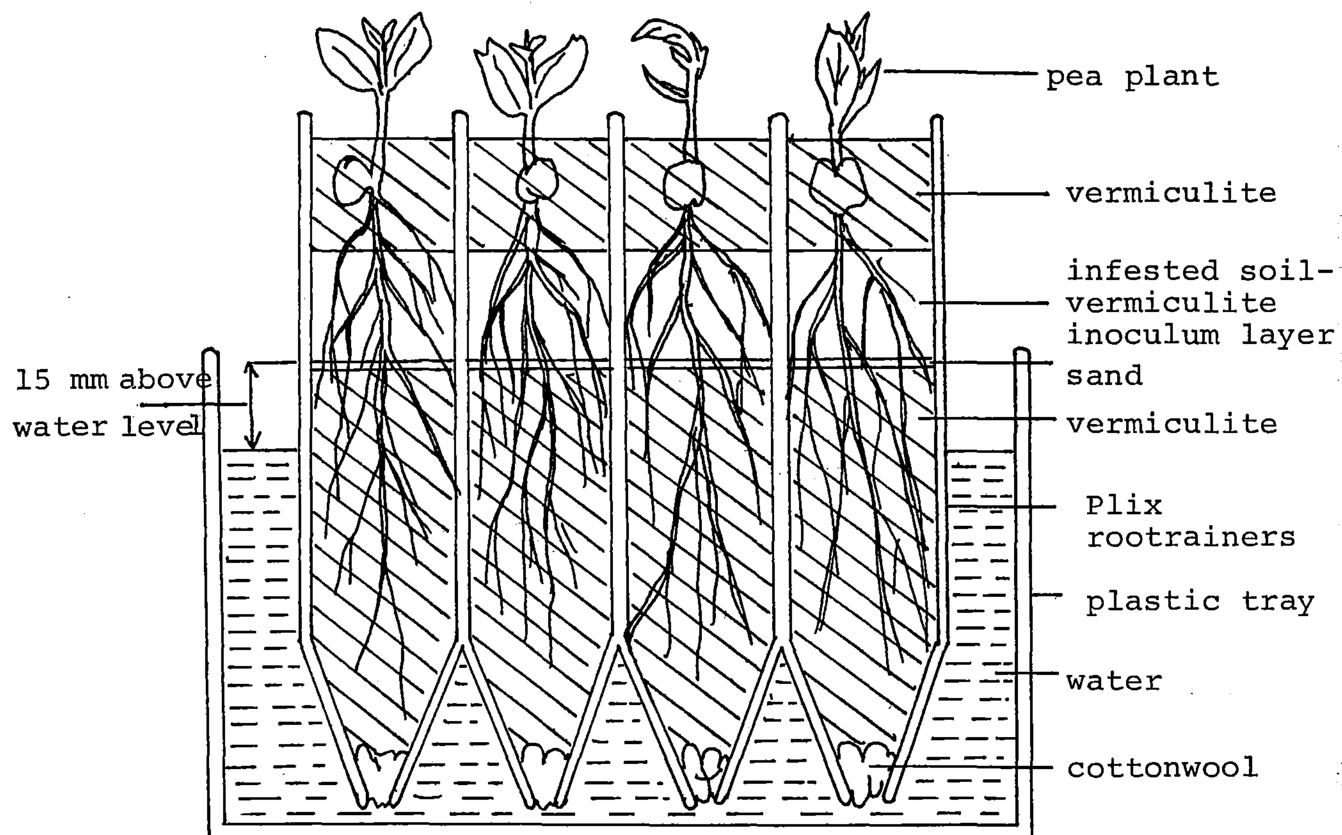


Figure 2.3: Cross-section view of the apparatus used in 'Most Probable Number' Bioassay of inoculum potential of *Aphanomyces euteiches* (Figure at ½ scale: 1 cm to 2 cm).

honey-yellow colour lesions (an easily distinguished symptom) were scored as positive or negative for infection. The plants showing disease symptoms at each dilution level were used to calculate the MPN estimates of infective *Aphanomyces euteiches* inoculum density in the undiluted soil based on Table 2.2.

2.4 RESULTS

2.4.1 Testing of Soil Samples

Table 2.3 shows the DSI of soil samples obtained in Canterbury from 18 fields, each described in Table 2.1.

The values for the DSI ranged from 7 to 100 and appeared to be related to the cropping history of each field. since the longer the time since a field was sown with peas the lower the DSI, as shown in Figure 2.4. Fields with a break of 4 years or more had DSI values of <50 except three fields (L1 (6 yrs), L2 (4 yrs) and W (4 yrs), with DSI values of 73.5, 75.3 and 65.3 respectively. Soil types indicated in Table 2.1 did not appear to affect DSI though there are only a few results of each.

When results of the inoculum density experiment (cross ref. to 2.3.3) expressed as number of oospores per 100 g of soil, were correlated with DSI of the same soil samples, they showed a curvilinear relationship (Figure 2.5).

Table 2.2: MPN index and 95% confidence limits for various combinations of positive and negative results of infection when five 100 g portions, five 10 g portions and five 1 g portions of *Aphanomyces euteiches* infested soil are used.

No. of plants showing positive reaction out of			MPN index per 100 ml	95% confidence limits	
5 of 100 g each	5 of 10 g each	5 of 1 g each		Lower	Upper
0	0	0	< 2		
0	0	1	2	< 0.5	7
0	1	0	2	< 0.5	7
0	2	0	4	< 0.5	11
1	0	0	2	< 0.5	7
1	0	1	4	< 0.5	11
1	1	0	4	< 0.5	11
1	1	1	6	< 0.5	15
1	2	0	6	< 0.5	15
2	0	0	5	< 0.5	13
2	0	1	7	1	17
2	1	0	7	1	17
2	1	1	9	2	21
2	2	0	9	2	21
2	3	0	12	3	28
3	0	0	8	1	19
3	0	1	11	2	25
3	1	0	11	2	25
3	1	1	14	4	34
3	2	0	14	4	34
3	2	1	17	5	46
3	3	0	17	5	46
4	0	0	13	3	31
4	0	1	17	5	46
4	1	0	17	5	46
4	1	1	21	7	63
4	1	2	26	9	78
4	2	0	22	7	67
4	2	1	26	9	78
4	3	0	27	9	80
4	3	1	33	11	93
4	4	0	34	12	93
5	0	0	23	7	70
5	0	1	31	11	89
5	0	2	43	15	110
5	1	0	33	11	93
5	1	1	46	16	120
5	1	2	63	21	150
5	2	0	49	17	130
5	2	1	70	23	170
5	2	2	94	28	220
5	3	0	79	25	190
5	3	1	110	31	250
5	3	2	140	37	340
5	3	3	180	44	500
5	4	0	130	35	300
5	4	1	170	43	490
5	4	2	220	57	700
5	4	3	280	90	850
5	4	4	350	120	1,000
5	5	0	240	68	750
5	5	1	350	120	1,000
5	5	2	540	180	1,400
5	5	3	920	300	3,200
5	5	4	1,600	640	5,800
5	5	5	>2,400		

Table 2.3: Disease severity index, determined in May 1981, of soil samples from 18 fields in Canterbury (previous 4 crops grown in each field before sampling and number of years since last pea crop are indicated).

Soil sample	Previous four crops				No. of yrs since last pea crop	Disease severity index
	1977	1978	1979	1980		
B1	Wheat	Pea	White clover	Wheat	3	100.0
B2	Ryegrass/white clover	Ryegrass	Pea/white clover	"	2	81.5
B3	" " " "	White clover	Wheat	Ryegrass/White Clover	6	50.4
B4	Ryegrass	" "	Ryegrass/white clover	Wheat	6	40.8
B5	White clover	Ryegrass	Wheat	Barley	5	47.7
B6	Ryegrass/white clover	Wheat	Ryegrass/white clover	White clover	6	44.2
B7	White clover	Ryegrass/white clover	Wheat	Barley	6	7.1
H1	Wheat	Peas	White clover	Wheat	3	61.5
H2	"	"	" "	"	3	63.3
L1	White clover	Wheat	Wheat	Barley	6	73.6
L2	Peas	Ryegrass	White clover	Wheat	4	75.3
L3	Ryegrass	White clover	Wheat	"	5	49.8
L4	Wheat	Barley	Barley	"	6	32.0
M1	Peas	White clover	Wheat	Ryegrass	4	18.2
M2	"	" "	"	"	4	17.3
T	Ryegrass	" "	"	Peas	1	100.0
T5	"	Wheat	Peas	Wheat	2	90.0
W	Peas	Ryegrass	White clover	"	4	65.3

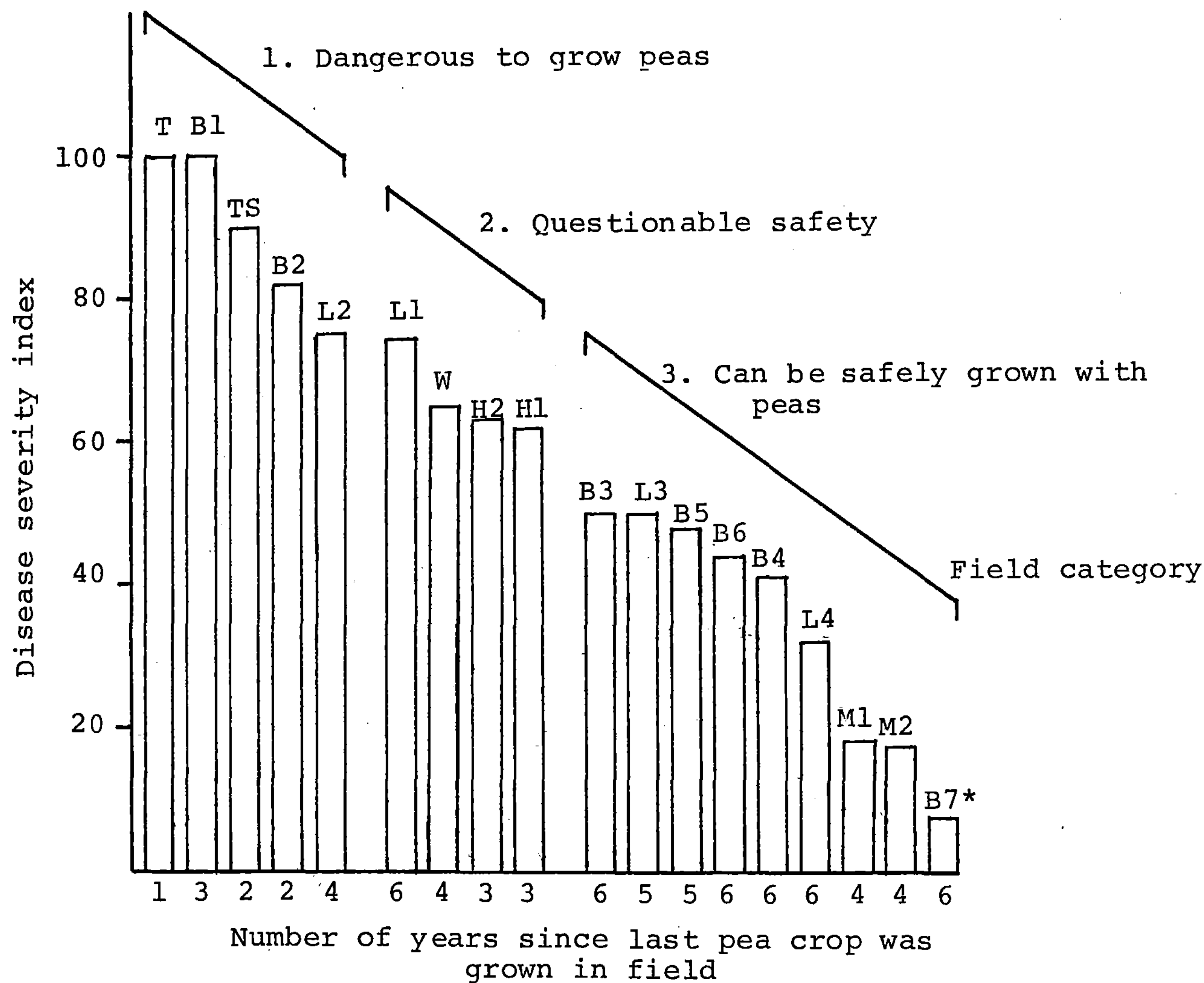


Figure 2.4: Relation between disease severity index and the cropping history of the 18 fields (*soil symbols as described in Table 2.3) infested with *Aphanomyces euteiches*.

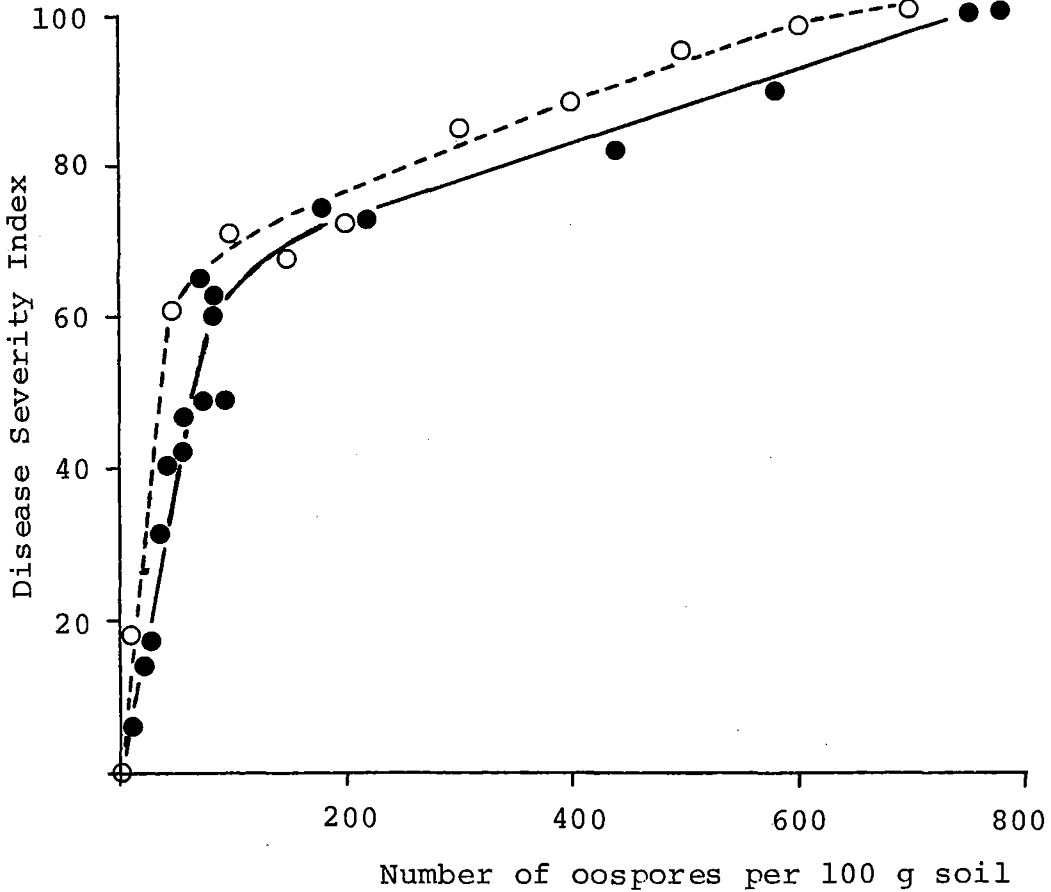


Figure 2.5: Correlation between Disease Severity Index of aphanomyces root rot of pea and i) number of oospores per 100 g infested field soil (●) and ii) number of cultured oospores added to non-infested soil (○).

At low densities, the increase in DSI was high with increasing number of oospores. However, at densities above 100 oospores per 100 g of soil, the increase in DSI was relatively low and a very high population of about 700 oospores per 100 g of soil was required to reach the maximum DSI.

A similar relationship between DSI and number of cultured oospores added to 100 g of non-infested soil was also obtained (Figure 2.5). In general, a higher DSI value was obtained at each inoculum level compared with those found in soil samples from the field.

Linear regressions of DSI and inoculum density of both experiments by logarithm probability transformations are set out in Figure 2.6. The regression coefficients ($r_1 = 0.97$ and $r_2 = 0.99$ at $P < 0.01$) were highly significant, indicating that the DSI predicted with accuracy, the inoculum density in the field and thus the amount of aphanomyces root rot which could develop.

2.4.2 MPN Tests

The effect of the three media used to dilute *Aphanomyces euteiches* infested soil on MPN estimates of inoculum density is shown in Table 2.4.

The diluents, though causing differences in MPN estimates of infective oospores of *Aphanomyces euteiches* did not prevent the pathogen from infecting the host plants. Soil diluted with sterilised vermiculite had the highest

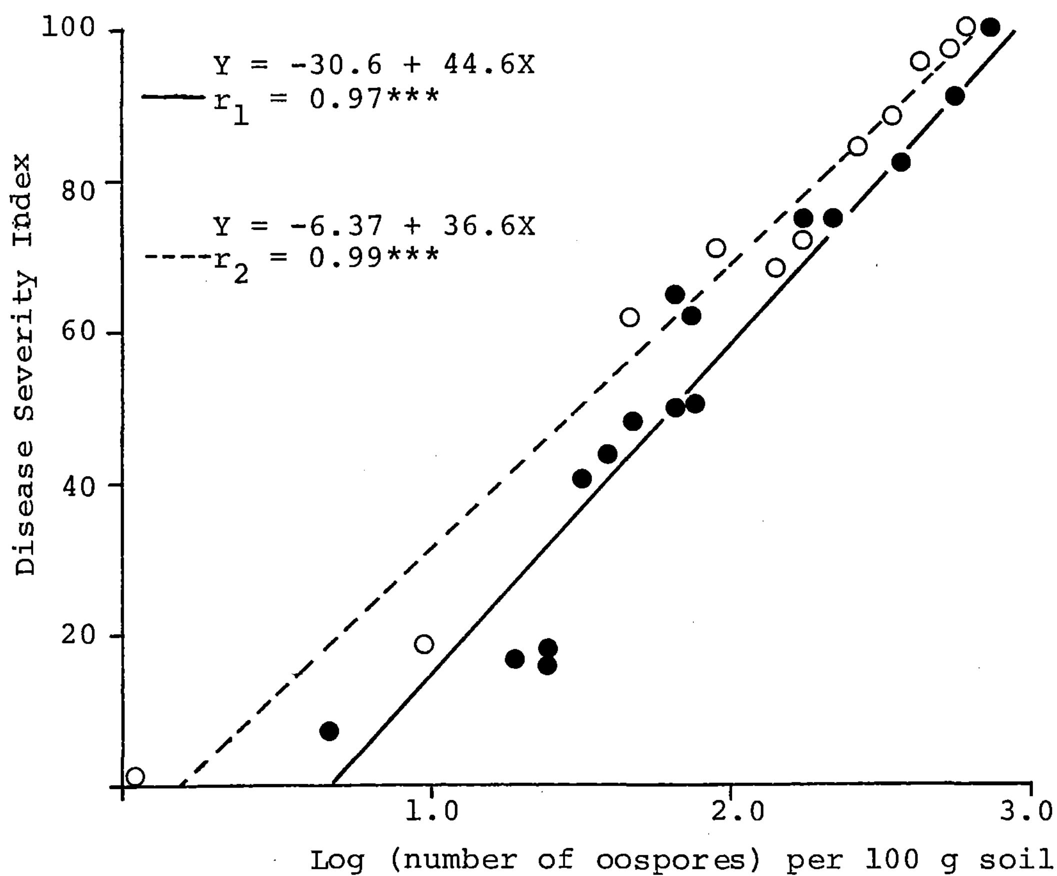


Figure 2.6: Linear regression of Disease Severity Index of aphanomyces root rot of peas and i) number of oospores per 100 g infested field soil (●) and ii) number of cultured oospores added to non-infested soil (○) by logarithm probability transformation.

Table 2.4: Effect of three diluents used to dilute aphanomyces-infested field soil for estimating 'Most Probable Number' (MPN) of infective oospores of *Aphanomyces euteiches*.

Soil type (infested soil with DSI of 100)	Soil diluents	MPN (infective no. of oospores per 100 g of soil)	95% confidence limits		Infectivity (%)
			Lower	Upper	
Barrhill silt loam (inoculum density of 783 oospores per 100 g of soil)	Non-infested soil	26	9	78	3.3
	Non-infested steamed soil*	79	25	190	10.1
	Sterilised vermiculite	350	120	1000	44.7
Templeton silt loam (inoculum density of 751 oospores per 100 g of soil)	Non-infested soil	33	11.1	93	4.4
	Non-infested steamed soil*	220	57	700	29.3
	Sterilised vermiculite	350	120	1000	46.6

*Steamed at 80° for 30 min.

infectivity value in detecting the presence of inoculum. On the basis of these results, it was decided to use the vermiculite diluent for estimating MPN of infective oospores of soil samples.

MPN estimates of infective inoculum density in field soils are shown in Table 2.5. In general, the proportion of infection of host pea plants caused by *Aphanomyces euteiches* decreased with increasing dilution rates of a given soil, as expected. MPN estimates increased with increasing DSI as shown in Table 2.2, although the test did not measure the total inoculum density of the pathogen as indicated by the low estimates of infective oospores and per cent infectivity. There was no significant discrimination in per cent infectivity among DSI below 50 and MPN estimates were not obtained below DSI of 10.

2.5 DISCUSSION

It was concluded that the wet-sieving flotation direct microscopic counting of oospores was the best method for estimating inoculum density of *Aphanomyces euteiches* and this would be the most satisfactory and reliable method to use in experiments on the populations of the pathogen. The validity of this method for estimating inoculum density was shown by the similarity of the curvilinear relationships between DSI of *Aphanomyces* root rot and the number of oospores per 100 g infested field soil and the number of cultured oospores added to non-infested soil (Figure 2.5).

Table 2.5: 'Most Probable Number' (MPN) estimates and % infectivity (MPN estimated inoculum density divided by number of oospores in soil samples obtained by wet-sieving flotation method) of *Aphanomyces euteiches* inoculum in soil samples obtained from 18 fields in Canterbury, collected in May 1981.

Soil sample	DSI from* bioassay (Table 2.3)	MPN (infective no. of oospores per 100 g of soil	95% confidence limits		Infectivity %
			Lower	Upper	
B1	100.0	350.0	120.0	1000.0	44.6
B2	81.5	49.0	17.0	130.0	22.5
B3	50.4	1.7	0.5	4.6	2.2
B4	40.8	0.8	1.0	1.9	2.2
B5	47.7	1.3	0.3	3.1	2.6
B6	44.4	1.1	0.2	2.5	2.5
B7	7.1	<0.2	-	-	-
H1	61.5	3.3	1.1	9.3	4.1
H2	63.3	3.4	1.2	9.3	4.3
L1	73.5	13.0	3.0	31.0	5.9
L2	75.3	11.0	3.1	25.0	5.9
L3	49.8	1.7	0.5	4.6	2.4
L4	32.0	0.5	<0.05	1.3	1.6
M1	18.2	0.5	<0.05	1.3	2.7
M2	17.3	0.5	<0.05	1.3	2.4
T	100.0	350.0	120.0	1000.0	46.6
T5	90.0	170.0	43.0	490.0	29.3
W	65.3	3.3	1.1	9.3	4.2

*Data from Table 2.3 obtained by plant tests.

This result is conclusive evidence to confirm the suggestion by Boosalis and Scharen (1959) who predicted that the disease potential of aphanomyces root rot in the field could be possible using this method. The value of the method lies in its capability and reliability in the discrimination between the soils from different fields.

Mitchell *et al.* (1968) and Pfender *et al.* (1981) claimed that the original method of counting oospores (Boosalis and Scharen, 1959) was tedious and time-consuming. However, these problems were overcome by modifying the original method. First, the soil suspension was homogenised in a Waring Blender which improved the distribution of infected fragments of plant debris and improved the randomness of sampling. Secondly, the membrane filter method (Adams, 1967) which replaced the counting of oospores in petri dishes greatly sped up the procedure.

Mitchell *et al.* (1969) and Pfender *et al.* (1981) stressed that although this method measured a precise level of *Aphanomyces euteiches* inoculum density, it could not be used to assess the viability of oospores. Any assessment of inoculum potential, however, requires a knowledge of the virulence of the pathogen. No attempts were made to test the viability of individual oospores. However, Scharen (1960) showed that 40% of oospores embedded in plant tissue germinated and produced zoospores that could infect pea roots. Although viability is certainly essential, viability alone cannot define a population of infective oospores of the

pathogen (Mitchell *et al.* 1969). Inoculum potential of oospores (Garett 1960) and disease potential of the host (Baker 1978) are not expressed by inoculum density. Therefore, inoculum-host interactions assessed by the DSI method, together with results from the wet-sieving flotation method, would provide a suitable technique for quantifying the inoculum density in soil and the likely level of disease in a pea crop planted in that soil (Figure 2.5). This is supported strongly by the highly significant correlations between DSI of *Aphanomyces* root rot of peas and number of oospores per 100 g infested field soil and number of cultured oospores added to non-infested soil by logarithm probability transformation (Figure 2.6). Boosalis and Scharen (1959), using their bioassay, also showed that the number of plant debris fragments infected with oospores had a highly positive correlation with the severity of aphanomyces root rot of peas in soil from which the plant residue was obtained.

The results of MPN estimates of infective oospores, as set out in Table 2.4, showed that this technique could not accurately assess the changes in a population of *Aphanomyces euteiches* when subjected to experimental soil treatments especially with soils with low DSI values, i.e. values of <50. Furthermore, MPN is a statistical estimate of organisms in a given amount of sample (Geldreich *et al.* 1967) and cannot be regarded as an actual number. Thus, the approximate 95% confidence limits lie between 31% to 289% (from data in Table 2.6, approximately 1/3 to 3 times

the MPN).

Pfender *et al.* (1981) reported that low MPN estimates of infective inoculum density could be due to dormancy or non-viability of oospores. However, Jones and Drechsler (1925) showed in their experiments that oospores had no dormancy and germinated immediately. The cause of the low estimates may be due to mutual interference of oospores at high inoculum concentrations (Garett 1956). This explanation was based on the concept that the *Aphanomyces euteiches*-plant interaction was a motile inoculum invaded by a moving infection count, i.e. root tips (Baker *et al.* 1967) would be required when efficiency of penetration and infection by abundant zoospores (about 300-400), the primary units which can develop from a single oospore (Jones and Drechsler 1925).

CHAPTER 3

WATER POTENTIAL, MOISTURE CONTENT AND TEMPERATURE OF SOIL AS FACTORS IN THE DEVELOPMENT OF APHANOMYCES ROOT ROT OF PEAS

3.1 INTRODUCTION

The influence of soil moisture and temperature on the development of root rot diseases has been emphasised by many workers. Jones and Drechsler (1925) reported that among the root rot diseases of plants, few showed "a greater degree of erratic irregularity" than aphanomyces root rot. They observed that in fields known to be very severely infested, the disease was usually worse in areas where the soil remained wet, but at other times, there was no clear relation between disease occurrence and any obvious soil differences. In their review article, Papavizas and Ayers (1974) concluded that the severity of aphanomyces root rot depended on high soil moisture for infection and rapid development. The pathogen preferred soil temperatures from 22° to 28°. The effects of soil moisture and temperature on the relationships between inoculum levels of *Aphanomyces euteiches* and disease severity have never been fully investigated.

In ecological studies on *Aphanomyces euteiches*, soil moisture has been described solely in terms of moisture content. However, Griffin (1963) stated that, although water was a major environmental variable of soil affecting fungal

growth, there have been only a few clear studies on the way in which water affects the growth of fungi in soil. The concept that soil water potential influenced microbial activity in soil has been well established and micro-organisms are well-known to differ in their ability to remove water from soil (Cook *et al.* 1972) and that their response to water potential may vary with temperature (Griffin 1970).

This chapter describes and presents the results of experiments to clarify the influences of water potential, moisture and temperature and the effects of these soil factors on the quantitative relationships between inoculum levels and the development of aphanomyces root rot. The literature on the concept and terminology of water potential and its influence in micro-organisms is reviewed.

The following experiments were designed to investigate several aspects:

1. Laboratory studies *in vitro* on the effect of water potential and temperature on the pathogen.
2. Glasshouse studies on the effect of soil moisture and temperature on infection.
3. Glasshouse studies on the effect of soil moisture on the development of aphanomyces root rot, plant growth and yield of peas.

3.2 REVIEW OF LITERATURE

Research on plant-soil-water relationships has been complicated by the use of many terms such as suction force, waterholding capacity and diffusion pressure deficit (Cook and Papendick 1972). Water, like heat, when described in terms of its potential energy, is defined as the capacity to do work, relative to the work capacity of pure, free water, measured in bars or atmospheres (Anon. 1965). In the reviews of Griffin (1961, 1963), and Cook and Papendick (1971, 1972), the terms used to describe soil water potential and their significance in plant pathology and soil microbiology are discussed. A glossary of all terms used is in Appendix 1.

According to Aslyng (1963), total water potential may be divided into a) osmotic potential due to solutes (always negative); b) gravitational potential caused by changes in elevation (positive or negative); c) matric or capillary potential which included both adsorption and capillary effects of the solid phase (always negative), and d) pressure potential which resulted from external pressure applied to the soil water (positive or negative).

Cook (1972) classified the effect of water potentials on soil-borne fungal pathogens into three categories as follows:

i) restrictive effects on pathogen growth;

ii) effects on pathogen-antagonist interactions;

iii) effects on host-pathogen interactions.

Griffin (1963) reported that an organism must use energy to overcome the osmotic and matric components of water potential which tended to oppose uptake of water from soil. Every micro-organism has optimal and minimal water potentials for growth and these can be altered by temperature (Scott 1957; Griffin 1963; Bruehl and Cunfer 1971). Some respond differently to osmotic and matric water potentials (Adebayo and Harris 1971; Cook and Papendick 1972). It is well known that most root diseases occur in wet soil as the water potential is too low for growth of the pathogen in drier soils. Examples of this generalisation include *Phytophthora cinnamoni* Rands (Sommer *et al.* 1970), *Ophiobolus graminis* Sace (Cook *et al.* 1972), both of these fungi grow optimally at -5 bars or wetter, and the growth rate is reduced by half or more at -20 to -25 bars with minimal growth at water potentials below -50 to -60 bars. The root, foot, stem and seedling diseases caused by *Fusarium solani* (Mart) Apel and Wr. emend. Snyd and Hans are among the few pathogen problems associated with dry soils. For this species, soil water potential appeared to be optimal at -10 to -30 bars, with growth being reduced by half at -40 to -60 bars and prevented only if water was below -100 bars. These observations indicate important differences in the physiology of these fungi (Cook *et al.* 1972).

Effects on Pathogen-Antagonist Interactions

Changes in water potential may give the pathogen a greater or lesser competitive advantage, relative to other organisms. Baker and Cook (1973) referred to this effect as

the 'Relative Competitive Advantage' of the organisms. A water potential unfavourable to the antagonists but more unfavourable to the pathogen would enhance biological control while if it were the opposite, a disease outbreak caused by the pathogen would occur. Cook and Papendick (1970) reported that the luxuriant growth of *Fusarium roseum* f. sp. *cerealis* 'Culmorum' in dry soil was an excellent example escape from antagonism by virtue of the ability of this fungus to grow at very low water potential. They suggested that lack of continued growth under wetter conditions (below -8 to -10 bars) was due to the effects of soil bacteria which were inactive in the drier conditions because the bacteria were able to compete more successfully for nutrients than the fungus.

Effects on Host-Pathogen Interactions

There has been few experiments on the influence of plant water potential on host-pathogen interactions. According to Slayter (1967), results based on the water potential concept have shown that water uptake by roots, movement in the vascular system and transpiration (the flow of water vapour from a high energy status within the leaf to a lower energy status in the atmosphere) occurred in response to a water potential gradient. The internal water deficit was determined by the soil water potential which set the base level of the plant water potential and by the extent to which the flow of water within the plant lagged behind evaporative demand. The relationship of plant water potential to disease development was affected by the

transpiratory capacity of the plant (Cook and Papendick 1972). Cook (1972) suggested that fungi that caused cortical decay were exposed primarily to osmotic potential of the necrotic host tissue but that vascular pathogens were exposed primarily to matric potential of the vascular fluid. Thus, with pathogen-antagonist interactions, if the particular water potential was adverse to the host, but did not impose comparable constraints on the pathogens, then disease could be severe, but if the restriction on the pathogen physiology was relatively more than that on the host, then disease might be absent or mild.

An additional effect discussed by Cook and Papendick (1972) was the limitation on movement of the pathogen by the size of soil pores affected by matric potential. They suggested that maximum movement of a micro-organism dependent on the matric potential that allowed the greatest frequency of water-filled pores, large enough to accommodate the organism. They suggested this factor would vary with soil type. Wallace (1958) showed that the movement of larvae of *Heterodera schachtii* was optimal at matric potential of -25 to -40 millibars where the pore diameters were 40-60 μm. Stolzy *et al.* (1965) suggested that zoospores of *Phytophthora* spp. required water-filled pores at least 40-60 μm in diameter before they could move freely.

Little is known concerning the response of *Aphanomyces euteiches* to water potential and temperature interactions. The literature reviewed by Papavizas and Ayers (1974) clearly

showed the importance of soil moisture and temperature on the development of aphanomyces root rot of peas.

3.3 EXPERIMENTAL

3.3.1 Laboratory Studies *in vitro* on the Effect of Water Potential and Temperature on the Pathogen

Introduction - The use of substrates osmotically maintained at various water potentials to study the relations of these factors to fungi was developed by Sommers *et al.* (1970). Cook *et al.* (1972) and Wilson and Griffin (1979) found the method appropriate for their studies on effect of water potential on two root-rot fungi of cereals and some soil basidiomycetes. Thus the effect of five osmotic water potentials on *Aphanomyces euteiches* growth at four temperatures was investigated using a similar method.

Materials and Methods - Two media, Potato Dextrose Agar (Difco), (PDA), and a synthetic agar medium referred to as Basal Medium (BM) were used as substrates (both described in Appendix 2). The osmotic water potential was controlled by the addition of appropriate amounts of KCl or NaCl based on the equation S = -RTCs (Hanks and Ashcroft 1980) in which S is solute potential or osmotic water potential, R is the universal gas constant (82 bars cm^3/mol K), T is the absolute temperature (K) and Cs is the solute concentration (mol/cm^3). After the salts were dissolved, the media

were sterilised by autoclaving, with precautions against evaporation. Any loss in volume of amended media was restored with sterile distilled water. The osmotic water potentials of non-amended media were determined psychometrically to be -3.4 bars and -1.2 bars for PDA and BM respectively at 25°. Thus water potentials were maintained at -3.4 bars (PDA) or -1.2 bars (BM), -5 bars, -10 bars, -20 bars and -30 bars and temperatures were at 10°, 15°, 20° and 25°.

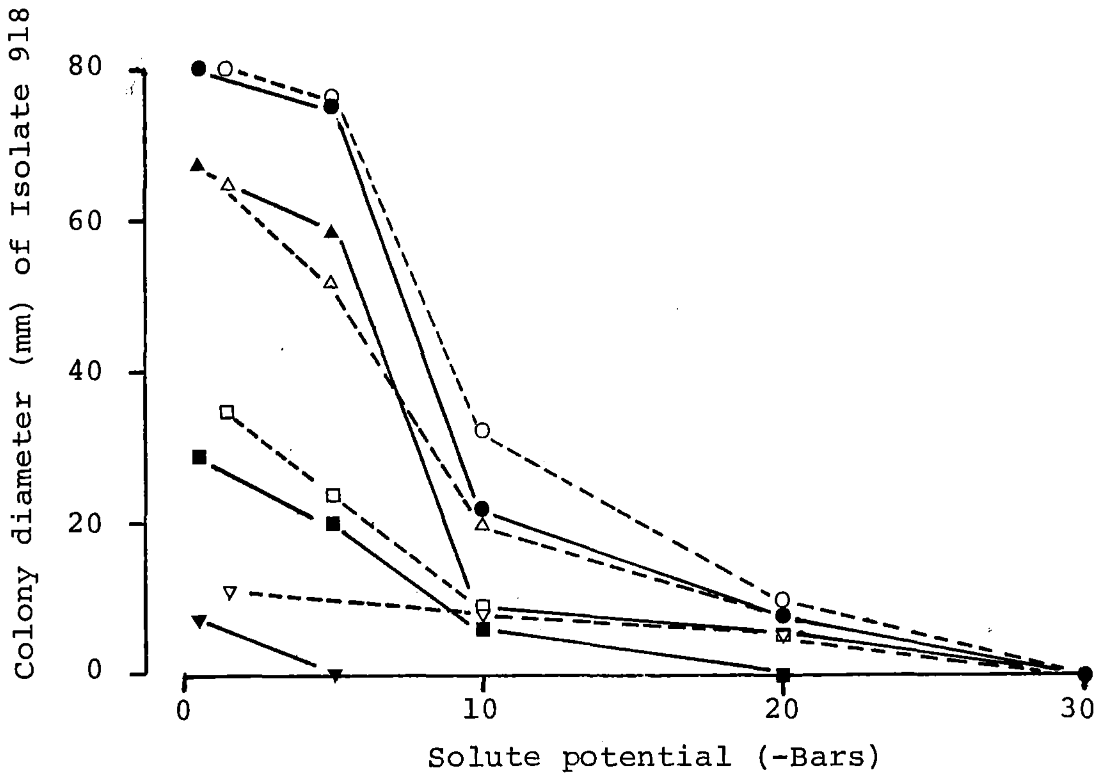
Two isolates of *Aphanomyces euteiches*, Numbers 918 and 919, were obtained from Plant Disease Division, Department of Scientific and Industrial Research, Auckland. The isolates were grown on PDA for 3 days when growth covered three-fourths of the agar surface in petri dishes. Disks (5 mm diameter) were cut from the periphery of the colonies with a sterile cork borer and placed in the centre of an 80 mm diameter plastic petri dish containing 20 ml of osmotically adjusted agar medium. Five replicates at each isolate X potential X temperature of incubation were used. All plates of a given isolate X temperature group were placed together in a sealed plastic bag and the plates incubated in an inverted position. Colony diameters were measured daily for 5 days of incubation when the most rapidly growing colonies had reached the edge of the plates. Rate of growth, i.e. mm/h, was then calculated.

Results - The two isolates of *Aphanomyces euteiches* responded similarly to increasing solute potential. They also reacted similarly to temperature of incubation as shown in Figure 3.1. Maximum growth occurred at the highest solute potential (-1.2 to -3.4 bars) and was reduced to approximately 50% at -10 bars at temperatures 15°-25°. Over the five-day growth period, cessation of growth occurred at about -10 bars at 10° and at -30 bars at 15°, 20° and 25°. Fungal growth was slowest at 10°; it increased as temperature increased.

There were no interactions between solute potential and temperature on fungal growth.

The effects of solute potential and temperature on rate of colony growth are shown in Table 3.1. The radial extension of the colony of both isolates was maximum at the highest water potential and temperature. Growth rate was considerably reduced at < -10 bars and zero at -30 bars.

Discussion - *Aphanomyces euteiches* was shown to require high water potential and warm temperature for maximum growth. As a consequence, decreasing potential and temperature altered the fungal growth by lowering growth rate and prolonging the lag before growth (Scott, 1957).



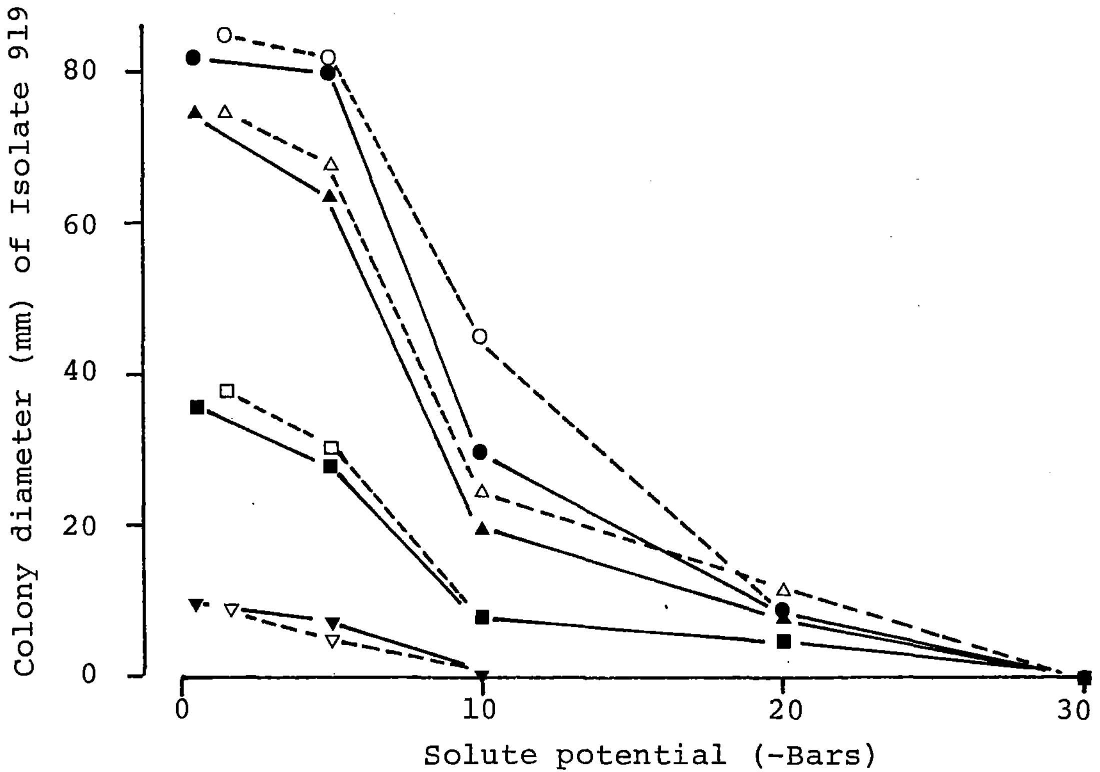


Figure 3.1: Relationship between solute potential and colony growth of *Aphanomyces euteiches* Isolates 918 and 919 on Basal Medium at 25° (●); 20° (▲); 15° (■); 10° (▼) and Potato Dextrose Agar at 25° (○); 20° (△), 15° (□); 10° (▽).

Table 3.1: Effect of solute potential on rate of colony growth of *Aphanomyces euteiches* isolates 918 and 919 on Basal Medium and Potato Dextrose Agar at four temperatures. (*0 solute potential was -3.4 bars for PDA or -1.2 bars for BM.)

