SPECIES OF PYTHIUM ASSOCIATED WITH BARLEY IN SOUTH AUSTRALIA

by

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Thesis submitted to The University of Adelaide
in fulfilment of requirements for the
degree of Doctor of Philosophy

July, 1985

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DECLARATION

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma in any university. To the best of my knowledge and belief, no material described herein has been previously published or written by another person except when due reference is made in the text.

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SUMMARY

A field survey of barley soils conducted at three sites in South Australia : Roseworthy and Virginia, north of Adelaide, and Strathalbyn, south of Adelaide, revealed that Pythium species are common inhabitants of barley soils in South Australia. Population levels are higher in the 0-10 cm layer of the soil that in the 10-20 cm layer. A seasonal variation was found in the Pythium population of barley soils over 12 months. Monthly assays suggested that activity of Pythium in soil was directly influenced by temperature in two sites, whereas rainfall affected the Pythium population in only one site.

Eleven species of Pythium, isolated from soils and from infected barley seedlings, were identified and characterized. P. irregulare was found to be the predominant species. P. volutum was recorded for the first time in Australia.

The influence of environmental factors influencing Pythium species on barley was studied. There was a high positive correlation between soil moisture and the incidence of disease on barley seedlings caused by P. irregulare, P. graminicolum and P. volutum. The relationship between soil water and inoculum density of P. irregulare on the growth of barley revealed that, for this fungus, inhibition in growth of barley seedlings is associated with high population levels in soils with a high level of moisture.

The relationship between pH of the medium and growth rate of P. irregulare showed that the fungus can grow linearly and in weight at relatively high and low extremes with an optimum growth at neutral pH.

Experiments carried out to evaluate the influence of temperature on mycelial growth of Pythium irregulare and on disease severity indicated that the optimum temperature for mycelial growth was between 25-30°C, whereas the pathogenicity test showed that root length of barley seedlings was significantly reduced by the fungus at 13°C.

The influence of Pythium irregulare and the nematode Pratylenchus thornei was assessed to test the hypothesis that a statistical interaction occurred between the two organisms in their effect on host plants (barley and wheat). P. thornei seems to be equally pathogenic in wheat and barley, unlike Pythium irregulare which is more damaging in barley than in wheat. Nematode and fungus appear to act independently in terms of plant response to infection ; Pythium irregulare more so in barley than in wheat.

Pythium volutum, was recorded for the first time in Australia. It was consistently isolated from infected barley roots and from soil in the survey of barley soils. Furthermore, P. volutum occurred in another 10 barley and wheat fields, with pH values and clay contents quite different from each other. Some aspects of fungus biology were studied in vitro such as the influence of temperature, pH and soil water on growth. Three barley cultivars commonly grown in South Australia were sown in artificially infested soil to assess varietal resistance and susceptibility to P. volutum. There were no differences between cultivars in terms of response to infection. All cultivars tested were susceptible to infection by P. volutum.

Studies on possible control measures for Pythium species were conducted. Metalaxyl (Ridomil 25 W P) was tested in vitro, in pots and

in the field. Metalaxyl, a fungicide specific against Pythium spp. and other oomycetes, produced a marked increase in growth when applied as a seed treatment.

Pythium oligandrum was tested in vitro and in pots to control other species of Pythium which were found to be pathogenic to barley seedlings. Clear evidence of control was achieved.

ACKNOWLEDGEMENTS

I wish to thank Professor H.R. Wallace, who supervised me during this work ; his encouragement and direction are gratefully acknowledged. Also, I thank Mr. M. Bumbieris for his help in this project and Dr. J.H. Warcup for providing constructive criticism.

Appreciation is due to Mr. P.E. Geytenbeek and Dr. D.H.B. Spparow, for their help with the field experiments.

The contribution from the Biometry and Photography sections in the Waite Agricultural Research Institute is gratefully acknowledged.

I wish to thank The University of Adelaide for the award of a University Research Grant Scholarship throughout this work.

I express my gratitude to staff and fellow students in the Department of Plant Pathology, Waite Institute, for their assistance and to my parents, for their encouragement and understanding.

CHAPTER 1

GENERAL INTRODUCTION

The distribution of *Pythium* species is world wide (Rangaswami, 1962 ; Tompkins, 1975). They occur in tropical, temperate even cold regions. They occur most abundantly in cultivated soil in the root zone in the surface layers of the soil. *Pythium* species have been isolated from arable land, pastures, forests and nurseries and are known to cause disease and reduce yields in cereal crops ; several reviews on these diseases have been published (Subramaniam, 1928 ; Vanterpool & Truscott, 1932, 1938 ; Noble, 1934 ; Millikan, 1938 ; Bisby *et al.*, 1938 ; Savulescu, 1940 ; Buchholtz *et al.*, 1942, 1947 ; Sprague, 1950 ; Hampton, 1957 ; Barton, 1958 ; Warcup, 1957 ; Hirane, 1960 ; Teakle, 1960 ; Sharma & Jain, 1967 ; Saladini and White, 1975 ; Lipps, 1978, 1980 ; Waller, 1979 ; Cook *et al.*, 1980). Studies on disease in barley caused by *Pythium* (Erikson, 1912 ; Gram, 1921 ; Chabrolin, 1927 ; Subramaniam, 1928 ; Vanterpool & Truscott, 1932 ; Asuyama, 1935 ; Simmonds *et al.*, 1935 ; Drechsler, 1936 ; Elliott *et al.*, 1937 ; Ho *et al.*, 1941 ; Haskett, 1953 ; Bruehl, 1953 ; Summer, 1958 ; Freeman, 1966 ; Kilpatrick, 1968 ; McKeen, 1977 ; Waller, 1979) contain little information on the occurrence, etiology and control of *Pythium* under Australian conditions. Thus in studying *Pythium* in barley crops in South Australia it was first necessary to determine what species occurred there, which species were common and which were the most pathogenic. Further information was required on the influence of the South Australian environment on *Pythium*. Thus factors such as pH, temperature and rainfall and their influence on the incidence, mycelial growth and reproduction of *Pythium* spp. were studied. The effect of and interaction between soil water and the pathogenicity of *Pythium* seemed a particularly important aspect to investigate to answer the question : Is

there a particular level of soil water at which Pythium is most damaging? Furthermore, the influence of inoculum density in the etiology of the disease was considered worthy of study. Such etiological studies are a prelude to control ; at least they should indicate where further research might usefully go.

In this project two approaches to control have been taken : the effect of the fungicide Ridomil and the parasitic effect of Pythium oligandrum on pathogenic species of Pythium on barley.

Most of South Australia is arid or semi-arid, and cannot be used for crop production. Cereal production is confined to the more southerly regions whose climate is indicated by that for Adelaide (Figure 1). This severe natural limitation means that only 6 million of 63 million hectares in rural establishments are devoted to cropping or permanent pasture. Figure 2 indicates the areas sown with the three major cereal crops in South Australia since 1937. The South Australian area under barley and wheat in recent years (1980-1984) has varied between 2.4 and 2.6 million hectares (Table 1). The cereals - wheat, barley and oats sown for grain each year occupy 92 per cent of the total area cropped in South Australia. The area sown to barley in South Australia between 1980-1984 represent 39 per cent of the Australian total and the production was 38 per cent of the total grain produced. The total gross value of barley produced in South Australia in 1983-1984 of 292 million dollars represented 35 per cent of the total gross value of Australian barley. These data indicate clearly the economic importance of barley in South Australia and justify a programme of research on diseases in this crop.