Isolate no.	Medium	Temperature (°)	Mean radial growth rate (mm/h)				
			Solute potential (-Bars)				
			0*	5	10	20	30
918	Basal	25	0.67	0.63	0.18	0.07	0.00
		20	0.57	0.50	0.08	0.05	0.00
		15	0.25	0.17	0.05	0.00	0.00
		10	0.07	0.00	0.00	0.00	0.00
	Potato Dextrose	25	0.67	0.63	0.27	0.06	0.00
		20	0.54	0.44	0.17	0.07	0.00
		15	0.29	0.20	0.08	0.05	0.00
		10	0.10	0.07	0.05	0.00	0.00
919	Basal	25	0.68	0.67	0.25	0.08	0.00
		20	0.63	0.54	0.17	0.07	0.00
		15	0.31	0.23	0.07	0.05	0.00
		10	0.09	0.06	0.00	0.00	0.00
	Potato Dextrose	25	0.71	0.67	0.38	0.07	0.00
		20	0.63	0.57	0.22	0.10	0.00
		15	0.32	0.26	0.07	0.05	0.00
		10	0.07	0.05	0.00	0.00	0.00
		$S\bar{x}$	0.004				
		LSD (0.05)	0.05				
		LSD (0.01)	0.09				
		CV%	7.7				

The nutrient status had an effect on the growth - water relations as indicated by the upward displacement of the growth - water potential curve (Figure 3.1) and the increased growth rate at all water potential levels (Table 3.1) with PDA. This nutrient - water potential relation was also observed by Scott (1953) for *Staphylococcus aureus*. Sommer *et al.* (1970) showed that with adequate nutrition, *Phytophthora parasitica* grew well even though water stress was apparent. This suggested that the increased growth of *Aphanomyces euteiches* was more likely a response to the richer media of PDA than to decreased water potential.

3.3.2 Glasshouse Studies on the Effect of Soil Water Potential and Temperature on Infection

Introduction - Results from laboratory studies (Section 3.3.1) had shown the susceptibility of *Aphanomyces euteiches* to water stress and high water potential and warm temperature were required for maximal growth on solid media. These responses may account for the greater incidence of disease caused by the pathogen in wet and warm conditions (Papavizas and Ayers, 1974). Thus two experiments were conducted to investigate the effect of soil water potential and temperature on infection by aphanomyces root rot of peas.

The Soil - The soils used was a Barrhill sandy loam collected from Rakaia in Canterbury on 30 September, 1982. Some physical and chemical properties of this soil are given in Table 3.2.

Table 3.2: Some chemical and physical properties of Barrhill sandy loam (0-20 cm depth) from Rakaia, Canterbury (NZ Soil Bureau Bull. 1968)

Soil properties	Soil zone	
	0 - 75 cm	7.5 cm - 20 cm
pH	6.0	5.8
Organic carbon %	4.0	2.7
Cation exchange capacity meg/100 g	14.6	9.8
Mechanical analysis:		
Sand %	50.0	47.0
Silt %	28.0	34.0
Clay %	13.0	19.0
Dry bulk density g/cm^3	1.16	1.28
Total porosity %	54.5	51.0
Macro porosity %	5.8	5.4

The DSI, inoculum density and previous 4 crops grown in the three soils, naturally infested with *Aphanomyces euteiches*, and number of years since last pea crop are given in Table 3.3.

A non-infested soil, to be used as control, was collected from the same location from a field in which peas had not been grown for almost 20 years. As a further check for the presence of *Aphanomyces euteiches*, it was examined microscopically as described in Section 2.3.3 in Chapter 2.

Table 3.3: Disease severity indices (DSI) and inoculum densities of three Barrhill sandy loam soils, naturally infested with *Aphanomyces euteiches*, and obtained from Rakaia, Canterbury on 30 September 1982 (previous four crops grown in each field before sampling and number of years since last pea crop are indicated).

Soil sample (DSI)	Inoculum density (no. of oospores per 100 g soil)	Previous four crops				Number of years since last pea crop
		1978	1979	1980	1981	
100	783	pea	white clover	wheat	barley	4
75	219	ryegrass/ white clover	ryegrass	pea/ white clover	wheat	2
50	77	white clover	wheat	ryegrass/ white clover	pea	1

<u>Determination of Soil Water Potential and Moisture Content</u> - Water potentials between -0.2 bars and -0.02 bars were determined by Tension Tables (Hall *et al.* 1977) and between -15 bars and -0.5 bars by ceramic pressure plates (Soil Moisture Equipment Corporation, California, USA). The tension tables, a form of tensiometer, were adjusted at a height of 200 cm, 170 cm, 100 cm, 50 cm and 20 cm above reservoirs of water contained in beakers to obtain water potentials of the soil placed on the tension tables of -0.2, -0.17, -0.10, -0.05 and -0.02 bars. Similarly, the gas pressure in the ceramic pressure plates apparatus was maintained at either 0.5, 1.0 or 15 bars, so that the water content of the soil placed on the plates would be at similar potentials. To obtain the drying boundary curve of the soil moisture characteristic curve, the soil sample of about 10 g was spooned into a circular rubber ring (5 cm diameter and one cm depth), placed over a square nylon gauze on the porous plates of the tension tables or ceramic pressure plates. The soil samples were saturated with distilled water and water was withdrawn by the potential treatments until an equilibrium was reached a week later. The water held in the soil sampler, subject to the different potentials, was measured gravimetrically (% water), i.e. as g of water per g of soil oven-dried for 72 h at 105°. Three replicates of each soil sample were determined at each water potential.

The relationship between water potential and moisture content of Barrhill sandy loam from Rakaia, Canterbury, is shown in Figure 3.2. When the water potential was expressed in logarithmic form, there was a significant relationship between water potential (expressed in -Log Bars) and the moisture content of the soil with a correlation coefficient of r = 0.98 (Figure 3.3). This logarithmic relationship was used to determine water potential corresponding to the moisture levels used as treatments in subsequent experiments.

Determination of Soil Holding Capacity - The water-holding capacity of the soil was determined using Keen-Raczkowski boxes made of brass (manufactured after Keen and Raczkowski 1921 and modified by Coultz 1930). They were cylindrical with a diameter of 50 mm and were 25 mm deep with perforated bottoms (holes of 0.75 mm in diameter and 5 mm apart). A circular filter paper cut to the internal measurement of the base was placed in each unit which was weighed and then filled with air-dried soil sieved through 1.70 mm mesh. The test was replicated three times for each soil sample. Approximately 10 g of soil was added at a time and the box tapped on the bench after each addition. When it was nearly full sufficient soil was added to allow the surface to be struck off flat (with a spatula) and the whole box with soil then was weighed. The box and contents were placed in a flat bottomed dish containing about 8 mm depth of distilled water and left overnight. When a number of boxes were placed in the same dish, additional water had to be added at intervals to keep the level constant. The

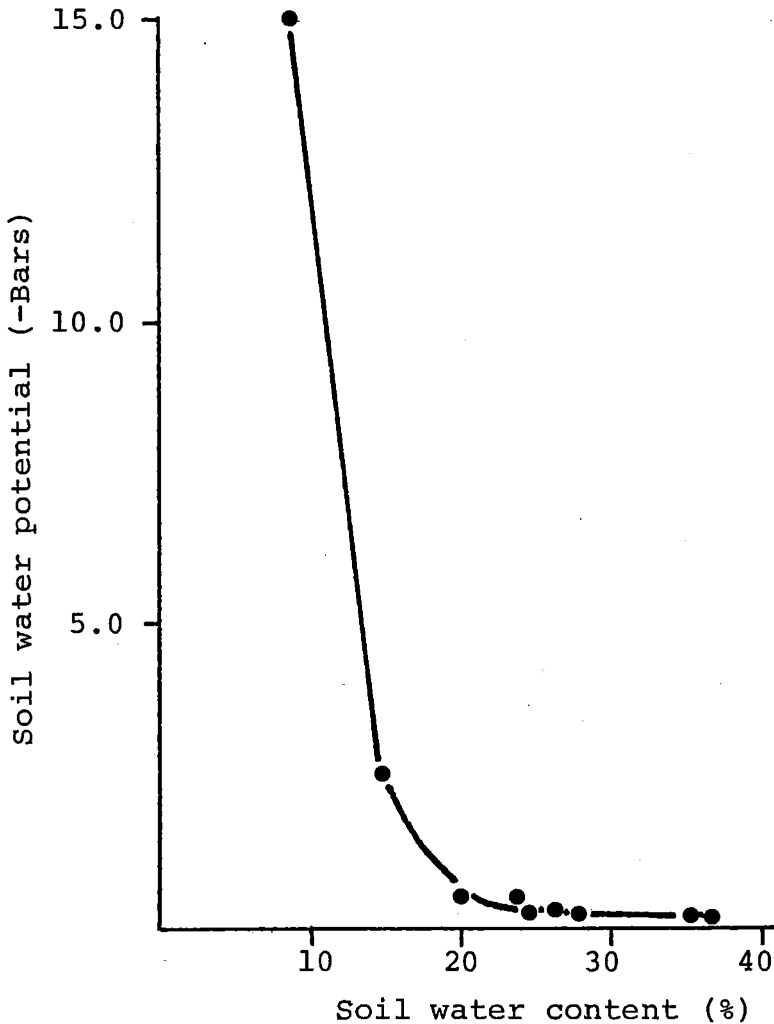


Figure 3.2: Soil water potential and water content relationship for Barrhill sandy loam from Rakaia, Canterbury. The curve shown is the drying boundary curve determined by Tension Tables (Hall *et al.* 1977) and ceramic pressure plates (Soil Moisture Equipment Corporation, California, USA).

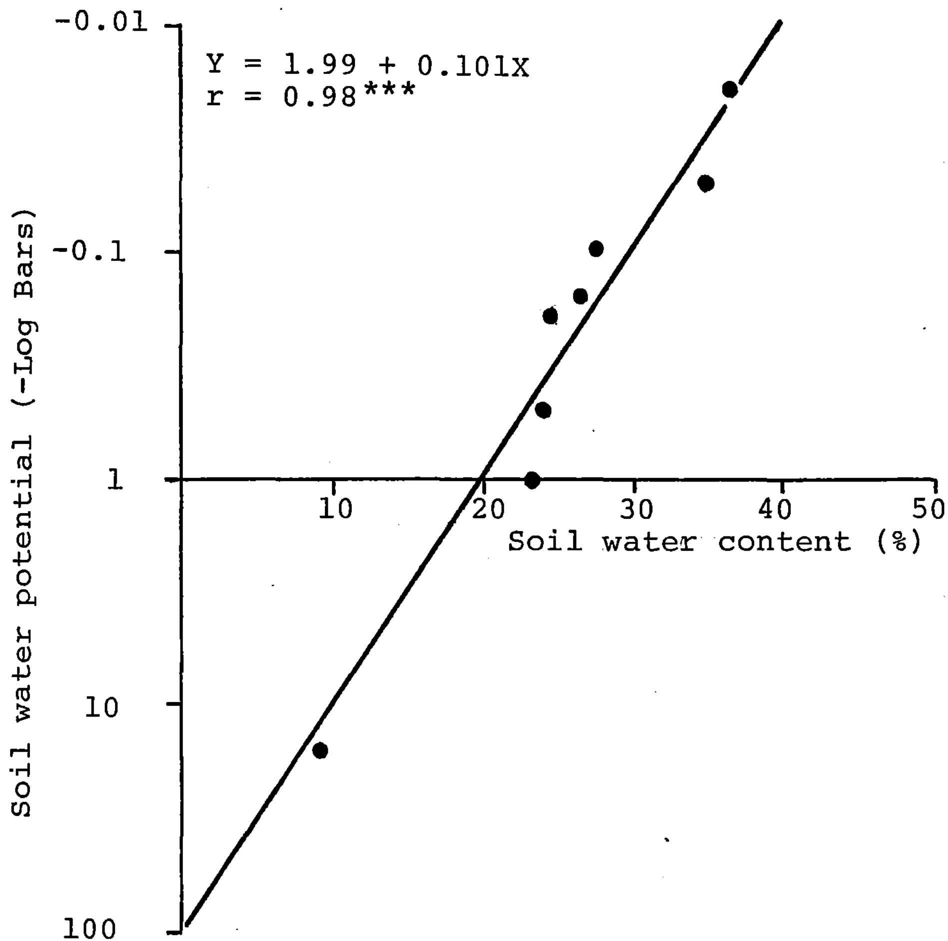


Figure 3.3: The relationship between logarithmic transformation of soil water potential (-Log Bars) and soil water content (%) of Barrhill sandy loam from Rakaia, Canterbury (method same as Figure 3.2).

boxes were removed and placed on a stainless steel rack in another flat-bottomed dish to allow excess gravitational water to drain from the samples. Each dish was covered with aluminium foil to prevent evaporation of water from the soil. After 48 h, the boxes were dried on the outside, weighed and oven-dried at 105° for 72 h. At the end of this period, they were cooled in a desiccator and reweighed. Waterholding capacity was calculated by the amount of water held in the soil expressed as percentage of weight of oven-dried soil.

Table 3.4 shows the moisture contents corresponding to the soil matric potentials (Figure 3.3) at 50%, 65%, 70%, 80% and 90% of the waterholding capacity of Barrhill sandy loam. The water content at 100% waterholding capacity when determined by Keen-Raczkowski boxes method was found to be 55%.

Table 3.4: Water content and water potential of moisture regimes of Barrhill sandy loam from Rakaia, Canterbury, determined by Tension Tables (Halls *et al.* 1977) and ceramic pressure plates (Soil Moisture Equipment Corporation, California, USA) with a waterholding capacity of 55% determined by Keen-Raczkowski boxes method.

Percentage of water-holding capacity	Soil water content (%)	Soil water potential (- millibars)
50	27.5	200.0
65	35.8	28.0
70	38.5	14.0
80	44.0	3.0
90	49.5	1.0
100	55.0	0.0

According to Hodgson (1978), where soil was categorised as wet (-10 millibars), moist (-1000 millibars) and dry (-10,000 millibars), the treated soil maintained at 50% waterholding capacity was classified as between moist and dry; 65% and 70%, moist; and 80% and 90%, wet.

Preparation of Pots - For all experiments, the soil was packed in pots, 85 mm in diameter and 180 mm in depth and without holes in the bottom. The soil was packed so that the bulk density was similar to field conditions. The bulk density was obtained by using a graduated soil core sampler (2.54 cm diameter). The core sampler was inserted, always to a depth of 20 cm where soil was being collected. Each soil sample of 101.4 cm^3 from the core sampler was placed in a plastic bag and taken to the laboratory to be weighed. Bulk density was calculated and expressed as percentage of weight of soil divided by volume after it had been oven-dried at 105° for 72 h.

The Wisconsin Soil Temperature Tanks - The experiments were conducted in Wisconsin soil temperature tanks in the Microbiology Department glasshouse at Lincoln College. The design of the Wisconsin tanks was based on that originally used at Rothamsted, England, and modified by Plant Diseases Division of the Department of Scientific and Industrial Research, Mount Albert, Auckland (Menzies and Manning (pers. comm.). An overall view of the tanks is in Figure 3.4. The unit consisted of 8 tanks, 4 in each of two glasshouse units. Each tank fitted into a frame at

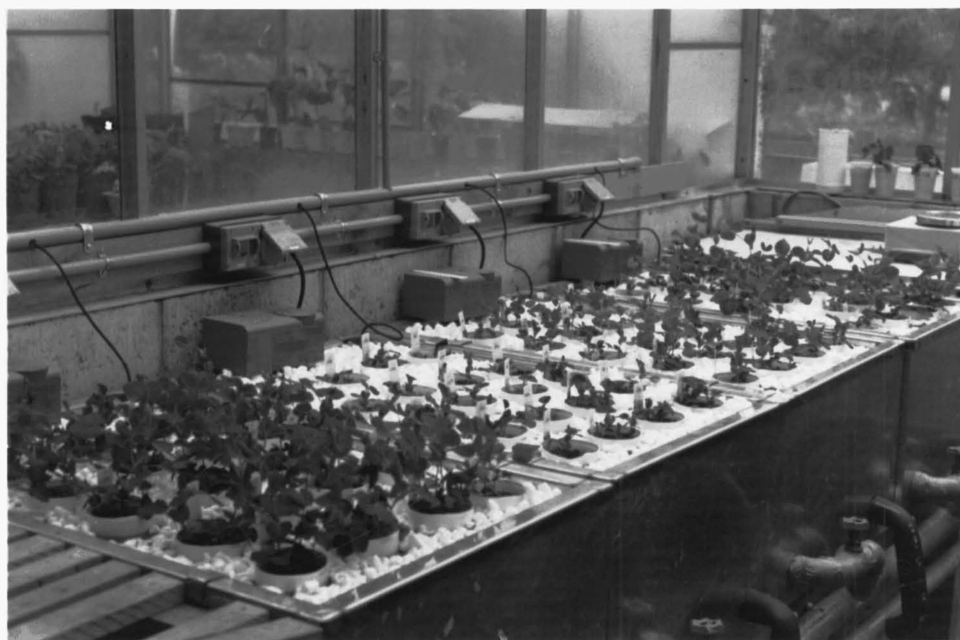
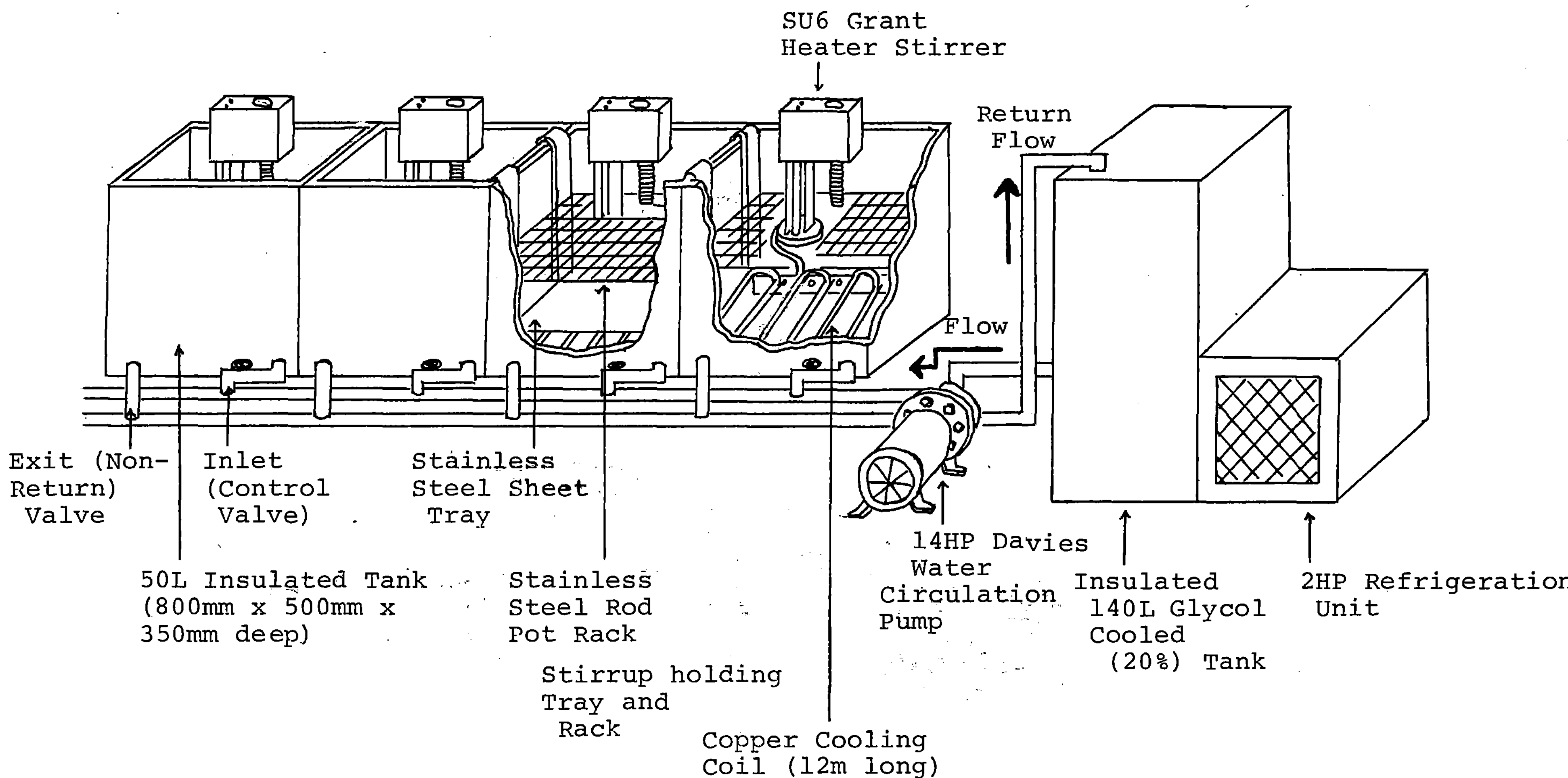
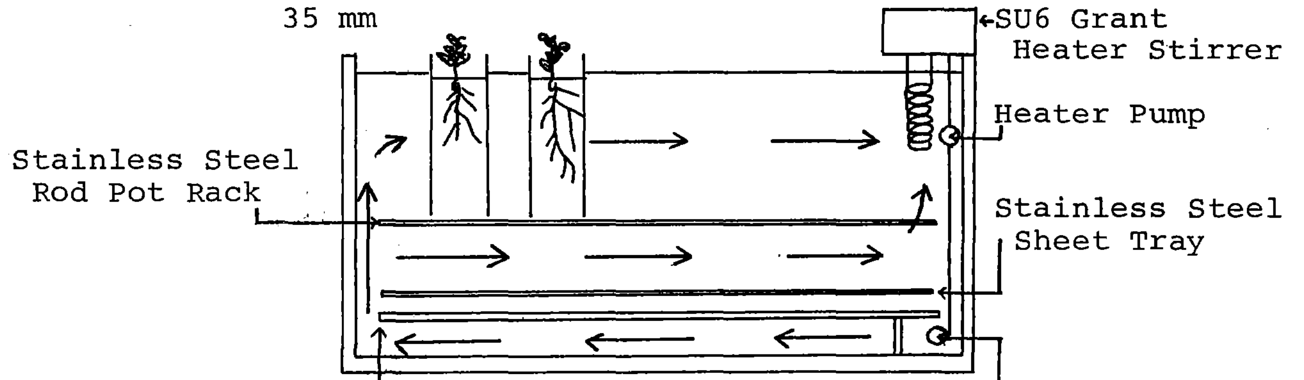


Plate 3.4: The Wisconsin soil temperature tanks located in the Microbiology Department glasshouse in Lincoln College. Plan views of the 8-tank unit are also included.



GENERAL VIEW OF WISCONSIN SOIL TEMPERATURE TANKS IN MICROBIOLOGY DEPARTMENT GLASSHOUSES AT LINCOLN COLLEGE.

SIDE VIEW OF 50L INSULATED TANK



PLAN OF AN 8-TANK UNIT

SU6 Grant Heater Stirrer

500 mm

800 mm

12-Print Chart Honeywell Versaprint Recorder

Insulated 140L Glycol Cooled (20%) Tank

2HP Refrigeration Unit

¼HP Davies Water Circulat-ing Pump

Copper Cooling Pipe (12m long)

Inlet Control Valve

Flow of Glycol

Exit (Non-Return) Valve

bench height. Each tank was made of stainless steel with an internal dimension of 800 x 500 x 350 mm, was surrounded by 35 mm thickness of insulation, provided with a drain for emptying and with suitable inlet and outlet apertures. These were connected to copper coils, 12 m long x 10 mm internal diameter, in the base of each tank, for cooling the water. A pot rack made from stainless steel rod was fitted in each tank. The water in the tank was kept circulating, at 10 litres per min, by a SU6 Grant heater stirrer (Grant Instrument, Cambridge, Ltd, England). All tanks were cooled by cold glycol (2°-5°) circulating through the copper coiling coils; the glycol originated from a main tank, 450 mm diameter x 600 mm height insulated with polystyrene beads. Glycol was pumped using a Davies Water Circulating Pump to the individual water tanks entering through a needle valve and circulating in the tank through the copper cooling coils. The rate of flow was adjustable so that the temperature of the tank would remain constant when air temperature exceeded that of water in the tank. The refrigeration unit reduced the temperature of the glycol to 2°-5°. The Wisconsin tanks were heated by the SU6 Grant Heater Stirrer which had a one kw element. The temperature of water was controlled by a proportionating thermostat with an accuracy of $\pm$ 0.05^{o} where power to the element was pulsed. Shorter pulses were indicated when the tank was closer to the desired temperature. The effect of air temperature on the tank water temperature was controlled by wall insulation at the sides and bottom of the tanks and polystyrene beads floating on the water surface around the pots.

A 12-print chart Honeywell Versa print recorder equipped with a print cycle timer, which controlled the chart speed at 15 mm/h (MSI Honeywell Ltd, Christchurch) was installed to monitor the temperature of water in each tank, the glycol in the refrigeration tank and air temperature above the plants.

EXPERIMENT ONE: Effect of Soil Moisture and Temperature of Aphanomyces Root Rot

Introduction - This experiment was conducted in Wisconsin soil temperature tanks to investigate the effect of soil moisture (expressed in soil water potential) and temperature on infection of peas with aphanomyces root rot, and on subsequent disease development.

Materials and Method - A split plot design was used for the experiment which consisted of two blocks of main treatments (temperatures of 8°, 12°, 16° and 20°) and subtreatments, moisture levels at -200 millibars (soil water content % (SWC of 27.5), -28 millibars (SWC = 35.8) and -3 millibars (SWC = 44.0) with soil of DSI 100. Each block consisted of four Wisconsin tanks randomly set at 8°, 12°, 16° and 20° in one glasshouse unit with air temperature maintained at about 25°. The experiment was established on 7 December, 1982.

Four pea seeds of cultivar Canterbury 39 were sown at a depth of 2 cm in each pot (as described in 'Pot Preparation'

section). Pots were watered each day for 3 days as required to maintain the soil moisture at -3 millibars in order to promote vigorous growth and seedling establishment. Moisture regimes were begun on the fourth day after sowing. The weight of each pot and the soil in it were recorded and the water required was calculated for each of the moisture regime treatments. Pots were checked daily at 2pm by weighing and watered to replace water lost by evapotranspiration.

The experiment was terminated at four weeks after sowing when the plants were removed from the pots and the soil removed from them by thorough washing. The number of nodes on each plant and the whole plant dry weights (obtained by oven-drying at 70° for 48 h) were recorded. In addition, all plants were rated for disease severity according to the Disease Classes in Figure 2.2 (Chapter 2).

Results - The main effects and interactions of moisture and temperature on disease severity, growth stage, dry matter weight and top:root (T/R) ratio of the pea plants grown in soil DSI of 100 are shown in Table 3.5.

Disease severity was shown to be enhanced with increasing temperature and moisture. There was a significant linear increase (P = 0.05) with increased temperature. No visual symptoms were observed at 8° and disease severity was recorded zero. Root rot development was not severe at 12° and 16°. However, at 20°, the disease severity increased to 44.9%, three times more severe than at 16°.

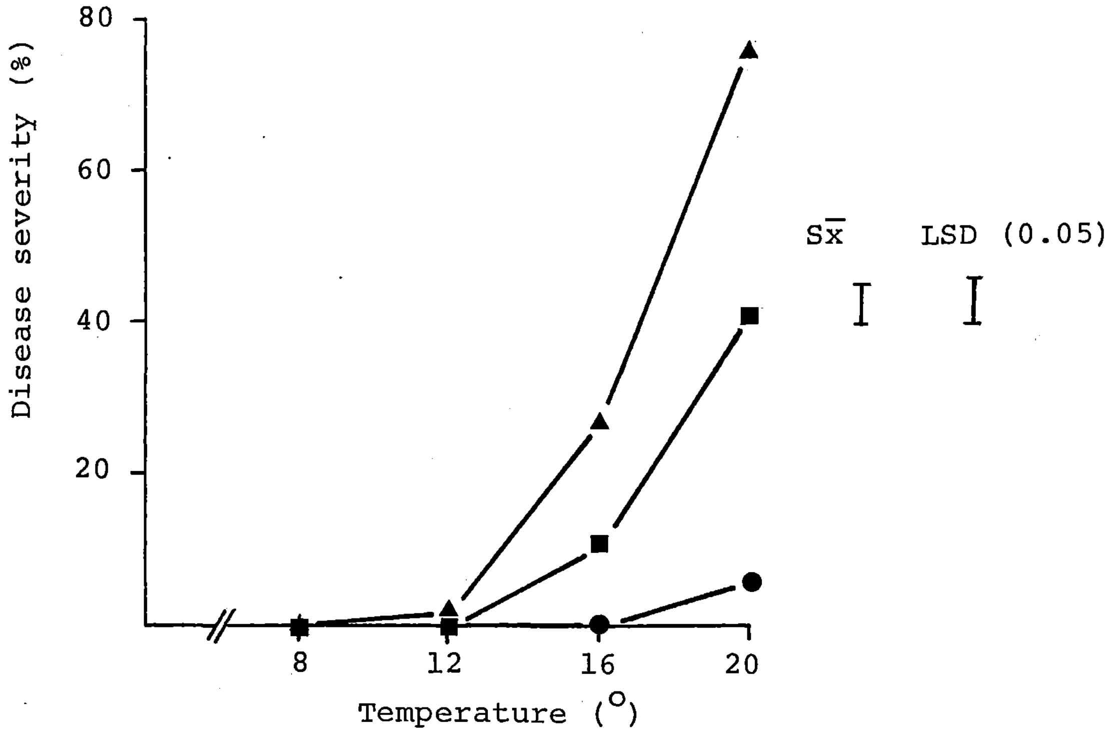
Table 3.5: The effect of soil temperature and moisture on severity of *Aphanomyces euteiches* root rot at four weeks of growth as shown by growth stage (number of nodes), dry weight and top:root ratio of pea cultivar Canterbury 39 grown in Barrhill sandy loam soil of DSI 100 (*mean weight of four plants).

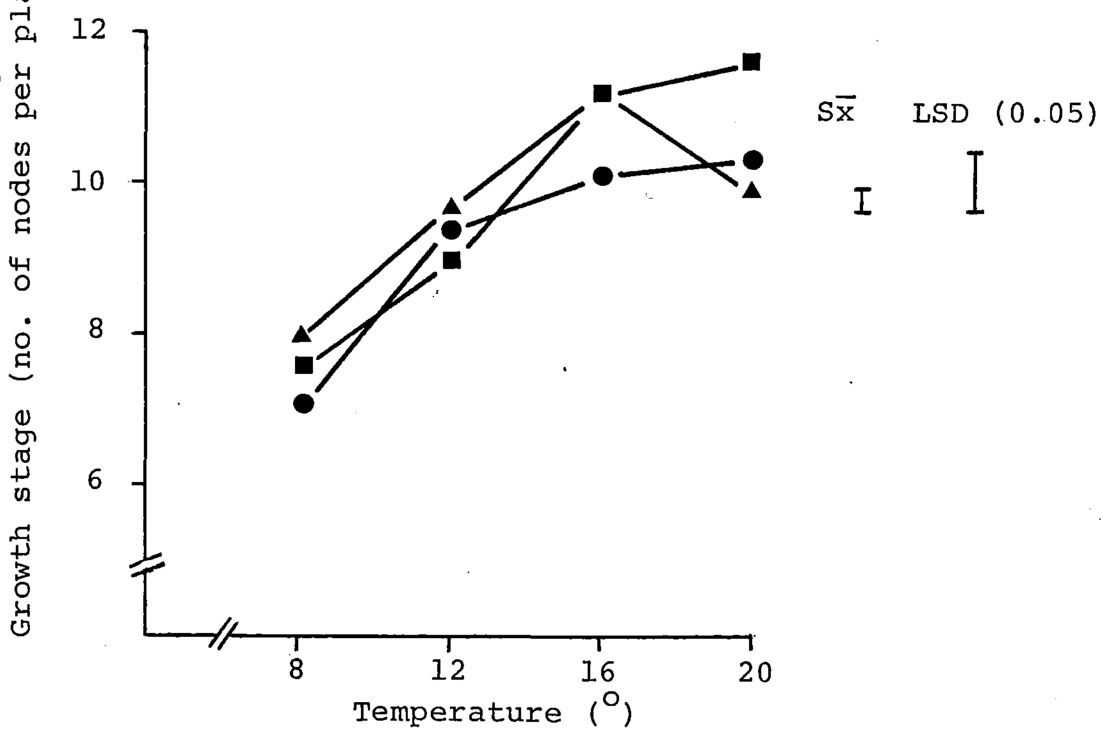
Factors		Disease severity (%)	Growth stage	*Dry matter weight (mg per plant)			Top:root ratio
				Top	Root	Total	
Temperature ° (T)	8	0.0	7.6	140	177	318	0.87
	12	0.5	9.4	218	195	413	1.21
	16	12.5	10.8	309	199	508	1.57
	20	44.9	10.6	382	121	503	3.50
	$S\bar{x}$	4.85	0.23	29.6	25.9	4.3	0.101
Significance		*L	**L	*L	NS	NS	**L
Moisture (-millibars) (M)	-200	1.7	9.2	218	188	406	1.36
	- 28	15.6	9.8	273	179	452	1.61
	- 3	26.1	9.7	296	152	448	2.39
	$S\bar{x}$	0.86	0.11	13.5	9.4	19.6	0.150
Significance		**L	*L	**L	NS	NS	**L
Significant interaction							
TXM		**L	**L	*L	NS	*	*L
CV%		16.8	3.3	14.6	15.4	12.7	23.8

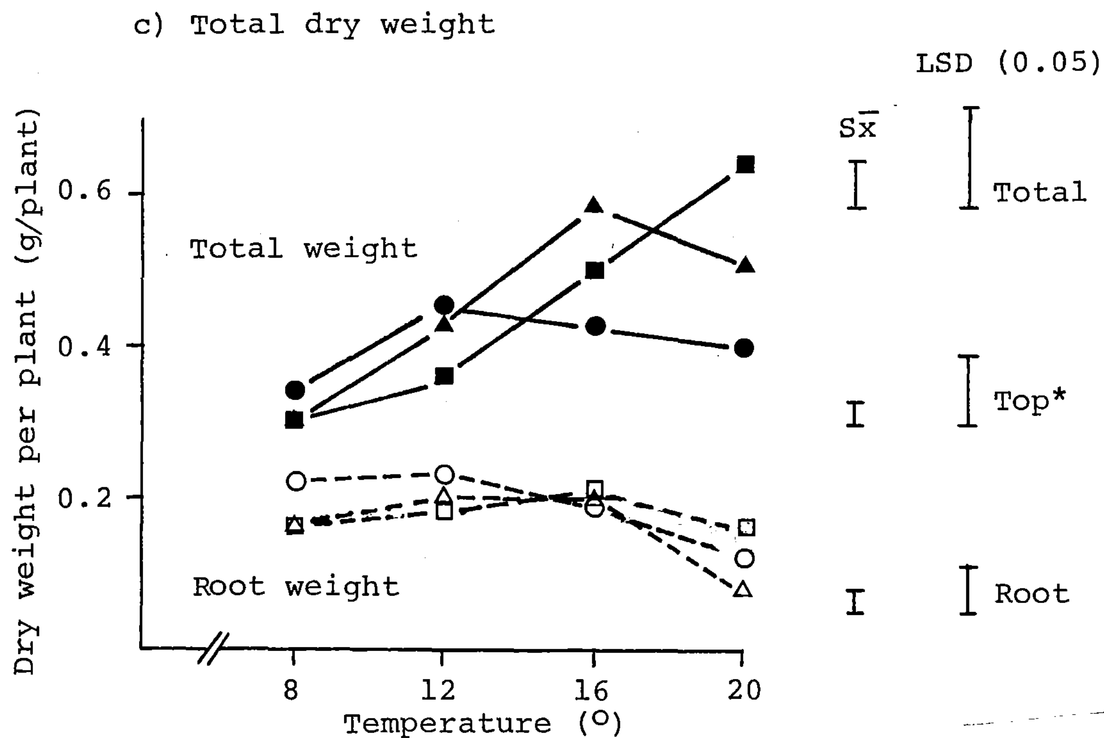
Moisture effects showed a linear increase in root rot development at a highly significant level (P = 0.01). Infection in plants maintained at -200 millibars was not severe, but had increased to 15.6% at -28 millibars and 26.1% at -3 millibars. A highly significant linear interaction between these two factors was also observed as shown in Figure 3.5a. At a temperature of 8°, there were no disease symptoms at all moisture levels. There were slight symptoms at 12°, only at -3 millibars. Highly significant differences were recorded between the moisture levels at temperatures above 16°. At -200 millibars, disease symptoms only occurred at 20°.

Growth of the pea plants was not only affected by colder temperature and drier conditions, but also by the root rot infection at warmer temperatures and wetter conditions. This was shown by the highly linear response (P = 0.01) of growth stage with an increasing number of nodes between 8° and 16° and a slight reduction at 20°. Similarly, a linear response (significant at P = 0.05) to increasing moisture was obtained. At -3 millibars, growth was affected by infection. Interaction effects between temperature and moisture shown in Figure 3.5b clearly showed the extent of these factors on growth.

Higher temperatures resulted in a linear increase (significant at P = 0.05) in top dry matter yields. Plants accumulated 55%, 120% and 172% more top dry matter at 12°, 16° and 20° respectively than at 8°. Higher moisture levels also caused an increase in top dry matter linearly, at a more


a) Disease severity
Disease severity (%)
80
60
40
20
8
12
16
20
Temperature (°)
Sx̄
LSD (0.05)


b) Growth stage
Growth stage (no. of nodes per plant)
12
10
8
6
8
12
16
20
Temperature (°)
Sx̄
LSD (0.05)



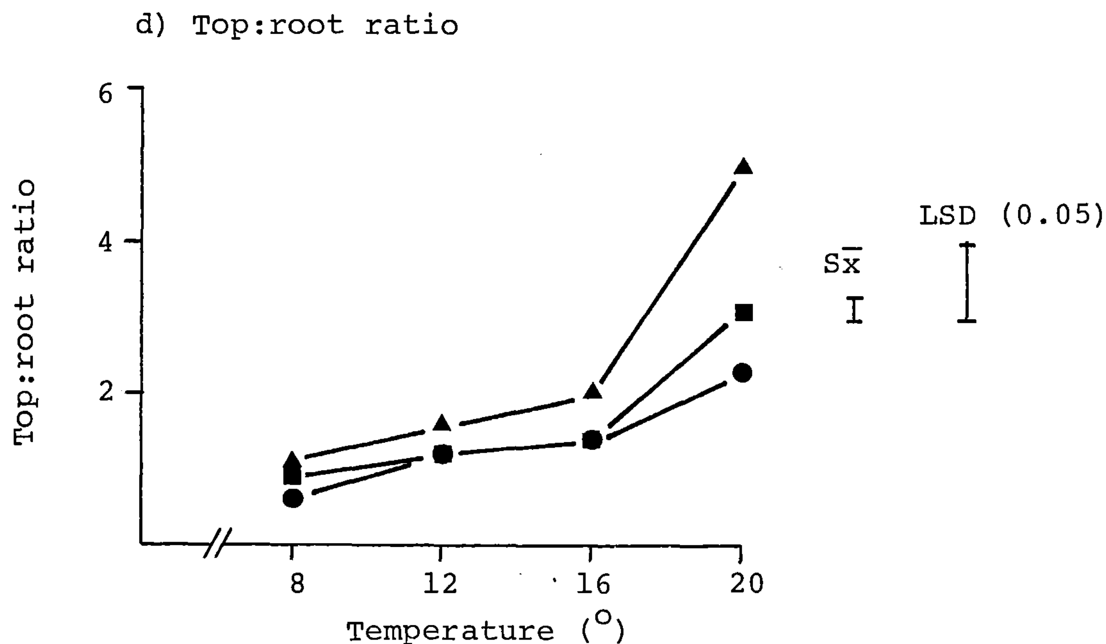


Figure 3.5: Effect of temperature and moisture interactions (-200 millibars ●; -28 millibars ■; -3 millibars ▲) on a) disease severity of aphanomyces root rot, b) growth stage, c) total dry weight per plant and d) top:root ratio of pea cultivar Canterbury 39 grown in Barrhill sandy loam with Soil DSI 100.

*Top dry weight is the difference between total and root dry weights).

significant level ($P = 0.01$). There were 25% and 35% differences in yield between plants grown in soils with the moisture maintained at -28 millibars and -3 millibars compared with those plants held at -200 millibars. Figure 3.5c shows a linear significant response ($P = 0.05$) to interactions between temperature and moisture on top dry matter. Higher yields were obtained at warmer temperatures and wetter conditions with the exception that at 20°, there was an 11% higher yield at -28 millibars than at -3 millibars.

Root dry matter increased as temperature increased from 8° to 16° with a reduction at 20° and at increased moisture levels due to root rotting. However, differences between temperatures and moisture levels were not significant. No interaction between the treatment factors was observed.

Total dry matter yield was related to temperature in a similar way to root dry matter. The response of total dry matter to moisture levels resulted in higher yields at -28 millibars and -3 millibars than at -200 millibars (by 10%). Significant interaction ($P = 0.05$) between temperature and moisture on total dry matter yield is shown in Figure 3.5c. Yield increased with temperature changes from 8° - 12° at all moisture levels, and started to decrease beyond 12° at -200 millibars and 16° at -3 millibars while no decrease was observed at -28 millibars as temperature increased.

When the ratio of top:root dry weight (T/R) was calculated, there was a highly significant linear response

(P = 0.01) to temperature and moisture. Temperature increased the T/R ratio from 0.87 at 8° to 1.21 at 12° (a 39% increase), to 1.57 at 16° (80%) and to 3.50 at 20° (300%). The T/R ratio was increased from 1.36 at -200 millibars to 1.61 at -28 millibars (+ 18%) and 2.39 at -3 millibars (+ 76%). As is shown in Figure 3.5d, a significant linear response (P = 0.05) was obtained to the temperature-moisture interaction.

EXPERIMENT TWO: Effect of Temperature and Moisture on Severity of aphanomyces Root Rot at Four Soil Inoculum Levels

Introduction - In this experiment, the effect of temperature and soil moisture on severity of aphanomyces root rot at four soil inoculum levels was investigated using the Wisconsin soil temperature tanks.

Materials and Method - The experiment was of a split plot design and consisted of four blocks of main treatments (soil temperatures of 20° and 25°) and subtreatments (three moisture levels at -200 millibars, -28 millibars and -3 millibars x 4 soil inoculum levels of DSI 0 (Control), 50, 75 and 100). Each subtreatment was replicated 6 times. The experiment was established on 28 February, 1983.

Four pea seeds of cultivar Canterbury 39 were sown at a depth of 2 cm in each pot (as described in 'Pot Preparation' section). Water treatments maintained throughout the experi-

ment were similar to those described in Experiment One. The experiment was terminated on 1 April, 1983, 32 days after sowing. Plants were removed from the pots and the soil was removed by thorough washing. The severity of aphanomyces root rot was assessed according to the Disease Classes (Figure 2.2, Chapter 2), and the number of nodes of the plants counted. Total dry weight was obtained by oven-drying at 70° for 48 h.

Results - The main effects of temperature and soil moisture on disease severity, growth stage, dry matter weight and T/R ratio of pea plants at the four soil inoculum levels are shown in Table 3.6.

There were no significant differences that could be attributed to temperature. The responses to soil moistures and inoculum levels were highly significant (P = 0.01) and severity increased linearly as level of moisture increased (-200 millibar to -3 millibar) or inoculum increased. A significant quadratic response to temperature and inoculum interaction was obtained as shown in Figure 3.6a. Disease severity increased linearly with increased inoculum at 20°. At 25°, a linear increase was observed only between 0 and 75 DSI. Above 75 DSI, a diminishing curve response was obtained. There was a highly significant interaction (P = 0.01) between moisture and inoculum on disease severity as shown in Figure 3.7a. At inoculum levels below 50 DSI, there were no significant differences in disease severity at the three moisture levels. However, above that inoculum level, there was a

Table 3.6: Effect of temperature and moisture level on severity of aphanomyces root rot, 32 days after sowing, as shown by dry matter weight (mean weight of 4 plants), number of nodes and top:root ratio of pea. The cultivar used was Canterbury 39 grown at 4 inoculum levels of Barrhill sandy loam.

Factors		Disease severity %	Number of Nodes	Dry matter weight (mg/plant)			Top:root ratio
				Top	Root	Total	
Temperature (°) (T)	20	49.7	10.9	322	179	500	2.30
	25	55.9	10.4	304	139	444	3.22
	$S\bar{x}$	0.73	0.22	5.7	8.0	12.7	0.446
Significance		NS	NS	NS	NS	NS	NS
Moisture (-millibars) (M)	-200	43.6	10.7	292	161	453	2.18
	- 28	54.0	10.9	325	171	496	2.60
	- 3	60.8	10.4	322	145	467	3.50
	$S\bar{x}$	1.56	0.13	9.6	7.2	13.3	0.339
Significance		**L	*L	*L	NS	NS	*L
Inoculum level (DSI) (IL)	0	0.0	11.9	381	250	630	1.53
	50	48.6	11.4	361	205	566	1.80
	75	72.1	10.4	298	115	413	3.39
	100	90.5	9.0	212	67	279	4.33
	$S\bar{x}$	1.81	0.15	1.1	8.4	15.3	0.392
Significance		**L	**L	*L	**L	**L	**L
Significant interaction	TXM	NS	NS	NS	NS	NS	NS
	TXIL	*Q	NS	NS	*Q	*Q	NS
	MXIL	**L	*L	*L	*L	**L	NS
	TXMXIL	NS	NS	NS	NS	NS	NS
	CV%	11.8	4.8	12.3	18.2	11.2	49.2

a) Disease severity

Disease severity (%)

100
80
60
40
20
9

LSD (0.05)
$S\bar{x}$

0 50 75 100

Inoculum level (Soil DSI)

b) Total dry weight

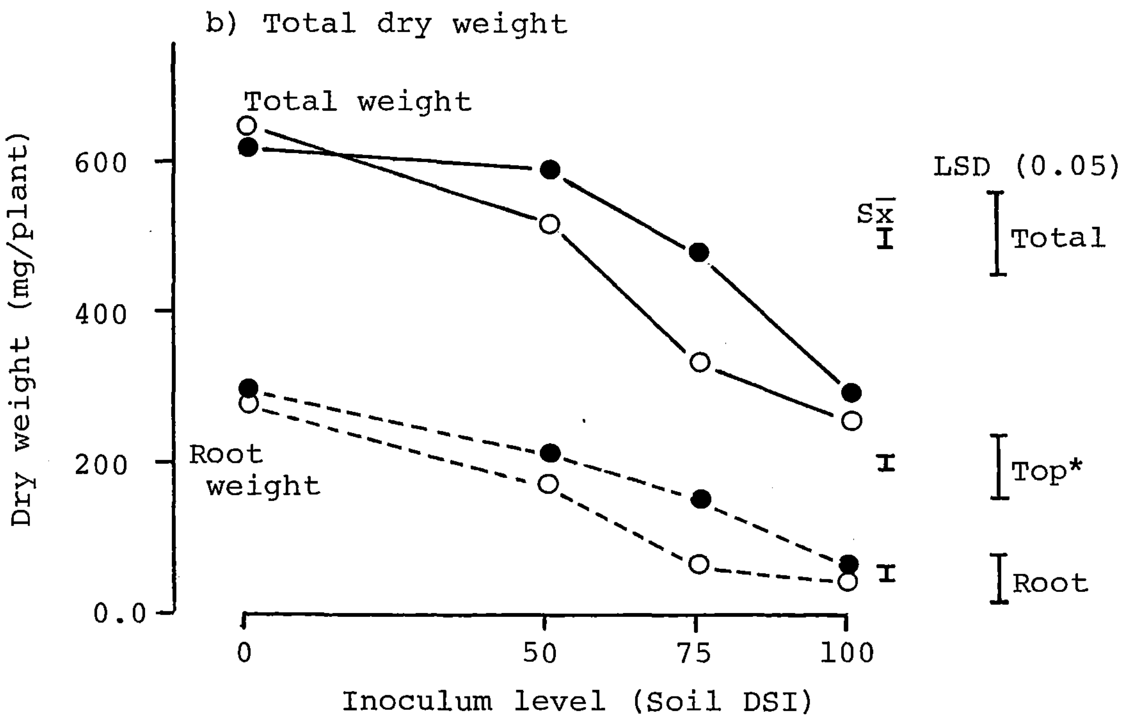


Figure 3.6: Effects of temperature (20° ● ; 25° ○) and inoculum interaction on aphanomyces root rot as shown by a) disease severity and b) dry weight of pea cultivar Canterbury 39 grown in Barrhill sandy loam in the experiment conducted in Wisconsin Tanks at 32 days after sowing.

*Top weight is the difference between total and root weights.

a) Disease severity

100
80
60
40
20
0

Disease severity (%)

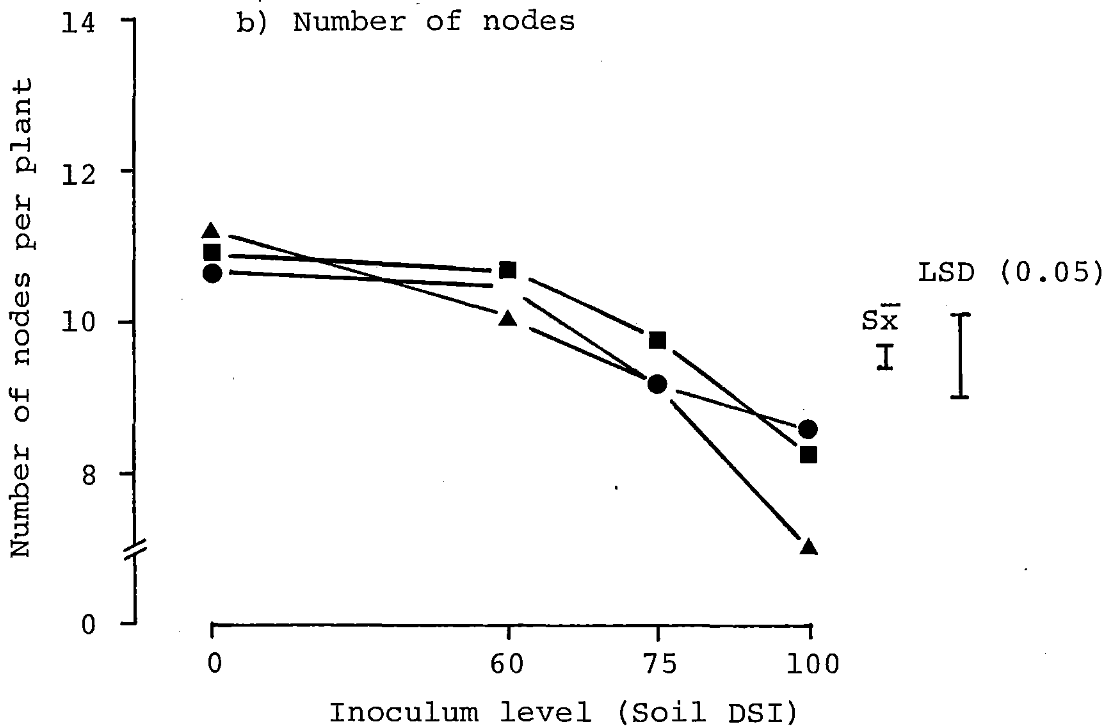
LSD (0.05)

Sx̄

▲ -3 millibars
■ -28 millibars
● -200 millibars

0 50 75 100

Inoculum level (Soil DSI)



c) Dry weight (mean weight of 4 plants)

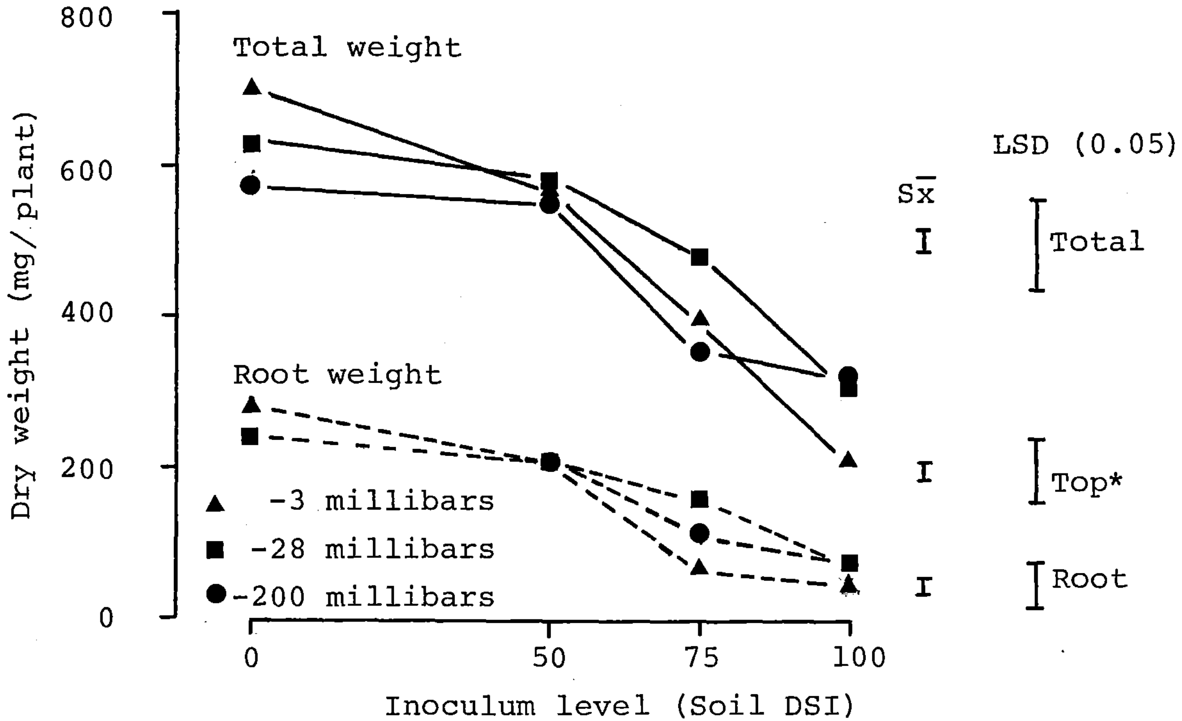


Figure 3.7: The effect of moisture (-3 millibars, -28 millibars, -200 millibars) and inoculum level (DSI = 0, 50, 75, 100) interaction on aphanomyces root rot at 32 days after sowing as shown by a) disease severity, b) number of nodes per plant and c) dry weight of pea cultivar Canterbury 39 grown in Barrhill sandy loam in an experiment conducted in Wisconsin Soil Temperature Tanks.

*Top weight is the difference between total and root weights.

significant increase of 60% at inoculum level 75 DSI and 45% at 100 DSI in disease severity between -200 millibars and -3 millibars. No significant interactions among the three main factors were observed, but generally, disease development was more severe at higher soil moisture levels and temperaturss at all inoculum levels.

There were no significant differences in number of nodes on plants growing at 20° and 25°; however, more nodes were recorded on plants grown at 20°. There was a negative significant linear response ($P = 0.05$) to the effect of moisture on the number of nodes per plant. The number decreased in wetter soils. Likewise, the number of nodes decreased (highly significant at $P = 0.01$) in response to increased inoculum. A significant reduction in the number of nodes was observed only at inoculum levels above 50 DSI, where there was a 13% and 25% decrease in node number with inoculum levels of 75 DSI and 100 DSI. There were no interaction effects between temperature and moisture and temperature and inoculum levels. However, interactions between moisture and inoculum levels showed a highly significant linear effect on number of nodes as shown in Figure 3.7b. The reduction in number of nodes was more dramatic at -3 millibars than at -200 millibars and -28 millibars at increased inoculum levels. At 100 DSI, the highest number of nodes was recorded at -200 millibars. No significant interactions occurred between the three main factors. Not only did the higher moisture at both temperatures favour growth as indicated by plants grown in inoculum of 0 DSI, but it also enhanced

severe infections in infested soil.

Top dry matter yields were not affected by temperature changes in the soil (Table 3.6). There was a significant moisture effect (P = 0.05) on top dry matter with a positive linear response to increasing moisture. However, no differences occurred between -28 millibars and -3 millibars. Yield decreased significantly (P = 0.01) at increasing levels of inoculum. Plants accumulated less top dry matter in DSI 50 (5%), 75 (21%) and 100 (44%) compared to DSI 0. The only significant interaction occurred between moisture and inoculum levels as shown in Figure 3.7c. Higher yield was observed at inoculum levels less than soil DSI 75 when there was more moisture. However, less yield was obtained at -3 millibars with soil DSI 100.

There were no differences in root dry matter yield due to temperature and moisture levels. However, root dry matter yielded 22% more at 20° than at 25° and about 10% more in soil at -200 millibars than that at -28 millibars. A highly significant linear decrease (P = 0.01) in root dry matter resulted from increased inoculum levels. Plants accumulated 18%, 54% and 75% less in soils of DSI 50, 75 and 100 than in soil of DSI 0. There was a significant quadratic response (P = 0.05) to temperature and inoculum interactions as shown in Figure 3.6b. Root dry matter decreased as inoculum levels increased with greater reductions at 25°. At the warmer temperature of 25°, root dry matter did not differ significantly between soils of DSI 75 and DSI 100.

A similar response was found with moisture and inoculum level interaction on top dry matter as shown in Figure 3.7c.

The effects of temperature, moisture and inoculum on top dry matter were similar to root dry matter.

Temperature had no significant effect on T/R ratio. Increased moisture caused an increase of the T/R ratio significantly (P = 0.05) from 2.18 at -200 millibars to 2.60 (+ 20% increase) at -28 millibars. There was also a highly significant response (P = 0.01) of T/R ratio to increased inoculum levels. T/R ratio was increased from 1.53 at DSI 0 to 1.80 at DSI 50 (an increase of 18%), to 3.39 at DSI 75 (+ 122%) and to 4.33 at DSI 100 (+ 183%). No significant response was shown by any of the interactions between and among main factors.

Discussion - The results of these experiments support the hypothesis that the erratic occurrence of aphanomyces root rot of peas may be attributed to the interaction of soil temperature and moisture on the pathogen. Experimental results showed that warm temperatures and wet soil conditions favoured disease development.

As in previous studies on the diseases, soil moisture expressed as percentage waterholding capacity was shown to be inadequate to explain the influence of availability of water on growth of *Aphanomyces euteiches*. The most appropriate manner in which water content could be expressed was

in terms of water potential. This conclusion is in agreement with that of Emberger and Welthy (1982) in their studies on the effect of water matric potential on resistance to *Fusarium oxysporum* f. sp. *medicagines* in lucerne. They found that the percentage moisture holding capacity could not be equated to matric potential.

In soil, disease development was considerably reduced at a higher potential (-200 millibars) than was the growth of organism on agar media (-30 bars). The failure to grow at higher water potentials in soil could not be related to insufficient water for fungal growth but to some indirect effect of soil water as suggested by Cook and Papendick (1970).

Development of aphanomyces root rot was shown to occur at the range of soil temperatures (8-25°) at which the peas were grown. Disease symptoms were not severe nor did invasion proceed rapidly at temperatures below 15°. Below the optimum temperature reported by Papavizas and Ayers (1974), 22° to 28°, the progress of the disease was favoured in soils with the highest moisture content (Figure 3.5a). There was no evidence of disease at 8° but some effects were observed at 12° at the highest moisture level. Laboratory studies showed that at 10°, growth of cultures on agar media occurred at water potential > -10 bars (Figure 3.1). This result agreed with that of Jones and Drechsler (1925) who reported a small amount of disease to occur at 10°. They also reported that zoospores were produced at 8° from sporangia on the mycelium of the fungus and germination of

oospores occurred as low as 6.5^{o}. Since Burke *et al.* (1969) reported that maximum temperature for infection was 16° provided the soil moisture was near saturation point, infection should be expected below 16°, i.e. at 8° to 10°, the minimum for growth of *Aphanomyces euteiches*. When the temperature was optimum for the pathogen, disease development was significantly related to soil moisture level (Figure 3.5). Higher disease severity was observed in infected plants growing in wetter soils than in those in drier soils, although disease incidence was equal in most cases. This was similar to the finding reported by Jones and Drechsler (1925). The effect of moisture stress was generally not on initial establishment of the pathogen in the host but rather on the development of established infections as suggested by Cook and Papendick (1972).

Growth of a healthy pea plant as shown by dry weight and the number of nodes at 4 weeks of growth (Experiment Two) was best in the wettest soil treatment and was progressively reduced in drier soils and cooler temperatures. Infection by *Aphanomyces euteiches* altered the soil moisture-growth relationship. The increased yield responses to increased soil moisture was no longer observed in diseased plants when disease severity was high. Dry matter yield of plants in the presence of the pathogen were reduced in response to soil moisture. The faster growth rate of the plant roots under wetter conditions suggested that primary infections occurred at a rate determined largely by the rate at which roots grew through the infested soil. This conclusion was

supported by Baker *et al.* (1967) who classified *Aphanomyces euteiches* pathogen-host relationship as an epidemic model where motile fungal spores were attracted to moving root tips. Thus the major limiting process in this type of epidemic, according to Huisman (1982), was considered to be root growth. Pfender and Hagedorn (1983) arrived at a similar conclusion and suggested that the rate of spread was severely constrained by an apparent requirement for the contact or at least proximity of the host plants' roots to the inoculum. Spread by the pathogen was limited as shown by Haenseler (1925) who reported that zoospores did not migrate in the soil more than 1.2 cm.

High inoculum levels in combination with warm temperatures and wet soil conditions clearly had an effect on the initiation of rapid disease development (Table 3.6). Higher disease severity occurred at high inoculum levels, as plants could have been expected to come in contact with the inoculum earlier as roots grew through the soil and initial infection led to the colonisation of a root system. It is possible that an increase in inoculum may permit movement of a pathogen to neighbouring plants, either by mycelial growth between roots in contact with one another or by short distance zoospore movement between roots (Pfender and Hagedorn 1983). They found the spread of pathogen was limited to a distance of 5 plants (18 cm) from the initially infected plant. This was consistent with their observation that lateral roots of one plant grew far enough to contact lateral roots of the fourth or fifth plant away from the row. Their findings also showed that the disease development was quite

similar at all inoculum levels after disease incidence reached about 5% and that lower disease severity occurred at low inoculum levels due to the longer duration of disease development at the beginning of the epidemic which had the effect of reducing the overall disease severity throughout growth.

The effect of a temperature and moisture may be examined separately at each inoculum level or in combination (Table 3.6 or Figures 3.6 and 3.7). Both temperature and moisture were shown to enhance disease development. The growth of plants and dry weight yield at warmer temperatures and wetter conditions were decreased, similar to the findings of Experiment One.

3.3.3 Glasshouse Studies on the Effect of Soil Moisture on the Development of Aphanocymes Root Rot, Plant Growth and Yield of Pea

Introduction - Salter (1962, 1963) showed that flowering and pod swelling were the critical stages of pea growth when adequate soil water was essential for high yields. Stoker (1977) has recommended irrigation of pea crops to restore the full rooting zone to field capacity especially at flowering and podding stage. As *Aphanomyces euteiches* has been shown to attack the host plant over a period of time (Pfleger *et al.*, 1976), infection by the pathogen at any growth stage would affect the uptake of soil moisture and the growth of pea plants.

To investigate the relationship between soil water and disease development, an experiment was set up in the glasshouse. Four moisture regimes were applied at three growth stages of peas, i.e. applying a high water-holding capacity at the three growth stages to ensure maximum infection at each stage.

<u>Materials and Method</u> - The peas were grown in plastic containers (170 x 170 x 180 mm deep) without holes, each contained 4 kg of soil on an oven-dried weight basis. Forty-eight pots were prepared, 16 of each soil inoculum level, and equally spaced out on glasshouse benches. Six seeds were sown per pot on 17 February, 1983. They were thinned to 4 plants per pot a week later.

The moisture level in each pot was maintained by adding water to give a weight based on water deficit of the soil checked once a day at 2pm, as described in Experiment One in Section 3.3.2. The glasshouse temperature was set at 25° and daily temperature was recorded by a 12-point Honeywell Chart Recorder. The pots were rotated every other day to obtain a uniform temperature regime and light. No artificial light was provided.

The experiment was a 3 x 4 factorial design, arranged in a complete randomised block replicated four times and consisted of four water treatments on peas grown in the three levels of soil inoculum, DSI 0 (control), 50 and 75 of Barrhill sandy loam (described in Tables 3.2 and 3.3).

In Treatment 1, the soil moisture was maintained at -28 millibars throughout the 12 weeks growth period of the pea crop, and in the other treatments, the soil moisture was raised to field capacity (from -28 millibars) for a period of three days at three weeks growth stage (Treatment 2), at flowering stage (Treatment 3) and at podding stage (Treatment 4). In assessing the flowering and podding stages, the system defined by Zain *et al.* (1983) was used. Flowering was when 50% of the plants showed at least one open flower and podding was when 50% of the plants had one pod greater than 20 mm in length on the first two flowering nodes.

At 12 weeks after sowing, the plants were harvested and assessed for disease severity (assessed according to the Disease Classes in Figure 2.2, Chapter 2), pod numbers and dry matter yield of roots, tops and pods.

<u>Results</u> - The effects of water treatments in relation to soil inoculum levels on the parameters measured are shown in Table 3.7.

Disease severity was found to increase linearly in a highly significant manner (P = 0.01) as soil inoculum level increased. The response to the water treatments was also significant (P = 0.05) but with a quadratic trend. Treatment 2 increased the disease severity (by 30%) while Treatments 3 and 4 did not produce any statistical differences from Treatment 1.

Table 3.7: Effect of moisture on the development of aphanomyces root rot, pod number and dry weight of pea cultivar Canterbury 39 grown in Barrhill sandy loam at inoculum levels DSI 0, 50 and 75. Water treatments were: Treatment 1: soil held at -28 millibars throughout the growth period; Treatments 2, 3 and 4: soil held at -28 millibars and watered to field capacity for a period of 3 days at 3 weeks after sowing (2); at flowering stage (3) and at podding stage (4).

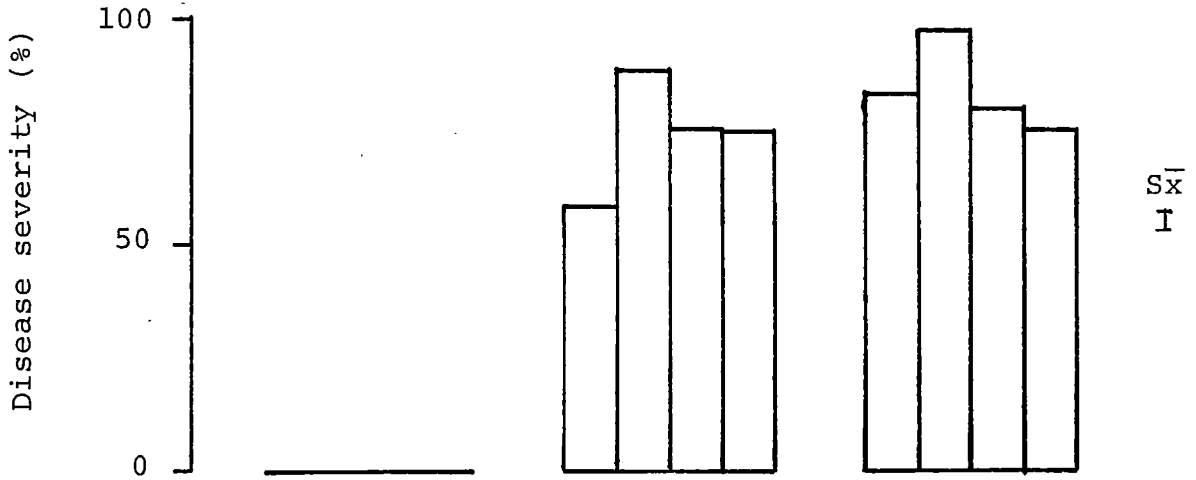
Factors		Disease severity %	Pod number per plant	*Dry weight (mg per plant)			
				Root	Top	Pod	Total
Soil inoculum level							
(DSI)	0	0.0	1.6	235	1319	783	2337
	50	75.0	1.1	126	1062	425	1613
	75	84.0	0.9	102	746	349	1198
	$S\bar{x}$	3.08	0.07	17	58.9	57.2	103.6
Significance		**L	**L	**L	**L	**L	**L
Water treatments							
	1	47.9	1.3	18.6	1053	549	1788
	2	62.1	1.1	118	803	331	1251
	3	52.1	1.2	140	1088	604	1832
	4	50.0	1.3	174	1225	592	1991
	$S\bar{x}$	3.55	0.08	19.9	68.0	66.1	119.6
Significance		*Q	NS	NS	**Q	*Q	**Q
Interaction		NS	NS	NS	NS	NS	NS
CV%		23.2	23.5	44.8	22.6	44.1	24.2

*Mean weight of four plants, from four replications.

Pod number per plant was affected by soil inoculum levels being reduced from 1.6 in DSI 0 to 1.1 in DSI 50 (-31%) and to 0.9 in DSI 75 (-45%). There were no significant differences in pod number in response to the water treatments.

Increased inoculum levels resulted in highly significant linear reductions (P = 0.01) of total dry weight (composed of the root, top and pod dry weights). Plants grown in soil of inoculum levels DSI 50 and 75 only accumulated half the amount of dry matter of plants grown in soil of DSI 0. Treatment 2 caused a reduction in root dry matter, but this was not significant. On the other hand, top and pod dry weights were related to water treatments significantly with a quadratic trend. Plants accumulated the highest yield in Treatment 4, followed by Treatment 3 and the lowest yield in Treatment 2. However, pod dry matter accumulation was highest in Treatment 3, though not significantly different from Treatment 4. Total dry matter followed a similar pattern to top dry matter.

There were no significant interactions between the soil inoculum levels and water treatments. The results of these effects are illustrated in Figure 3.8. In all cases, high soil moisture levels at the seedling stage reduced growth and with infested soils caused severe disease with dry weight yields being reduced. Watering to field capacity at flowering and podding had a less effect on disease severity as shown by the dry weight of the plant parts (roots, tops and pods).



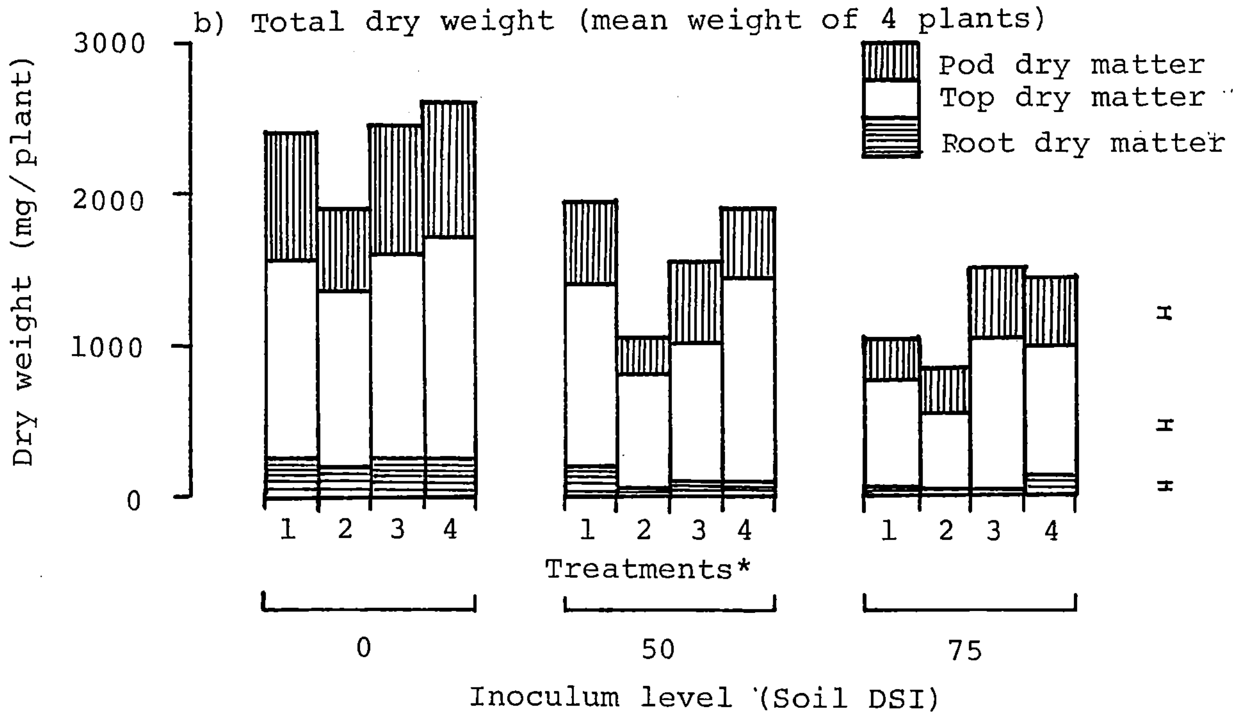


Figure 3.8: Effect of moisture on development of aphanomyces root rot as shown by a) disease severity and b) total dry weight of pea cultivar Canterbury 39 grown in Barrhill sandy loam at 3 inoculum levels DSI 0, 50 and 75.*Treatments were: (1) Soil held at -28 millibars throughout growth period; (2), (3) and (4) soil held at -28 millibars but watered to field capacity for a period of 3 days at 3 weeks of growth; at flowering stage and at podding stage respectively.

Discussion - The favourable effect of high soil moisture on aphanomyces root rot development was observed at all growth stages of the pea crop (Table 3.7). However, the conventional practice in which soil water level was raised to field capacity at flowering and podding stages (Stoker 1977) did not cause severe enough disease to appreciably reduce yields. This suggested that pea losses in yield due to root rot might not be proportional to the amount of inoculum in soil, but depended on the time of infection in relation to stage of pea growth and amount of soil moisture. Evidence was provided by Lockwood and Ballard (1959) and Lockwood (1960), who reported that the older the pea plant was at the time of infection, the lower would be the disease development and amount of root rot. Thus, significant yield reduction obtained from Treatment 2 when soil moisture was raised to field capacity at 3 weeks growth stage of the pea crop resulted from poor crop establishment due to severe root damage by aphanomyces root rot. The delay of rapid disease development at later growth stages (Treatments 3 and 4) suggested that root rotting did not cause sufficient damage so that the conducting tissue of the plant was left intact by the pathogen (Haenseler, 1925).

CHAPTER 4

THE ROLE OF LEGUMES AND OTHER WEEDS AS ALTERNATE HOSTS FOR *Aphanomyces euteiches*

4.1 INTRODUCTION

The factors involved in the survival of *Aphanomyces euteiches* between pea crops have not been fully understood. Once a field had become highly infested, the DSI was known to decline slowly if peas were not grown (Papavizas and Ayers 1974). Boosalis and Scharen (1959) recovered oospores from plant debris the season after an infected pea crop. Scharen (1960) concluded that in the absence of the main host plants (peas), oospores were probably the means by which the pathogen was perpetuated. This pathogen was shown to be able to resist adverse conditions by Sherwood and Hagedorn (1962) who reported that when oospores either in sterile or naturally infested soil were kept dry, moist or saturated with water, they remained infective even after two years of either alternate or continuous freezing.

It has been suggested that infection of plants other than peas may play a role in perpetuation of the fungus. Haenseler (1926), Linford (1927), Geach (1936) and Sherwood and Hagedorn (1962) have been among the investigators who have found that several other leguminous plants (listed by Papavizas and Ayers, 1974) were susceptible to *Aphanomyces euteiches* although none was as severely affected as peas.

However, as Papavizas and Ayers (1974) pointed out, some of the hosts were evaluated by growing plants in inoculated sterilized media and that they were not infected when grown in field soil infested with the pathogen. Sherwood and Hagedorn (1962) suggested that antagonism to pathogen by other micro-organisms could affect infection and reported that some non-leguminous plants were not suitable hosts.

Linford (1927) found oospores that he thought were those of *Aphanomyces euteiches* in plant debris. Later they were identified by Drechsler (1954) as *Aphanomyces campostyles* Drechs. Thus isolation and pathogenicity testing must be used to determine if species other than peas have been infected in the field with *Aphanomyces euteiches*.

This chapter reports the results of experiments to cases in New Zealand, the role of legumes and other weeds in infested areas as alternate hosts for *Aphanomyces euteiches*.

4.2 EXPERIMENTAL

4.2.1 Evaluation of Plants from Aphanomyces-infested Fields as Possible Hosts

Materials and Method - In May 1981, soil samples containing seeds were obtained from 5 fields in Mid-Canterbury which were known to be highly infested with *Aphanomyces euteiches* (DSI of 100). The soil sampling method used was described in Section 2.3.1. Ten plastic pots (150 mm diameter and 200 mm deep) were filled with soil from each

field, labelled and placed randomly on a bench in a glass-house with temperature maintained at 24° to 28°. During seed germination and emergence, soil moisture was kept at 65% of waterholding capacity based on gravimetric measurement as described in Section 3.3.2.

A week after emergence of the seedlings, the soil moisture was raised to field capacity for a week, and then maintained at 65% of waterholding capacity. At 5 weeks after emergence, the soil was again raised to field capacity for one week and then lowered to 65%. At 7 weeks after emergence, all of the emerged plants were removed from the soil and roots washed thoroughly under running water. The plants were identified and the root systems examined microscopically for symptoms and signs of infection. Infected root cortical tissue revealed oospores, a conspicuous diagnostic characteristic which distinguishes *Aphanomyces euteiches* from other root rot pathogens (Figure 2.2).

Results - Table 4.1 lists those plants that grew in the soil samples collected from the field. Oospores with characteristics typical of *Aphanomyces euteiches* were observed in the root systems of *Medicago sativa, Trifolium repens,, Capsella bursa-pastoris, Stellaria media* and *Viola arvensis*. The latter three have not previously been reported as hosts. None of these infected plants showed any symptoms up to the time they were removed from the pots.

Table 4.1: Results of a pot trial evaluating plants as possible hosts of *Aphanomyces euteiches* grown in soil naturally infested. Infection was indicated by the presence of oospores (+) in the root system examined microscopically after 7 weeks of growth.

Plant species*	Common name*	Family	Presence of oospores
Stellaria media	Chickweed	Caryophyllaceae	+
Chenopodium album agg.	Fathen	Chenopodiaceae	-
Cirsium arvense	Californian thistle	Compositae	-
Cirsium vulgare	Scotch thistle	"	-
Crepis capillaris	Hawksbeard	"	-
Senecio vulgaris	Groundsel	"	-
***Sonchus oleraceus*	Sow thistle	"	-
Capsella bursa-pastoris	Shepherd's purse	Cruciferae	-
Erodium cirutanum	Storksbill	Geraniaceae	-
***Triticum aestivum*	Wheat	Gramineae	-
***Medicago sativa*	Lucerne	Leguminosae	+
***Trifolium repens*	White clover	"	+
***Trifolium subterranean*	Subterranean clover	"	-
***Vicia sativa*	Common vetch	"	-
***Malva rotundifolia*	Round-leaved mallow	Malvaceae	-
Polygonium aviculare	Wireweed	Polygonaceae	-
***Rumex acetosa*	Sorrel	"	
Rumex obtusifolius	Broad-leaved dock	"	
Viola arvensis	Field pansy	Violaceae	+

* The species and common names are as listed in Healy (1984).
** Plant species reported as hosts in the literature.

4.2.2 Pathogenicity Studies

Introduction - To verify that the oospores, observed in the roots of infected plants from the pot trial (Section 4.2.1) were those of *Aphanomyces euteiches*, Koch's postulates were carried out.

Materials and Method - On PDA and maltose-peptone agar which were used extensively for routine propagation of the pathogen (Scott 1961), isolation of the pathogen directly from infected plants showing typical signs with oospores present in the root tissue were not successful due to the presence of the faster growing secondary pathogens like *Fusarium* spp. which suppressed the primary pathogen. The technique of baiting described by Manning and Menzies (1980) was used to isolate the pathogen. However, sterilized vermiculite inoculated by infected root fragments of possible hosts reported in Section 4.2.1 were used instead of aphanomyces infested soil. Fifty g of infected root fragments (fresh weight) from plants grown in aphanomyces infested soil (Section 4.2.1) were washed thoroughly under running water for 2 h and then mixed with sterilized vermiculite. Each sample was put into a plastic pot (150 mm diameter and 200 mm deep). Eleven sterilized captan treated pea seeds (cultivar Canterbury 39) were sown in each pot. The experiment was conducted in a glasshouse with temperatures at 24°-28°. Pots were watered to full vermiculite moisture capacity and covered with paper towels until the peas emerged. After a week, the seedlings were carefully removed and washed under tap water. Roots with

honey coloured lesions were excised and surface sterilized in 10% sodium hypochlorite, a solution containing 0.2% available chlorine, for one min, then washed 5 times in sterile distilled water. These roots were cut into 4 mm segments and placed on PDA plates. The plates were incubated at 20° for 48 h after which time the tips of hyphae from developing colonies were transferred to Corn Meal Agar (CMA). Serial transfers of hyphal tips to fresh CMA plates were made to rid cultures of contaminating bacteria.

Zoospores from isolates of *Aphanomyces euteiches* from infected plants of *Capsella bursa-pastoris*, *Medicago sativa*, *Stellaria media*, *Trifolium repens* and *Viola arvensis* were used as inocula in pathogenicity tests. Isolates 918 and 919 were used as controls. Preparation of zoospore inoculum was based on the methods described by Llanos and Lockwood (1960) and Mitchell and Yang (1966). One hundred ml of maltose-peptone broth (1.0 g of peptone and 3.0 g of maltose per 1000 ml) per Erlenmeyer flask (250 ml) was autoclaved for 15 min at 121° and inoculated with a 5 mm plug of a PDA culture of the fungus. After 4 days incubation at 24°, the mycelial mats were rinsed in sterile tap water; this was decanted and enough sterile distilled water was added to cover the mats. The washing process was repeated 2 h and 4 h later. The zoospores produced 16-20 h after the final wash were highly motile.

a) <u>Pathogenicity of isolates to peas</u>

Pea seeds (cultivar Canterbury 39) were surface sterilized in 10% sodium hypochlorite (0.2% available chlorine) for 3 min and sown in sterile vermiculite. Plants were inoculated 7 days after germination with zoospores from the five isolates of the pathogen from the infected plant species and isolates 918 and 919 to ensure that each isolate produced the same disease on the inoculated plants. Five ml of a zoospore suspension from each isolate which had a concentration of 10^4 spores per ml of sterile distilled water at the base of one stem of each 5 pea plants in a pot and there were 3 pots per replicate. The pots were placed in a glasshouse of 24°-28° for a month for aphanomyces root rot to develop. The moisture content of the vermiculite was maintained at 80% of its waterholding capacity.

b) <u>Pathogenicity of isolates to other host plants</u>

Plants of potential host species were grown in sterilised vermiculite. Ten seedlings of each species were established per pot from surface sterilized seeds. When they were approximately equivalent in size to 7 day old peas, 2×10^9 zoospores as used by Sherwood and Hagedorn (1962) suspended in 10 ml of sterilized distilled water were pipetted alongside the base of each plant. There were 3 pots of each species. The pots were kept in a glasshouse at 24°-28°. The moisture of the vermiculite was maintained at 80% of its waterholding capacity. A month after inoculation, the plants were removed from the vermiculite and roots washed thoroughly under running water. Sections of roots were placed on microscopic slides, crushed and stained with cotton

blue lactophenol stain (Appendix 2) for identification of oospores of *Aphanomyces euteiches*. Controls were also set up with uninoculated plants.

Results - When pea plants were grown in vermiculite which had been inoculated with root fragments of infected plants of the 5 species: *Capsella bursa-pastoris*, *Medicago sativa*, *Stellaria media*, *Trifolium repens* and *Viola arvensis*, then the peas showed symptoms of aphanomyces root rot and the typical oospores of the pathogen. The morphological characteristics of mycelial growth on CMA were similar in appearance to those described by Drechsler and Jones (1925) and Manning and Menzies (1980). Mycelial growth of isolates from the 5 plant species was sparse and granular in appearance and could not be distinguished from growth produced by Isolates 918 and 919.

a) Pathogenicity of isolates to peas

Inoculated pea plants had honey coloured lesions on the roots, whereas the roots of control plants were healthy. There was no difference in disease severity caused by each isolate on the pea plants.

b) Pathogenicity of isolates to other host plants

The 5 plant species inoculated with zoospores did not show any sumptoms but oospores, similar to those in plants taken from naturally infested soil and Isolates 918 and 919, were observed in their roots. Table 4.2 shows oospore frequency in the root systems of the plant species infected by *Aphanomyces euteiches* inoculated with zoospores. Plant

species (*Medicago sativa* and *Trifolium repens*) belonging to the leguminosae showed higher oospore frequency in the root system.

Table 4.2: Oospore frequency in species infected by *Aphanomyces euteiches* inoculated with zoospores of isolates of *Capsella bursa-pastoris, Medicago sativa, Stellaria media, Trifolium repens* and *Viola arvensis.* (No cross-inoculation of zoospores of isolate from one plant species to another was carried out.)