Figure 1 : Climatological data, Adelaide, South Australia ; mean monthly rainfall based on all years of records (A), temperature (B), and relative humidity (C) based on composite records of Greenwich Stand and Stevenson screen observations.

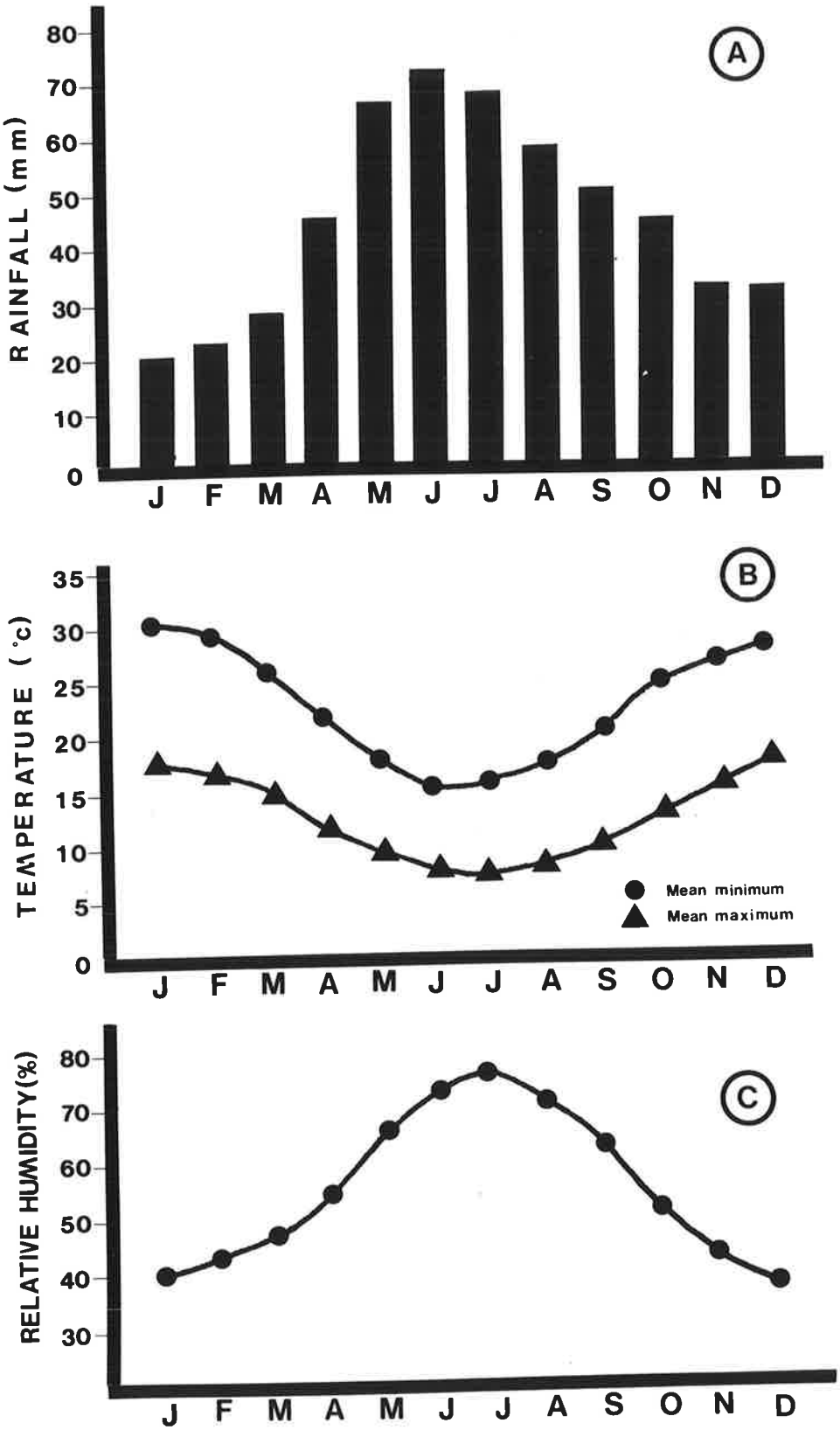

A
RAINFALL (mm)
80
70
60
50
40
30
20
10
0
J F M A M J J A S O N D
B
TEMPERATURE (°c)
35
30
25
20
15
10
5
0
Mean minimum
Mean maximum
J F M A M J J A S O N D
C
RELATIVE HUMIDITY(%)
80
70
60
50
40
30
J F M A M J J A S O N D

Figure 2 : Area under cereals for grain in South Australia from 1937 to 1980.

(Australian Barley Board, Bureau Census and Statistics)

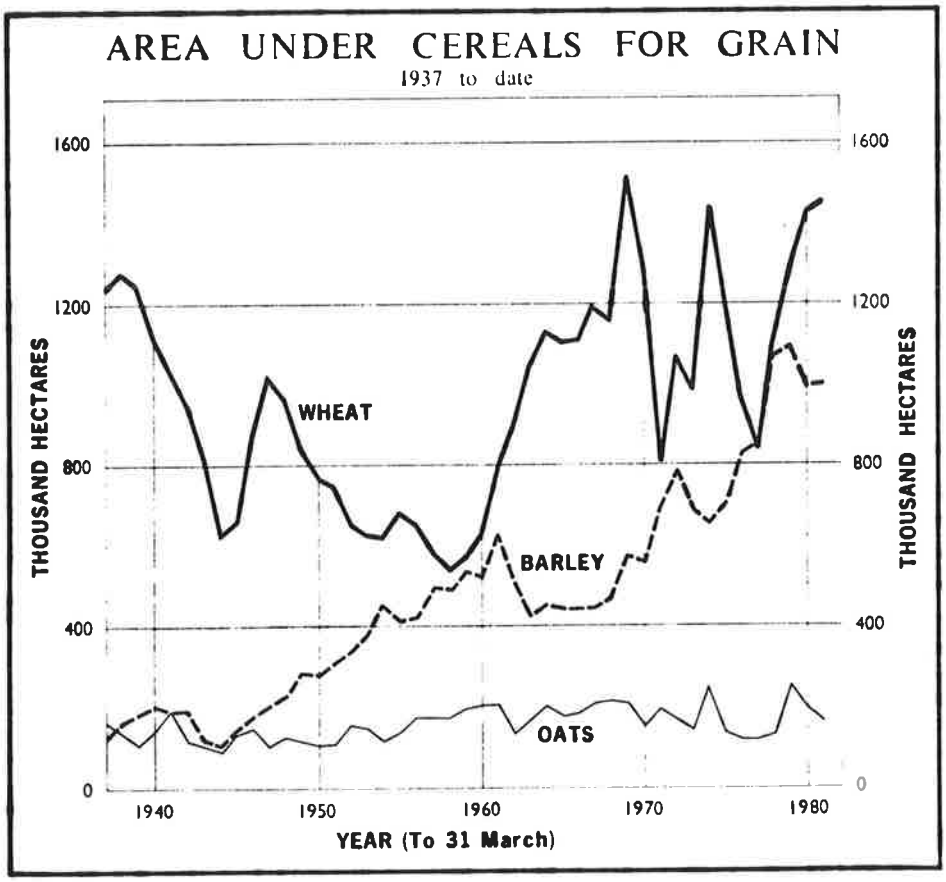

AREA UNDER CEREALS FOR GRAIN
1937 to date
1600
1200
800
400
0
THOUSAND HECTARES
WHEAT
BARLEY
OATS
1940
1950
1960
1970
1980
YEAR (To 31 March)