Host		Abundance of oospores of isolate per plant*		
Botanical name	Common name	Plant species**	918	919
Capsella bursa-pastoris	Shepherd's purse	+	+	+
Medicago sativa	Lucerne	++	++	++
Stellaria media	Chickweed	+	+	+
Trifolium repens	White clover	+++	+++	+++
Viola arvensis	Field pansy	+	+	+

*Mean numbers of oospores per 10 plants
+ - indicates 5 oospores or less in root system
++ - indicates 5-10 oospores in root system
+++ - indicates more than 10 oospores in root system

**Isolate from each plant species.

4.2.3 Field Studies

A field survey for possible host plants of *Aphanomyces euteiches* involved collecting plants from fields in Mid-Canterbury known to have a high DSI as shown by tests performed by the Plant Health Diagnostic Station, Ministry of Agriculture and Fisheries, Lincoln, Canterbury (Braithwaite pers. comm.).

Materials and Method - Plant samples were collected in May, 1981; December, 1981; May, 1982; and September, 1982. One plant of each species was collected from 25 sites in each of 5 fields using a diagonal sampling pattern across the field, similar to that described in Section 2.3.1. Roots were washed thoroughly under running water and examined microscopically for symptoms and signs of aphanomyces root rot. A similar survey in November, 1982, used a W-shaped sampling (Figure 4.1). The number of sampling sites per field was reduced from 25 to 10, but the number of each plant species collected at each sampling site was increased from one to three plants. At each site, the number of each plant species within a 0.1 m^2 quadrat was recorded.

Results - In Table 4.3, the results of the field survey studies from May, 1981 to November, 1982 are set out. Of the 16 species collected from the fields, *Viola arvensis*, *Stellaria media*, *Capsella bursa-pastoris*, *Trifolium repens* and *Medicago sativa* were observed to be infected by *Aphanomyces euteiches* as indicated by the presence of oospores in the root system. When the mean disease incidence of each plant species in 5 fields was calculated based on the percentage of the number of plants infected in a sample of 25 plants per field on each sampling date, the pathogen appeared to parasitize the alternate hosts most extensively during warm temperatures and wet conditions as the incidence of the disease was higher in spring than summer or autumn. *Trifolium repens* was the most susceptible species as it was observed to have the highest disease incidence at any time and the only

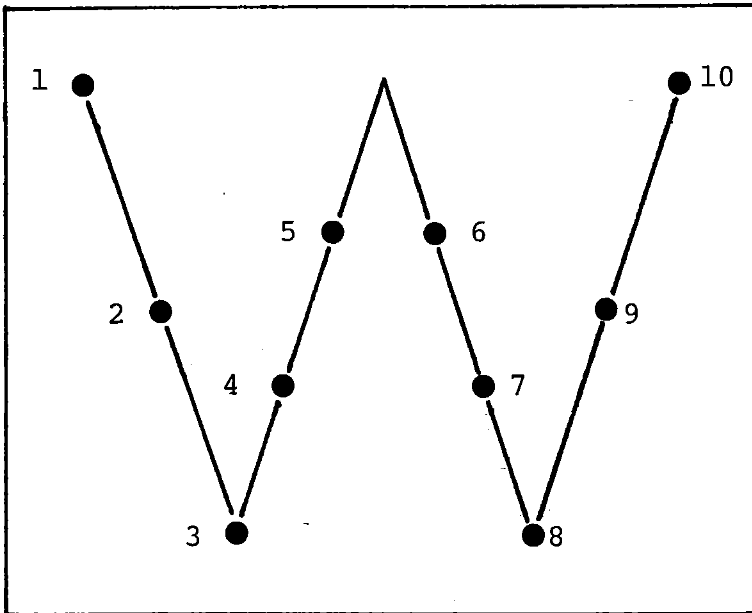


Figure 4.1: Relative positions of sampling sites in a W-shaped path (after Basu *et al.* 1977).

Table 4.3: Results of the 1981-1982 field survey in Mid-Canterbury evaluating field plants as alternate hosts for *Aphanomyces euteiches* collected from 5 highly infested fields with soil DSI of 100.

Hosts		Incidence* (%)				
		Date of sampling				
Botanical name	Common name	Autumn May 81	Summer Dec 81	Autumn May 82	Spring Sept 82	Summer Nov 82
Cirsium arvense	Californian thistle	0	0	0	0	-
Stellaria media	Chickweed	0	0	0	40.0	18.4(675)
Viola arvensis	Field pansy	0	37.6	0	28.0	64.2(4633)
***Malva rotundifolia*	Round-leaved mallow	0	0	0	0	-
Cirsium vulgare	Scotch thistle	0	0	0	0	-
Capsella bursa-pastoris	Shepherd's purse	0	11.4	0	27.8	25.6(417)
Sonchus oleraceus	Sow thistle	0	0	0	0	-
Erodium cicutarium	Storksbill	0	0	0	0	0(0)
***Trifolium repens*	White clover	0	40.4	22.0	75.0	32.5(1175)
***Trifolium angustifolia*	Vetch	0	0	0	0	-
***Vicia faba*	Broad bean	0	0	0	0	-
Rumex obtusifolium	Narrow-leaved dock	0	0	0	0	-
***Rumex acetosa*	Sheep's sorrel	0	0	0	0	-
Ranuculus repens	Buttercup	0	0	0	0	-
Crepis capillaris	Hawksbeard	0	0	0	0	-
***Medicago sativa*	Lucerne	0	0	0	4.0	-

* Incidence is the mean incidenceof 5 fields calculated as number of plants with oospores in root systems in a sample of 25 plants of each species per field, except in November 1982 survey where a sample consisted of 30 plants and populations of plant species per 0.1 m^2 were indicated within parenthesis; - indicates that plants were not collected for assessment.

**Plant species that had been reported as hosts in literature.

species infected during the cooler autumn temperature (May, 1982).

4.3 DISCUSSION

Pathogenicity tests (Section 4.2) showed that *Aphanomyces euteiches* parasitized two leguminous plant species, *Medicago sativa* and *Trifolium repens* and three other weeds, *Capsella bursa-pastoris, Stellaria media* and *Viola arvensis* belonging to the families Cruciferae, Caryophyllaceae and Violaceae respectively. The latter three plant species have not been reported as hosts in Literature.

The importance of these hosts to the survival and perpetuation of the pathogen was not assessed in this study. Evidence from glasshouse and field studies (Tables 4.1 and 4.3) showed that these hosts were parasitized at wet conditions similar to those that favoured disease development in peas.

These hosts could be harmful to pea crops as the infected roots could act as a source of inoculum for following pea crops by ensuring the maintenance of the inoculum level by production of new oospores. Such conclusion is supported by findings of Temp and Hagedorn (1967) who found that substantial number of fields showed increased or static disease indices even in the absence of pea plants, suggesting involvement of other hosts.

The finding that non-leguminous plant species could act as hosts contradicted the conclusion of Sherwood and Hagedorn (1962) that these species were not suitable hosts. They concluded this from the fact that non-leguminous plant species that had been reported as hosts were results of pure culture inoculated infection. This did not provide sufficient evidence to show that they were hosts as they did not get infected in naturally infested soil.

Because the alternate hosts of *Aphanomyces euteiches* examined in this study showed no symptoms at all, thus known as 'symptomless carriers' (Garett, 1960), persuading farmers to pursue weed control programmes with sufficient zeal may be difficult. Control of the disease by not growing leguminous crops which acted as alternate hosts like *Medicago sativa* and *Trifolium repens* would not prove practical in Canterbury arable crop rotations for the cash return and nitrogen fixing.

CHAPTER 5

STUDIES ON THE CONTROL OF APHANOMYCES ROOT ROT CAUSED BY *Aphanomyces euteiches*

5.1 INTRODUCTION

Since *Aphanomyces euteiches* was first reported by Jones and Drechsler (1925), several studies have been reported on chemical or cultural control of root rot of peas caused by this pathogen. Economic methods of control of this disease are not available and resistant cultivars have not yet been developed. Control in New Zealand has paralleled experience elsewhere (Jermyn *et al.* 1982) in that disease avoidance has been accepted as the only reliable method.

Jermyn *et al.* (1982), in New Zealand, have investigated fungicides reported to be effective against Oomycetes in the field and they found them to be ineffective. Part of the present study was to determine the effects of fungicides on control of the pathogen both in the laboratory and glasshouse to verify the conclusions of Jermyn *et al.* (1982).

Research by Papavizas (1966, 1967) on the cultural control of *Aphanomyces euteiches* has suggested that the root rot disease caused by this pathogen could be reduced by cruciferous amendments to infested soil. In this chapter, the literature on chemical control and cultural control by

cruciferous amendments is reviewed. This provides the background for the experiments which included:

1. Laboratory studies on the effects of fungicides on *Aphanomyces euteiches in vitro*.
2. Glasshouse studies on the effects of seed treatment fungicides on disease development.
3. Glasshouse studies on the effects of cruciferous amendments on aphanomyces root rot of peas.

Further information on pesticides included in this chapter is included in Appendix 3.

5.2 LITERATURE REVIEW

Chemical Control

In their review, Papavizas and Ayers (1974) stated that the literature was almost completely devoid of information on pea seed treatments to control root rot caused by *Aphanomyces euteiches*. However, there have been several reports on the effects of seed treatments on root rot severity. Delwiches *et al.* (1939) observed that seed treatments were of no benefit in the control of root rot and Johnson (1953, quoted by Papavizas and Ayers 1974) noted that heavy rates of seed-protecting materials increased plant survival in infested soil, but seed treatment was not shown to decrease root rot severity. Papavizas and Lewis (1974) observed in glasshouse experiments that pea root rot was suppressed for up to 4 weeks from planting by combinations of fungicides used as seed treatments. An attempt to use copper sulphate/methocel

by Pfleger *et al.* (1976) as a seed treatment was not successful and was furthermore found to be phytotoxic. The beneficial effects of hymexazol against the pathogen were reported by Kotova and Tsvetkova (1980). The fungicide mixture fenaminosulf plus benomyl resulted in a small but significant reduction in root rot with some cultivars of peas, but the effect did not persist (Jermyn *et al.* 1982). However, Mitchell and Hagedorn (1969) stated that the only practical measure of the effectiveness of a specific treatment in reducing root rot was the effect on the subsequent yield of the crop.

In the 1960s and early 1970s, considerable research was on the control of aphanomyces root rot with soil fungicides. Most of the fungicides tested either completely failed to control the disease or gave mediocre results. Lockwood (1961) found that only six of the many different fungicides tested in the glasshouse and field reduced root rot. Haglund (1968) reported that in-furrow application of chloronitropropane was effective in increasing pea yields in the field by delaying the progress of the disease. This fungicide, however, was lachrymatory, of high toxicity (acute oral LD_{50} of the rat at 197 mg per kg) and with no tolerances permitted, thus was not given clearance for use for peas. Papavizas and Lewis (1971) found that the soil sterilants metham-sodium tetrahydro-3,5-dimethyl-2H-1; Basamid; carbon disulfide; dimethydisulfide; methyl isothiocyanate and methanethiol reduced pea root rot in the glasshouse. They also reported that an application of fenaminosulf

reduced root rot in the field. These results supported the findings of a number of other investigators (Mitchell and Hagedorn 1966, 1969, 1971; Papavizas 1967), who reported that soil treatments could suppress the disease if properly applied to soil. Further evidence was provided by Pfleger *et al.* (1976) who reported that pyroxychlor was effective only when applied in a granular form placed directly in the furrow at sowing. However, although these chemical controls were achieved in the glasshouse and in limited field trials, none of the chemicals has been recommended for field application (Papavizas and Ayers 1974) because it was not economic to use soil chemicals for control of pea root rot.

Several workers have demonstrated that dinitroaniline herbicides can reduce root rot both in glasshouse and field trials. Examples were trifluralin (Harvey *et al.* 1975), dinoseb (Jacobsen and Hopen 1975), dinitramine (Grau and Reiling 1977; Grau 1977) and combination of oryzalin and trifluralin (Teasdale *et al.* 1978). Harvey *et al.* (1975) reported that higher yields of peas, obtained in soils treated with the herbicides, were due to a reduction of root rot severity by herbicides in both field and glasshouse conditions. They obtained direct evidence that trifluralin was inhibitory to hyphal growth and zoospore formation of the pathogen. This suggested that this would delay the onset of the severe root disease and enhance seedling vigour and, as a result roots would be more tolerant of *Aphanomyces euteiches* when they grew out of the herbicide zone (Grau and Reiling 1977). Teasdale *et al.* (1979) therefore concluded

that dinitroaniline herbicides could be expected to reduce disease severity and increase pea growth under those conditions which frequently resulted in aphanomyces root rot.

Cultural Control by Organic Amendments

Repeated cropping of a field with peas causes an increase of *Aphanomyces euteiches* inoculum in the soil. Martins (1925) reported that 100 per cent of the pea crop became infected with the pathogen when peas followed peas in root rot infested fields. Only 26% of plants were infected when peas followed alfalfa, 14% oats and 4% after one year of fallow. However, Reinking *et al.* (1945) reported that rotations did not eliminate but only reduced the severity of the disease. The value of crop rotations as a control measure was questioned by Olofsson (1967) who noted that once a heavy infestation of the pathogen was established in a field, growing peas became uneconomic. He postulated that it might take 10 to 15 years to reduce inoculum density in soil to a safe level. Temp and Hagedorn (1967) made the first systematic study of the effect of cropping practices on aphanomyces root rot of the peas. They found a larger decrease of severity in fields that had been planted with more than half their cropping rotation in corn, grain or vegetables (excluding peas) than in fields cropped primarily with forage crops. Their results showed conclusively that it was the type and quantity of the crop residues associated with each crop sequence that was important in the decrease of root rot. They agreed with Olofsson (1967) that cropping rotations without peas for as long as 10 years were not always effective in

eradicating root rot. However, long rotations could reduce inoculum density to such an extent that a profitable pea crop could be grown.

Prior to 1960, there was little information on the effects of organic amendments to soil on *Aphanomyces euteiches* inoculum. Davey and Papavizas (1961) noted that mature oat straw and corn stover, with or without supplemental nitrogen, could reduce root rot of peas. Interest in organic amendments was renewed by the findings of Papavizas (1966, 1967) who showed that cruciferous amendments such as stems and leaves of *Brassica oleraceae* (cabbage, kale and brussel sprouts) and *Sinapis alba* (mustard), when added to soil, reduced root rot of peas. In later trials, Papavizas and Lewis (1971) and Lewis and Papavizas (1971) found that several cruciferous amendments added to soil three weeks before sowing were very effective in the glasshouse against root rot. In the field, incorporated kale reduced pea root rot by 50%, and cabbage reduced it by 40%. Papavizas (1967) showed that water extracts of decomposing cabbage leaves and stems in soil, did not suppress, reduce or prevent mycelial growth, sexual reproduction, zoospore production and release, zoospore germination or infectivity of germinating zoospores. He postulated that sulphur-containing volatiles were produced during decomposition of amendments in soil and those might adversely affect vital phases in the life cycle of the pathogen before or after host penetration. This author used evidence provided by Dateo *et al.* (1957), Clap *et al.* (1959), Bailey *et al.*

(1961) and Lichtenstein *et al.* (1964) all of whom reported that cruciferous plants were characterised by high contents of sulphur-containing compounds such as methanethiol, dimethyl sulfide, and isothiocyanates which were liberated in volatile forms by chemical or enzymatic degradation. Lewis and Papavizas (1970) obtained direct evidence that cruciferous species decomposed in soil with the formation of the volatile sulfur-containing compounds and later Lewis and Papavizas (1971) reported that commercially-produced sulfides and isothiocyanates especially dimethyl disulfide and methyl isothiocyanate were extremely toxic to *Aphanomyces euteiches* even at concentrations as low as 0.04 ppm. They suggested that sulfur-containing volatiles might be implicated in the mechanism of control of aphanomyces root rot of peas by organic amendments as they found that vapours from decomposition of cabbage tissues adversely affected the morphology of the pathogen, development of oospores and mycelial growth in comparison to vapours arising from the decomposition of corn which had no effect on the pathogen. These findings indicated that the potential for root rot diseases of peas, caused by *Aphanomyces euteiches*, could be reduced by adding organic amendments to infested soil. However, Papavizas and Lumsden (1980) maintained that the use on a field scale of cruciferous amendment was likely to be not feasible as peas were a crop of low financial return. They also stated that research should be directed to the use of commercial fumigants, e.g. Vapam, Vorlex or Basamid containing inhibitory sulfur-compounds. In view of earlier comments by Papavizas and Ayers (1974) that economics did not permit use of soil

chemicals in the control of pea root rot caused by the pathogen, research in the development of commercial fumigants containing sulphur-compounds should take into account the economic reality of the prevailing agricultural system.

5.3.1 Laboratory Studies on the Effect of Fungicides on *Aphanomyces euteiches in vitro*

Introduction - The objective of these experiments was to ascertain whether the systemic fungicides, hymexazol, metalaxyl and pyroxyfur, investigated by Jermyn *et al.* (1982) and found to be non-effective against *Aphanomyces euteiches* in field conditions, inhibited the pathogen *in vitro*.

Table 5.1: Fungicides used for the chemical control studies on the pathogen.

Common name	Trade name	Formulation
Pyroxyfur	Dowco 444 XRM 4408	75% a.i. emulsifiable concentrate
Metalaxyl*	Apron 35	35% a.i. wettable powder
Hymexazol*	Tachigaren	70% a.i. wettable powder

*Names as from Anon. (1983).

Pyroxyfur

Some of the information is from data supplied by Dow Chemicals via Ivan Watkins-Dow.

1. Nomenclature and Development - The chemical name of this systemic fungicide is 2-chloro-6-(2-furanylmethoxy)-4-(Trichloromethyl) pyridine. It was introduced by Dow Chemical

Company with code number DOWCO 444. The trade mark is thus Dowco 444.

2. <u>Properties</u> - The fungicidal properties were described by Frick (1981). It is a straw-coloured liquid with a vapour pressure of 6.4×10^3 mPa. Its solubility is 1.2 ppm in water at 24.4^o and it is readily miscible in most solvents. It is non-mobile in the soil and non-persistent in the environment.

3. <u>Uses</u> - The fungicide is capable of downward movement in plants with accumulation in the root-system and can provide long residual disease control. Frick (1981) reported that it had been fully tested in laboratory, glasshouse and field trials and found to be highly effective for control of lower stem and root rot diseases caused by the genera *Pythium*, *Phytophthora* and *Aphanomyces euteiches* root rot of peas. Additional data showed that the fungicide could control other root rots like *Fusarium oxysporum* wilt of carnation and bulb rot of tulips and seed-borne diseases like *Pyrenophora graminea* leaf stripe of barley.

4. <u>Formulation</u> - Four formulations of the fungicide are available:

a) Emulsifiable seed treatment concentrate containing dye 800 g a.i./*l*.

b) Emulsifiable concentrate 800 g a.i./*l*.

c) 5% dust formulation.

d) Stem treatment formulation 250 g a.i./*l*.

Metalaxyl

Some data are obtained from Anon. (1983).

1. Nomenclature and Development - The chemical name of metalaxyl is methyl N-C2-methoxyacetyl)-N-(2,6-xylyl)-DL-alaninate. It was introduced by Ciba-Geigy Aktiengesellschaft (Company) as code number 'CGA 48988'. Trade marks are 'Ridomil', 'Apron' and 'Fubol' (mixture with mancozeb).

2. Properties - Its fungicidal properties were described by Urech (1977). Pure metalaxyl forms colourless crystals. The melting point is 71.8-72.3^{o} and vapour pressure 293 μPa at 20°. The density is 1.21 g/cm^3 at 20°. Solubility, at 20°, was 71. g/*l* of water; 550 g/*l* benzene; 750 g/*l* dichloromethane; 650 g/*l* methanol; 130 g/*l* octan-1-ol and 270 g/*l* propan-2-ol. Fifty per cent hydrolysis (calculated) at 20° occurs in > 200 days at pH 1, 115 days at pH 9 and 12 days at pH 10. The fungicide remains stable at < 300°.

3. Uses - Metalaxyl is a systemic fungicide suitable for the control of diseases caused by air and soil borne peronosporales, in a wide range of temperate, subtropical and tropical crops. Foliar sprays with mixtures of metalaxyl and conventional protectant fungicides are recommended for the control of air-borne diseases such as that caused by *Pseudoperonospora humuli* in hops, *Phythophthora infestans* in potatoes, *Peronospora tabacina* in tobacco and *Plasmopara viticola* in vines. Metalaxyl alone is used as a soil application for control of soil-borne pathogens causing root and lower stem rots in crops such as avocado and citrus. This

method of use is also recommended in primary systemic infections of downy mildew in hops and tobacco seed beds. It is used as a seed treatment for the control of systemic downy mildews as well as damping-off caused by *Pythium* spp.

4. Formulations - For soil applications, 'Ridomil' 5G, granules at 50 g a.i./kg is available. Seed treatments include 'Apron SD35' at 350 g a.i./kg; 'Apron FW350' in suspension concentrate (flowable) form at 350 g a.i./l. Fungicide combinations for foliar use include 'Acylon Super F', 'Ridomil combi' (metalaxyl + folpet); 'Apron 70 SD' (350 g metalaxyl + 350 g captan/kg); 'Fubol', 'Ridomil MZ' (metalaxyl + mancozeb); 'Ridomil M' (Metalaxyl + maneb) and 'Ridomil plus' (metalaxyl + copper oxychloride).

Hymexazol

Some data are obtained from Anon. (1983).

1. Nomenclature and Development - Hymexazol or hydroxyisoxazole, its chemical name: 5-methylizoxazol-3-ol, was introduced by Sankyo Co. Ltd as code No. 'F-319' or 'SF-6505'. The trademark of the systemic fungicide is Tachigaren.

2. Properties - Its fungicidal properties were described by Iwai and Nakamura (1966). The technical grade (98% pure) forms colourless crystals with melting point at 86° and vapour pressure at < 133 mPa at 25°. Its solubility is 85 g/l water at 25° and it is readily soluble in most organic solvents. It is stable and non-corrosive under alkaline conditions, comparatively stable under acidic con-

ditions, comparatively stable under acidic conditions and is not affected by light.

3. Uses - It is effective against soil-borne diseases caused by *Aphanomyces*, *Corticium*, *Fusarium* and *Pythium* spp. and other fungi when applied as a soil drench. It is also used as a seed treatment for sugar beet. It also has been found to have some activity as a plant growth regulator.

4. Formulation - These include liquid (30 g a.i./*l*); dust (40 g a.i. g/kg) and seed treatment (700 g a.i./kg).

Experiment One: Effect of Fungicides on Hyphal Growth *in vitro*

Introduction - The effects of pyroxyfur, metalaxyl and hymexazol on hyphal growth of three isolates of *Aphanomyces euteiches*, Isolates 918, 919 and 9203 (described in Section 3.3.1), were investigated.

Materials and Method - Potato dextrose agar (Difco PDA) was used as an assay medium to investigate the effect of fungicides on growth and development of the three isolates.
The autoclaved medium was allowed to cool to 40° before the fungicides, pyroxyfur, metalaxyl or hymexazol were added at concentrations of 1, 5, 10 and 100 ppm. The amended media were poured into plastic petri dishes (85 mm diameter).
Five dishes (20 ml per dish) were poured of each amended medium of each fungicide at the prescribed concentration and used immediately after the medium had set. A plug of inoculum, 5 mm diameter, cut from the margin of an actively

growing 3-day old colony of each isolate, was placed on the medium at the centre of each dish. After four days incubation at 25° in the dark, when the colony of the no fungicide, of Isolate 919 had reached the edge of the petri dish, the experiment was terminated. Two diameters of the colony in each dish at right angles were measured.

Results - Table 5.2 shows the results of the *in vitro* assay experiment. Pyroxyfur completely inhibited growth of all isolates. Reductions of growth by metalaxyl and hymexazol were not consistent. Significant reductions of 25% and 35% by metalaxyl were recorded only with Isolates 918 and 9203 respectively, while 16% and 25% reductions by hymexazol were recorded with Isolates 918 and 919. Metalaxyl treatments reduced growth of Isolate 918 significantly with increasing rates; however, this was not observed with Isolate 919 and 9203 where effective reductions were only obtained at 10 ppm and 100 ppm with Isolate 919 and a non-statistical difference between 5 ppm and 10 ppm with Isolate 9203. With Isolates 918 and 9203, hymexazol did not reduce growth significantly at concentrations of one to 10 ppm but effectively reduced the growth by 47% and 29% at 100 ppm respectively. Hymexazol treatments had no effect on Isolate 919 at the concentration of one ppm, but reduced growth significantly at increasing rates from 5 ppm to 100 ppm.

Although colony diameter was not markedly reduced with hymexazol, morphological changes were observed in the mycelium (figure 5.1). In the nil fungicide medium,

Table 5.2: Effect of pyroxyfur, metalaxyl and hymexazol at 4 concentrations on mycelial growth of 3 isolates of *Aphanomyces euteiches* on Potato Dextrose Agar (Difco). Measurements were taken after 4 days of incubation at 25° when colony of Isolate 919 in the nil treatment reached the edge of petri dish.

Fungicide		Colony diameter (mm) of isolate		
		918	919	9203
Nil		75.0	85.0	56.0
Pyroxyfur		0.0	0.0	0.0
Metalaxyl		56.0	79.5	36.5
Hymexazol		63.3	64.0	52.0
$S\bar{x}$		0.8	0.2	0.3
Significance		**	**	**
Metalaxyl (ppm)[a]	0	75.0 a	85.0 a	56.0 a
	1	70.0 b	85.0 a	41.0 b
	5	60.0 c	85.0 a	39.0 c
	10	51.0 d	80.0 b	38.0 c
	100	43.0 e	68.0 c	28.0 d
	$S\bar{x}$	0.5	0.3	0.2
Hymexazol (ppm)[a]	0	75.0 a	85.0 a	56.0 a
	1	73.0 a	85.0 a	56.0 a
	5	70.0 b	72.0 b	56.0 a
	10	70.0 b	64.0 c	56.0 a
	100	40.0 c	35.0 d	40.0 b
	$S\bar{x}$	0.7	0.1	0.2
	CV%	7.0	2.0	3.0

[a]Means followed by the same letter do not differ significantly (P = 0.05) using Duncan's Multiple Test.

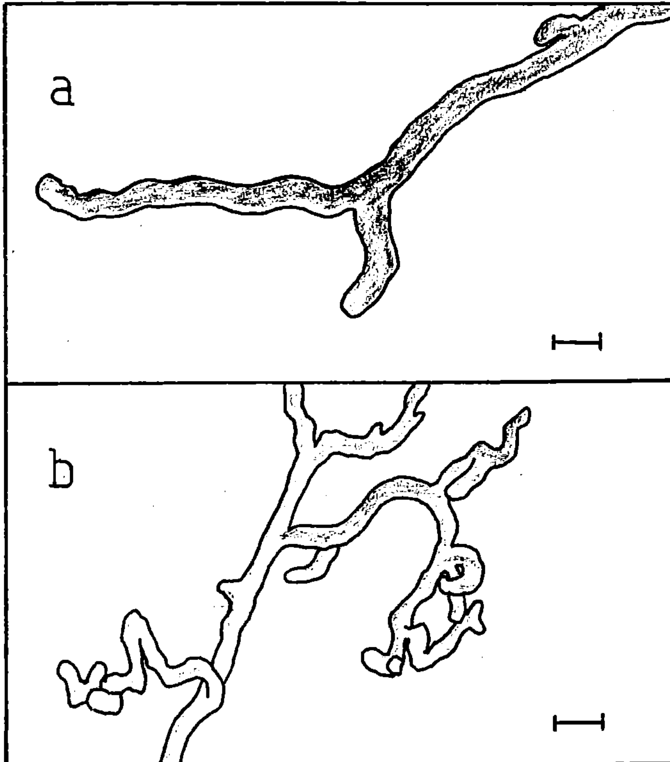


Figure 5.1: Comparison of hyphae of *Aphanomyces euteiches* grown in Potato Dextrose Agar amended with a) no fungicide and b) hymexazol at 100 ppm.

(Drawn and enlarged with aid of a compound microscope at a magnification of 400X as hyphae appear in culture stained with cotton blue lactophenol stain under a cover glass on a microscopic slide for lucidity; Bar = 8 μm).

whitish aerial mycelium was prominent in each dish and young vigorous hyphae with 3-10 μm diameter, destined to become zoosporangia, were normally packed with coarsely granular cytoplasm with irregularly scattered vacuoles and small refractive oil droplets. In the PDA containing pyroxyfur, metalaxyl or hymexazol, the hyphae, (< 5 μm in diameter), branched abundantly with no aerial growth and contained almost 40-50% less granular cytoplasm. The hyphal tips were curved or enlarged or globose.

Discussion - Results in Table 5.2 indicate that pyroxyfur, metalaxyl and hymexazol were effective in suppressing or preventing growth of *Aphanomyces euteiches* with different effects on each of the three races. The deformed hyphae due to fungicidal effects, especially noticeable in the presence of hymexazol, was similarly reported in the hyphae of the pathogen in the presence of volatile sulfur compounds evolved during decomposition of cruciferous amendments (Lewis and Papavizas, 1970) and dinitroaniline herbicides (Grau, 1977). Lewis and Papavizas (1970) suggested that hyphae in such conditions might be more predisposed to lysis in soil.

Experiment Two: Effect of Fungicides on Zoospore Formation and Motility *in vitro*

Experiment One showed that hyphal growth of *Aphanomyces euteiches* was either inhibited or reduced by pyroxyfur, metalaxyl and hymexazol. Available circumstantial evidence suggests that the mycelium has a minor role in infections in natural soil (Papavizas and Ayers, 1974). Scharen (1960,

cited by Papavizas and Ayers 1974) presented evidence that oospores of the pathogen provided the primary inoculum for new outbreaks of pea root rot and many of these oospores did not germinate in soil, but those that did especially those adjacent to plant roots did so by means of zoosporangia and zoospores. Subsequently, the zoospores were reported by Cunningham and Hagedorn (1961) to be the primary unit of infection and had a chemotactic response to pea root exudates especially strongest in the region of elongation or immediately behind the root cap. Thus it was important to investigate the effects of pyroxyfur, metalaxyl and hymexazol on zoospore formation and motility of two isolates of *Aphanomyces euteiches* Isolates 918 and 919.

Because studies in Experiment One used solid media of PDA, this made it difficult to separate the effects on growth of mycelium and the formation and motility of zoospores. To induce asexual reproduction in juvenile mycelium of this fungus, Jones and Drechsler (1925) followed the practice common then, as now, with water molds of replacing the nutrient medium with water and replacing this successively a few times at brief intervals. Visible evidence of asexual reproduction occurred some 5-6 h later. Llanos and Lockwood (1960) confirmed the validity of this practice and also pointed out the importance of aeration and the occurrence of strain differences in the formation of zoospores.

Materials and Method - The method developed by Llanos and Lockwood (1960) was used to produce zoospore inoculum in maltose and peptone broth consisting of 1.0 g of peptone and

3.0 maltose per litre of distilled water. Boiling tubes (24 mm x 150 mm) were filled each with 15 ml of the broth. The tubes were plugged with non-absorbent cotton wool, covered with caps and autoclaved at 121° for 15 min. After cooling, each tube was inoculated with a 6 mm diameter plug from the margin of 3-day old colonies of *Aphanomyces euteiches* Isolates 918 or 919. The tubes were incubated for 4 days at 25°, kept inclined to provide maximum mycelial growth. At the end of the incubation period, nutrient medium was replaced with salt solution at pH 6.5 (adjusted with 0.01 M HCl) consisting of 1.75×10^{-3} M $CaCl_2$, 10^{-3} M KCl and 10^{-3} M $MgSO_4$ which Mitchell and Yang (1966) had shown to favour abundant zoospore production. The washing procedure was repeated immediately and again 2 and 4 h later. At the final washing, the salt solution contained 4 concentrations of fungicides at 1, 5, 10 and 100 ppm. The nil treatment included was the salt solution alone.

The tubes containing the treated mycelial mats in salt solution were further incubated in a water bath with a reciprocal shaker (Grant Instruments, Cambridge Ltd, England) at 25° and shaken at 150 strokes per min for 16 h. Mycelial mats were stored at 4° after incubation to stop sporulation and induce encystment of zoospores to facilitate counting.

Each treatment was replicated 3 times and 2 subsamples of each replicate were used for counting using a counting chamber (Levy Double Neubauer, Clay Adams, Becton Dickinson and Company, USA).

The effect of the fungicides on zoospore motility was tested by introducing motile zoospores, produced in a fungicide-free environment, into a salt solution containing the fungicides. Twelve h after final mycelial wash, zoospores in salt solution were added to equal volume of salt solution amended with fungicides, final concentrations similar as defined. The zoospores were incubated in the water bath at 25° and shaken at 150 strokes per min for one h. The percentage of zoospores showing motility was determined by counting the non-motile zoospores initially present in the preparation and subtracting the number from the zoospore total population which was counted after applying heat from a bunsen flame to the counting chamber to eliminate motility for 15 sec.

Results - Figure 5.2 shows the results obtained on zoospore formation. Isolate 918 produced 34% fewer zoospores than Isolate 919. Pyroxyfur was the most effective fungicide, inhibiting zoospore formation of Isolate 918 completely, even at one ppm, and Isolate 919 at concentrations higher than 5 ppm. Metalaxyl was the least effective fungicide, reducing zoospore formation by both isolates effectively only at concentrations higher than 10 ppm. At 100 ppm, there was no zoospore produced by Isolate 918, while 6% was produced by Isolate 919 compared to the nil treatment. On the other hand, one ppm of metalaxyl increased zoospore formation by Isolates 918 and 919 by 83% and 31% over the nil treatments respectively. Zoospore formation by both isolates was reduced at increasing concentrations of hymexazol.

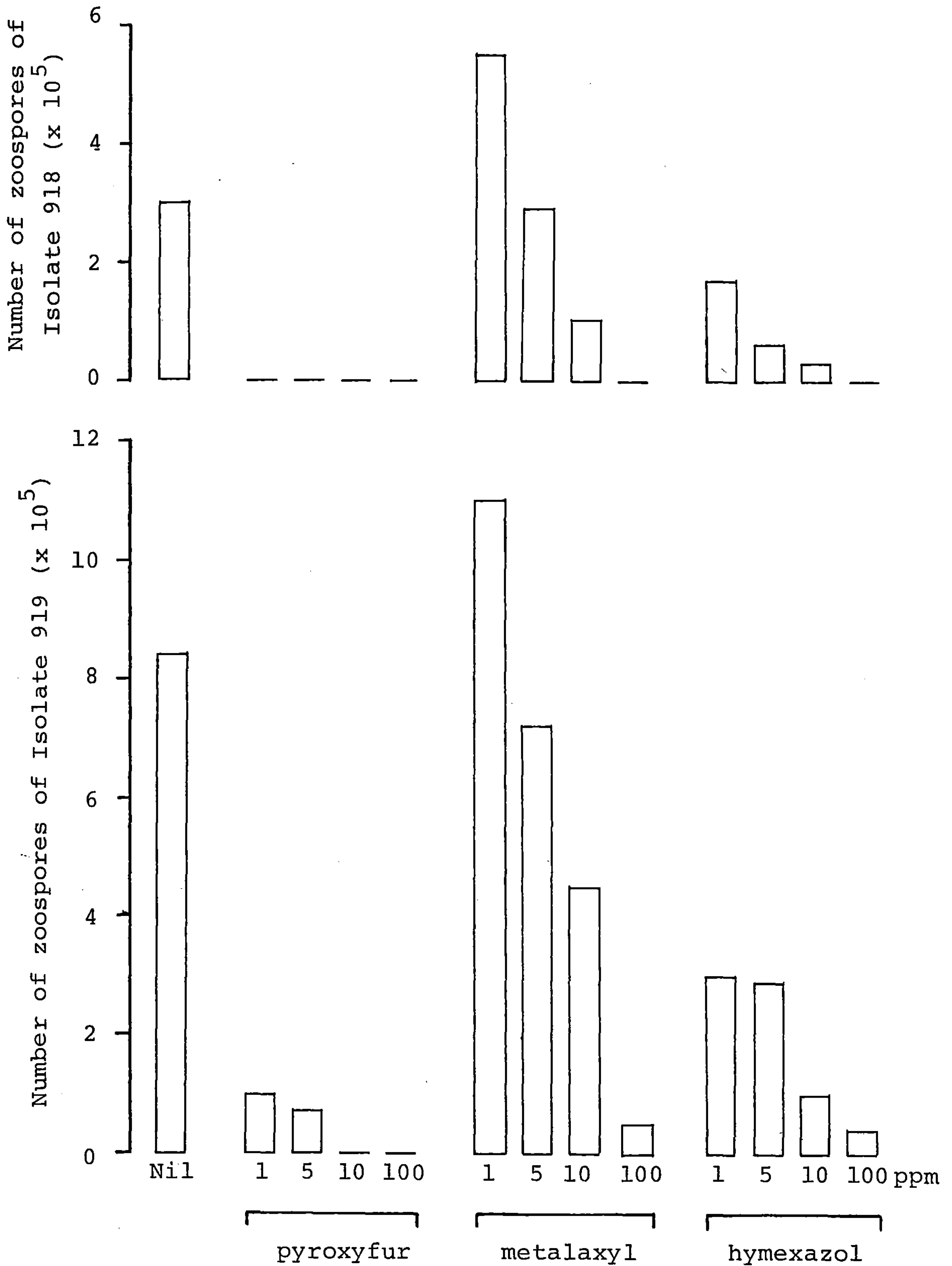


Figure 5.2: Effect of fungicides on zoospore formation of Isolates 918 and 919 of *Aphanomyces euteiches*, 4-day old colonies at 25°.

Zoospore motility was effectively inhibited by pyroxyfur and hymexazol at all concentrations. In the metalaxyl treatments, inhibition only occurred at concentrations higher than 5 ppm and there was no inhibition of zoospore motility of both isolates at one ppm and 5 ppm.

Discussion - Results in Figure 5.2 showed that pyroxyfur, metalaxyl at high concentrations and hymexazol were effective in suppressing formation of zoospores. Other results showed them to inhibit motility of zoospores. The effects of these fungicides could be related to two factors: 1) the direct effect on the morphology of the hyphae as shown in Experiment One where either complete or partial inhibition of mycelial growth was obtained; 2) the presence of fungicide, after the activation of asexual reproduction by mycelial washing, might have affected the sequences of asexual sporogenesis as described by Jones and Dreschler (1925). Evidence was provided by Mitchell and Yang (1966) that the medium in which mycelium was exposed determined the production of zoospores. They reported that the ionic composition of medium had substantial effect once the processes leading to asexual reproduction were fully activated by the washing procedure and once the mycelium was suspended in the basic salt solution, the sequence of events continued through to completion with the formation of motile zoospores. They also found that activation was completely reversible as when fresh peptone of glucose broth was added to activate mycelium, normal vegetative growth was stimulated.

The increased zoospore formation of both Isolates 918 and 919 by the low concentrations of metalaxyl could be due to the death of some hyphae caused by the fungicide. This phenomenon has been described as 'hyphae density' effect by Mitchell and Yang (1966) who showed that the response to mycelial washing varied with density of hyphal growth. They observed that in areas of the fungal colony where the mycelium was thin, asexual spores differentiated and were extruded. In denser areas, spores were differentiated but none were extruded. Cochrane (1958), in his discussion of metabolism and reproduction, pointed out the possibility that some factor in normal hyphae might be released on their death to reach a threshold of concentration high enough to induce sporulation.

Experiment Three: Effect of fungicides on oospores formation *in vitro*

Because pyroxyfur, metalaxyl and hymexazol were shown to have considerable effects on growth of mycelium and asexual sporogenesis of *Aphanomyces euteiches* (Experiments One and Two), effective control of root rot of peas would be expected. However, Jones and Drechsler (1925) reported that the formation of oospores, the sexual stage and the major factor in the survival between pea crops (Sherwood and Hagedorn, 1964), was generally considered to occur when the thallus of the pathogen was exposed to adverse conditions or environmental stress. Thus a third experiment investigated the effect of pyroxyfur, metalaxyl and hymexazol on the ability of the mycelium of the pathogen to produce oospores when exposed to the fungicides at concentrations of one, 5, 10 and 100 ppm.

Materials and Methods - The effects of pyroxyfur, metalaxyl and hymexazol on oospore formation were determined by the number of oospores produced in mycelial mats of the fungus suspended in fungicide at one, 5, 10 and 100 ppm. Mycelial mats were prepared using cellophane to separate the fungus from the agar medium, a technique developed by Fleming and Smith (1944). Moisture permeable discs of cellophane (plain transparent of 600 ø thickness) were cut to 80 mm in diameter to fit 85 cm plastic petri dishes. The discs were placed between sheets of filter paper soaked in distilled water in a glass petri dish and autoclaved at 121° for 20 min. The discs were then transferred to the surface of PDA in petri dishes, avoiding air spaces being formed as much as possible. A plug of inoculum, 5 mm in diameter cut from the margin of 3-day old colony of each isolate of the fungus was placed in the centre and on the top of the disc on the PDA. During the 3-day incubation at 25°, colonies in each plate were checked at 12 h intervals for oospore production so that only colonies without oospores were used for treatment. Cellophane discs with colonies growing on them were removed from the plates and the mycelial roots, separated from cellophane discs, were transferred to plastic petri dishes containing 20 ml of fungicide solution or sterile distilled water. Four replicates were prepared for each treatment. These were incubated for 48 h, after which time the number of oospores produced was counted microscopically. Twenty counts at random were made in each colony and the number of oospores produced in the fungicide treatment was calculated as the average number of oospores per cm^2 of the area of the colony of each isolate.

Oospores produced were greater in number nearer the source of inoculum.

Results - After 3 days of growth on PDA, colony diameters of Isolates 918 and 919 reached 42 mm and 62 mm respectively. Figure 5.3 shows that the nil treatment, Isolate 918 was more prolific in oospore production per cm^2, ($650/cm^2$), than Isolate 919 ($32/cm^2$). However, the overall percentage number of oospores produced by each isolate in all fungicide treatments did not differ statistically (35% and 36% compared to nil by Isolates 918 and 919 respectively).

The effect of pyroxyfur on the two isolates was similar with the number of oospores being markedly reduced by 5 and 100 ppm, but reductions also occurred at one and 10 ppm. Fewer oospores were produced at one ppm of metalaxyl than at the higher concentrations with both Isolates 918 and 919, but large numbers of motile zoospores were observed only in the one ppm concentration. Hymexazol increased oospore production of Isolate 918 by 18% at one ppm. Only effective reductions were obtained at 10 ppm and 100 ppm concentrations. Progressive inhibition of oospore formation with increasing concentrations was obtained with Isolate 919, although concentrations at one ppm and 5 ppm were equally effective.

Discussion - It has been suggested by Jones and Drechsler (1925) that oospore formation occurred when the mycelium of *Aphanomyces euteiches* was exposed to adverse conditions or environmental stress. Results in Figure 5.3 showed that Isolate 918 was more capable of survival than 919 because of

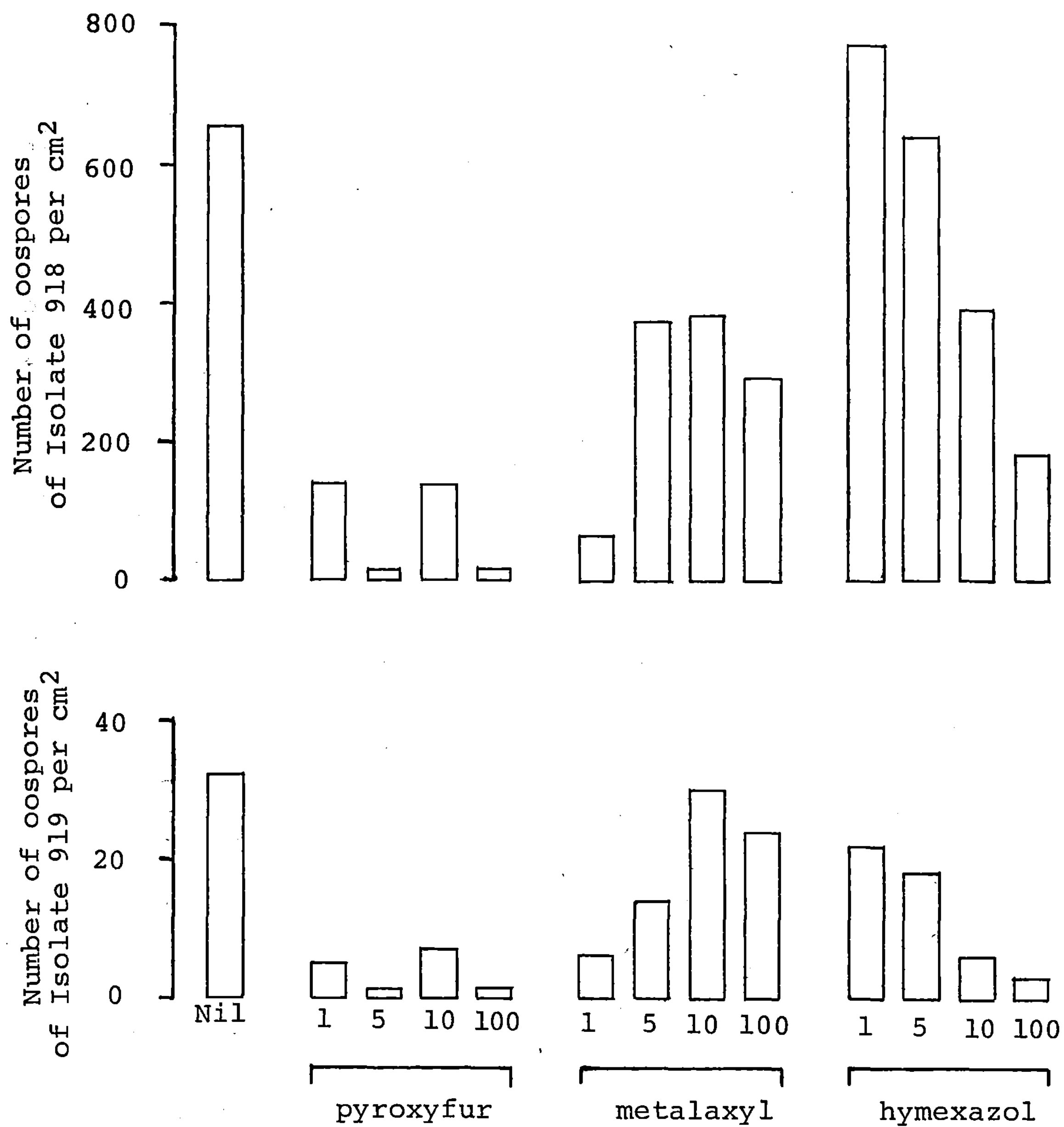


Figure 5.3: Effect of fungicides on oospore formation of Isolates 918 and 919 of *Aphanomyces euteiches* 3-day old colonies at 25°.

greater oospore production. When each was exposed to pyroxyfur, metalaxyl and hymexazol, the overall reduction in oospore numbers was similar with each isolate. Thus the ability of the pathogen to produce oospores, the survival units (Jones and Drechsler, 1925) in the presence of fungicides, even in high concentrations may help to reduce severity of root rot of peas, but not reduce inoculum in the infested soil.

5.3.2 Glasshouse Studies on the Effects of Fungicide Seed Treatments on Disease Development

Introduction - Laboratory studies (Section 5.3.1) showed that fungicides listed in Table 5.1 significantly reduced the growth and development of *Aphanomyces euteiches* in bioassay tests. In this section, a series of glasshouse experiments were carried out to investigate the effect of fungicide seed treatments on disease development.

Experiment One: Effect of fungicide seed treatments

The first glasshouse experiment investigated the effectiveness of fungicides when applied as seed treatments and the interactions when used as mixtures.

Materials and Method - Fungicide treatments (Table 5.3) were applied to 100 g samples of pea seed (cultivar Canterbury 39), already treated with the standard protectant fungicide Captan, in 500 ml sealed erlenmeyer flasks by vigorous shaking on a Griffin Flask Shaker (Griffin and George Ltd, Britain). The

chemicals were applied with one ml of water except when combined with the liquid pyroxyfur formulation. The treated seeds were dried in trays.

Table 5.3: Fungicide treatments used to investigate the effectiveness of fungicide seed treatments.

Fungicide	Treatments (a.i. g/kg seed)	
	Trial One	Trial Two
Untreated (Captan) PO, MO, or HO	-	-
Pyroxyfur (PL)	1.5	1.5
Pyroxyfur (PH)	3.0	3.0
Metalaxyl (ML)	3.0	-
Metalaxyl (MH)	6.0	-
Hymexazol (HL)	-	5.0
Hymexazol (HH)	-	10.0
PL + ML	1.5 + 3.0	-
PL + MH	1.5 + 6.0	-
PH + ML	3.0 + 3.0	-
PH + MH	3.0 + 6.0	-
PL + HL	-	1.5 + 5.0
PL + HH	-	1.5 + 10.0
PH + HL	-	3.0 + 5.0
PH + HH	-	3.0 + 10.0

The experiment was split into two trials: Trial One with pyroxyfur and metalaxyl as one treatment combination, and Trial 2 with pyroxyfur and hymexazol as the other treatment combination. This design was to reduce unnecessary combinations that could occur. A 2 x 3 x 3 factorial design with randomised complete block design replicated four times

was used for each trial. Two soil inoculum levels (DSI 50 and 100) of a Barrhill sandy loam (soil description and cropping history described in Section 3.3.2) obtained from Rakaia, Canterbury on 30 September 1982, were used. Treated seeds were sown on 29 November 1982 at a rate of one per cavity in the 36 cavities of rootrainers (described in Section 2.3.4) per tray containing the soil. Each tray consisted of one treatment and isolated in individual plastic containers. Seventy-two trays were prepared for each trial and equally spaced on glasshouse benches with temperatures maintained between 24°-28°. Soil moisture was kept at -3 millibars throughout the experiment.

Fifteen plants per replicate were harvested at four weeks (27 December 1982) and eight weeks (17 January 1983) after sowing for disease severity assessment (Figure 2.1 in Section 2.3.2) and dry matter yield of roots and tops of plants.

Results

Trial One The main effects and interactions of seed treatment with pyroxyfur and metalaxyl on severity of disease development as shown by dry weight of pea plants at two soil inoculum levels are shown in Table 5.4.

a) Disease Severity

Disease severity differences were highly significant (P = 0.01) between inoculum levels DSI 50 and DSI 100 throughout the growth period. There were no above-ground symptoms in the four weeks old plant in inoculum level DSI 50.

Table 5.4: Effect of pyroxyfur and metalaxyl seed treatments on severity of aphanomyces root rot as shown by DSI and dry matter yield of pea plants at four weeks and eight weeks of plant growth at two soil inoculum levels. The pea cultivar Canterbury 39 was used.

Treatments		4 weeks				8 weeks			
		DSI %	Dry matter yield (g/plant)			DSI %	Dry matter yield (g/plant)		
			Root	Top	Total		Root	Top	Total
Inoculum level									
(I)	50	2.8	0.21	0.35	0.56	49.8	0.26	0.41	0.47
	100	47.2	0.10	0.23	0.34	66.1	0.26	0.40	0.66
	Sx̄	0.45	0.003	0.004	1.09	0.010	0.015	0.020	
Significance		**	**	**	**	**	NS	NS	NS
Pyroxyfur	PO	20.8	0.15	0.27	0.41	61.3	0.24	0.42	0.66
	PL	28.1	0.18	0.35	0.53	54.1	0.30	0.04	0.69
	PH	26.1	0.15	0.26	0.41	58.5	0.24	0.41	0.65
	Sx̄	0.55	0.004	0.005	0.008	1.34	0.012	0.018	0.024
Significance		**Q	**Q	**Q	**Q	**Q	**Q	NS	NS
Metalaxyl	MO	34.4	0.12	0.26	0.37	57.6	0.27	0.39	0.66
	ML	21.9	0.15	0.28	0.43	56.1	0.26	0.43	0.70
	MH	18.8	0.20	0.34	0.54	60.1	0.25	0.40	0.64
	Sx̄	0.55	0.004	0.005	0.008	1.34	0.012	0.018	0.024
Significance		**L	**L	**L	**L	NS	NS	NS	NS
Interactions:									
I X P[a]		**	**	**	**	**	**	NS	NS
I X M[a]		**	**	**	**	NS	NS	NS	NS
P X M[a]		**	**	**	**	**	**	NS	**
I X P X M[b]		**	**	**	**	**	**	NS	**
	CV%	9.4	10.1	7.6	7.1	9.8	20.1	19.2	18.1

[a]All of these data with significant interactions are presented later in graphs.

[b]All of these data with significant interactions are presented later in tables.

Disease development was very rapid from four weeks to eight weeks of plant growth, with an increase from 2.8% to 49.8% with soil of DSI 50 and from 47.2% to 66.1% with soil of DSI 100.

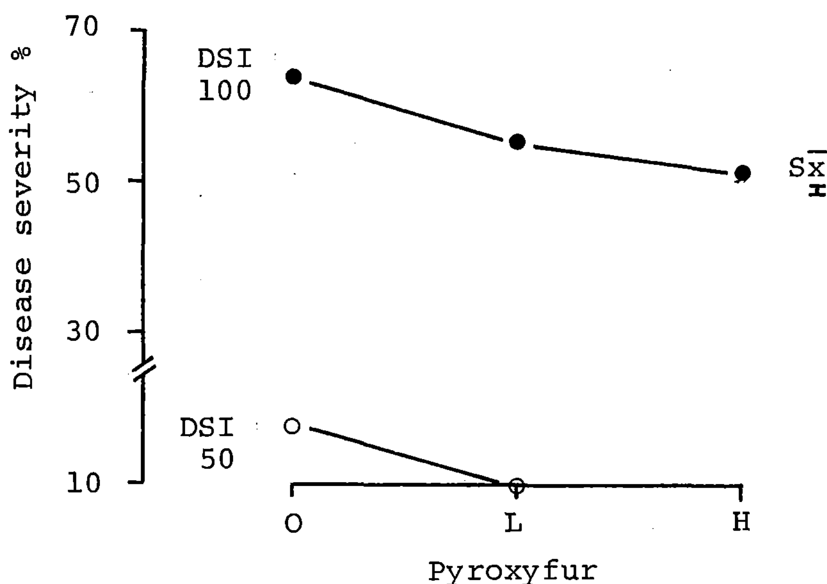
Plants from seed treated with pyroxyfur at both levels showed a significant quadratic increase (P = 0.01) in disease severity at four weeks. Severity was observed to be 35% and 25% worse in plants from seed treated with PL and PH respectively than in plants from untreated seed. However, at eight weeks, pyroxyfur treatments suppressed disease severity quadratically at a highly significant level (P = 0.01). PL was more effective in reducing disease by 11% than PH by 5%, which did not differ statistically from nil treatments.

Metalaxyl treatments were shown to be highly effective (significant at P = 0.01) in reducing disease severity with a linear response to increasing rate at four weeks of plant growth. There was a 36% decrease by ML and a 45% decrease in disease severity by MH. At eight weeks, both metalaxyl treatments showed a nil effect on disease development as there was no significant differences in disease severity.

There was a highly significant interaction (P = 0.01) between inoculum level and pyroxyfur as shown in Figure 5.4. At four weeks of plant growth, pyroxyfur treatment completely controlled disease development at inoculum level DSI 50 and continued its effectiveness at eight weeks reducing DSI with increased rates of the fungicides. At inoculum level DSI 100, disease was reduced significantly by the high rate of pyroxyfur at four weeks, but at eight weeks the only effect-

a) Pyroxyfur

4 weeks



8 weeks

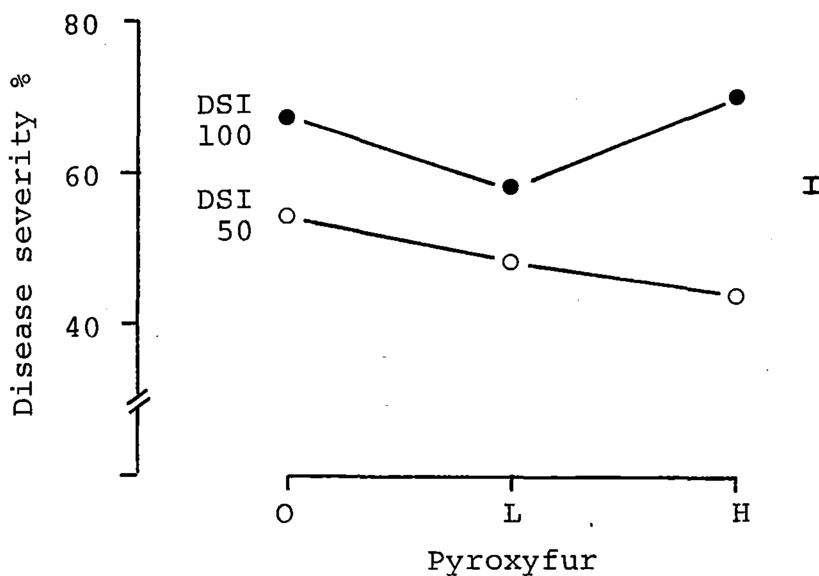
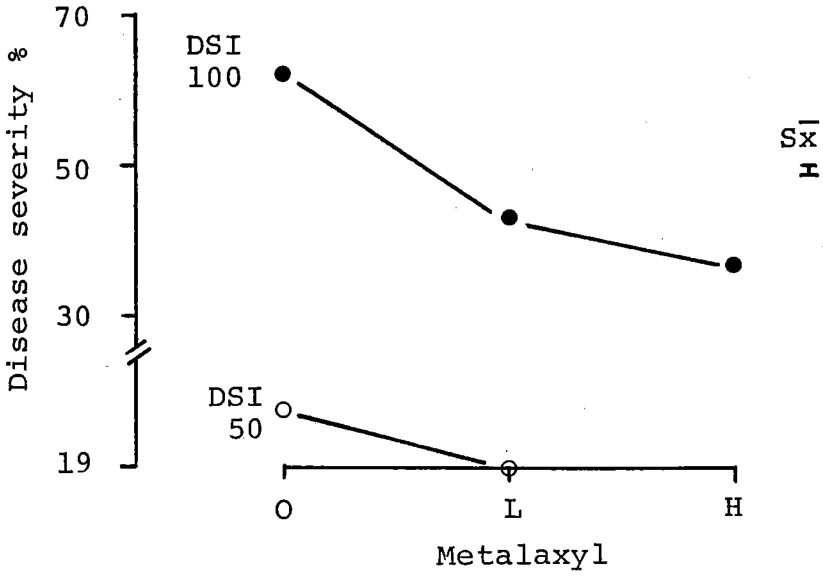


Figure 5.4: Interaction between a) pyroxyfur or b) metalaxyl and two soil inoculum levels DSI 50 and DSI 100 on severity of aphanomyces root rot of pea cultivar Canterbury 39 at 4 weeks and 8 weeks after sowing. (Mean data derived from results of 3 rates of each fungicide.)

b) Metalaxyl
4 weeks



ive response to treatment was at M_L where disease severity was reduced by 13%.

Significant interaction (P = 0.01) between inoculum levels and metalaxyl treatment only occurred at four weeks of plant growth (Figure 5.4). Disease development was completely controlled at DSI 50, while at DSI 100, disease severity was reduced significantly by 27% and 34% respectively by ML and MH treatments.

There was a highly significant interaction (P = 0.01) between pyroxyfur and metalaxyl treatments on disease severity as shown in Figure 5.5. All fungicide treatments effectively reduced disease severity at four weeks of plant growth. There was no significant difference in disease severity between treatments PL and PH (a reduction of 30% and 36% respectively). Metalaxyl alone at both rates was shown to be most effective reducing disease severity by 71% with ML and 87% with MH. There was a reduction in effectiveness of ML when combined with PL and PH. Similarly, the effectiveness of MH on its own was also reduced when combined with pyroxyfur at either rates. At eight weeks, only the PH application by itself significantly reduced disease severity by 20%, while PL and metalaxyl at either rates were no longer effective. However, the effectiveness of PL or ML was improved when applied as a mixture, resulting in the lowest disease severity obtained though not significantly different from the PH application. The other fungicide combinations did not result in any beneficial effects.

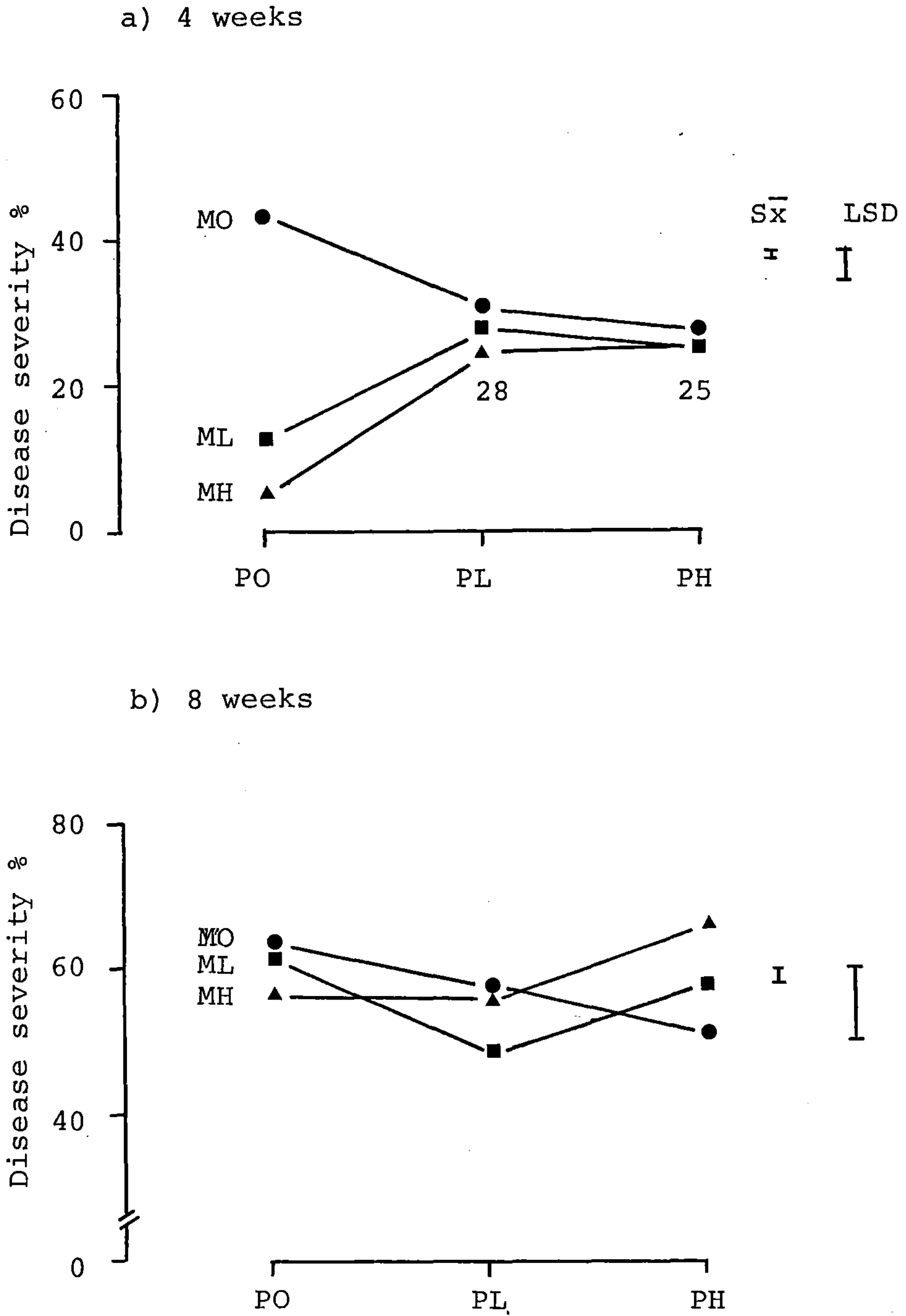


Figure 5.5: Interaction between pyroxyfur (P) and metalaxyl (M) at three rates on severity of aphanomyces root rot of pea cultivar Canterbury 39 at a) 4 weeks and b) 8 weeks of growth. Overall means of data derived from DSI 50 and 100.

A highly significant interaction among the three main factors was obtained (Table 5.5).

Table 5.5: Effect of pyroxyfur and metalaxyl seed treatments at two soil inoculum levels interaction on disease development of aphanomyces root rot of pea cultivar Canterbury 39 at four weeks and eight weeks of growth (disease severity %).

Soil inoculum (DSI)	Pyroxyfur (rate)	4 weeks			8 weeks		
		Metalaxyl (rate)			Metalaxyl (rate)		
		MO	ML	MH	MO	ML	MH
50	PO	25.0	0.0	0.0	55.4	50.0	47.6
	PL	0.0	0.0	0.0	50.3	39.3	50.0
	PH	0.0	0.0	0.0	47.6	50.0	50.0
100	PO	62.5	62.5	56.3	73.9	64.6	54.2
	PL	25.0	56.3	50.0	65.3	76.4	64.6
	PH	12.5	50.0	50.0	50.0	62.5	83.3
	$S\bar{x}$	2.34			5.69		
	LSD (0.05)	3.88			9.42		

At inoculum level DSI 50, all treatments effectively controlled disease development at four weeks. However, at eight weeks, the only fungicide treatment which was significantly effective was the mixture of PL and ML, reducing disease severity by 29%. At inoculum level DSI 100, both rates of pyroxyfur effectively reduced disease severity by 60% with PL and 80% with PH at four weeks, but only metalaxyl at the higher rate reduced disease severity significantly by 10%. Fungicide combinations were not beneficial as compared with pyroxyfur application alone at either rates and in fact reduced the effectiveness of pyroxyfur. At eight weeks, the higher rates of both pyroxyfur

and metalaxyl was shown to be equally effective as they did not differ significantly. Although treatment combinations PH and ML reduced disease severity significantly by 15%, the combination was not as effective as PH application alone.

b) Root Dry Matter

Root dry matter yield was highly significantly (P = 0.01) affected by inoculum levels with a 52% difference between DSI 50 and DSI 100 at four weeks of plant growth. However, at eight weeks, there was no statistical difference (Table 5.4).

A quadratic response (highly significant at P = 0.01) to pyroxyfur treatment by root dry matter yield was due to the 20% at four weeks and 25% increase at eight weeks by PL treatment. At four weeks, yield increased linearly at a highly significant level (P = 0.01) with increased rate of metalaxyl with 25% with ML and 67% with MH. At eight weeks, no statistical difference in yield was obtained.

There was a highly significant interaction (P = 0.01) between inoculum levels and pyroxyfur on root dry matter at four and eight weeks of plant growth (Figure 5.6). At four weeks, at inoculum level DSI 50, pyroxyfur treated plants accumulated more yield than the non-treated and the increase was slightly more with PL (21%) than with PH (10%). At inoculum level DSI 100, a 30% increase in yield was obtained with PL treated plants, but 20% decrease occurred with PH treated plants. At eight weeks, both treatments of pyroxyfur decreased yield by 14% at DSI 50, while at DSI 100, the treatments increased yield by 32% and 26% with PL and PH respectively.

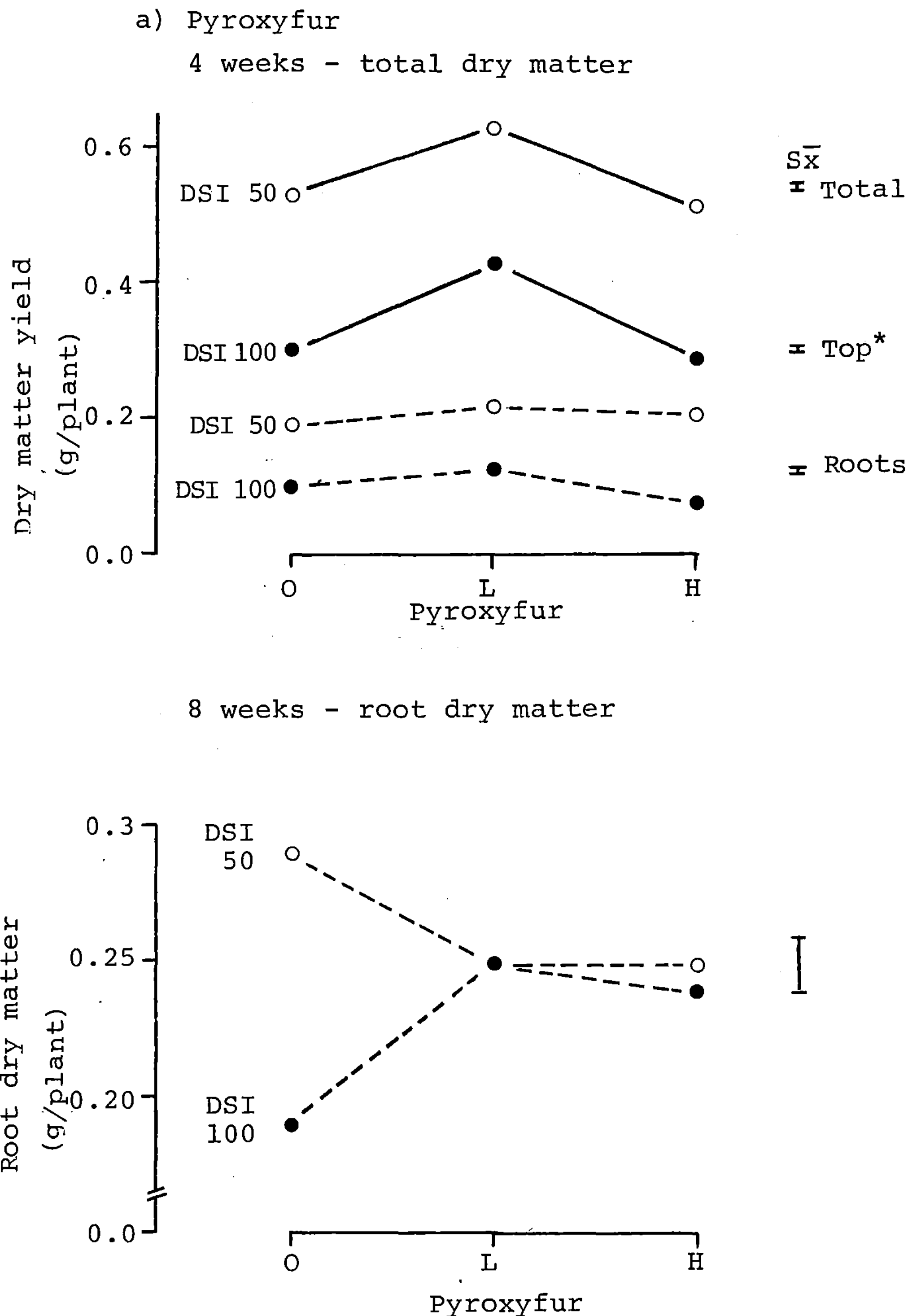
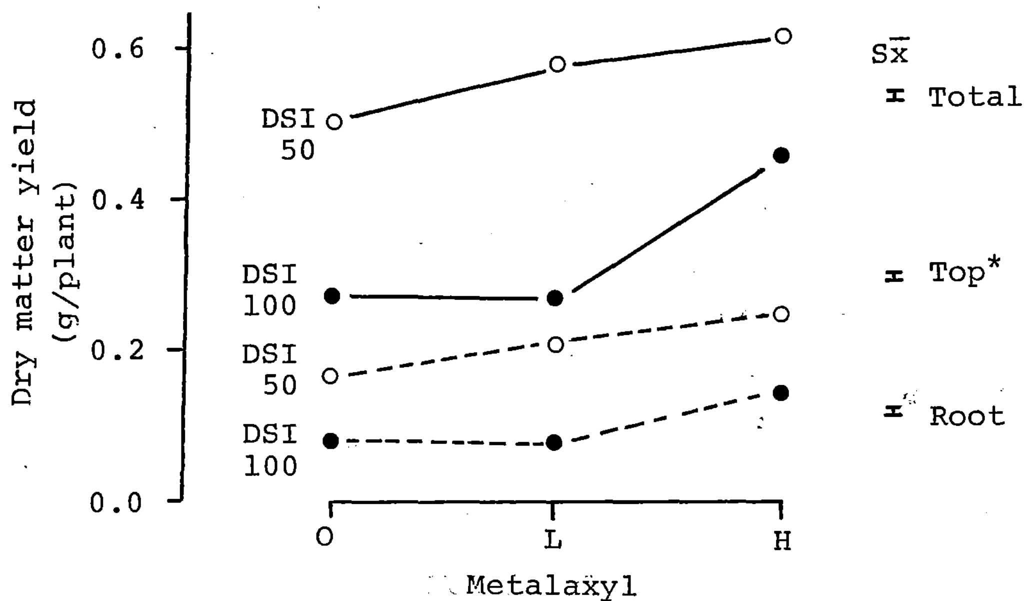


Figure 5.6: Interaction between pyroxyfur (a) or metalaxyl (b) and soil inoculum levels DSI 50 and DSI 100 on total dry matter (——) and root dry matter (---) of pea cultivar Canterbury 39 at 4 weeks and 8 weeks after sowing. Means data derived from 3 rates of each fungicide.

*Top dry matter is the difference between total and root dry matter.

b) Metalaxyl

4 weeks - total dry matter.



Interaction between inoculum levels and metalaxyl only occurred at four weeks (Figure 5.6). In DSI 50, yield was increased with increased rates (38% with ML and 56% with MH). In DSI 100, no statistical difference was obtained between the nil-treated and treated plants.

There was a highly significant interaction (P = 0.01) between pyroxyfur and metalaxyl treatments as shown in Figure 5.7. At four weeks, both rates of pyroxyfur were equally effective, increasing yield by 40%. Although there was no statistical difference between ML and nil treatments, there was a slight increase of 10%. The highest root dry matter accumulation was with plants treated with MH (140% over MO). Fungicide combinations did not show any increase in efficacy as compared with either one of the fungicides used in the mixtures. At eight weeks, the only treatment which increased yield significantly (61%) was the combination of PL and ML.

A highly significant interaction (P = 0.01) among the three main factors on root dry matter yield was obtained as shown in Table 5.6.

At four weeks of plant growth, at inoculum level DSI 50, all treatments increased yield. Both pyroxyfur and metalaxyl alone were equally effective in increasing yield at increasing rates (25% with the low rates and 70% with the high rates). Fungicide combinations increased the efficacy of pyroxyfur and metalaxyl except PH + MH which was not significantly different from each fungicide alone at the high rate. The combination of PL and ML increased the effectiveness of both fungicides at

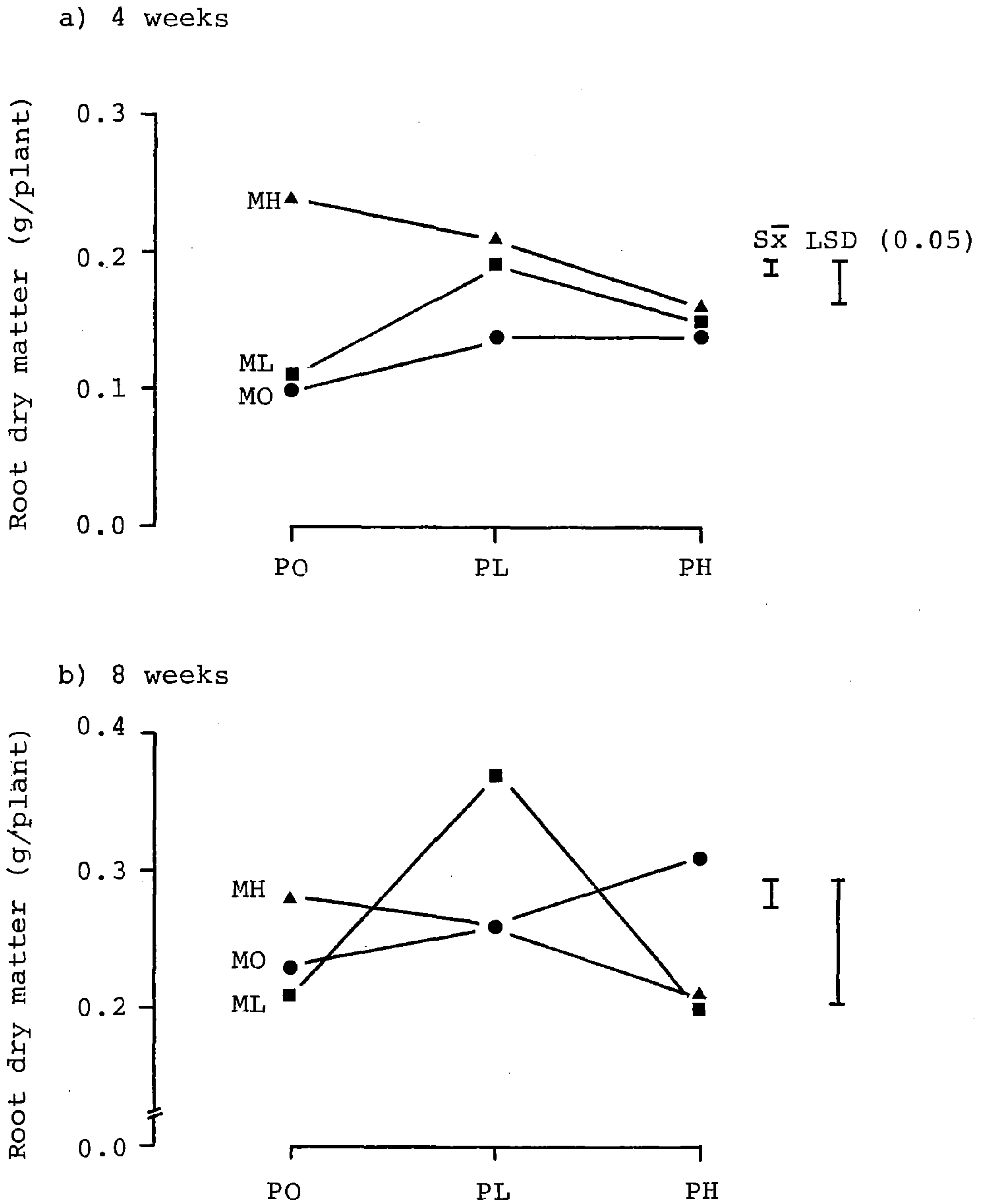


Figure 5.7: Interaction between pyroxyfur (P) and metalaxyl (M) at three rates on root dry matter yield of pea cultivar Canterbury 39 at a) 4 weeks and b) 8 weeks of growth. Overall means of data derived from DSI 50 and 100.

low rate by 33%. Combinations of PH + ML and PL + MH showed marked increases of 116% and 158% respectively as compared with the nil treatment. At eight weeks, treatments PH and combination of PL and MH increased yield by 3% which was not significant from nil treatment, while the other treatments reduced yield significantly especially the low rates of pyroxyfur (34%), metalaxyl (31%) and the treatment combination of PH + ML (38%).

Table 5.6: Effects of pyroxyfur and metalaxyl seed treatments at two soil inoculum levels on root dry matter yield of pea cultivar Canterbury 39 affected by aphanomyces root rot at four weeks and eight weeks (g/plant).

Soil inoculum (DSI)	Pyroxyfur (rate)	4 weeks			8 weeks		
		Metalaxyl (rate)			Metalaxyl (rate)		
		MO	ML	MH	MO	ML	MH
50	PO	0.12	0.15	0.21	0.32	0.22	0.23
	PL	0.15	0.20	0.31	0.21	0.26	0.33
	PH	0.20	0.26	0.19	0.33	0.20	0.26
100	PO	0.07	0.13	0.06	0.13	0.22	0.41
	PL	0.07	0.10	0.07	0.30	0.13	0.22
	PH	0.16	0.16	0.12	0.42	0.32	0.16
	$S\bar{x}$	0.016			0.052		
	LSD (0.05)	0.03			0.09		

At inoculum level DSI 100, at four weeks, PH application increased yield by 129% while PL did not differ significantly from the nil treatment. On the other hand, only ML was effective, increasing yield by 86%. At eight weeks, pyroxyfur increased yield with increased rates although root accumulations in PL and PH treated plants did not differ significantly. A similar trend was observed with metalaxyl with yield increased by increased rates (70% with ML and 223% with MH). Throughout

plant growth there was no indication of beneficial usage of fungicide combinations as compared with the fungicides alone.

c) Top and Total Dry Matter

The top and total dry matter responses to the effect of pyroxyfur and metalaxyl seed treatments at two inoculum levels DSI 50 and 100 followed a similar pattern to that of the responses of root dry matter at four weeks (Table 5.4 and Append. 4 to 7). However, at eight weeks, the responses of the top and total dry matter to pyroxyfur treatment differed from that of root dry matter, for there were no significant differences between either rate of pyroxyfur and the nil treatments. Consequently, there were no significant interactions between inoculum levels and pyroxyfur on top and total dry matter as occurred in the root dry matter.

Trial Two The effects of pyroxyfur and hymexazol on the severity of aphanomyces root rot with two soil inoculum levels and on the dry matter yield of pea plants at four weeks and eight weeks are shown in Table 5.7.

a) Disease Severity Index

There was a highly significant difference ($P = 0.01$) in disease severity between inoculum levels both at four weeks and eight weeks. With soil of DSI 50, disease severity increased from DSI 2.8% to 47.6% throughout the growth period while with soil of DSI 100, the increase was from 30.4% to 52.3%.

Table 5.7: Effects of pyroxyfur and hymexazol seed treatments on severity of aphanomyces root rot as shown by DSI and dry matter yield of pea plants at four and eight weeks after sowing at two soil inoculum levels. The pea cultivar Canterbury 39 was used.

Treatments		4 weeks				8 weeks			
		DSI %	Dry matter yield (g/plant)			DSI %	Dry matter yield (g/plant)		
			Root	Top	Total		Root	Top	Total
Inoculum level (I)	50	2.8	0.18	0.34	0.52	47.6	0.26	0.40	0.66
	100	30.4	0.12	0.25	0.37	52.3	0.28	0.48	0.76
	S$\bar{x}$	0.57	0.003	0.004	0.006	1.14	0.012	0.015	0.020
Significance		**	**	**	**	**	NS	**	**
Pyroxyfur	PO	20.8	0.12	0.26	0.38	54.3	0.26	0.44	0.70
	PL	15.4	0.17	0.31	0.48	49.6	0.26	0.45	0.71
	PH	13.6	0.17	0.32	0.49	46.8	0.29	0.43	0.72
	S$\bar{x}$	0.70	0.004	0.005	0.007	1.39	0.014	0.019	0.024
Significance		**L	**L	**L	**L	**L	NS	NS	NS
Hymexazol	HO	29.2	0.11	0.26	0.38	54.7	0.25	0.40	0.65
	HL	13.6	0.16	0.30	0.46	49.0	0.30	0.46	0.77
	HH	7.0	0.18	0.32	0.50	46.2	0.25	0.45	0.71
	S$\bar{x}$	0.70	0.004	0.005	0.007	1.39	0.014	0.019	0.024
Significance		**L	**L	**L	**L	*L	*L	**Q	**Q
Interactions:									
I X P[a]		*	*	**	*	*	NS	NS	NS
I X H[a]		**	**	*	*	NS	NS	**	*
P X H[a]		**	**	**	**	**	NS	NS	NS
I X P X H[b]		NS	**	**	**	NS	*	*	*
	CV%	17.8	11.8	7.2	7.2	11.8	22.2	18.3	14.5

[a]All of these data with significant interactions are presented later in graphs.

[b]All of these data with significant interactions are presented later in tables.

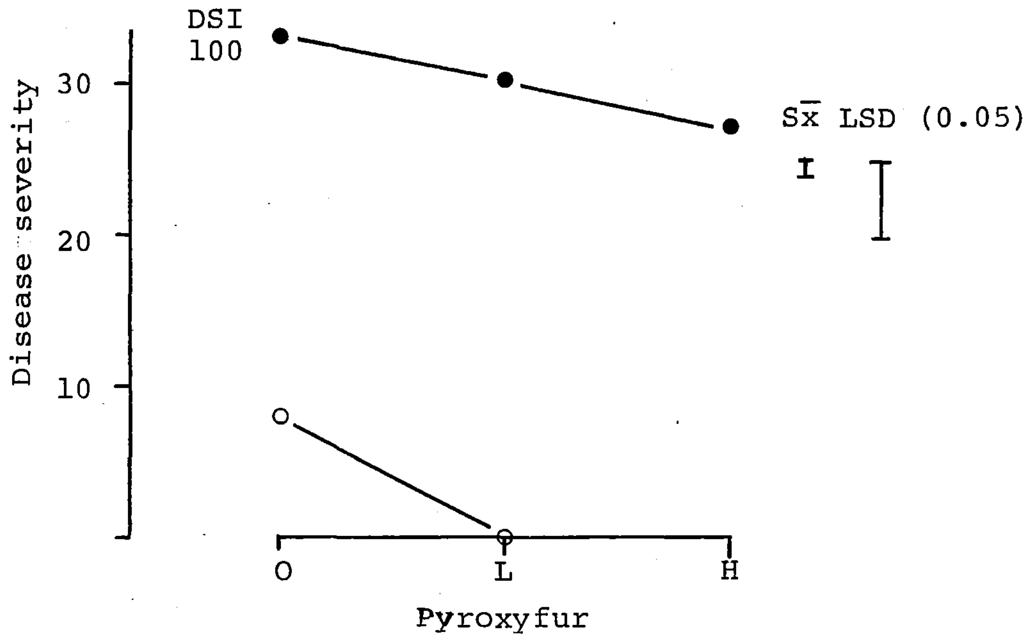
There was a linear response with increase rates of pyroxyfur and hymexazol with the results being highly significant. Hymexazol was more effective than pyroxyfur at four weeks, but at eight weeks both treatments did not show any differences with their low and high rates.