Table 1 : Area under barley and wheat sown in South Australia between 1980–1984

	AUSTRALIA				SOUTH AUSTRALIA			
	BARLEY		WHEAT		BARLEY		WHEAT	
	Area ,000 Ha	Production ,000 Ton	Area ,000 Ha	Production ,000 Ton	Area ,000 Ha	Production ,000 Ton	Area ,000 Ha	Production ,000 Ton
1980 - 1981	2,451	2,682	11,283	10,856	989	1,158	1,445	1,650
1981 - 1982	2,685	3,450	12,041	16,360	1,032	1,227	1,427	1,695
1982 - 1983	2,495	1,858	11,546	8,818	1,005	668	1,398	692
1983 - 1984	3,163	4,937	12,909	22,064	1,122	1,815	1,569	2,678

Data provided by the South Australian Statistics Bureau

Many diseases of barley occur in South Australia of which scald and powdery mildew are considered the most serious (Barr and Mayfield, 1979). Scald (Rhynchosporium secalis (Oudem) J. Davis) is common every year in most barley crops throughout South Australia. Barr and Mayfield, (1979) have shown that all commonly grown cultivars are susceptible with disease being most severe in crops sown early in May and particularly in years with wetter growing seasons. They found losses in yield of up to 25% due to scald in field trials following inoculation of barley with R. secalis. Powdery Mildew (Erysiphe graminis D.C. f. hordei Marchal), according to Barr and Mayfield (1979), is widespread and apparently severe when long periods of dry, warm weather occur during tillering of crops. In districts where the growing season is cooler and longer, powdery mildew has been most severe during the period when grain is filling.

Leaf diseases of barley of less importance in South Australia are net blotch (Drechslera teres (Shoem)), stem rust (Puccinia hordei (Otth)), loose smut (Ustilago hordei (Pers/Lagerh), and leaf spot (Drechslera verticilata (O'Gora) Shoem).

The root disease take-all caused by Gaeumannomyces graminis was ranked as second in importance after stem rust (Garrett, 1942), and it is generally acknowledged (Kirby, 1925 ; Garrett, 1942) that take-all has proved most destructive in areas where intensive cereal cropping programmes have been pursued with little or no reference to rational practice. Under such conditions individual crop losses of the order of 25–75 per cent have been recorded in the past in Australia (Richardson, 1910 ; Hynes, 1935). Purple or bare patch caused by Rhizoctonia solani Kuhn, can be of major concern in South Australia (McKnight, 1960) and

periodically causes appreciable yield losses on the Central and South Western slopes of New South Wales.

Common root rot of barley, is a complex disease caused by a number of fungi the most important of which are Helminthosporium sativum (Pammel, King, and Bakke) and various species of Fusarium especially F. culmorum (W.G. Sm.) Sacc. Under Australian conditions Curvularia spp. notably C. ramosa (Bainier) Boedijm, may also cause disease in barley (Hynes, 1935 ; Millikan, 1942). Among the virus diseases of cereals, Barley yellow dwarf virus, has been recorded on barley (Warcup and Talbot, 1981).

Heterodera avenae, Wollem. and Pratylenchus neglectus (Rensch) Filipjev and Schuurmans Stekhoven, are the most prevalent plant parasitic nematodes recorded in South Australia on barley.

Although many of the root diseases mentioned probably reduce barley yields their relative importance, and how they compare with Pythium spp. is unknown. This project aims to answer some of these questions.

CHAPTER 2

FIELD SURVEY FOR *PYTHIUM* IN BARLEY CROPS IN SOUTH AUSTRALIA

A. Introduction

The distribution of a pathogen is largely determined by its environment. The presence of its host and the environmental factors that influence its survival, determine whether the pathogen will be common or rare, and whether it will be economically important. Vaartaja and co-workers (Vaartaja and Bumbieris, 1964 ; Agnihotri and Vaartaja, 1967a ; Vaartaja and Agnihotri, 1967) have concluded that environmental factors chiefly determine severity of seedling root diseases caused by *Pythium* in forest nursery beds. Campbell and Hendrix (1967) concluded that *Pythium spp.* are damaging only during or following periods of excessive soil moisture, but that disease severity may be less dependent upon soil moisture when *Pythium* populations become excessively high. A number of investigators have reported increase in disease caused by *Pythium* following addition of residues (Trujillo and Hine, 1965), seed (Singh, 1965) or high-carbohydrate soil amendments (Barton, 1960, 1961 ; Lin and Vaughn, 1960 ; Yarwood, 1966). Kendrik and Wilbur (1965) concluded that more than 500 propagules of *Pythium irregulare*/g soil were needed for severe pre-emergence kill of lima bean, after the population of *Pythium* had increased with repeated plantings of lima bean seed. In most studies (Schmitthenner, 1962 ; Vaartaja and Bumbieris, 1964 ; Campbell and Hendrix, 1967) the population density of *Pythium spp.*, in particular that of the pathogenic species e.g. *P. ultimum*, *P. irregulare*, and *P. aphanidermatum*, was found to be less than 100 propagules/g soil. *Pythium spp.* are of economic importance in cereal crops (Cook, Sitton and

Waldher, 1980) and in Queensland, Teakle (1960) has described fourteen species of which P. aphanidermatum was pathogenic on barley. However, apart from this, little research has been done on the influence of Pythium on cereals in Australia.

B. Characteristics of the field survey

A field survey of barley fields was conducted at three sites in South Australia : Roseworthy (50 km north of Adelaide), Virginia (35 km north of Adelaide) and Strathalbyn (70 km south of Adelaide) as shown in Figure 3. The meteorological characteristics of the three sites are shown in Table 2 and the characteristics of the soil from each field site are presented in Table 3. From these data it is evident that the two northerly sites at Virginia and Roseworthy tend to have higher temperatures, less rainfall, and less clay in the soil than Strathalbyn in the south. Thus, the two northerly sites might be expected to present the barley crops and Pythium with a harsher environment.

The survey was conducted from September 1981 to August 1982 and set out to test the hypothesis, based on observations in many agricultural soils in South Australia, that seasonal fluctuations in Pythium populations are influenced by environmental factors ; that, because of temperature differences, populations at the soil surface are more abundant and fluctuate more widely than those at depth ; that temperature and rainfall determine the long range changes in population levels of Pythium. Such effects should be reflected in differences between the northerly and southerly sites which have different climatic conditions.

Figure 3 : Location of field survey areas

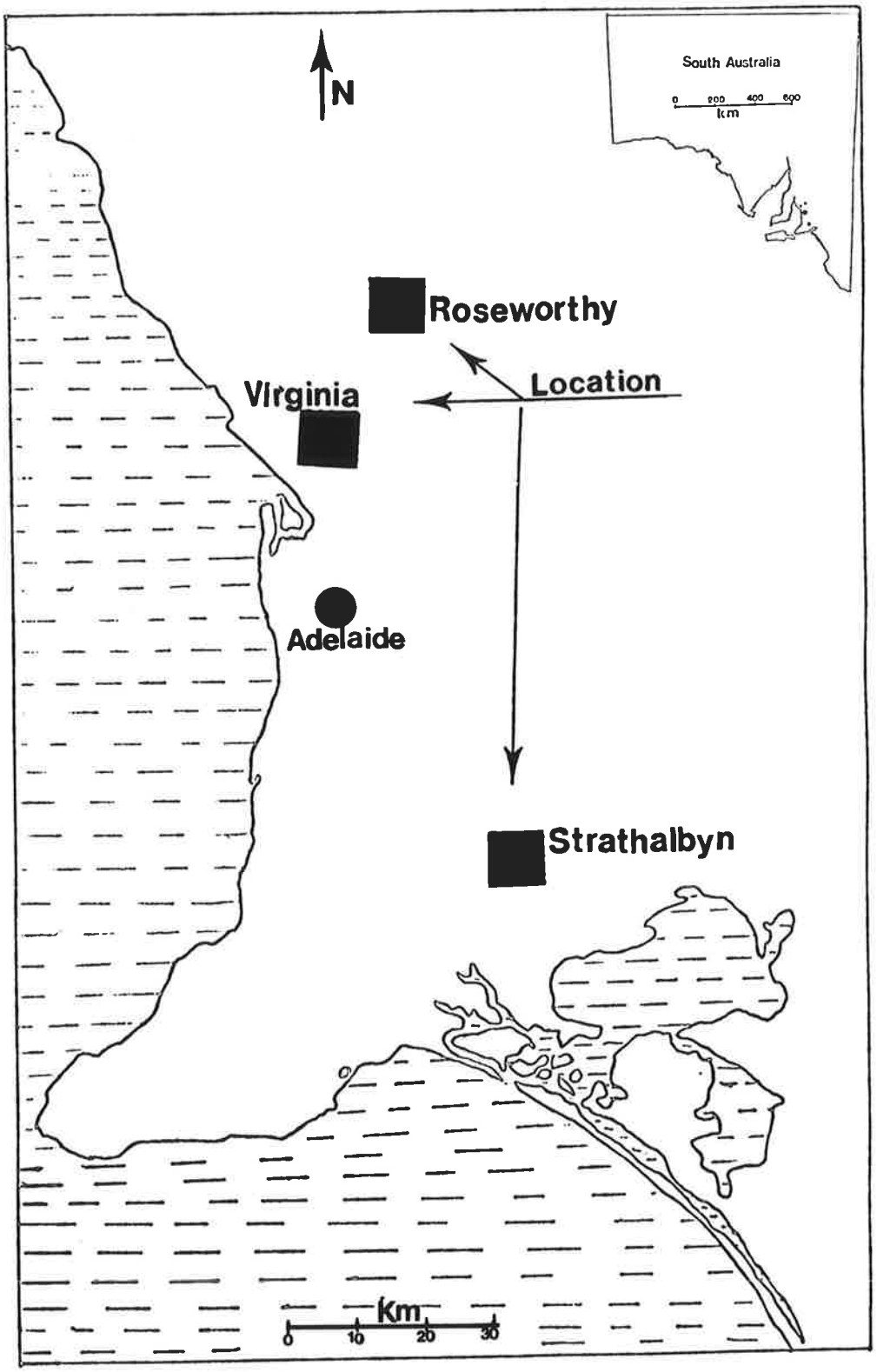

N
South Australia
0 200 400 600
km
Roseworthy
Location
Virginia
Adelaide
Strathalbyn
Km
0 10 20 30

Table 2 : The meteorological characteristics of the three field survey sites

	ROSEWORTHY	VIRGINIA	STRATHALBYN
Mean annual rainfall (mm)	440	446	494
Mean rain days (no.)	106	117	125
Mean minimum temperature (°C)	10.5	11.0	9.4
Mean maximum temperature (°C)	22.3	22.4	21.2
Mean minimum winter temperature (°C)	6.5	6.1	6.1
Mean maximum winter temperature (°C)	15.6	16.3	15.7
Mean minimum summer temperature (°C)	14.8	16.1	12.9
Mean maximum summer temperature (°C)	28.9	29.3	26.6

Data provided by the Meteorological Bureau, Adelaide

Table 3 : Characteristics of the soils at the sampling sites.

Site	Soil Type	Soil Texture 0–10 cm				Soil Texture 10–20 cm		
		pH	Clay %	Silt %	Sand %	Clay %	Silt %	Sand %
Strathalbyn	Sandy Loam	7.8	19.28	5.64	75.08	17.86	3.89	78.25
Virginia	Loamy Sand	8.0	11.52	3.84	84.64	9.22	3.70	87.08
Roseworthy	Loamy Sand	7.9	12.35	3.87	83.78	10.84	3.92	85.24

C. Materials and Methods

C.1. Soil samples

Samples were collected monthly from September 1981 to August 1982 from three sites containing barley crops, Virginia and Roseworthy in the north and Strathalbyn in the south (Figure 3). Four areas, 50 m square, were sampled at each site. Thirty sub-samples, each of 200 ml, were collected at random within each square and bulked to form one sample. Long handled trowels were used to collect the composite samples from 0-10 cm, and 10-20 cm deep. No soil from upper layers was allowed to drop to lower layers. The sampling trowels were cleaned and immersed in a 3 per cent formaldehyde solution prior to taking each sample.

The soil in each sample was sieved through a 1.68 mm mesh sieve and mixed thoroughly. Soil samples were placed individually in clean plastic bags, and were stored at 4°C and processed within a few days of collection to assess Pythium populations, pH, and texture.

C.2. Assessment of Pythium populations

Two methods were compared, Warcup's soil plate method (Warcup, 1950), and a soil dilution plate method.

C.2.1 The Soil plate method

Soil plates were prepared by transferring a small amount of air dry soil (10 mg) into a sterilized Petri dish. 10 ml of cooled medium were added and the soil particles dispersed throughout the agar. Due to the

high content of clay in some soils, the particles of soil were mixed with a drop of sterile distilled water in the plates, before the medium was added. Soil particles were dispersed by gentle rotation before the agar solidified. Selective medium was used (Ocana and Tsao, 1966 ; Tsao and Ocana, 1969). There were four replicate plates per sample. Plates were incubated in the dark at 25°C and numbers of colonies counted 24 and 48 h later and recorded as the number of colonies per g of oven dried soil.

C.2.2 Dilution plate method

This method, widely used in soil mycology has been adapted from the method originally developed for general isolation of soil bacteria (Waksman, 1927). 10 g of soil, from the same sample used for the soil plate method, were placed in a graduated cylinder. Sterile distilled water was added to give a total volume of 100 ml. The suspension was stirred and poured into a 1000 ml Erlenmeyer flask. The flask containing the suspension was shaken on a mechanical shaker for 30 min. 10 ml of this suspension was immediately drawn (while in motion) into a sterile 10 ml pipette and transferred to 90 ml of sterile water. Each suspension was shaken by hand for 5 sec and was in motion while being drawn into the pipette. The soil dilution used was 1:100. Half of the final soil dilutions used, were allowed to settle for 5 minutes, the other half for 60 minutes.

Both soil dilutions were separated into top (A), middle (B), and bottom (C) levels. Each of the three levels A, B and C were allowed to settle for 5 and 60 minutes, and then 1 ml of suspension was tranferred aseptically into Petri dishes (four Petri dishes for each dilution

transferred). The dishes were rotated by hand in a broad swirling motion so that the diluted soil was dispersed in the agar. The same selective medium (Ocana and Tsao, 1966 ; Tsao and Ocana, 1969) was used. According to Tsao (unpublished data) this selective medium gives the highest recovery of Pythium from natural soil. Plates were incubated in the dark at 25°C and examined at 24 and 48 h. Any Pythium colonies located were transferred to corn-meal agar. The number of Pythium colonies was recorded as number of colonies per gram of oven dried soil. There were seven treatments with four replicates, arranged in a randomised complete block design.