Interactions between inoculum levels and pyroxyfur were shown to be significant (P = 0.05) throughout the growth period while the interactions between inoculum level and hymexazol only occurred at four weeks at a highly significant level (P = 0.01). This is illustrated in Figure 5.8. Severity in the nil treatment increased from 8.3% at four weeks to 52% at eight weeks in soil inoculum DSI 50 and from 33% to 55% in DSI 100. At four weeks, complete control of disease development was obtained by all fungicide treatments in DSI 50. In DSI 100, both pyroxyfur and hymexazol reduced disease severity with increased rates. However, PL application was not significantly different from nil treatment and PH, while PH significantly reduced severity by 18% compared to nil treatment. In the hymexazol treatments, only HH was effective in reducing severity. At eight weeks, PH continued its effectiveness, resulting in severity significantly less than PL and nil treatments in DSI 50. There were no significant differences in severity in DSI 100 with PL showing a better effect than PH.

The pyroxyfur and hymexazol interaction was highly significant (P = 0.01) as shown in Figure 5.9. At four weeks, pyroxyfur reduced severity by 50% as both rates. Hymexazol

a) Pyroxyfur

4 weeks



8 weeks

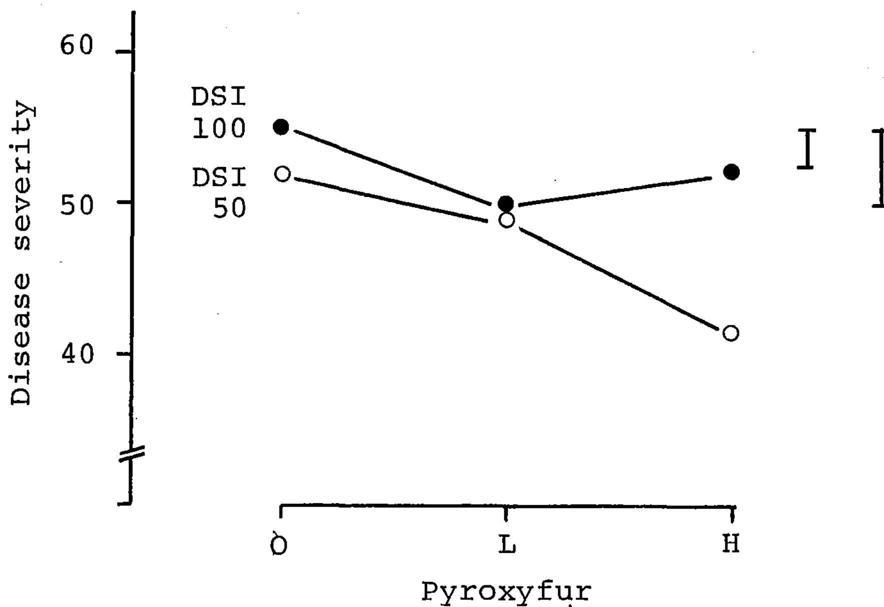
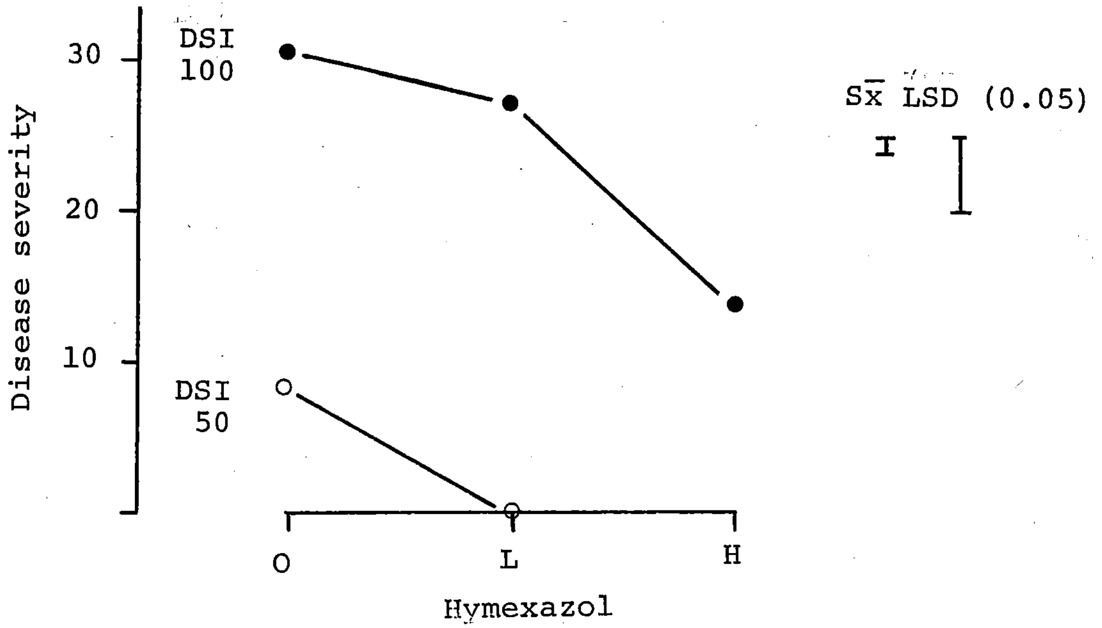


Figure 5.8: Interaction between a) pyroxyful or b) hymexazol and two soil inoculum levels DSI 50 and DSI 100 on severity of aphanomyces root rot of pea cultivar Canterbury 39 at 4 weeks and 8 weeks after sowing. (Mean data derived from results of 3 rates of each fungicide.)

b) Hymexazol
4 weeks



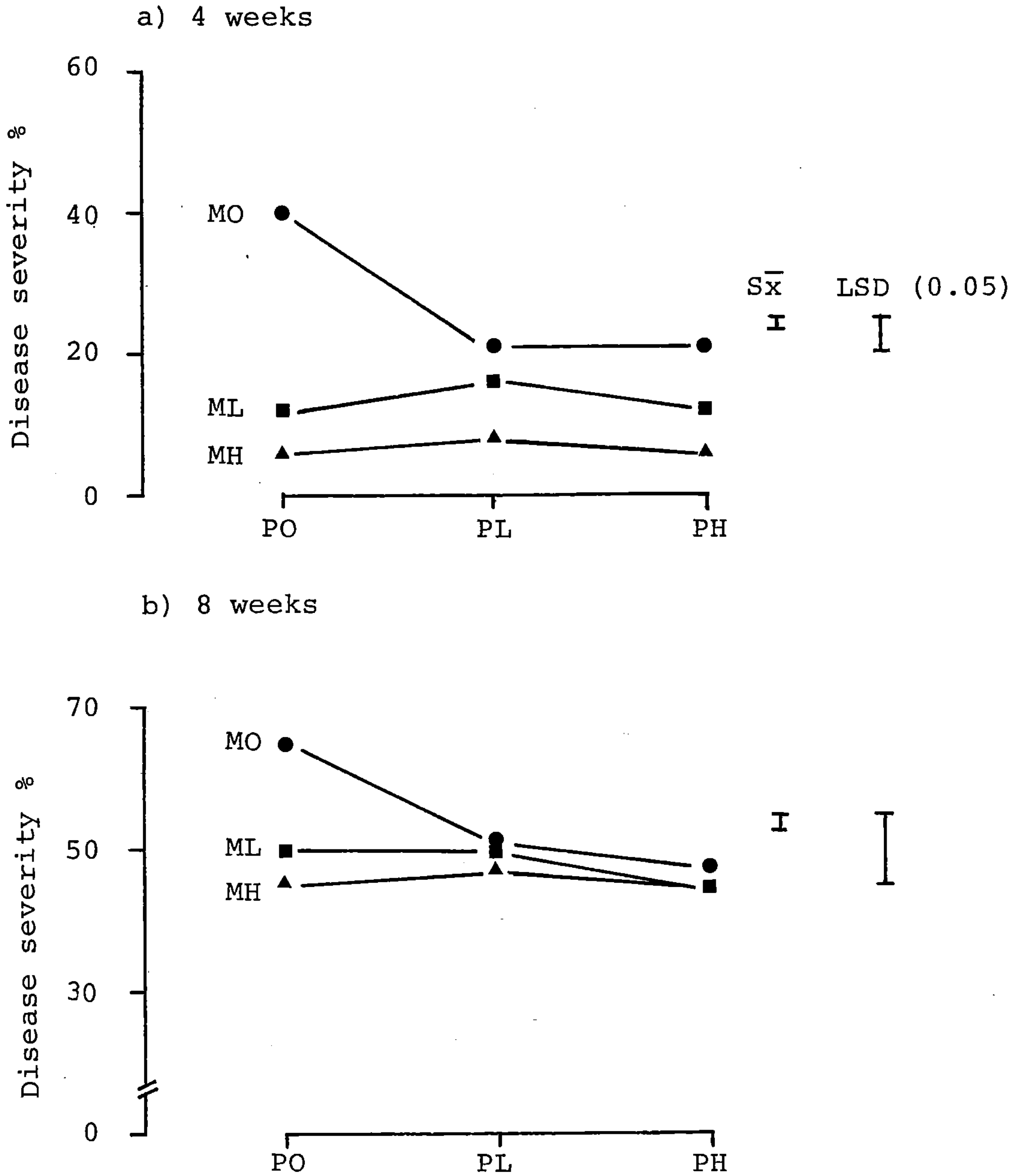


Figure 5.9: Interaction between pyroxyfur (P) and hymexazol (H) at three rates on severity of aphanomyces root rot of pea cultivar Canterbury 39 at a) 4 weeks and b) 8 weeks of growth. Overall means of data derived from DSI 50 and DSI 100.

was more effective compared to pyroxyfur reducing severity by 70% and 87% with HL and HH respectively. Severity observed in treatment combinations did not differ statistically from HL or HH. At eight weeks, both rates of both fungicides were equally effective as the severity was not significantly different. Treatment combinations also did not differ statistically from each fungicide application on its own.

With respect to disease severity, there were no significant interactions among the three main factors.

b) Root Dry Matter

At four weeks, root dry matter yield was affected by the inoculum level with a 50% difference between DSI 50 and DSI 100. This difference was no longer present at eight weeks.

At four weeks, there was a linear response to pyroxyfur with increased rates at a highly significant level (P = 0.01). Both rates increased yield by 42%. At eight weeks, there was no significant difference between the two treatments.

The response to hymexazol was linear (highly significant at P = 0.01) at four weeks with a 45% and 64% increase in root accumulation in plants treated with HL and HH respectively. At eight weeks, the trend was changed to a quadratic response (significant at P = 0.05) with only HL increasing yield by 20%.

Interactions between inoculum and fungicides on root dry matter occurred at four weeks as shown in Figure 5.10. At both inoculum levels, both rates of pyroxyfur were equally effective as there was no significant difference between root matter yield. However, higher yield was obtained in soil inoculum level DSI 50 (43% increase with both rates of pyroxyfur over nil treatment). In soil inoculum level DSI 100, plants treated with PL and PH increased root accumulation by 40% and 30% respectively. In the case of hymexazol treatments, both rates were equally effective in DSI 50 although higher yield was obtained in the HL application (43%) than HH (36%). In DSI 100, a linear response with increased rates was observed with 33% in the HL application and 78% in the HH application.

Root accumulation was affected by an interaction between pyroxyfur and hymexazol occurring at four weeks, and this is shown in Figure 5.11. The low rates of pyroxyfur and hymexazol did not differ significantly in yield as compared with nil treatment. However, the high rates of these two fungicides proved to be equally effective, both increasing yield by 60%. Fungicide combinations of PL + HL increased the effectiveness of the fungicide in the absence of the other by 100%, while the other three combinations did not show any beneficial effect.

The effect of the three main factors interaction on root dry matter yield, shown in Table 5.8, was significantly higher (P = 0.01) at four weeks than at eight weeks (P = 0.05).

a) Pyroxyfur
4 weeks

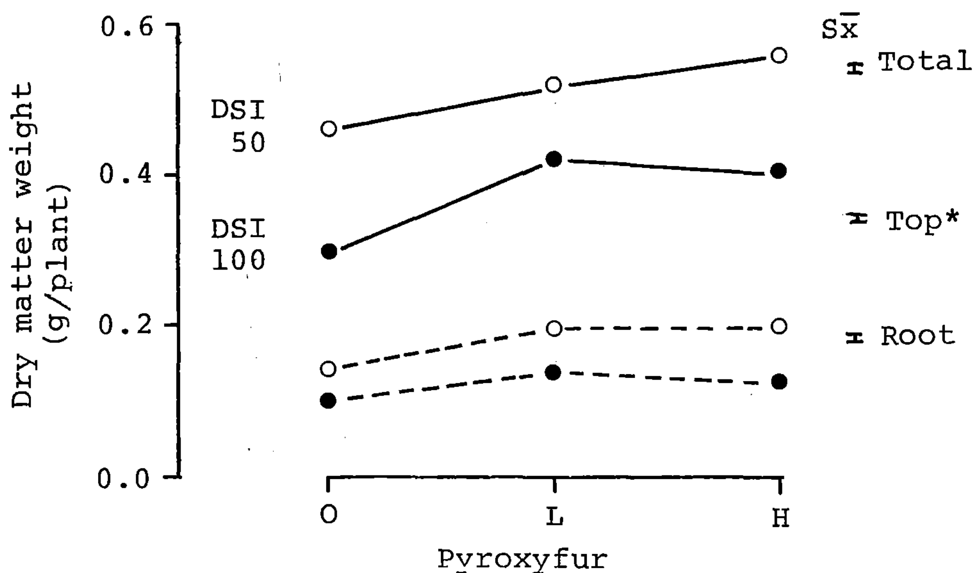
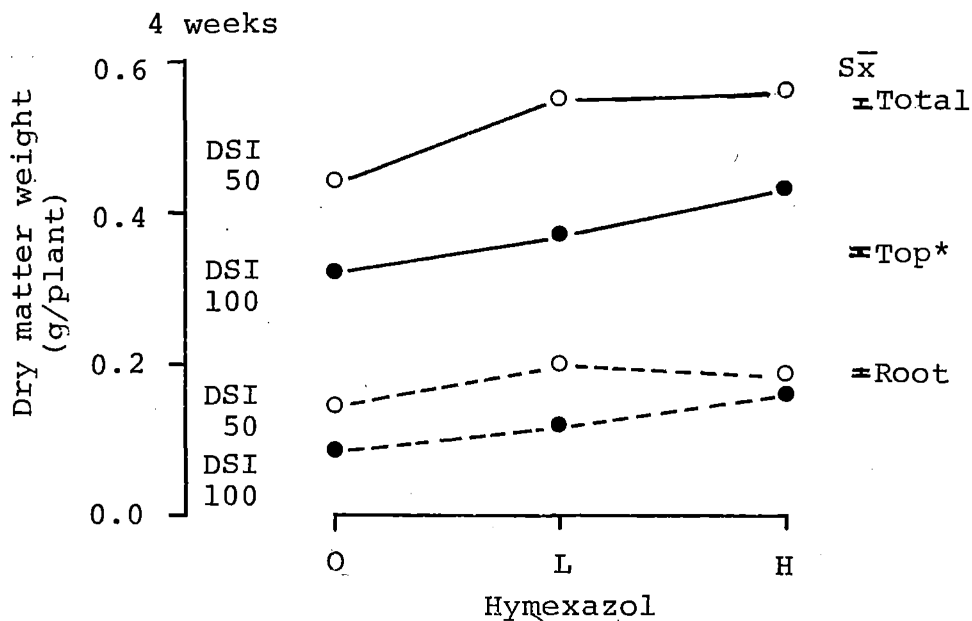
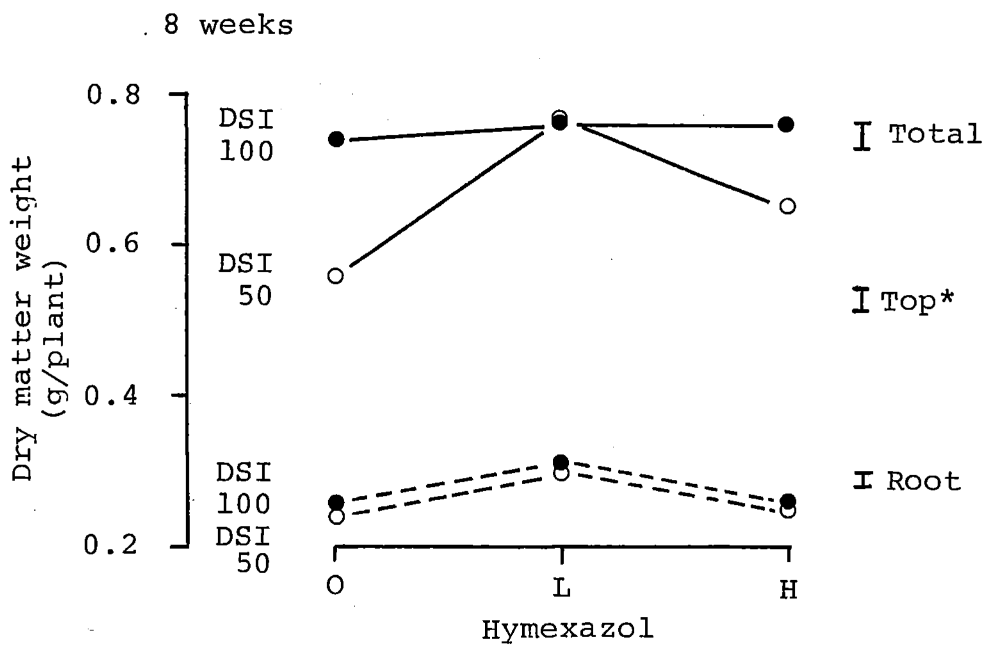


Figure 5.10: Interaction between pyroxyfur (a) or hymexazol (b) and soil inoculum levels DSI 50 and DSI 100 on total dry matter (——) and root dry matter (---) of pea cultivar Canterbury 39 at 4 weeks and 8 weeks after sowing. (Mean data derived from 3 rates of each fungicide.)

*Top dry matter is the difference between total dry matter and root matter.

b) Hymexazol





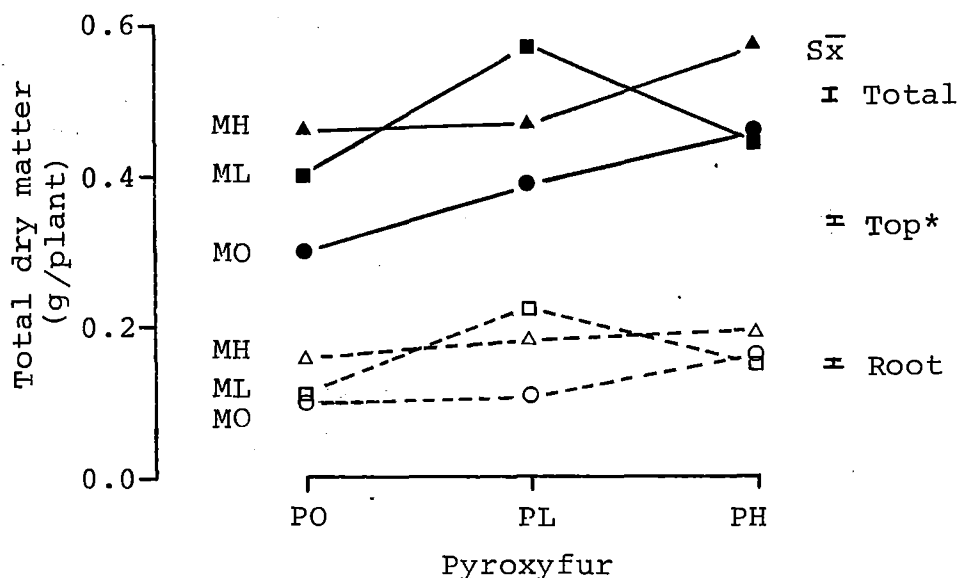


Figure 5.11: Interaction of pyroxyfur (P) and hymexazol (H) at three rates on dry matter yield (total dry matter —— and root dry matter ---) of pea cultivar Canterbury 39 at 4 weeks after sowing. Mean data derived from DSI 50 and DSI 100.

*Top dry matter is the difference between total dry matter and root dry matter.

Table 5.8: Effects and interactions of pyroxyfur and hymexazol seed treatments at two soil inoculums on root dry matter of pea cultivar Canterbury 39 affected by aphanomyces root rot at four weeks and eight weeks of growth (g/plant).

Soil inoculum (DSI)	Pyroxyfur (rate)	4 weeks Hymexazol (rate)			8 weeks Hymexazol (rate)		
		HO	HL	HH	HO	HL	HH
50	PO	0.10	0.13	0.20	0.24	0.21	0.26
	PL	0.20	0.17	0.15	0.46	0.33	0.19
	PH	0.21	0.21	0.22	0.22	0.31	0.24
100	PO	0.07	0.09	0.11	0.13	0.30	0.42
	PL	0.07	0.16	0.13	0.22	0.15	0.22
	PH	0.16	0.16	0.16	0.41	0.32	0.16
	$S\bar{x}$	0.018			0.060		
	LSD (0.05)	0.03			0.10		

At four weeks with soil inoculum DSI 50, both rates of pyroxyfur yielded 100% over nil treatment. Treatment HL increased yield by 30% while HH was similarly effective to pyroxyfur application. None of the fungicide combinations improved the effectiveness of pyroxyfur alone. With soil of DSI 100, yield increases occurred only with the higher rates of fungicide, 129% with PH and 57% with HH. However, the effectivness of the low rate treatments were improved when they were used as a mixture PL + HL increasing yield by 129% equally effective to the higher rate of pyroxyfur.

At eight weeks, with DSI 50, the only significant increase in yield was with PL treated plants. The effectiveness of this treatment was considerably reduced when combined with HL by 28% and HH by 59%. On the other hand, the PH + HL mixture increased the effectiveness of the fungicide in the

the absence of the other by approximately 44%. With soil of DSI 100, yield was increased with increased rate of both fungicides. PL and HL applications showed an increase of 69% and 130% in root accumulation, while the high rates of both fungicides resulted in similar increase of 219%. Fungicide combinations significantly reduced the effectiveness of fungicide in the absence of the other at any rate.

c) Top and Total Dry Matter

At four weeks, a trend similar to that of root dry matter was observed on top and total dry matter responses to the effect of pyroxyfur and hymexazol treatments at two inoculum levels (Table 5.7 and Append. 8 to 9). However, at eight weeks, the responses of the top and total dry matter to inoculum levels was highly significant and different from the non-significant response in root dry matter. There were significant interactions between inoculum levels and hymexazol (I X H) treatment effects on top and total dry matter, but no interaction occurred between inoculum levels and pyroxyfur (I X P).

Discussion

Root rot severity as measured by the dry matter yield of pea plants grown from treated seed showed that *Aphanomyces euteiches* was suppressed to varying degrees by pyroxyfur, metalaxyl and hymexazol in glasshouse trials. At four weeks of growth, significant control was obtained by all the fungicides with significant increases in yield over nil treated plants. However, at eight weeks of growth, the

effectiveness was reduced to a non-significant level in the metalaxyl treatment as shown by disease severity and root and top dry matter yield. The significance in the disease severity with either pyroxyfur or hymexazol was less at eight weeks as compared with four weeks, and hence there was no difference in dry matter between pyroxyfur and nil treated plants. Similar findings by Papavizas and Lewis (unpublished data reported by Papavizas and Ayers 1974) were also reported that in their fungicide trial, considerable protection of peas was afforded for up to four weeks after sowing. Jermyn *et al.* (1982) reported that fungicidal effect on root rot in their field trials occurred while plants were in the pre-flowering stage (12-15 nodes), but the effect did not persist. *Aphanomyces euteiches* attacks host plants over a period of time (Pfleger *et al.* 1976) and for this reason, seed treatment might not be expected to be very successful against the pathogen when roots could quickly grow away from the area of fungicidal influence. However, the action of seed treatments was shown to retard root infection by the pathogen. The speculation that any delay of root infection would enhance seedling vigor over seedlings under immediate disease stress, increased seedling vigor might make roots more tolerant for the pathogen when they grew out of the fungicidal zone (Grau and Reiling 1977). The delayed onset of severe root rot would therefore result in less apparent root rot at any point in time and thus higher yield obtained.

Determination of chemical rate was important. Rate should be regulated to avoid phytotoxicity such as observed for pyroxyfur at 3 g a.i./kg seed. Root severity was reduced but so was yield. On the other hand, metalaxyl and hymexazol gave better controls when rates were doubled to those used by Jermyn *et al.* (1982).

Fungicide combinations gave inconsistent effect on disease severity as indicated by dry matter yield. In general, they did not show any increase in efficacy of increasing yield over each fungicide in the absence of the other.

Farmers have been advised to avoid planting peas in soil with DSI greater than 70, whereas they were advised that soils with DSI between 50 and 70 were potentially risky (Sherwood and Hagedorn 1958). The results here have shown that chemical treatments in plants to be sown in the lower index range of soil could result in beneficial economic yield, while the fungicides were not effective in DSI 100 soil.

Experiment Two: The effect of three fungicide seed treatments on disease development at two inoculum levels over a period of 12 weeks.

Introduction - The glasshouse experiment was conducted to investigate the persistence of the three seed treatment chemicals, pyroxyfur, metalaxyl and hymexazol, on disease development over a period of 12 weeks at two soil inoculum levels.

<u>Materials and Method</u> - The four seed treatments consisted of pyroxyfur (3.0 g a.i./kg seed), metalaxyl (6.0 g a.i./kg seed), hymexazol (10 g a.i./kg seed) and untreated seed. Two soil inoculum levels were used and the experiment was arranged in a 4 x 2 factorial complete randomized design replicated 4 times. Similar soils, as defined in Experiment One, were used. Five treated seeds (fungicides were applied as described in Experiment One) were sown per pot (150 mm diameter x 200 mm deep) on 29 November, 1982 and 16 pots were prepared for each soil inoculum level. Each pot was isolated in individual plastic trays to prevent inoculum moving from one pot to the next. The pots were rotated every other day so that each received a similar temperature and light regime in the glasshouse maintained between 24°-28°. Soil moisture was kept at -3 millibars throughout the experiment. At 12 weeks after sowing, the plants were harvested (7 February, 1983) for disease assessment and dry matter yield of roots, tops and pods.

<u>Results</u> - The results of the experiment are presented in Table 5.9.

a) <u>Disease Severity Indices (DSI)</u>.

As expected, there was a highly significant (P = 0.01) difference between the two soil levels. All fungicide treatments were significantly different (P = 0.01) from untreated, and the hymexazol also was significantly different from the pyroxyfur and metalaxyl. Interaction between inoculum levels and fungicides arose mainly because of the significant differences in disease severity between treatments in DSI 50 but not in DSI 100 (Table 5.9). All treatments

Table 5.9: Effect of seed treatments on disease development at two soil inoculum levels of *Aphanomyces euteiches* as shown by DSI and dry matter yield of peas cultivar Canterbury 39 at 12 weeks of growth.

Treatments		Disease Severity Index (DSI)	Dry matter yield (g/plant)			
			Root	Top	Pod	Total
Inoculum level	DSI (50)	67.8	0.24	0.57	0.43	1.24
	DSI (100)	96.6	0.12	0.49	0.43	1.04
	$S\bar{x}$	2.56	0.021	0.048	0.043	0.100
Significance		**	**	NS	NS	NS
Fungicide	F_1 (untreated)	93.3	0.13	0.43	0.35	0.92
	F_2 (pyroxyfur)	80.8	0.18	0.51	0.50	1.19
	F_3 (metalaxyl)	81.9	0.19	0.72	0.52	1.43
	F_4 (hymexazol)	73.0	0.22	0.46	0.34	1.02
	$S\bar{x}$	3.62	0.030	0.068	0.067	0.141
Significance		**	NS	*	NS	NS
Interactions:						
Inoculum level X Fungicide		**	NS	NS	*	*

	DSI 50 S1	DSI 100 S2	Root	Top	Pod S1	Pod S2	Total S1	Total S2
F_1	86.6	100.0			0.26	0.45	0.80	1.04
F_2	73.4	88.1			0.42	0.59	1.15	1.24
F_3	63.8	100.0			0.69	0.40	1.89	0.97
F_4	47.5	98.5			0.34	0.33	1.10	0.93
$S\bar{x}$	5.13				0.095		0.199	
LSD (0.05)	15.0				0.28		0.58	
CV%	12.5		47.1	36.2	44.2		35.0	

reduced disease severity in DSI 50 but only metalaxyl and hymexazol were significantly different from nil treatment. There were no significant differences between pyroxyfur and metalaxyl treatments and both differed significantly from hymexazol.

b) Root Dry Matter

Root dry matter yield (Table 5.9) was twice as much in DSI 50 than in DSI 100 (highly significant at P = 0.01). Although yield was increased by fungicide treatment (38%, 46% and 69% with pyroxyfur, metalaxyl and hymexazol applications respectively) the difference between all treatments including untreated were not significant. There was no significant interaction between the two main factors on root dry matter.

c) Top Dry Matter

There was no significant difference in top dry matter yield between the two inoculum levels. There was a significant response (P = 0.05) to fungicide treatments. Metalaxyl was the only treatment that significantly increased yield by 67% over nil treatment, but it was not significantly different from pyroxyfur and hymexazol treatments which increased yield by 19% and 7% respectively. The interaction between inoculum levels and fungicides on top dry matter was not significant.

d) Pod Dry Matter

Similar pod dry matter yields were obtained in the two inoculum levels. The response to fungicide treatments was not significant although pyroxyfur and metalaxyl applications increased yield by 42% and 48% respectively. There was a

slight reduction of 3% in yield in the hymexazol treatment. A significant interaction (P = 0.05) between inoculum levels and fungicides on pod dry matter yield was obtained. The increase in yield by metalaxyl in the overall fungicide treatment was due to the significant increase of 165% in the metalaxyl treated plants in DSI 50. However, this increase did not differ statistically from yield obtained in the pyroxyfur treatment (62%). Hymexazol only yielded 31% more than nil treatment. In DSI 100, yields obtained were not significantly different from each other.

e) Total Dry Matter

The differences in total dry matter yield were not significant between the two inoculum levels although there was 18% more dry matter in DSI 50 than in DSI 100. The response to fungicides was also not significant. However, all treatments increased yield with metalaxyl producing the highest yield followed by pyroxyfur and hymexazol (55%, 29% and 10% respectively). The interaction between inoculum levels and fungicides was similar to that shown by pod dry matter yield. In DSI 50, soil metalaxyl application resulted in a significant difference in yield from all other treatments (136% increase over nil treatment). In DSI 100, yield differences were not significant from each other.

Discussion - The results in Table 5.9 confirmed the unsuccessful control by seed treatments of pyroxyfur, metalaxyl and hymexazol at high inoculum levels of *Aphanomyces euteiches* (DSI 100) due to their non-persistence effect against the pathogen reported in Experiment One (Section 5.3.2) and field

trial conducted by Jermyn *et al.* (1982). However, with soil of DSI 50, there was a reduced DSI and significant differences in pod and total yields among treatments with metalaxyl being significantly different. The hymexazol treatment, although reducing the DSI, also caused a reduction in dry matter yield and this is likely to be due to phytotoxicity effect on the treated plants.

Two factors that have to be considered in comparing results from the glasshouse trials and field trials (Jermyn *et al.* 1982) are: first of all, the rates of fungicides used were twice as high in the glasshouse trials as in the field trials; secondly, there is the relevant environmental conditions. In the glasshouse, conditions were conducive to aphanomyces root rot development whereas in the field, the condition during the trial was dry and not favourable to disease development. Thus, further research should study the factors and conditions required for a specific chemical-plant disease interaction to be expressed.

Experiment Three: Effect of pyroxyfur seed treatment at two soil temperatures and three soil water potentials.

Introduction - Results from experiments on the effects of soil temperature and water potential on disease development of aphanomyces root rot (Chapter Three) showed that growth of pea plants was not only affected by colder temperatures and drier conditions, but also by root rot infection at warmer temperatures and wetter conditions. Field trials by Jermyn

et al. (1982) showed that none of the chemicals at the rates tested provided effective control of the disease in a season of dry conditions generally not conducive to the development of the disease. Previous experiments in this chapter showed that effective control by pyroxyfur, metalaxyl and hymexazol occurred during early growth of plants but not at later growth stages. However, these experiments were in conditions conducive to the development of aphanomyces root rot.

The objective of this experiment was to investigate the effect of pyroxyfur seed treatment on aphanomyces root rot of peas at two soil temperatures and three soil water potentials in Wisconsin soil temperature tanks in the glasshouse maintained at 24-28°.

Materials and Method - The pot trial was a 2 x 2 x 3 split plot design with temperatures (20°, 24°) as the main plots arranged in a complete randomised block design and replicated four times. The subplots were two seed treatments (nil and treated) and three water potential levels (-200 millibars, -28 millibars and -3 millibars) replicated 8 times within each main plot. This experiment was conducted with DSI 100 soil.

The trial was similar to that described in Section 3.3.3. Four untreated (captan) seeds or seeds treated with pyroxyfur at 3.0 g a.i./kg seeds were sown per pot on 11 May, 1983. Each pot was watered daily according to weight loss to maintain the required water potential levels.

The plants were harvested and assessed every two weeks (two replicates of each treatment within a main plot) for disease severity and dry matter yields of roots and tops over a period of 8 weeks. The final harvest was on 21 June, 1983.

Results

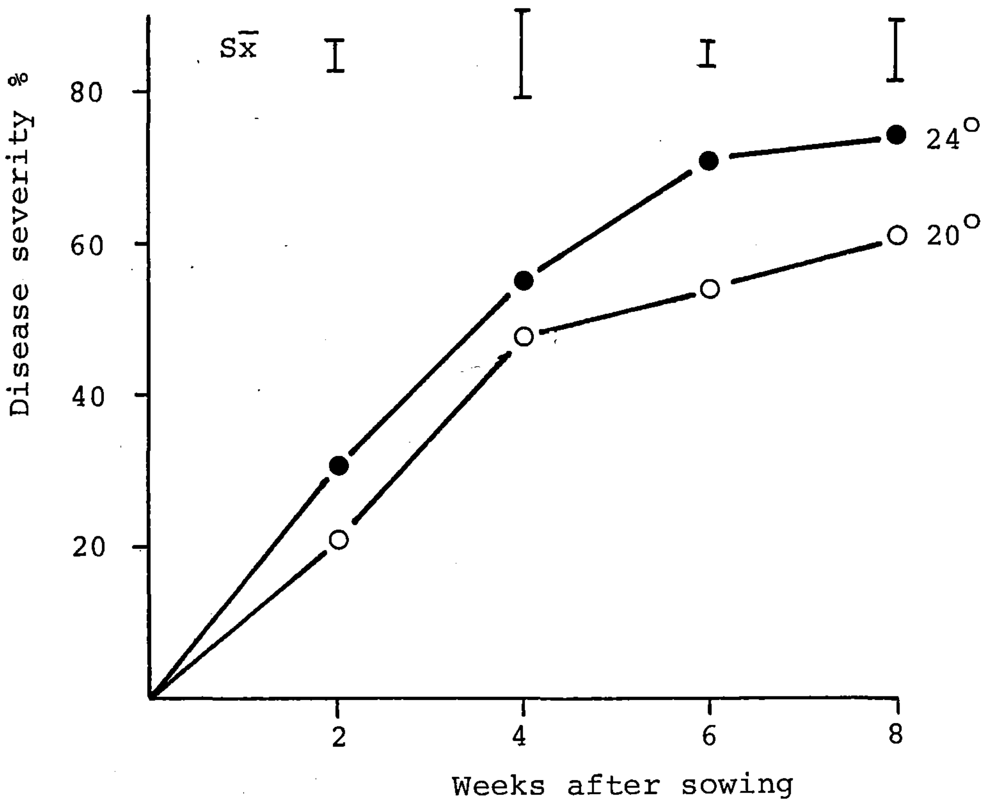
a) Disease Severity

There was no significant difference in disease severity between the two soil temperatures throughout plant growth (Figure 5.12a). The rate of increase followed a similar trend although disease was more severe at 24° than at 20°.

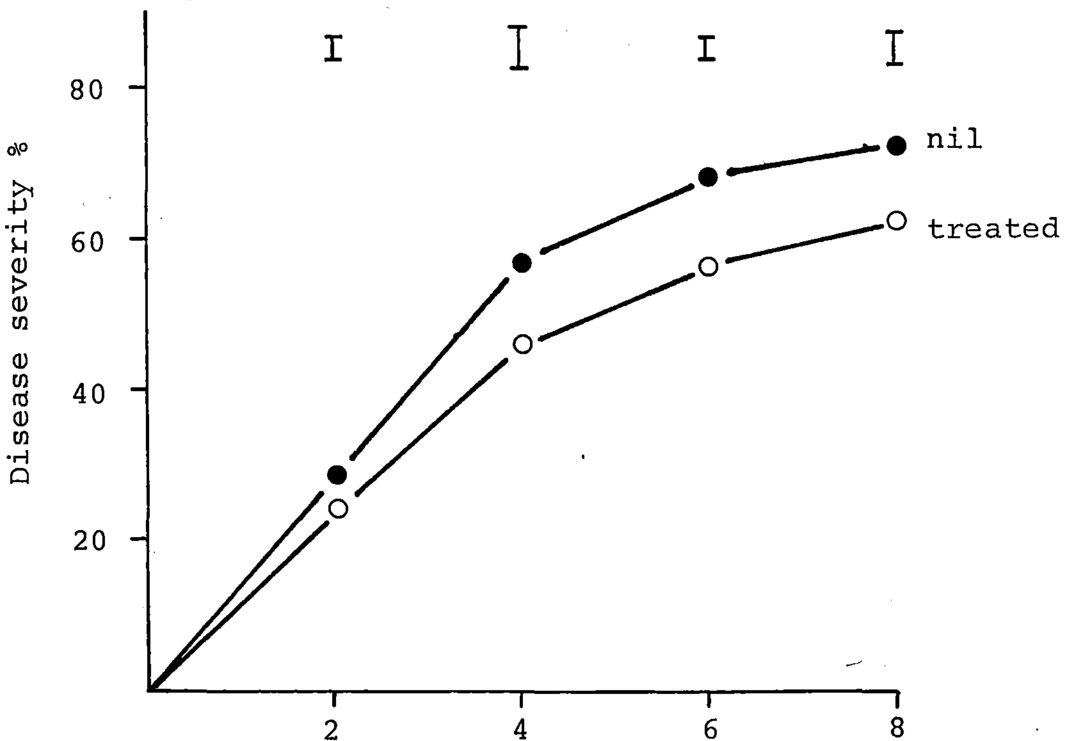
Although disease severity was reduced by pyroxyfur seed treatment (Figure 5.12b), the only significant effect was at 6 weeks of growth where an 18% reduction occurred.

The response of disease severity to increasing water potentials was highly significant (P = 0.01) throughout the 8 weeks of growth. In general, an increase in water potential resulted in an increase in disease severity (Figure 5.12c). At two weeks, there were significant differences between the water potential effects. However, as growth progressed, a significant difference was no longer observed between disease severity at -28 and -3 millibars and the rate of increase in both conditions was rapid. In water potential -200 millibars, disease severity was low and did not increase significantly throughout growth.

a) Effect of temperature on disease severity



b) Effect of pyroxyfur on disease severity (significant at P = 0.05 at 6 weeks)



c) Effect of water potential on disease severity (highly significant at $P = 0.01$ throughout growth)

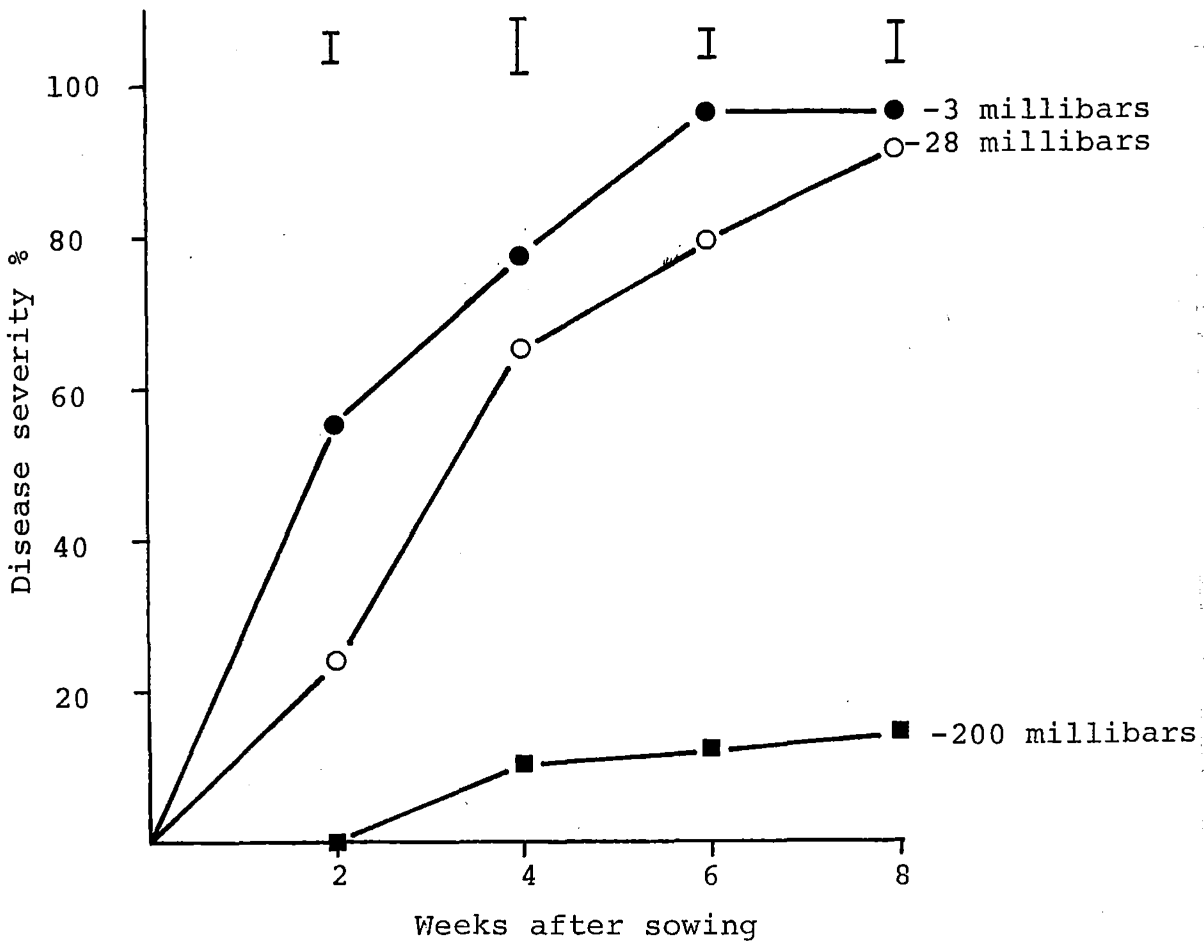


Figure 5.12: The overall effect of a) soil temperature (20° and 24°), b) pyroxyfur seed treatment and c) water potential levels (-200, -28 and -3 millibars) on severity of aphanomyces root rot of pea cultivar Canterbury 39 over 8 weeks of plant growth.

There were no significant interactions between temperature, soil moisture level and seed treatments on disease severity. Hence the results are presented as the overall effects.

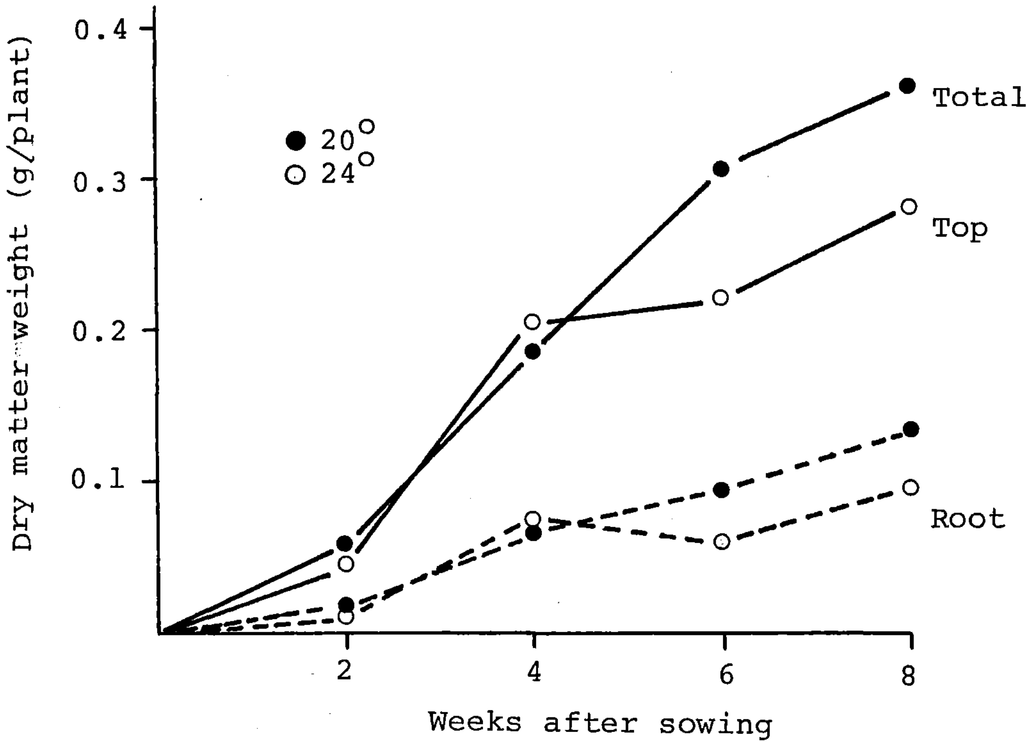
b) <u>Dry Matter Yield</u>

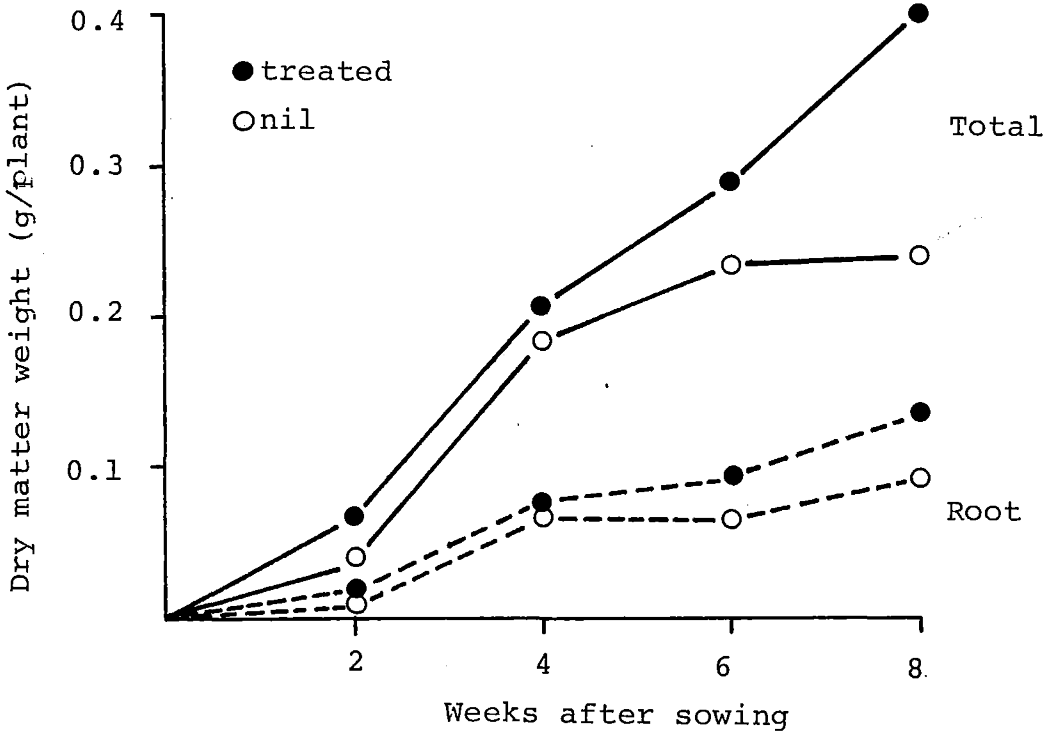
The overall effect of temperature on dry matter yield is shown in Figure 5.13a. Root dry matter yield did not differ statistically between the two temperatures throughout plant growth. The effect of disease started to show from four weeks onwards when plants were accumulating less root matter at 24° than at 20° (34% and 26% less at 6 and 8 weeks respectively).

Similarly, differences in total dry matter yield between the two soil temperatures was not evident until after four weeks of growth. However, a significant difference was not observed until 8 weeks when yield obtained was 27% more at 20° than 24° (significant at $P = 0.05$).

The difference between total dry matter and root dry matter in Figure 5.13a is the top dry matter, which followed a similar pattern to that of total dry matter. At 8 weeks, the difference between the two temperatures was 30% more at 20° than 24° (significant at $P = 0.05$).

The effect of pyroxyfur seed treatment is shown in Figure 5.13b. Root dry matter accumulation in pyroxyfur treated plants only differed significantly from nil treated


a) Effect of temperature on dry matter weight
0.4
0.3
0.2
0.1
Dry matter weight (g/plant)
● 20°
○ 24°
Total
Top
Root
2
4
6
8
Weeks after sowing


b) Effect of pyroxyfur on dry matter weight
0.4
0.3
0.2
0.1
Dry matter weight (g/plant)
● treated
○ nil
Total
Root
2
4
6
8
Weeks after sowing

c) Effect of water potentials on dry matter yield.

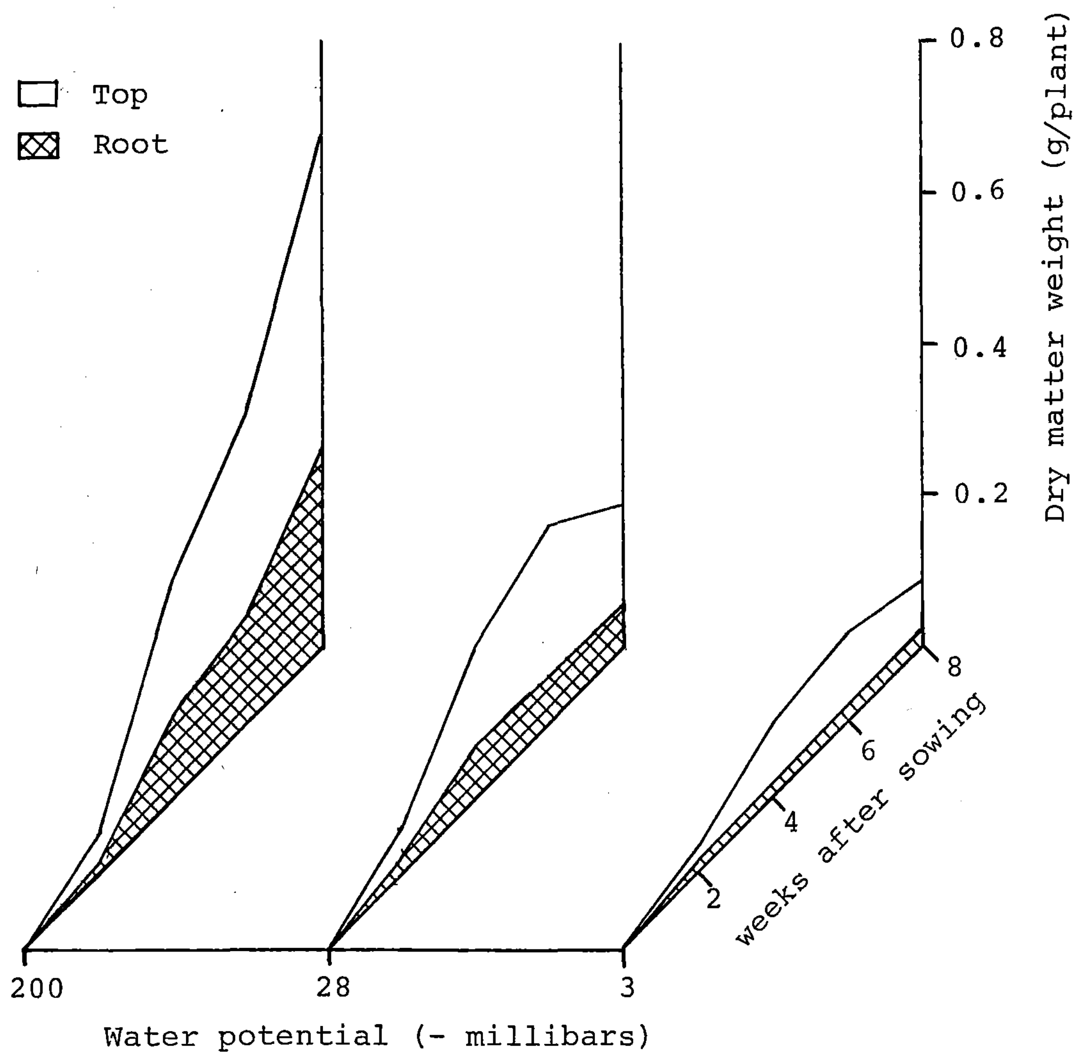


Figure 5.13: The overall effect of a) temperature (20°, 24°); b) pyroxyfur seed treatment; and c) water potential levels (-200, -28 and -3 millibars) on severity of aphanomyces root rot as shown by dry matter yield of pea cultivar Canterbury 39 over a period of 8 weeks of plant growth.

plants (39% higher in yield) at 6 weeks. However, treated plants generally yielded more than the nil treated plants.

At two weeks, the effect of treatment on total dry matter resulted in a highly significant (P = 0.01) increase in yield (49%). At four weeks, there was no significant difference between treated and non-treated plants. However, significance (P = 0.05) was observed at 6 weeks where there was a 23% increase in total dry matter accumulation by the treated plants. At final harvest, yield was not statistically different from nil treatment even though there was 66% more dry matter from the pyroxyfur treatment.

At two weeks, top dry matter yield (the difference between total and root dry matter in Figure 5.13b) was significantly more, 44% in the treated plants than the nil treated plants. But this significance in differences was not observed either at four weeks or six weeks, even though a higher yield of 16% was obtained from treated plants. However, at 8 weeks, pyroxyfur effectively resulted in a significant 78% (P = 0.05) higher top matter yield.

Figure 5.13c shows the effect of soil water potentials on dry matter yield. The overall differences in root yield at two weeks (significant at P = 0.05) increased from four weeks onwards (highly significant at P = 0.01). At two weeks, similar root dry matter yields were obtained at -200 millibars and -28 millibars and these differed significantly (171%) from that at -3 millibars. At -200 millibars, root

dry matter yield continued to increase as growth progressed. However, at -28 and -3 millibars, after a further yield increase at four weeks, root accumulation decreased as disease started to have an effect. At the same time, yield between -200 and -28 millibars was observed to differ significantly at an increasing rate as growth progressed.

Top dry matter accumulations, at two weeks, did not show any significant differences in response to the three water potential levels. At four weeks, yield was significantly ($P = 0.05$) different and by six weeks, highly significant ($P = 0.01$) between the levels of soil water potentials with top matter accumulation increasing as growth progressed at -200 millibars. This trend was observed at -28 and -3 millibars only up to six weeks where decrease in yield was obtained at 8 weeks.

Response of total dry matter yields to water potential followed a similar pattern to top dry matter yield.

The only significant interaction on dry matter yield occurred between temperature and water potential levels at four weeks (Figure 5.14). At 20°, there was no significant difference in the yield in root, top and total dry matter in response to water potential levels. At 24°, root dry matter yield at -28 and -3 millibars did not differ statistically but both were significantly different from yield obtained at -200 millibars (+ 180% and +186% respectively). Total dry matter yield at -28 millibars at 20° did not differ statistic-

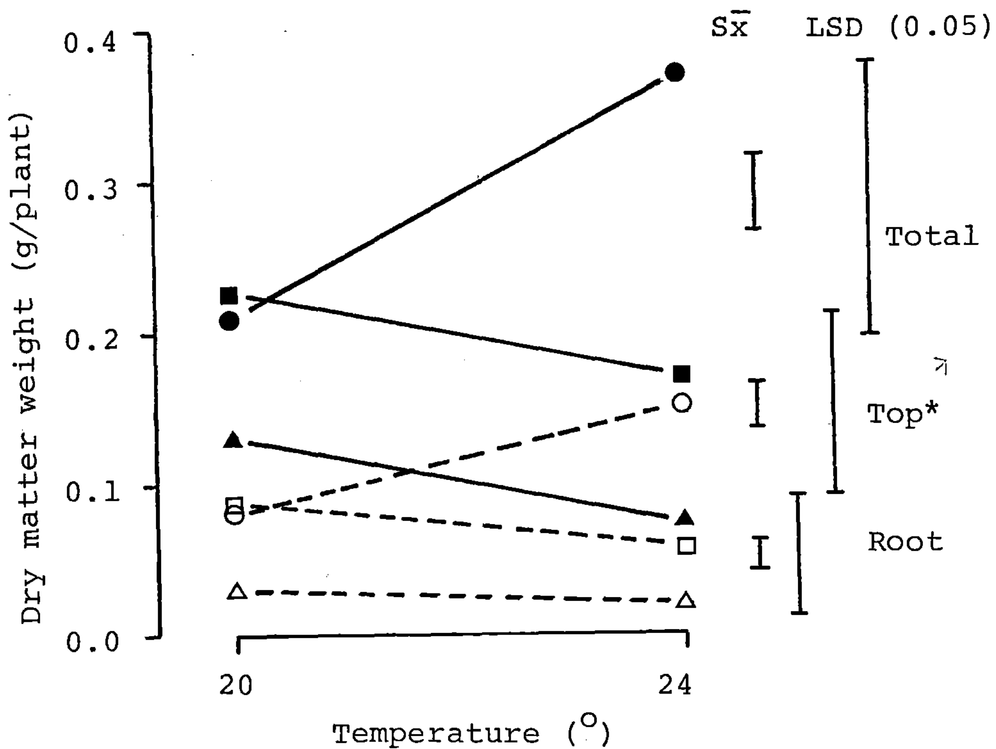


Figure 5.14: Interaction between temperatures and water potentials on dry matter weight of pea cultivar Canterbury 39 affected by aphanomyces root rot.

(root (○) and total dry matter (●) at -200 millibars;

root (□) and total dry matter (■) at -28 millibars;

root (△) and total dry matter (▲) at -3 millibars).

*Top dry matter is the difference between total and root dry matter.

ally from either yield at -200 millibars or -3 millibars but a significant increase of 267% was obtained at -200 millibars over -3 millibars in yield at 24°. Top dry matter response followed a similar pattern to that of root dry matter.

Discussion - Except at six weeks, seed treatment by pyroxyfur did not reduce the overall disease severity significantly (Figure 5.12b). However, although disease severity was reduced, this effect was not always accompanied by significant increases in pea yield as shown in Figure 5.13. The significant differences in disease severity, as shown by dry matter yield, in the water regimes were mainly due to the pathogen's response to the availability of water for root rot infection and development as reported in Section 3.0.

The potential in using chemical seed treatment against aphanomyces root rot will be limited in soil with a high inoculum level. A similar conclusion was also drawn by Papavizas and Ayers (1974) and Jermyn *et al.* (1982). The faster growth rate of the plant roots, under wetter conditions (Section 3.0), would result in a faster rate of root growth out of the fungicide zone (Experiment One in Section 5.3.2 and Pfleger *et al.* 1976). On the other hand, in dry and warm conditions, not favourable to this pathogen, disease severity was not sufficient to reduce yield significantly and to warrant chemical seed treatment.

5.3.3 Glasshouse Studies on the Effect of Cruciferous Amendments on Infested Soil by *Aphanomyces euteiches*

Introduction - The effect of chemical seed treatments was evident until about six weeks after sowing, but the effect did not persist at later growth stages (Section 5.3.2). These results confirmed the extensive chemical testing against this disease in the United States (Papavizas and Ayers 1974) and the report of Jermyn *et al.* (1982). Without resistant cultivars or effective chemicals, avoidance appears to be the only reliable method of control.

Earlier work by Lewis and Papavizas (1971) and Papavizas and Lewis (1971) showed that adding several cruciferous amendments to infested soil was effective against aphanomyces root rot of peas. The cruciferous crops were believed to act on the pathogen because of their high levels of volatile organic sulphur compounds (Lewis and Papavizas 1970).

In this section, the objective was to conduct a series of experiments in which cruciferous crops were grown or incorporated in infested soils and then to monitor the level of pathogen in the soil and the severity of the disease in subsequent pea plants.

Experiment One: Effect of growing non-host and host plants on the Disease Severity Index (DSI) of soil infested with *Aphanomyces euteiches*

Introduction - A preliminary glasshouse trial studied the effect of non-host and host plants on the DSI of soil infested with *Aphanomyces euteiches*.

Materials and Method - Three Templeton silt loam soils and two Barrhill sandy loam soils, naturally infested with *Aphanomyces euteiches*, were collected from fields in Canterbury in May, 1981 (soil description in Table 2.1 in Chapter 2). The DSI, inoculum density and previous four crops grown in each soil before sampling are given in Table 5.10.

The experiment was a complete randomised design consisting of five treatments replicated six times. The treatments consisted of growing four plant species over a period of four months in each soil sample: pea (pea cultivar Canterbury 39); *Trifolium repens* (white clover cultivar Huia); *Triticum aestivum* (wheat cultivar Rongotea) and *Brassica napus* (Wairangi rape).

The fifth treatment was soil not sown with plants. One hundred and fifty plastic pots (150 mm diameter and 200 mm deep) were prepared and equally spaced on the glasshouse benches. The pots were watered daily to keep the soil moist and glasshouse temperature was maintained at 24° - 28°. At the end of four months, the plants were removed from the soil. The soil was tested for DSI based on Section 2.3.2.

Table 5.10: Disease severity indices and inoculum densities of three Templeton silt loam soils and two Barrhill sandy loam soils naturally infested with *Aphanomyces euteiches* obtained from Canterbury in May, 1981. Previous four crops grown in each field before sampling and number of years since last pea crop are indicated.

Soil sample (DSI)	Inoculum density (no. of oospores per 100 g soil)	Previous four crops				Number of years since last pea crop
		1977	1978	1979	1980	
Templeton silt loam						
Soil 1 (100)	751	Ryegrass	White clover	Wheat	Peas	1
Soil 2 (80)	441	Ryegrass/ white clover	White clover	Pea/white clover	Wheat	2
Soil 3 (70)	186	White clover	Wheat	Pea	Barley	2
Barrhill sandy loam						
Soil 4 (60)	79	Wheat	Pea	White clover	Wheat	3
Soil 5 (90)	581	Wheat	Pea	White clover	Wheat	3

<u>Results</u> - Table 5.11 shows the result of the trial.

Table 5.11: Effect of non-host and host plants grown in soil infested with *Aphanomyces euteiches* for four months on subsequent Disease Severity Indices (DSI) of pea root rot.

Treatment	Disease Severity Index*				
	Soil 1	Soil 2	Soil 3	Soil 4	Soil 5
No plants	100 a	81 b	72 b	58 b	96 a
Pea	100 a	98 a	100	94 b	100 a
White clover	100 a	82 b	76 b	54 b	97 a
Wheat	100 a	82 b	72 b	50 b	100 a
Rape	100 a	56 c	53 c	27 c	93 a
$S\bar{x}$	0.4	3.6	1.4	1.6	1.6
CV %	0.6	7.9	3.3	4.8	2.8

*Means followed by the same letter do not differ significantly (P = 0.05) using Duncan's Multiple Test.

There were no treatment effects using Soil 1 with an inoculum level DSI 100. Similarly, in Soil 5 (DSI 96), there were no significant differences in DSI after treatments. Significant reductions in DSI were obtained in soil with inoculum levels DSI 81 and lower, grown previously with rape (30%, 26% and 53% reduction in Soils 2, 3 and 4 respectively). Growing of pea plants significantly increased DSI to a dangerous level in those samples. Soil grown with white clover and wheat did not change the DSI.

<u>Discussion</u> - Results in Table 5.11 showed that the amount of infestions of soil by *Aphanomyces euteiches* was correlated with the crop grown previously and that repeated cropping with peas would increase the DSI to a high level. These findings were also reported by Reiling *et al.* (1960) who concluded that the direct relationship between the number of pea crops grown and root rot severity was one of the most widely recognised characteristic of the disease. Importance of other crops in the cropping sequence was also emphasized. DSI would not be expected to decrease after a crop of white clover as it had been proved to act as alternate host (Chapter 4) and similarly after wheat which was also reported as host (Papavizas and Ayers 1974). Development of new oospores in alternate hosts would not be abundant (Sherwood and Hagedorn 1962) but release of oospores in the soil matrix during the decomposition of the infected tissue might not only increase inoculum density but maintain it.

Of particular interest was the effectiveness of rape to suppress root rot development in soil of DSI below 80. Soil with DSI 50 or between 70-80 could be reduced to a level of no risk by one or two subsequent plantings of rape respectively. The potential of using brassica species as amendment to the soil was also investigated by Papavizas (1967), Davey and Papavizas (1961) and Papavizas and Lewis (1970). However, additional information was needed to monitor the level of inoculum in soil after brassica amendments to the soil.

Experiment Two: Effect of cruciferous amendments on *Aphanomyces euteiches*

Introduction - The glasshouse trial was conducted to investigate the effect of cruciferous amendments by incorporating leaves and stems of five *Brassica* crops in infested soil on oospore populations and the subsequent DSI.

Materials and Method - The trial was a 3 x 6 x 2 factorial in a complete randomised block design replicated four times. Three soil inoculum levels (DSI 50, 75 and 100) of a Barrhill sandy loam (soil description and cropping history described in Section 3.3.2) obtained from Rakaia, Canterbury on 30 September, 1982 were used. Each plastic pot (170 mm x 170 mm square x 180 mm deep) was filled with an equivalent of 4.05 kg of oven-dried soil. One hundred and forty-four pots were prepared.

The following *Brassica* species were used as amendments:
Brassica oleraceae - kale cultivar Kapeti,
Brassica oleraceae - cabbage cultivar Succession,
Raphano-brassica - fodder raddish cultivar Neris,
Sinapis alba - mustard cultivar mustard
Brassica napus - rape cultivar Wairangi.
Four-months-old plants grown in the field were harvested (from 9 December, 1982 to 31 March, 1983). The plant materials, consisting of matured leaves and stems, were air-dried for one week and then chopped up finely. The amount (based on oven-dried weight) of plant materials incorporated was 0.5% of the dry weight of soil on 11 April, 1983. A non-

amended soil treatment was set up as control. Soil moisture was maintained at -28 millibars during the three or six weeks period of decomposition at glasshouse temperatures of 24-28°. At the end of each time length specified (2 May, 1983 and 23 May, 1983 respectively), soil from each pot was mixed thoroughly and transferred to a plastic pot (150 mm diameter and 200 mm deep) to be tested for DSI based on Section 2.3.2. The soil was also collected for oospore count with materials and method based on Section 2.3.3. The soil pH of each of the six treatments was recorded twice using PHM 62 standard pH meter (Nicholas, Watson Victor Ltd). The pH was not affected by any of the treatments.

Results - The effects of cruciferous amendments in *Aphanomyces* infested soil at three inoculum levels in subsequent disease severity and inoculum density after three weeks and six weeks of incorporation are shown in Table 5.12.

a) Disease Severity Indices (DSI)

There was a highly significant difference (P = 0.01) between the DSI of soil with amendments added three weeks and six weeks before sowing. The overall DSI was 23% lower after six weeks than three weeks of amendments.

The overall DSI between inoculum levels remained highly significantly different (P = 0.01) after treatments.

All brassica amendments tested gave a considerable overall reduction of DSI (highly significant at P = 0.01). The cabbage treatment was rated as the most effective, sup-

Table 5.12: Effect of cruciferous amendments in *Aphanomyces euteiches* infested soil at three inoculum levels of Barrhill sandy loam on disease severity and oospore population after three weeks and six weeks of incorporation. Inoculum densities at DSI 100, 75 and 50 were 783, 219 and 77 oospores per 100 g soil respectively.

Main factors		Disease Severity Index (DSI)	No. of oospores per 100 g of soil
Weeks (wk)	3	61.1	194
	6	47.2	142
	$S\bar{x}$	1.35	6.9
	Significance	**	**
Inoculum level (IL)			
(DSI)	50	35.7	5.2
	75	54.9	121
	100	71.9	330
	$S\bar{x}$	1.65	8.4
	Significance	**	**
Incorporated material (IM)			
Nil		75.5	347
Cabbage		38.0 (50)[a]	82 (76)[a]
Kale		65.7 (13)	248 (29)
Rape		47.5 (37)	95 (73)
Fodder radish		46.9 (38)	119 (66)
Mustard		51.4 (32)	115 (67)
	$S\bar{x}$	2.33	11.9
	Significance	**	**
Significant interactions			
WK X IL[b]		*	**
WK X IM[b]		**	*
IL X IM[b]		*	**
WK X IL X IB		NS	NS
	CV%	21.1	30.0

[a]Per cent reductions from nil treatment.

[b]All data with significant interactions are presented in graphs.

pressing DSI by 50%. Rape, fodder radish and mustard amendments proved to be equally effective, reducing DSI by 37%, 38% and 31% respectively. The kale amendment was the least effective, resulting in only a 13% reduction which was not different from the nil and mustard treatments.

Significant interaction (P - 0.05) between time intervals of all amendments before planting and inoculum levels was obtained as shown in Figure 5.15. In both inoculum levels DSI 100 and DSI 50, there were significant differences in DSI between length of time of amendments before sowing. The differences in DSI between three weeks and six weeks were 36% at DSI 50 and 22% at DSI 100. The reduction of 12% obtained at DSI 75 was not significant.

There was a highly significant interaction (P = 0.01) between individual brassica amendments and length of time of amendments before planting as shown in Figure 5.16. Brassica amendments maintained for six weeks before planting resulted in greater reductions in DSI than the three weeks period. However, only significant differences were observed with the cabbage and rape treatments where there were 68% and 49% reductions in DSI at six weeks as compared to 32% and 25% reductions at three weeks. In the kale, fodder radish and mustard treatments, 18%, 27% and 13% differences in DSI were obtained respectively by delaying sowing for a further three weeks. At the three weeks period, 6%, 29% and 28% reductions in DSI were obtained as compared to values at the six week period of 20%, 47% and

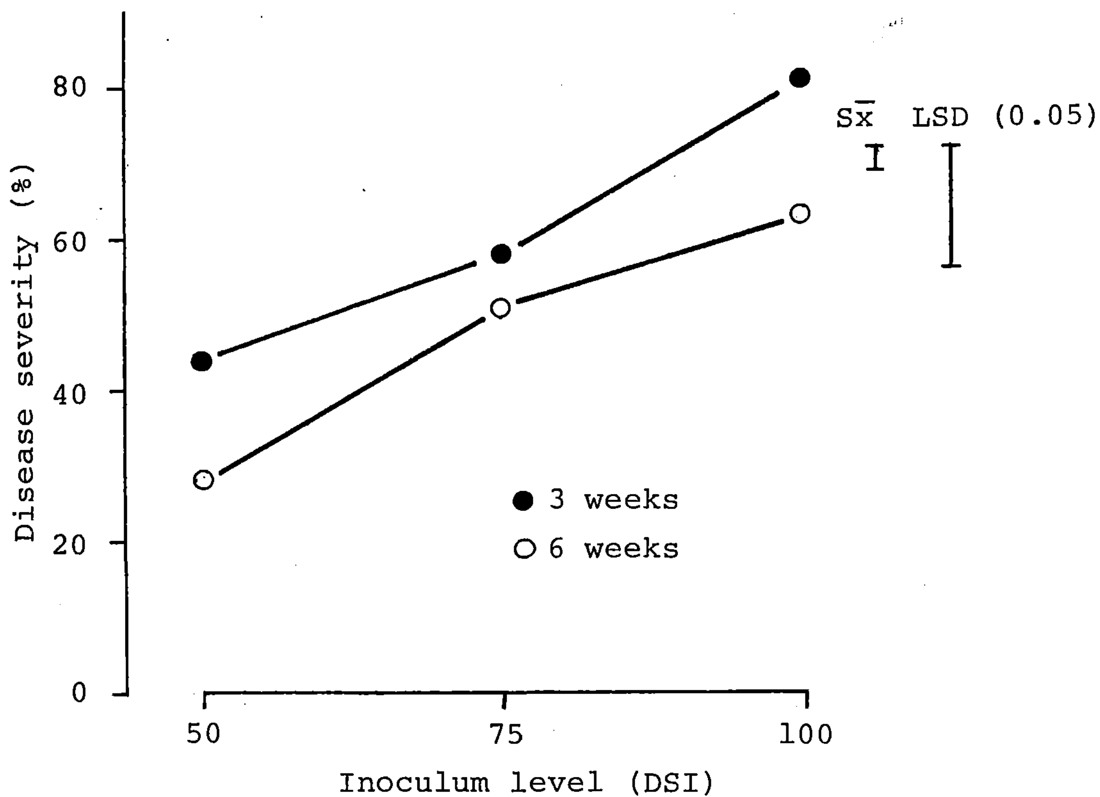


Figure 5.15: Effect of interaction between inoculum levels and length of time of brassica incorporation in aphanomyces infested soil on the disease severity index of pea root rot. (Mean data derived from all brassica treatments.)

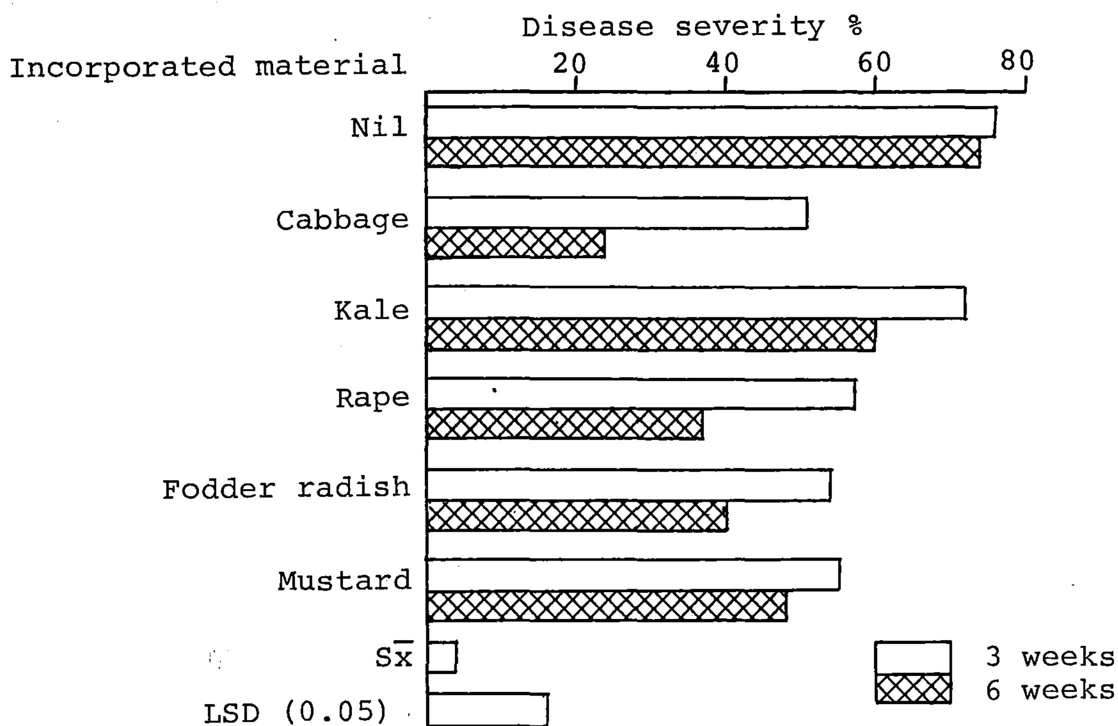


Table 5.16: Effect of interaction between incorporated brassica materials and length of time of incorporation in infested soil on disease severity index of aphanomyces root rot of pea. Mean data derived from three inoculum levels.

36% reductions over nil treatment with kale, fodder radish and mustard treatments respectively.

There were significant interactions (P = 0.05) between inoculum levels and brassica amendments as shown in Figure 5.17. In inoculum level DSI 50, DSI's were significantly reduced by 66% with the cabbage amendment, 50% with the rape, 41% with the fodder radish and 27% with the mustard. There was no significant reduction in DSI from the nil treatment in the kale amendment. In inoculum level DSI 75, lower significant reductions in DSI occurred as compared to DSI 50, where 52% reduction with the cabbage amendment, 25% with the rape and 39% with the fodder radish were observed. There was a 30% reduction with the mustard amendment. The kale amendment did not reduce DSI significantly. In inoculum level DSI 100, all the brassica amendments except kale were equally effective in reducing DSI significantly. The DSI were reduced by 39% with the cabbage amendment, 40% with the rape, 36% with the fodder radish and 37% with the mustard. With the kale amendment, there was a significant 16% reduction in DSI as compared with nil treatment.

b) Oospores in Soils

The main effects are shown in Table 5.12, which also shows the significant interactions.

The length of time of incorporated brassica materials in infested soil before sowing had a highly significant effect (P = 0.01) on numbers of oospores per 100 g of soil. Overall,

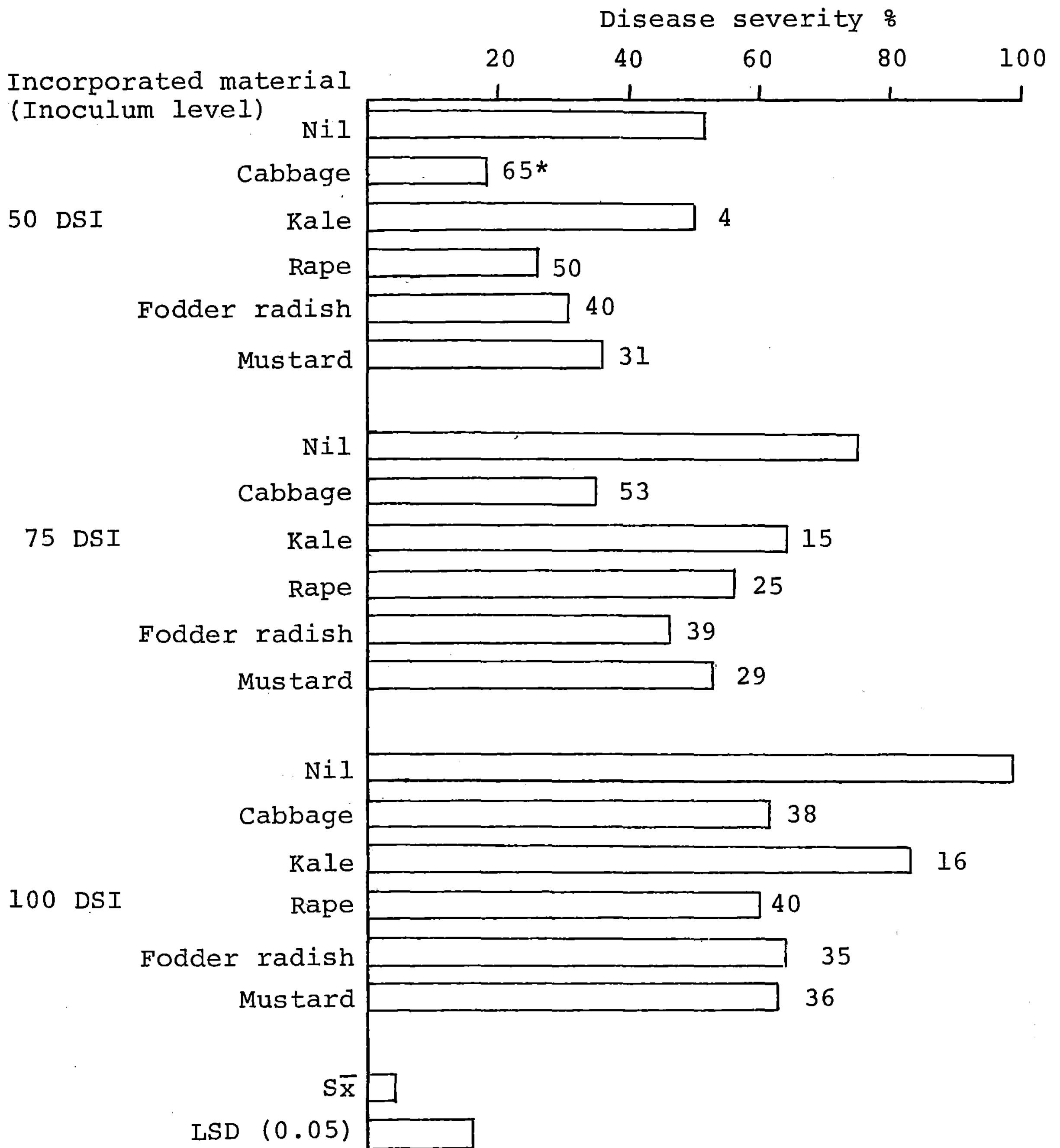


Figure 5.17: Interaction between inoculum levels and incorporated brassica materials in infested soil on disease severity index# of aphanomyces root rot of pea.

* Per cent reduction when compared to nil.

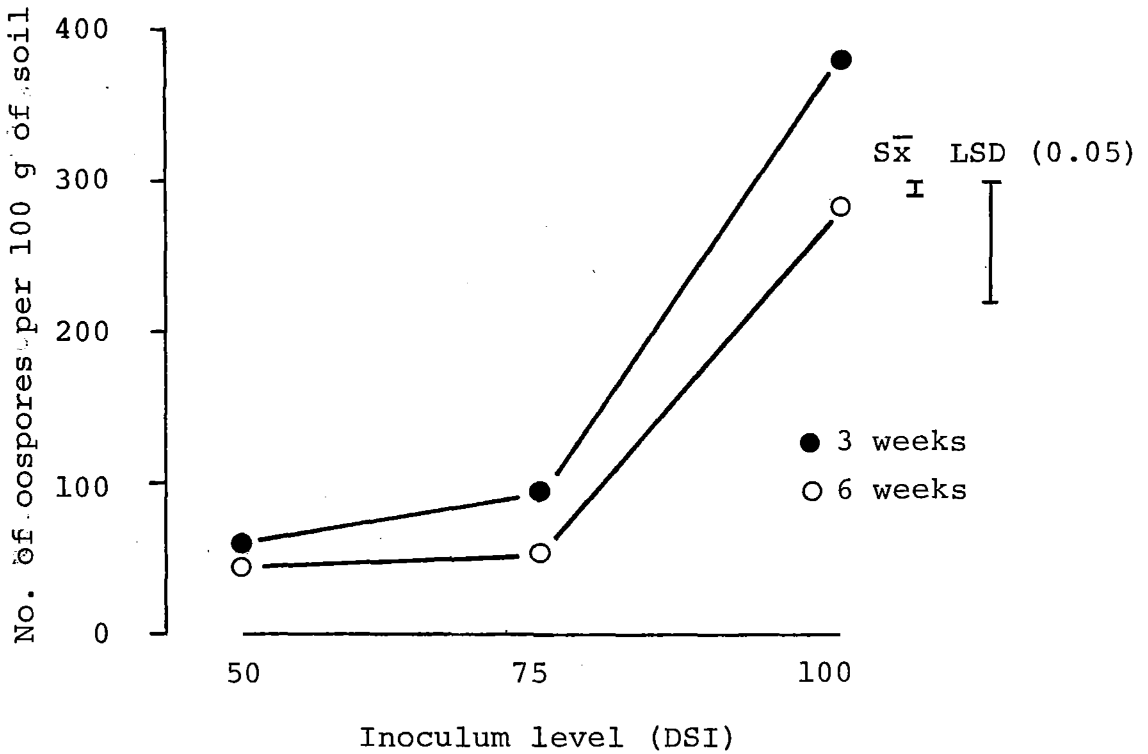
Means of 3 and 6 week data.

there was a 27% difference in population between the three and six weeks periods.

Inoculum levels had a highly significant effect (P = 0.01) on oospore population. However, there was no statistical difference in number of oospores per 100 g of soil between DSI 50 and DSI 75. Both were significantly different from DSI 100 when compared with DSI 100 where there were 85% less oospores in DSI 50 and 64% less in DSI 75.

The effect of the incorporated materials on oospore population was highly significant (P = 0.01). Cabbage and rape treatments were equally effective in reducing number of oospores as they did not differ significantly from each other (76% and 72% reduction respectively when compared with the nil treatment). Similarly, fodder radish and mustard treatments were equally effective reducing oospore populations by 66% and 67% respectively. Kale treatment was the least effective with only a 28% reduction.