C.2.3 Results

The numbers of Pythium propagules obtained by the two methods, soil plate and soil dilution, are shown in Table 4. There was no significant difference between the numbers of colonies obtained from the soil plates and from dilution plates except that samples taken from the top level (A) of the dilutions (allowed to settle for 5 and 60 minutes) clearly yielded significantly fewer.

C.2.4 Discussion

Usually, the detection or isolation of a pathogen from its living host is a simpler task than its detection in its non-pathogenic phase when it is living as a saprophyte in soil either actively or as inactive spores. The results of the experiment showed that both methods give essentially the same picture of the Pythium flora in soil. Garrett (1951) suggested that the dilution plate method favoured heavily-sporing fungi and evidence for this view was provided by Warcup (1955). Warcup

Table 4 : Numbers of *Pythium* colonies obtained by two methods : soil plate and soil dilution.

	TREATMENT	Mean value of Number of Pythium Colonies / 1 g dried soil
1	Soil plate method	425 a
2	Top level (A) of dilution allowed to settle 5 minutes	150 b
3	Middle level (B) of dilution allowed to settle 5 minutes	350 a
4	Bottom level (C) of dilution allowed to settle 5 minutes	350 a
5	Top level (A) of dilution allowed to settle 60 minutes	75 b
6	Middle level (B) of dilution allowed to settle 60 minutes	300 a
7	Bottom level (C) of dilution allowed to settle 60 minutes	350 a

L.S.D. = 226.23 (p = 0.01)

Values followed by similar letter are not significantly different.

Mean value of four replicates.

(1957) also showed that Pythia are isolated by dilution plates but are more common on soil plates.

It is interesting to note that both the soil plate and the dilution plate methods gave a higher number of Pythium propagules from the residue after preparation of soil suspension (Level C), than dilutions poured from the top (Level A). It would seem that soaking soil with distilled water does not allow all the Pythium propagules present to pass into suspension. Bainbridge (1966) showed that the dilution plate method, using water as a diluent, was unsatisfactory in that it resulted in a lower estimate of numbers than did the soil plate method.

The results in Table 4 show that when the soil dilution method is used, it is not necessary to wait for the soil to settle for more than 5 minutes after shaking ; if the suspension is left for more than 5 minutes, the samples should be taken from the bottom of the solution, because in the preparation of dilution plates many active hypha or spores of Pythium species can be discarded with the residue. The soil plate method allows the growth of those Pythium spp. embedded in humus or attached to mineral particles. Owing to variability within treatments the results in Table 4 fail to substantiate Warcup's (1957) conclusion that most pathogens which occur on dilution plates occur more frequently on soil plates. In both methods, use of a selective fungistatic antibiotic did not result in an increase in the number of Pythium colonies isolated, but was of use in eliminating other fungi which obscured observations on isolation plates.

Warcup's soil plate method for assessment of Pythium populations in soil possibly reflects more closely the real density of Pythium spp. in

soil and, because it was easier to use, this method was chosen. Twenty five ~~25~~ plates for each soil sample were used.

C.3. Isolation of *Pythium* species from the roots of infected barley seedlings

Several authors have commented on the difficulty in isolating *Pythium* from roots. Drechsler (1929) thought it might be due either to the rapid spread of bacteria over the surface of the tissue subsequent to plating, or to conditions within the diseased tissue itself. He was able to increase the frequency of isolation of the fungus from decaying tissue by first immersing roots in sterile water for 12 to 24 hours. Root pieces from which *Pythium* hyphae had grown during immersion were blotted dry before being planted on agar, where colonies of the fungus developed. Drechsler stressed the importance of careful drying to remove any moisture film in which bacteria might spread. He also thought it possible that immersion in water removed soluble substances from the pieces inhibitory to growth or germination of spores of *Pythium*. Vanterpool and Truscott (1932) also obtained an increased frequency of isolation of *Pythium* from wheat roots by Drechsler's method, as did Rands and Dopp (1934) from roots of maize.

The presence of other fungi, commonly thought to be secondary invaders, has been given as another reason for the difficulty in isolating *Pythium*. Robertson (1959), in isolating the fungus from decaying roots of oil palm (*Elaeis guineensis*) found that it was necessary first to suspend the pieces in running tap water for 24 h. If this was omitted procedures usually failed to recover *Pythium*, but gave instead a prolific growth of other fungi. Flentje (1964a) frequently isolated *Pythium* from rotted pea seeds only if they were

removed from the field within four days of planting ; after that time there was a marked decrease in the number yielding Pythium but an increase in those giving rise to Mucor, Rhizopus and Fusarium.

Attempts to find a sterilizing agent which would destroy most of the root surface contaminants, without killing Pythium within the tissue, have usually been unsuccessful. Baylis (1941) found that treatment with a 0.1 per cent solution of mercuric chloride or with a 0.1 per cent solution of silver nitrate followed by rinsing in a saturated sodium chloride solution, greatly reduced isolation of Pythium from pea seedlings. Flentje (1964a), using a 1:1500 solution of mercuric chloride, found that other fungi, notably species of Fusarium, were much more tolerant of it than was Pythium. Hawker, Harrison, Nicholls and Ham (1957), in isolating from Allium roots an endophythic fungus very similar to Pythium ultimum (Carre and Harrison, 1961), found sodium hypochlorite unsuitable as it almost completely prevented growth of the fungus when the roots were plated on water agar.

C.3.1 Selection of a root surface treatment

Following the Bainbrige (1966) method, silver nitrate and sodium hypochlorite were tested as root surface sterilants to see if the results obtained were similar to those of Baylis (1941) and of Hawker et al. (1957). Although the washing technique described by Robertson (1959) may not remove all contaminants from the root surface, it was tested to see if its use increased frequency of Pythium isolation.

To obtain infected roots for surface sterilization, barley seeds were planted in 1,000 g of uncultivated soil inoculated with Pythium

irregulare by mixing with the soil 3 g of fresh, washed, macerated mycelium grown on liquid neutral-Dox-yeast medium (NDY) ($NaNO_3$, 2 g ; $MgSo_47H_2O$, 0.5 g ; Sucrose, 30 g ; KH_2PO_4, 1.0 g ; $FeSO_4$, 0.01 g ; Water, 1000 ml ; KCl, 0.5 g ; Yeast extract, 0.5 g).

Eighty barley seeds were planted in the soil in an aluminium tray. Soil moisture was adjusted to 80 per cent of field capacity, and the tray covered with a sheet of polyethylene. Kaufman and Williams (1962) reported that water loss from soil kept in polyethylene bags was negligible whereas CO_2 exchange with the atmosphere was not impeded. The temperature was 15°C. After 14 days seedlings were harvested, roots were removed, washed free of soil in tap water and then batches of 20 roots were:

(a) Immersed for 3 minutes in 5 per cent aqueous solution of sodium hypochlorite then rinsed 3 times in sterile, distilled water.

(b) Immersed for 3 minutes in 0.1 per cent silver nitrate, for 10 minutes in a saturated sodium chloride solution, then rinsed 3 times in sterile distilled water.

(c) Rinsed 3 times in sterile distilled water. All roots were planted, two per dish on water agar. Water agar was used to keep bacterial growth to a minimum. Plates were incubated at 25°C and were examined after 24 and 48 hours for colonies of Pythium which were removed as they became visible. The percentage of roots giving P.irregulare in each treatment is shown in Table 5.

Washing in tap water for 18 hours as an aid in isolating Pythium was evaluated using the root systems of four 6 week old barley plants grown in field soil in pots in the glasshouse. At 6 weeks the roots had

Table 5 : The percentage of barley roots yielding P.irregulare after treatment with sodium hypochlorite or silver nitrate solutions.

TREATMENT	Roots yielding P.irregulare (per cent)
5 per cent hypochlorite for 3 minutes	25
0.1 per cent silver nitrate for 3 minutes then 10 minutes in saturated NaCl solution	0
3 minutes in sterile distilled water (controls)	100

been colonized by other fungi. On a large proportion of primary roots, lesions covered most of the root surface. Pieces, 1 cm long, were cut from the root systems and divided at random into two batches of 120 each. One hundred pieces from the first batch were washed for three minutes in changes of sterile water, blotted dry with filter paper and placed 5 per petri dish on water agar. The remainder were used to prepare squash slides for microscopic examination.

The second batch was placed in an upright filter funnel, covered with fine nylon gauze to prevent loss of pieces, and washed in a steady stream of tap water. After 18 hours washing, 100 root pieces were rinsed in sterile water, blotted dry with filter paper and placed on water agar. Plates were examined after 24 and 48 hours for Pythium colonies which were transferred to corn-meal agar for identification. The remaining 20 pieces from the batch were examined microscopically.

Bacterial development on the agar around the root pieces was much reduced by washing, but there was no noticeable difference in the growth of other fungi. The percentage of root pieces giving rise to colonies of Pythium irregulare is shown in Table 6.

Microscopic examination of squashed roots previously washed in running tap water for 18 hours, showed that all had fungal spores on the root surface or embedded in the tissue. Ten also contained spores which appeared to be oogonia or sporangia of P.irregulare. Of these root pieces seven contained aseptate hyphae similar in appearance to those of Pythium, the hyphal tips were filled with cytoplasm suggesting that they had been recently formed. Although oogonia and sporangia were present in

Table 6 : The percentage of root pieces yielding P.irregulare after washing for 18 hours in running tap water.

TREATMENT	Root pieces yielding P.irregulare (per cent)
Washed in tap water 18 hours	50
Washed in sterile water 3 minutes (control)	7

8 root pieces from the control treatment, cytoplasm filled hyphae were not seen. Suspending roots in water for an extended period thus seemed to stimulate germination of Pythium spores and growth of the fungus within and on tissue, which subsequently enabled it to grow into the agar ahead of other organisms.

That development of bacteria was reduced around washed root pieces on agar may be another reason why increased numbers of P.irregulare were isolated.

Treatment of roots with silver nitrate or sodium hypochlorite reduced the number of Pythium irregulare isolations to such an extent that the use of these chemicals as surface sterilizing agents was precluded. Washing for 18 hours increased the number of Pythium isolations. Although most hyphae were devoid of contents, the adhesion of these and other particles indicated that some Pythium colonies might arise from propagules on the root surface. Harley and Waid (1955) and Stenton (1958) have also reported the adhesion of soil particles and fungal propagules to roots after washing.

In the absence of a suitable means of sterilizing the external surface of roots, extended washing was selected as being the best method of removing many surface contaminants and at the same time of ensuring subsequent isolation of Pythium.

C.4 Isolation of Pythium from the root systems of barley seedlings taken from the field

Isolations were made from 10 root systems of 3 to 5 week old barley plants. The same number of barley seedlings showing symptoms of decline

or unthriftiness were collected from all three barley fields under survey. Each plant was lifted with as little disturbance as possible to the roots and transported to the laboratory in covered cans. Root systems could be removed from the cans without much loss of roots if the bulk of soil enclosing the roots was first saturated with tap water and the soil agitated with a jet of water from a flexible tube as the root system was lifted clear. Washing in fast flowing tap water for 18 hours was done in a large glass tank covered with a wire mesh lid. Samples from each root system showing discoloration or lesions were examined carefully in sterile water in a Petri dish and any adhering soil particles were removed with a stiff brush and cut into small pieces, 1 cm long, after being rinsed twice in sterile water. After removing surplus water, roots were planted one per Petri dish and covered with water agar at 45°C. Using the dissecting microscope, plates were examined after 18 and 42 hours for colonies of Pythium from which hyphal tips were transferred to corn-meal agar for subsequent identification.

C.5 Sporulation and identification of Pythium isolates

Isolates of Pythium species were grown at 25°C on cornmeal agar, and microscopic examination for the presence of oogonia was made at 5 to 7 days.

As the development of sporangia usually requires water to induce their formation, a small amount of a culture on cornmeal agar was placed in a Petri dish in a shallow layer of water to which a 1-2 cm piece of grass leaf was added. The water used consisted of one part of sterilized water and one part of distilled water. The grass leaf was boiled for 10 min. Changing the water favoured the production of sporangia and

discharge of zoospores. After a few hours to a number of days the Pythium colonized the grass leaf and developed zoosporangia along its margin, (Emerson, 1958 ; Webster and Denis, 1967). Measurements were made on slides in lactophenol-cotton-blue. Species were identified according to Middleton (1943) ; the criteria used were, diameter of hyphae size and shape of sporangia, size and shape of oogonia and oospores, and the morphology and point of origin of antheridia. All estimates of size were derived from a total of fifty measurements.

C.6 pH

pH of soil was measured using a pH meter. Ten grams of soil were placed in a 50 ml plastic container and 25 ml of 0.01 M $CaCl_2$ solution were added, stirred several times for about 10 minutes, and allowed to stand for 1 hour before pH was measured by immersing the glass electrode of the pH meter. Two measurements were made per sample taken from the field.

C.7 Soil texture

Soil texture was measured by using the hydrometer method to determine the proportion of clay, silt and sand. Fifty g of air-dried soil were put into 200 ml of distilled water, 20 ml of 10% Calgon solution and 3 ml of normal NaOH. The soil suspension was agitated vigorously for 20 minutes in an aluminium container, washed into a sedimentation cylinder and the volume made up to 1 l with distilled water. Measurements with the hydrometer were made at 5 minutes and 5 hours respectively after sedimentation began. The value at 5 hours gave the percentage of clay particles, then the percentage of silt and sand

particles was calculated. Corrections were made for temperature during the measurements.