A highly significant interaction (P = 0.01) occurred between length of time incorporated materials were in infested soil before sowing and inoculum levels (Figure 5.18). At inoculum levels DSI 50 and DSI 75, there were no statistical differences in oospore populations between three weeks and six weeks period, although there were more reductions at six weeks. At DSI 100, there was significantly fewer oospores at six weeks (26% less) than at three weeks.



Fiugre 5.18: Interaction between inoculum levels and length of time of brassica incorporation in aphanomyces infested soil on number or oospores per 100 g of soil. Mean data derived from all brassica treatments.

There was a significant interaction (P = 0.05) between the type of incorporated material and length of time materials were incorporated before sowing (Figure 5.19). In general, a greater reduction in oospore population occurred with the six week than with the three week period. However, the only significant difference in number of oospores between three week and six week period before sowing occurred in the cabbage treatment where the number was reduced by 88% after six weeks of incorporation as compared to 65% after three weeks. There was no statistical difference in oospore populations between length of time of incorporation in the other four brassica treatments. At three weeks, rape, fodder radish and mustard treatments were as effective as the cabbage treatments, resulting in 62%, 67% and 59% reductions in oospore numbers per 100 g of soil. Similarly, at six weeks, these three treatments were equally effective to the cabbage treatments reducing oospore numbers by 84%, 65% and 75% respectively. The kale treatment did not cause a reduction over nil treatment at either time of incorporation (19% and 39% reduction respectively at three weeks and six weeks).

The interaction between inoculum levels and incorporated materials in infested soils on oospore population was highly significant (P = 0.01). The data for each inoculum level is shown in Figure 5.20. In the DSI 50 soil, there was no significant reduction in oospore numbers with any amendment. However, a 64% reduction with the cabbage amendment, 26% with the rape, 29% with fodder radish and 33% with the mustard was observed. There was no reduction with the kale

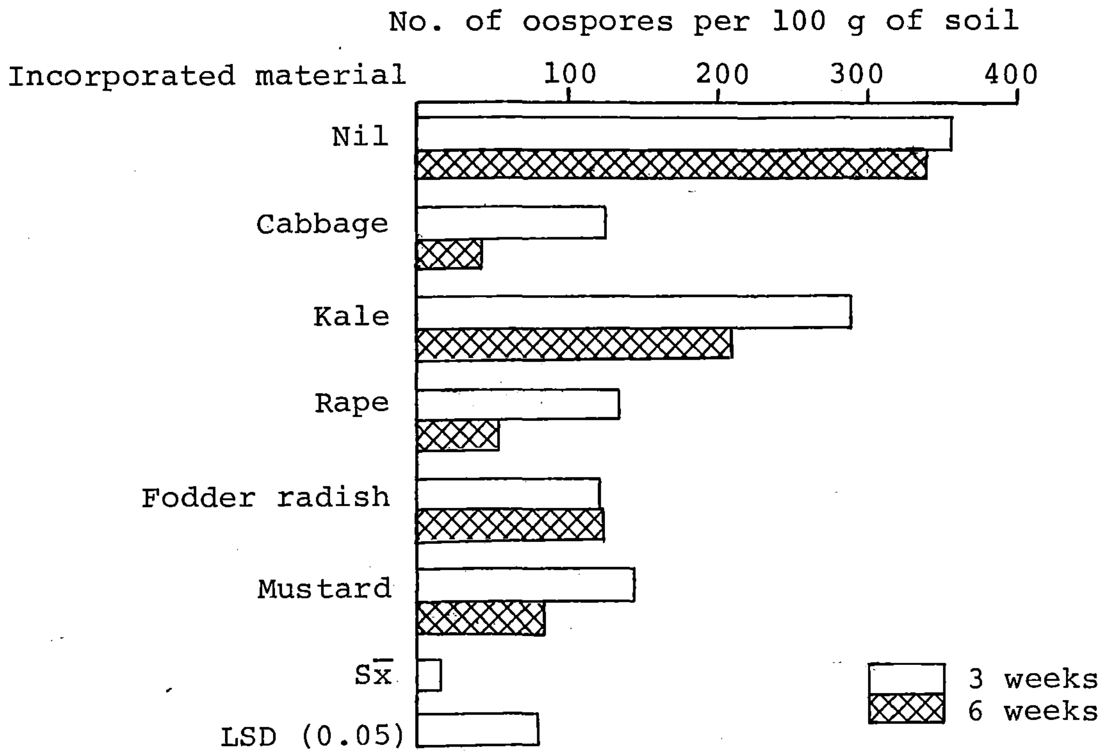


Figure 5.19: Interaction between incorporated brassica materials and length of time of incorporation in infested soil on number of oospores of *Aphanomyces euteiches* per 100 g of soil. (Mean data derived from all inoculum levels.)

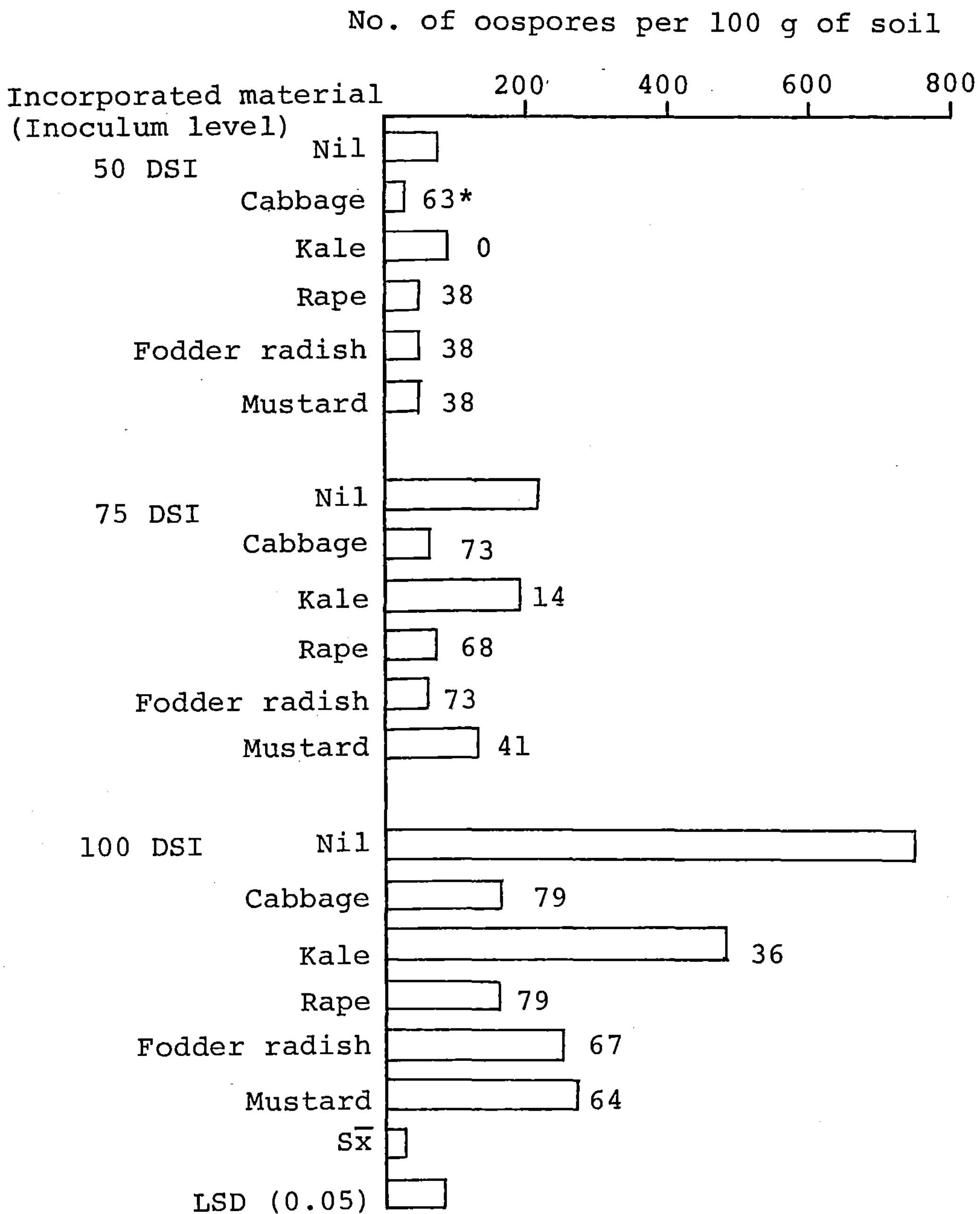


Figure 5.20: Interaction between inoculum levels and incorporated brassica materials in infested soil on number of *Aphanomyces* oospores# per 100 g of soil.

* Per cent reduction when compared to ml.

Means of 3 and 6 week data.

amendment. In the DSI 75 soil, only the kale amendment did not reduce oospore numbers significantly (14% less) as compared to nil amendment. The cabbage, rape, fodder radish and mustard amendments were equally effective, reducing oospore numbers significantly by 74%, 69%, 75% and 41% respectively. In the DSI 100 soil, all brassica amendments were significantly effective in reducing oospore numbers. The cabbage and rape amendments were the most effective, both reducing oospore numbers by 78%. There was no significant difference between the fodder radish and mustard amendments where reductions of 67% and 64% were obtained over nil treatments respectively. The kale amendment was the least effective, reducing oospore numbers by 36%.

There was no significant interaction between the three main factors (WK, IL and IB) on both DSI and oospore number per 100 g of soil.

Discussion - All cruciferous amendments tested with *Aphanomyces euteiches* infested soil were effective in suppressing subsequent root rot in the glasshouse trial. This effect may be due to the action of the volatile components of sulfides and disulfides arising from microbial decomposition of amendments of plant tissues and this explanation is based on evidence provided by Clapp *et al.* (1959), Bailey *et al.* (1961), Lichtenstin *et al.* (1964) and Lewis and Papavizas (1970). Many vital phases in the life cycle of the pathogen have been reported to be adversely affected (Lewis and Papavizas 1971).

The suppression of disease severity by the amendments could be ascertained to be due to the effect of these volatiles on the oospores' survival as indicated by the reduction in numbers of oospores found in amended soils. Lewis and Papavizas (1971) provided evidence that morphology and development of oospores were adversely altered.

The effectiveness of disease severity reductions by amendments was correlated with the inoculum level. With all three inoculum levels, the overall treatments did not significantly reduce the DSI from the original level. However, there was about a 28% reduction at all inoculum levels. This explained one of the factors causing the decrease in effectiveness of the cruciferous amendments in heavily infested soil in the field reported by Papavizas and Lewis (1971) and who suggested reduction of root rot of peas could only be expected in naturally infested soils where levels of infestations were not excessively high as cruciferous amendments might not eliminate the fungus. The amount of reductions of disease development must rely on the effectiveness of oospore reductions at any inoculum level. In high levels of inoculum, the number of oospores might not be sufficiently reduced enough to show any differences in DSI (Table 5.12 and Chapter 2).

More oospores were reduced the longer they were exposed to the decomposing amendments, resulting in further decline in disease severity (Figures 5.18, 5.19). Papavizas (1966) also showed that there was a general decline in DSI for peas

planted in soil 3, 6, 9, 12 and 15 weeks after incorporation of cabbage amendment. Similar findings were also reported by Papavizas and Lewis (1971) that the second planting at 6 months after incorporation showed greater effect on DSI than at 3 weeks.

The effectiveness of disease suppression by brassica amendment appeared to depend on the quality and quantity of the species (Table 5.12, Figure 5.17). This was also observed by Deans and Young (1956) in their studies on the influence of cropping sequences and their associated mycofloras on black root of *Beta vulgaris* (sugar beet). Lewis and Papavizas (1970) showed that cabbage evolved more sulfide volatile compounds than kale and mustard and the greatest amounts were produced during the second and third weeks of decomposition. This suggested that larger amounts of brassica material of the other species should be used to obtain similar effectiveness on disease suppression as cabbage. However, the rate of each brassica amendment should be determined as several undesirable effects were observed on peas grown in soil amended with high concentration of cabbage, e.g. stunting and browning of roots (Papavizas, 1966) and poor germination (Papavizas and Lewis, 1971).

Experiment Three: Effect of growing cruciferous plants and incorporating root material on the subsequent DSI of aphanomyces root rot and oospore numbers.

Introduction - This glasshouse trial investigated the effects of cruciferous crops grown in infested soil of three inoculum levels for six months before incorporating the roots for six weeks, on subsequent soil DSI and oospore population of *Aphanomyces euteiches*.

Materials and Method - The trial consisted of seven treatments replicated three times in a 7 x 3 factorial complete randomized block design. The soil used was described in Experiment Two in this section. The seventh treatment of *Lolium multiflorium* (ryegrass cultivar Tama) was included with the six treatments as in Experiment Two. Sixty-three plastic pots (each 170 mm x 170 mm square x 180 mm deep) were prepared and filled with an equivalent of 4.05 kg of oven-dried soil. They were equally spaced outside the Microbiology Department glasshouse. Five seeds of the six plant species were sown on 4 February, 1983, the seventh treatment was a fallow treatment. The pots were watered when necessary to keep the soil moist. When the weather became too wet, the pots were transferred into the glasshouse on 23 April and kept there until 25 July when the plants were six months old. At six weeks after sowing, brassica plants were thinned by removing the tops to two plants per pot to prevent plant competition. The plants were fed once a month with Phostrogen, a proprietary liquid nutrient which is a balanced formulation of major and minor elements marketed by

Phostrogen Ltd, Corwn, Clwydd LL21 OEE, United Kingdom. Weeds were removed from the fallow treatment. At the end of the growth period, the plant tops were harvested, leaving the roots in the soil. The soil and roots from each pot were mixed thoroughly and left for six weeks at 24-28° with soil moisture maintained at -28 millibars. At the end of six weeks (5 September 1983) oospores were counted and soil DSI tested similar to materials and method described in Experiment Two.

Results - The main effects of five cruciferous plant species and ryegrass in infested soil at three inoculum levels over six months on DSI and oospore population are shown in Table 5.13.

Table 5.13: Effect of five cruciferous plant species and *Lolium multiflorium* grown in *Aphanomyces euteiches* infested soil at three inoculum levels of Barrhill sandy loam for six months and incorporating root material on the subsequent DSI and oospore population. Initial inoculum densities at DSI 100, 75, and 50 were 783, 219 and 77 oospores per 100 g soil respectively.

Treatment		Disease Severity Index (DSI)	No. of oospores per 100 g of soil
Inoculum level (IL)			
(DSI)	50	36.2	41
	75	61.7	162
	100	74.0	490
	$S\bar{x}$	2.97	17.3
Significance		**	**
Plant species (P)			
Nil		77.0	362
Cabbage		38.1 (51)$^{+}$	156 (57)$^{+}$
Kale		58.8 (24)	183 (49)
Rape		53.1 (31)	161 (55)
Fodder radish		44.9 (42)	173 (52)
Mustard		56.6 (26)	244 (33)
Ryegrass		72.7 (6)	338 (7)
$S\bar{x}$		4.54	26.4
Significance		**	**
LSD (0.05)		22.5	130.6
Interaction: IL X P		NS	NS
CV %		24	34

$^{+}$Per cent reductions when compared with the nil treatment.

a) Disease Severity Indices (DSI)

The overall DSI between inoculum levels DSI 50 and DSI 75 or DSI 100 remained highly significantly different (P = 0.01) after treatment. However, DSI was reduced to a non-significant level between DSI 75 and DSI 100.

The effect of plant treatments on DSI was highly significant (P = 0.01). The cabbage, rape and fodder radish treatments were equally effective in reducing DSI as they did not differ from each statistically. Kale and mustard treatments reduced DSI by 23% and 26%, but these did not differ significantly from the nil treatment. The ryegrass treatment had no apparent effect.

There was no interaction between inoculum levels and plant species on DSI.

b) Oospores in Soil

There was a highly significant effect (P = 0.01) on number of oospores by levels of inoculum (Table 5.13). Treatments reduced the overall oospore numbers in DSI 50 and DSI 75 to a non-significant difference between the two inoculum levels. Both were significantly different from DSI 100 in oospore numbers.

Brassica treatments reduced oospores considerably (highly significant, P = 0.01). All, except mustard, were equally effective as they did not differ statistically, reducing oospore numbers from nil treatment by 57% with the cabbage

treatment, 49% with the kale, 55% with the rape and 52% with the fodder radish. The mustard treatment reduced oospore numbers by 33% but was not significantly different from nil treatment. Ryegrass treatment did not have any effect on oospore population.

There was no interaction between inoculum levels and plant species on oospore numbers.

Discussion - Growing brassicas in infested soils for six months before incorporation reduced the DSI in peas between 20% and 70% compared to 5% by ryegrass. Similarly, oospore numbers were reduced by 30-60% and 7% by ryegrass. This shows the potential for pea root rot caused by *Aphanomyces euteiches* to be reduced by growing a brassica crop and incorporating the roots of that crop into the ground before sowing the peas. Evidence provided by Papavizas and Lewis (1971) that disease suppression trends last for at least six months after incorporation suggest that brassica amendments could reduce root rot even the following season. This finding has been confirmed in a field trial by Cromey and Jermyn (pers. comm.) using fodder radish in which a DSI of 50 was recorded after amendment compared to 80 in the non-amendment treatment.

Therefore, restrictions on the use of fields with high index values (Sherwood and Hagedorn 1958) for growing peas could now be alleviated by adding a brassica crop to the rotation before the pea crop.

CHAPTER 6

GENERAL DISCUSSION AND CONCLUSION

In areas where it occurs, the root rot disease caused by *Aphanomyces euteiches* is likely to be serious and to restrict the economic production of pea crops. Because no reliable method has yet been found to control the disease in plants sown in heavily infested soil (Jermyn *et al.*, 1982), use of the DSI method (Sherwood and Hagedorn 1958) to avoid soils with high root rot potential appears to be the only practical solution to the problem. Thus one of the main aims of the research was to study the factors affecting development of aphanomyces root rot and to use this information in an evaluation of alternative control methods.

Many experimental studies of *Aphanomyces euteiches* have been concerned with inoculum density but the problem of assessing disease potential and severity in relation to control has been confounded by the differing methodology used by investigators (Sherwood and Hagedorn 1958; Boosalis and Scharen 1959; Mitchell *et al.* 1969; Burke *et al.* 1969; Pfender *et al.* 1981). From the results of laboratory and glasshouse studies to evaluate three existing methods: DSI method (Sherwood and Hagedorn 1958), direct counting of oospores by microscopic detection (Boosalis and Scharen 1959) and MPN method (Pfender *et al.* 1981), it was concluded that the DSI method, together with results from direct counting of oospores, were suitable techniques for quantifying the inoculum density in

soil. The correlation resulted in a highly significant regression coefficient of r = 0.97. The validity of these two assays was confirmed by determining the DSI in relation to known numbers of culture-produced oospores added to non-infested soils, in this case the regression coefficient was r = 0.99. Boosalis and Scharen (1959) also reported a direct relationship between either disease incidence or severity and inoculum density (oospores in soil). Despite reports (Mitchell *et al.* 1969; Pfender *et al.* (1981) that the original method of counting oospores (Boosalis and Scharen 1959) was tedious and time consuming, the two modifications (discussed in Section 2.5) have overcome the problems resulting in a quick and simple assay. The most important outcome of studying these two assay methods was to note their capability and reliability in the discrimination of disease potential between soils from different fields. The methods also could assess accurately the changes in a population of *Aphanomyces euteiches* when subjected to experimental soil treatments especially in soils with low DSI values where the MPN method (Pfender *et al.* 1981) was less satisfactory.

The severity of aphanomyces root rot is well known to be dependent on high soil moisture at temperatures from 22° to 28°. Ecological studies on the effect of soil moisture on the pathogen has been expressed in terms of water potential. Previous research has been described solely in terms of water content which did not explain the influence of availability of water on growth of fungi in soil (Griffin 1963). The conclusions from these studies that percentage

moisture holding capacity could not be equated to water potential, were in agreement with many workers (Griffin 1963, 1970; Cook *et al.* 1971, 1972; Adebayo and Harris 1971; Emberger and Welty 1982). *Aphanomyces euteiches* was shown to require high water potential and warm temperature for maximum growth. In laboratory studies (Section 3.3.1), maximum growth of the pathogen occurred at the highest water potential of 0 bars and warmest temperature of 25°. In glasshouse studies (Section 3.3.2), high water potential and warm temperatures also favoured diseases development. However, infection was shown to occur under drier conditions (at -200 millibars) and lower temperatures of 8°-12° especially with soil highly infested with the pathogen. This has suggested that it is not only wet soil and warm temperature which can initiate the fungus to active vegetative growth and to form zoospores in sporangia or mycelium from germinating oospores (Jones and Drechsler 1925). Results from laboratory studies (Section 3.3.1) showed that as a consequence of decreasing water potential and temperature, fungal growth was at a slower rate and the lag phase before growth commenced was prolonged (Scott 1957). According to Burke and Mitchell (1968) and Burke *et al.* (1969), in wet conditions optimum infection occurred at 16°, but remained latent and symptoms seldom developed unless the plants were subjected to higher temperatures. Thus provided the soil moisture was near saturation point, infection should be expected to occur between 8° and 10°, with 8° being close to the minimum growth of *Aphanomyces euteiches*.

The *Aphanomyces euteiches*-pea plant relationship can be considered as an epidemic model where mobile fungal spores are attracted to moving root tips (Baker *et al.* 1967). This suggests that the faster growth rate of plant roots under wetter conditions would determine the rate of infection which also could be influenced by the rate at which roots grew through infested soil. As a consequence, the yield responses of pea plants to increased soil moisture and temperature was altered, following infection by the pathogen. Thus according to Huisman (1982), the major limiting process in this type of epidemic was considered to be root growth. Pfender and Hagedorn (1983) also suggested that the rate of spread was severely constrained by an apparent requirement for contact or at least proximity of host plant roots to inoculum. Spread of the pathogen was limited as zoospores did not migrate in the soil more than 1.2 cm (Haenseler 1925). Therefore, high inoculum levels in soils would be expected to initiate rapid disease development as plants would be expected to come in contact with the inoculum earlier as roots grew through the soil (Section 3.3.2). Such speculation has come from the suggestion of Pfender and Hagedorn (1983) that initial infection led to the colonisation of a root system and the increase in inoculum permitted the more rapid contact to neighbouring plants either by mycelial growth between roots in contact with one another or short distance movement of zoospores between roots.

A recommended agronomic practice is to irrigate pea crops to field capacity especially at flowering and podding stages to obtain maximum yield (Stoker 1977). This practice

need not be altered in relation to control of aphanomyces root rot. According to the findings in Section 3.3.3, the availability of favourable conditions for rapid disease development during early crop growth caused severe damage to plants. This was not observed if such conditions occurred at later stages of plant growth, but there was an overall lower yield from crops grown in infested soils. This is probably due to occurrence of disease too late to cause severe root rotting in older plants (Lockwood and Ballard 1959; Lockwood 1960). As long as the water conducting system is left intact by the pathogen (Haenseler 1926) damage would not be sufficiently severe to appreciably reduce yield.

Although many areas of study have greatly increased the knowledge of *Aphanomyces euteiches*, they have not provided a satisfactory explanation for the mechanisms of survival and the decline of inoculum in soil when conditions are not favourable or in the absence of peas. Scharen (1960) has produced evidence that the pathogen survives as oospores in plant debris. No direct proof was provided to show the actual importance of alternate hosts for survival in field soils. Results from glasshouse and field studies on the role of alternate hosts (Chapter 4) showed that *Aphanomyces euteiches* parasitised two other legumes: lucerne and white clover, and three other weeds: chickweed, shepherd's purse and field pansy. No evidence of above-ground symptoms or root damage of these plants was observed. However, it is important to stress that the pathogen was able to produce new

oospores in the infected roots of these alternate hosts. Thus they, especially the annual weeds, would not only increase total inoculum density but each year would add a fresh batch of viable oospores into the soil (Papavizas and Ayers 1974). This has confirmed the findings of Linford (1927) who postulated that the life of *Aphanomyces euteiches* may be prolonged, not only by the ability of the oospores to remain viable in the soil for many years but also by the ability of the pathogen to parasitise many plants other than peas. It is appropriate to conclude that these alternate hosts are important in the survival of *Aphanomyces euteiches* and to cite Carley (1969) (cited by Papavizas and Ayers 1974) that survival "lies not only from additional quantities of oospores added to soil, but also on the continuous rejuvenation of the organism by passage through the alternate hosts". In relation to control measures, alternate hosts as 'symptomless carriers' (Garett 1960) pose two problems in trying to reduce the inoculum density of the pathogen in infested soil:

1. persuading farmers to pursue a weed control programme with sufficient zeal may be difficult; and
2. to refrain from growing the two important legumes, lucerne and white clover, would not prove practical in arable crop rotations where these plants are essential to the economy of the farm and for nitrogen-fixation.

Chemical control of disease can only be justified if the increased momentary return is greater than the cost

involved. On the basis of *in vitro* evidence that the fungicides pyroxyfur, metalaxyl and hymexazol could adversely affect mycelial growth, zoospore formation and motility and oospore formation, seed treatment as control measures using these fungicides could be effective against the pathogen. However, when these fungicides were used as seed treatments in a field trial, they were not effective as shown by final yield of the pea crop (Jermyn *et al.* 1982). Further investigations in the glasshouse (Section 5.3.2) indicated that the fungicides do have the ability to protect plants against the pathogen but this was evident only during the early stage of growth (up to 6 weeks after sowing). The *Aphanomyces euteiches*-pea plant relationship is based mainly on rate of growth of roots in infested soil and this determines the rate of infection (Huisman 1982). Thus roots could quickly grow away from the area of fungicidal influence and so infection could take place. This is because the pathogen can attack the host over a period of time (Pfleger *et al.* 1976) even though Lockwood (1960) showed that disease development tended to decrease with increasing age of plants at inoculation. In dry and warm conditions, unfavourable for rapid disease development, chemical control was not required as disease severity was not sufficient to reduce yield significantly (Experiment 3 in Section 5.3.2 and Jermyn *et al.* 1982).

Larger differences between untreated and fungicide treatment were observed with plants grown in the glasshouse compared to field-grown plants (Jermyn *et al.* 1982). This is because plants grown in glasshouse trial were evaluated

for root rot sooner after sowing than plants in field study. If field-grown plants had been evaluated by Jermyn *et al.* (1982) at an earlier date, larger differences might have been observed. In addition, the glasshouse trials used rates of fungicides which were twice those used by Jermyn *et al.* (1982). Mitchell and Hagedorn (1969) suggested that it was important to determine root rot within 3-4 weeks after sowing if the effect of chemical seed treatment on *Aphanomyces euteiches* was to be properly assessed, i.e. before any general colonisation of roots by weaker and relatively non-invasive pathogens. However, the potential use of seed treatment to delay root infection by the pathogen and enhance seedling vigour (when under immediate disease stress) is limited because poorly filled pods and low yields were obtained (Jones and Drechsler 1925; Jermyn *et al.* 1982) especially with soils of DSI greater than 70. In the lower DSI (50-70) range of soils, the chemicals were beneficial when conditions were conducive to disease development. However, the chemicals were not of value when used on seed sown in dry and warm conditions.

Cultural control by brassica amendments to aphanomyces-infested soil was found to be a promising option. In glasshouse trials, all brassica amendments (cabbage, kale, rape, fodder radish and mustard) to aphanomyces infested soils were effective in reducing root rot severity. The brassica crops were speculated to act on the pathogen because of high levels of volatile organic sulphur compounds arising from microbial decomposition. Lewis and Papavizas (1971) were

able to show that many vital phases in the life cycle of the pathogen could be adversely affected. This is evident from the correlation between the reduction of DSI and the decrease in inoculum levels (Section 5.3.3). However, it is not known whether the effects of the sulphur compounds on oospores were either stimulating them to germinate when survival was restricted due to its inability to live saprophytically (Smith 1931) or directly killing the oospores. The latter appears more likely as Lewis and Papavizas (1971) have shown that morphology and development of oospores were adversely altered. The effectiveness of disease suppression by brassicas will depend on the quality and quantity of species, as shown by Section 5.3.3, and Lewis (1971), where suppression trends lasted for at least 6 months after incorporation.

Growing a brassica crop and incorporating that crop into the soil has the potential to reduce the disease severity in fields which have been restricted from growing peas because of high levels of inoculum. Jermyn (1984) has suggested that the cost involved in incorporation of a brassica crop established in summer or early autumn would be worthwhile because peas are such an important crop in arable rotations for cash returns, and because peas have a short growing time and are able to fix nitrogen.

In conclusion, the potential of using brassica amendments in aphanomyces-infested soil to control the root rot disease has been demonstrated either in the glasshouse or

field (Jermyn 1984). However, future programmes should be included to clarify the effect of brassicas on oospores of *Aphanomyces euteiches*. This information will enable further research of the possibility of using sulphur compound extracts from a brassica crop, e.g. mustard oil manufactured commercially, to reduce the inoculum density and so assist in the control of aphanomyces root rot of peas.

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APPENDICES

APPENDIX 1

Glossary

The following terms used in the text in relation to soil moisture were taken from Aslyng *et al.* (1963).

1. Total potential of soil water

The amount of work that must be done per unit quantity of pure water in order to transport reversibly and isothermally an infinitesimal quantity of water from a pool of pure water at a specified elevation at atmospheric pressure to the soil water. It may be convenient to shorten the term to total potential or soil water potential and to divide it into divisions to be such that the sum of the divisions equal the total potential.

1.1 Osmotic potential - The amount of work that must be done per unit quantity of pure water in order to transport reversibly and isothermally an infinitesimal quantity of water *from a pool of pure water at a specified elevation at atmospheric pressure, to a pool containing a solution identical in composition with the soil water but in all other respects identical to the reference pool.*

1.2 Gravitational potential - The amount of work that must be done per unit quantity of pure water in order to transport reversibly and isothermally an infinitesimal quantity of

water *from a pool containing a solution identical in composition to the soil water at a specified elevation at atmospheric pressure to a similar pool at the elevation of the point under consideration.*

1.3 Matric or capillary potential - The amount of work that must be done per unit quantity of pure water in order to transport reversibly and isothermally an infinitesimal quantity of water *from a pool containing a solution identical in composition to the soil water at the elevation and the external gas pressure of the point under consideration to the soil water.*

1.4 Potential due to external gas pressure - This potential component is to be considered only when external gas pressure differs from atmospheric pressure, e.g. in a pressure membrane apparatus. A specific term and definition is not given.

2. Water content

The amount of water lost from the soil upon drying at 105°, expressed either as the weight of water per unit weight of dry soil or as the volume of water per unit volume g soil in bulk.

The following terms in the text in relation to soil properties were taken from Hanks and Ashcroft (1980).

2.1 Soil bulk density - Bulk density is defined as the mass (weight) of a unit volume of dry soil (including solids and pores).

2.2 Field capacity or holding capacity - The field capacity is the unique water content that a given soil reaches and maintains after it has been thoroughly wetted and allowed to drain freely for a day or two.

APPENDIX 2

Agar Media used in Laboratory Studies on *Aphanomyces euteiches*

1. Basal Medium (BM)

(ref. Sommers *et al.* 1970)

Na_2HPO_4	0.75 g
KH_2PO_4	0.75 g
$MgSO_4$	0.12 g
NaCl	0.10 g
NH_4PO_3	0.40 g
Glucose	1.80 g
Yeast extract	0.10 g
Malt extract	1.00 g
Bactor agar	15.00 g
Water	1000 ml
pH	6.4

2. Corn Meal Agar (CMA)

(ref. Gibco Diagnostics, Madison, Wisconsin, USA)

Cornmeal agar	17.0 g
Distilled water	1000 ml

3. Potato Dextrose Agar (PDA)

(ref. Difco Laboratory Incorporated, Detroit, Michigan, USA)

Potato dextrose agar	39.0 g
Distilled water	1000 ml

Preparation of Stain (CMI 1983)

Lactophenol	- Phenol (pure crystals)	20 g
	Lactic acid (SG 1.21)	20 g
	Glycerol	40 g
	Water	20 ml
Cotton Blue Lactophenol Stain		
	- Anhydrous lactophenol	6.70 ml
	Distilled water	20.0 ml
	Cotton blue or trypan blue	0.1 g

APPENDIX 3

List of Pesticides mentioned in the Text (Pest. Manual 1983)

Common name	Chemical name	Trade name(s)
benomyl	methyl 1-(butylcarbamoyl) benzimidazol-2-ylcarbamate	Benlate
carbon disulfide	carbon disulfide	Carbon disulfide
chloronitropropane	1-chloro-2-nitropropane	Lanstan
dazomet	tetrahydro-3,5-dimethyl-2H.1, 3,5-thiadiazine -2-thione	Basamid
dinitramine	N',N'-diethyl-2,6-dinitro-4-trifluoromethyl-m-phenylenediamine	Cobex
dinoseb	2-(1-methylpropyl)-4,6-dinitrophenol	'Premerge' (Dow)
fenaminosulf	sodium 4-dimethylaminebenzene-diazosulphonate	Lexan, Dexon, Bayer 5072
hymexazol	5-methylisoxazol-3-ol	Tachigaren
metalaxyl	methyl N-(2-methoxyacetyl)-N-(C2,6-xylyl)-DL-alaninate	TN Apron, Ridomil and Fubol (mixture with mancozeb)
metham-sodium	sodium methyl dithiocarbamate	Vapam
orxyzalin	3,5-dinitre N^4, N^4-dipropylsulfanilamide	Ryzdan or Surflan
pyroxychlor	2-chloro-6-methoxy-4-trichloromethylpyridine	Dowco 269
pyroxyfur	2-chloro-6-(2-furanylmethoxy) -4-(Trichloromethyl) pyridine	Dowco 444
Trifluralin	2,6-dinitro-N,N-dipropyl-4-trifluoromethyllaniline	Treflan'; Elancolan

APPENDIX 4

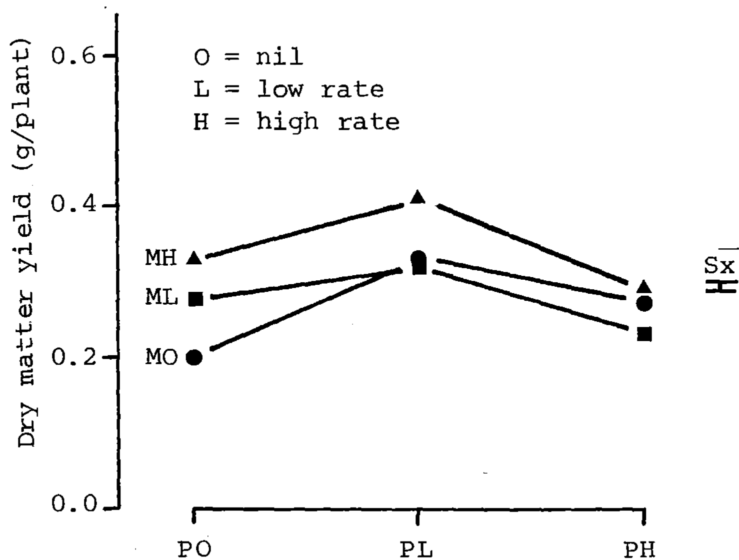
Effect of pyroxyfur and metalaxyl seed treatments at two soil inoculum levels on top dry matter yield (g/plant) of pea cultivar Canterbury 39 affected by aphanomyces root rot at four weeks after sowing.

Soil inoculum (DSI)	Pyroxyfur (rate)	Metalaxyl (rate)		
		MO	ML	MH
50	PO	0.24	0.38	0.38
	PL	0.38	0.29	0.39
	PH	0.04	0.42	0.28
100	PO	0.15	0.28	0.15
	PL	0.18	0.24	0.16
	PH	0.26	0.39	0.30
	$S\bar{x}$	0.022		
	LSD (0.05)	0.04		

APPENDIX 5

Effect of pyroxyfur and metalaxyl seed treatments at two soil inoculum levels on total dry matter yield (g/plant) of pea cultivar Canterbury 39 affected by aphanomyces root rot at four weeks and eight weeks after sowing.

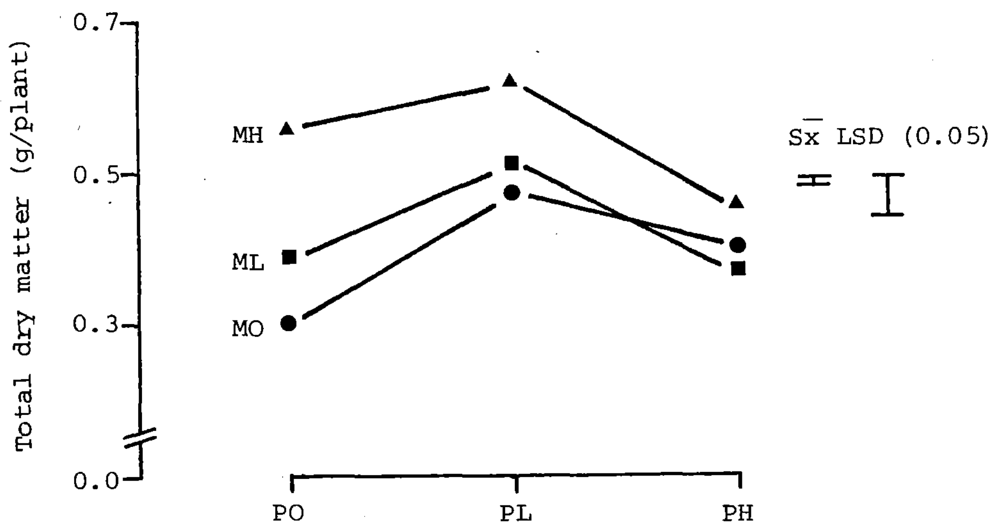
Soil inoculum (DSI)	Pyroxyfur (rate)	4 weeks			8 weeks		
		Metalaxyl (rate)			Metalaxyl (rate)		
		MO	ML	MH	MO	ML	MH
50	PO	0.36	0.53	0.59	0.68	0.65	0.66
	PL	0.53	0.49	0.70	0.74	0.79	0.72
	PH	0.60	0.68	0.47	0.69	0.56	0.69
100	PO	0.22	0.40	0.21	0.45	0.69	0.85
	PL	0.25	0.34	0.23	0.79	0.44	0.55
	PH	0.41	0.55	0.42	0.77	0.79	0.53
	$S\bar{x}$	0.032			0.103		
	LSD (0.05)	0.05			0.17		



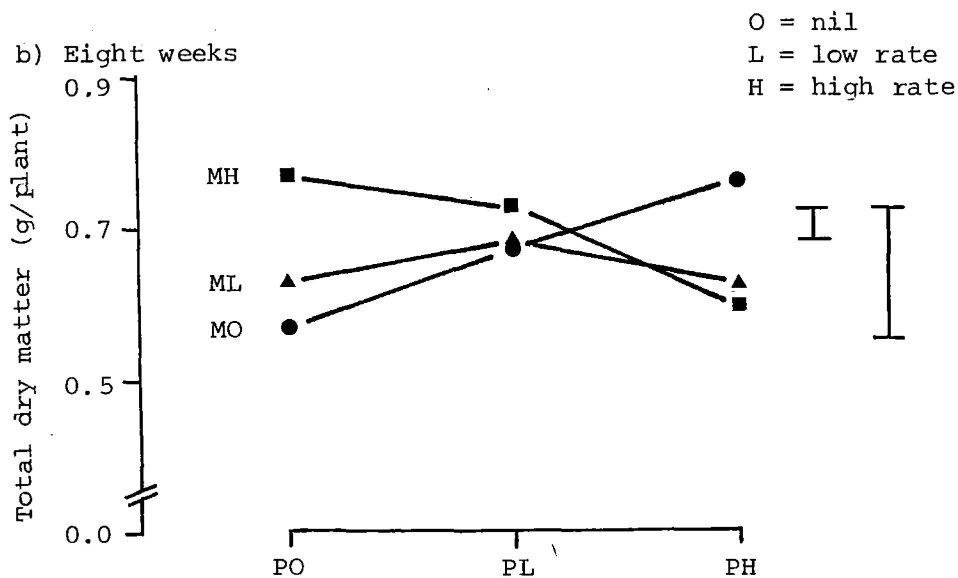
Appendix 6: Interaction between pyroxyfur (P) and metalaxyl (M) with three concentration levels on top dry matter yield of pea cultivar Canterbury 39 at four weeks after sowing.

(Overall means derived from DSI 50 and 100)

a) Four weeks



b) Eight weeks



Appendix 7 : Interaction between pyroxyfur (P) and metalaxyl (M) with three concentration levels on total dry matter yield of pea cultivar Canterbury 39 at four weeks and eight weeks after sowing.

(Overall means of data derived from DSI 50 and 100)

APPENDIX 8

Effect of pyroxyfur and hymexazol seed treatments at two soil inoculum levels on top dry matter yield (g/plant) of pea cultivar Canterbury 39 affected by aphanomyces root rot at four weeks and eight weeks after sowing.

Soil inoculum (DSI)	Pyroxyfur (rate)	4 weeks			8 weeks		
		Hymexazol (rate)			Hymexazol (rate)		
		HO	HL	HH	HO	HL	HH
50	PO	0.21	0.29	0.35	0.35	0.31	0.31
	PL	0.39	0.26	0.34	0.41	0.50	0.43
	PH	0.42	0.28	0.48	0.54	0.47	0.31
100	PO	0.15	0.28	0.26	0.32	0.38	0.43
	PL	0.18	0.29	0.29	0.57	0.30	0.33
	PH	0.26	0.29	0.29	0.35	0.47	0.41
	$S\bar{x}$	0.021			0.080		
LSD (0.05)		0.04			0.13		

APPENDIX 9

Effect of pyroxyfur and hemaxazol seed treatments at two soil inoculum levels on total dry matter yield (g/plant) of pea cultivar Canterbury 39 affected by aphanomyces root rot at four and eight weeks after sowing.

Soil inoculum (DSI)	Pyroxyfur (rate)	4 weeks			8 weeks		
		Hymexazol (rate)			Hymexazol (rate)		
		HO	HL	HH	HO	HL	HH
50	PO	0.31	0.42	0.55	0.59	0.52	0.57
	PL	0.59	0.43	0.49	0.87	0.83	0.66
	PH	0.63	0.49	0.70	0.76	0.75	0.55
100	PO	0.22	0.37	0.37	0.45	0.69	0.85
	PL	0.25	0.45	0.45	0.79	0.44	0.55
	PH	0.42	0.42	0.45	0.77	0.79	0.53
	$S\bar{x}$	0.031			0.103		
LSD (0.05)		0.05			0.17		