D. Results and Discussions

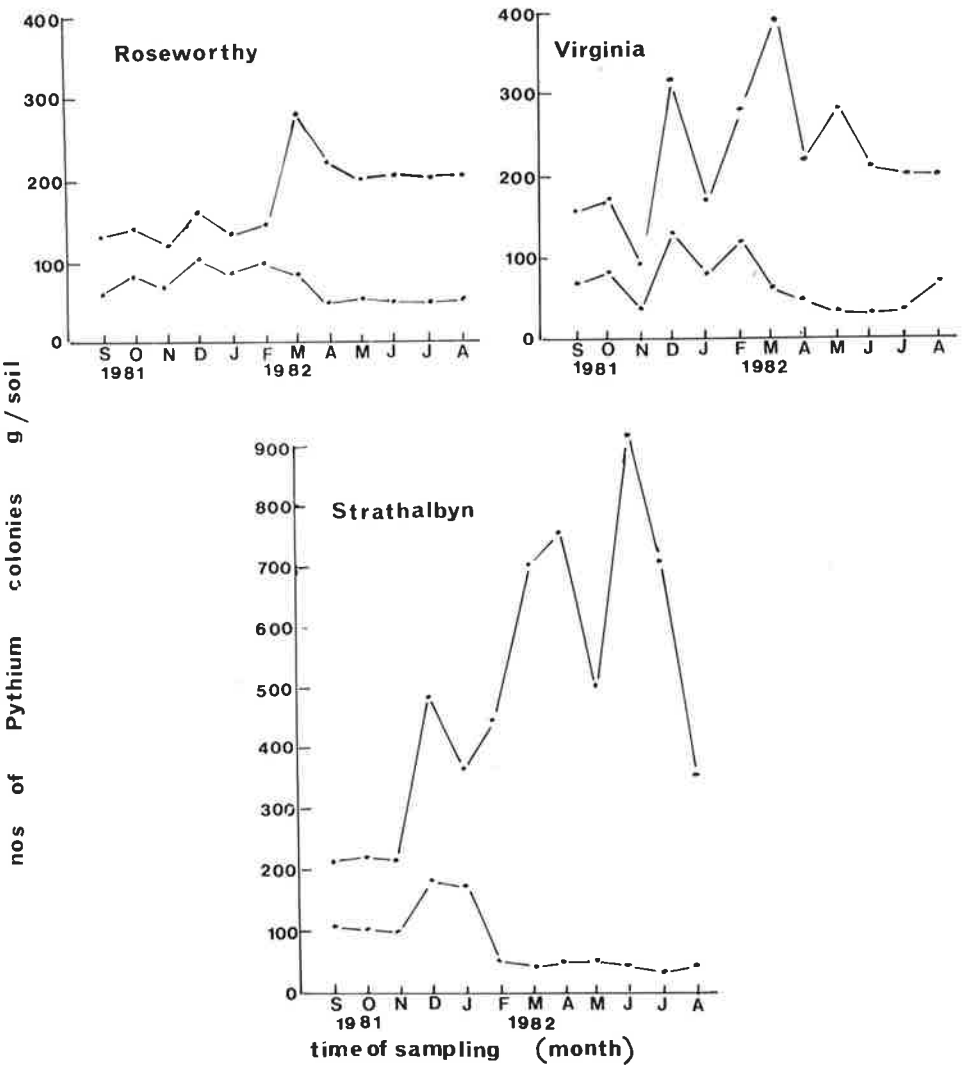
D.1 Soil pH and Soil texture

The analysis of soil pH and soil texture carried out in the laboratory, indicated that all three barley-field soils presented almost the same alkaline reaction, and the soil texture was a sandy loam at Strathalbyn and a loamy sand at Virginia and Roseworthy (Table 3).

D.2.1 Seasonal variation of Pythium populations

Soil samples, taken at two depths from barley sites from September 1981 to August 1982 were processed to determine population levels of Pythium species. The sites were chosen for their different climates. Virginia and Roseworthy have a lower mean annual rainfall and higher temperatures than the more southerly site at Strathalbyn (Table 2). As indicatd in Figure 4 populations in the top layer of soil 1 to 10 cm deep were consistently higher than those at 10 to 20 cm. Populations were also higher at Strathalbyn than at Roseworthy or Virginia. These observations suggest that Pythium probably has its greatest inhibitory effect on the growth of barley at germintion and emergence when roots of seedlings are growing through the upper soil layers that contain the highest densities of fungus. The results also suggest that the degree of inhibition of plant growth might be associated with the cooler and

Figure 4 : Population densities of Pythium at three sites, Roseworthy and Virginia in the north and Strathalbyn in the south of the barley growing region of South Australia. Populations were assessed monthly at two soil depths, 0-10 cm (upper graph) and 10-20 cm (lower graph). Letters on the obscissa denote the first letter of the month.


Roseworthy
Virginia
Strathalbyn
nos of Pythium colonies g / soil
time of sampling (month)
S O N D J F M A M J J A
1981
1982
0
100
200
300
400
800
900

Pythium populations

Table 7 : The influence of time (monthly samples from September 1981 to August 1982) temperature and rainfall at two depths in the soil a three sites Virginia, Strathalbyn and Roseworthy. The asterisk indicates significance at $p < 0.05$.

	VIRGINIA		STRATHALBYN		ROSEWORTHY	
	0–10 cm	10–20 cm	0–10 cm	10–20 cm	0–10 cm	10–20 cm
Time	N.S.	N.S.	*	*	*	*
Temperature	N.S.	*	N.S.	N.S.	*	*
Rainfall	N.S.	N.S.	N.S.	N.S.	*	N.S.

wetter conditions at Strathalbyn where populations are higher. Regression analysis of population levels of Pythium against time indicated that at Roseworthy and Strathalbyn populations increased from September 1981 to August 1982 in the 0 to 10 cm soil zone but decreased at 10 - 20 cm. No statistically significant regressions with time were evident at Virginia. Superimposed on such long term seasonal trends were monthly fluctuations. To try and account for such variations, regression analyses of population levels of Pythium against mean monthly temperature, and mean monthly rainfall at each site were done. There were no statistically significant regressions at Strathalbyn. At Virginia population levels in the 10 to 20 cm zone increased with temperature whereas at Roseworthy, population levels at 0 to 10 cm decreased with increasing temperature and rainfall. In the 10-20 cm layer populations increased with temperature as at Virginia. The results are summarised in Table 7.

D.2.2 Conclusions

1. Population levels are higher at Strathalbyn than at Virginia or Roseworthy.

2. Population levels are higher in 0 - 10 cm layer than at 10 - 20 cm at all sites.

3. There is no consistent picture for all three sites. In two sites there were trends in population change with time ; in two sites temperature had a significant influence on populations whereas rainfall only affected Pythium populations at one site and then at only one depth.

4. Barley was sown in all sites in April 1982 and harvested in November 1982. However, there were no major population changes during that time, hence, it is possible that the crop has little influence on populations as assessed by the soil plate method.

5. Higher levels of populations at Strathalbyn may be associated with lower temperature and higher rainfall there.

6. It is possible that recordings of soil water and soil temperature rather than meteorological data are required.

D.2.3 **Discusison**

Pythium species are evidently common inhabitants of barley soils in South Australia. A seasonal variation was found in the Pythium populations of barley soils over 12 months. Results of the monthly assay suggest that the activity of Pythium in soil was directly influenced by temperature in two sites, whereas rainfall affected Pythium populations at one site. Pythium populations were high during the winter and spring (low temperature) and low during summer (high temperature). Robertson (1973) in New Zealand found high populations of Pythium during winter to early spring when temperature was low and soil moisture content high. Hancock (1977) found that higher Pythium populations in California soils occurred during the autumn, winter and spring seasons, and lowest population levels invariably occurred during summer.

As all three barley soil sites have a similar pH and soil texture, differences in Pythium population between them probably can be ascribed to differences in climate.

The field survey raises questions that need to be answered by experiments:

1. Pythium has higher population levels at Strathalbyn where temperatures are lower and rainfall is higher than at the two other sites. To what extent does soil water influence Pythium? How does temperature influence the growth of Pythium?

2. The apparent absence of a relationship between population levels of Pythium and the age of barley crop was unexpected and raises the question whether plant root weight influences population density.

3. What is the effect of such factors as soil water and temperature on the pathogenicity of Pythium is barley?

4. What is the optimum pH of soil for mycelial growth of Pythium?

D.3.1 Occurrence of Pythium species on barley soils and diseased barley roots

(a) Barley soils

The soil samples taken at 0 - 10 cm depths from barley sites from September 1981 to August 1982, processed to determine the population level were also processed to determine the identity of Pythium species. The species of Pythium recovered and their relative abundance in the samples assayed are shown in Table 8.

Table 8 : The occurrence of *Pythium* species on barley soils

	% of *Pythium* in soil 0–10 cm		
	STRATHALBYN	VIRGINIA	ROSEWORTHY
P. irregulare	32.0	29.0	29.41
P. rostratum	16.0	14.33	16.85
P. debaryanum	9.16	13.08	13.66
P. oligandrum	10.0	9.83	9.25
P. echinulatum	9.58	7.08	7.83
P. volutum	3.66	5.66	5.33
P. acanthicum	5.0	6.83	6.08
P. paroecandrum	5.41	5.50	4.83
P. iwayamai	6.0	4.25	3.41
P. vexans	2.25	3.66	3.16

Pythium irregulare was the most commonly recovered species from all three sites under survey, 32% of the isolates at Strathalbyn, 29% at Virginia and 29.41% at Roseworthy. Pythium rostratum was the next commonly recovered species, followed by Pythium oligandrum, and Pythium echinulatum. Of the 10 species of Pythium isolated from barley soil samples (0 - 10 cm depth) 9 species had already been recorded in South Australia. Pythium volutum, not previously recorded in Australia, was isolated from all barley soil samples. (Table 8)

(b) **Diseased barley seedlings**

From 30 samples of 3-5 week-old barley plants from all three barley fields under survey, 103 populations of Pythium spp. were isolated. The percentage of plants with diseased roots yielding Pythium is shown in Table 9.

D.3.2 Discussion

The amount of soil per plate (10 mg) was a suitable compromise for counting both rare and common Pythium species at once. Increasing the amount would not greatly increase the records of rare species because most of their colonies would be masked by the common and fast growing species such as P. irregulare. The true numbers of Pythium propagules in soil were probably higher than shown in Figure 4 because two colonies occurring by chance close to each other, might be counted as one.

Table 9 : Survey of the *Pythium* species isolated from barley diseased roots

Pythium spp.	% of plants with diseased roots yielding Pythium
P. irregulare	38.7
P. graminicolum	21.7
P. debaryanum	18.1
P. volutum	7.5
P. paroecandrum	6.9
P. iwayamai	6.3
P. vexans	0.6
P. echinulatum	0.2
P. oligandrum	0
P. rostratum	0
P. achanticum	0

The result of outstanding importance is the generally high density of Pythium population regardless of the type soil, pH, history or amendments. A seedling can hardly avoid contact with Pythium propagules if every gram of surface soil contains more than 300 of them, many of them being P. irregulare, P. debaryanum, P. volutum which are pathogenic to barley seedlings. There is obviously a lower and an upper limit outside which the abundance of pathogen must strongly affect disease. Vaartaja, Cram and Morgan (1961) found that in experimental damping-off of conifers, the disease incidence largely depended on environmental factors if the inoculum was light ; if inoculum was heavy environment made little difference and even unusual pathogens were capable of causing high disease incidence. As seen from Table 8 and from the data of Warcup (1952) and Schmitthenner (1962) the proportion of different Pythium species probably varies between soils more than does the total incidence. However, the species found most commonly in soil, (P. irregulare, P. rostratum, P. oligandrum), appear largely the same in different parts of the world (Meredith, 1940 ; Middleton, 1943 ; Warcup, 1952 ; Schmittenner, 1962). P. iwayamai (Table 8) may have a restricted distribution (Middleton, 1943) whereas P. echinulatum may be more common in South Australia (Table 8) than elsewhere (Middleton, 1943).

CHAPTER 3

TAXONOMY OF THE GENUS PYTHIUM, DESCRIPTION AND IDENTIFICATION OF PYTHIUM SPECIES ISOLATED FROM BARLEY SOILS AND FROM INFECTED BARLEY ROOTS IN SOUTH AUSTRALIA

The genus Pythium has been treated systematically at least five times since its establishment in 1858. Pringsheim (1858) in his paper "DIE SAPROLEGNIEEN" created the genus Pythium which he included in the family SAPROLEGNIACEAE. Pythium monospermum and P. entophytum were the first described species. With the subsequent transfer of P. entophytum to Lagendium entophytum by Zopf (1890), P. monospermum remained as the lectotype species. The type species P. monospermum was described as a saprophyte on dead insects in water.

Cornu (1872) included Pythium in his treatise on the Saprolegniaceae published in 1872. In 1881 de Bary published a series of articles dealing with the genera Artrotrogus, Peronospora, Phytophthora and Pythium. He pointed out the similarity of the genera, indicated their probable inter-relationships and concluded that they should be placed in the family Peronosporaceae. Soon after, Berlese and de Toni in Saccardo's Sylloge Fungorum (1888) again placed this genus in the family Saprolegniaceae. Schroter (1897) placed the genus Pythium in a newly created family, the Pythiaceae, assigned to the order Saprolegniales. The subgenus Nematosporangium was elevated to generic rank and included in the Pythiaceae. He designated Nematosporangium as having filamentous sporangia and Pythium as having spherical or limoniform sporangia. Two subgenera, Aphragmium, Fischer and Eunematosporangium (Nematosporangium Fischer) were included in the newly elevated genus. Schroter also presented two new subgenera for his genus Pythium: Eupythium, including

species with smooth-walled oogonia, and Artotrogus, including species with echinulate-walled oogonia.

Butler (1907) in his monograph on Pythium followed Fischer's treatment in retaining the various species within this single genus. Butler considered the genus Nematosporangium of doubtful validity, grouping all species possessing filamentous sporongia within the genus Aphragmium. He did not accept Fischer's Nematosporangium as a distinct genus but included all the species under Pythium. His paper contains a long discussion of the relationships of Pythium to the other groups of fungi. Following this discussion there is a key with descriptions of all the species which had been described up to that time, and six plates illustrating nine different species. In 1930 Sideris (1930) proposed to revive the taxonomic plan proposed by Schroter. Sparrow (1931) published a criticism of Sideris' paper. He proposed either that Pythium spp. possessing spherical or limoniform sporangia and all Phytophthora spp. be placed in Sphaerosporangium, elevating it to generic rank, or that Pythium spp. be placed in Phytophthora.

Matthews (1931) and Middleton (1943) did not formally distinguish intrageneric taxa, although they used the same distinguishing criteria as the previous authors in their keys and arrangements. The following descriptions of species of Pythium found in South Australia are based on Middleton's keys. The taxonomic terms are Middleton's, the measurements are my own.

Pythium acanthicum (Drechsler)

Hyphae measuring 1.3 to 5.4 µm average 3.5 µm in diameter. Sporangia typically intercalary and occasionally terminal, then subspherical measuring 11.7–43.1 µm average 28.9 µm in diameter. Zoospores formed at 20°C biciliate, reniform measuring 8–9.5 µm in diameter. Oogonia terminal or intercalary, subspherical (Plate 1Aa) with an echinulate wall measuring 13.2 to 30 µm average 23.5 µm in diameter excluding the spines which are 1.3–5 µm average 2.7 µm in length and average 1.7 µm in basal diameter. Antheridia typically monoclinous (Plate 1Ab) 1–2 per oogonium, borne terminally on branches 10–15 µm long. The antheridial cell inflated clavate, oospores plerotic average 21.5 µm in diameter with moderately thickened wall, containing a single reserve globule and refringent body.

Pythium debaryanum (Hesse)

Hyphae 4.3–5.4 µm, average 4.7 µm in diameter, branched, septate in old cultures. Sporangia spherical, terminal or intercalary, 14.5 to 26 µm, average 18.7 µm in diameter. Oogonia spherical, smooth, (Plate 1B arrows) terminal or intercalary, 15.3 to 27.7 µm, average 21 µm in diameter. Antheridia 1–5 per oogonium, monoclinous and diclinous, when monoclinous arising some distance below the oogonium, not adjacent to it. Oospores smooth, aplerotic 11.9 to 19.8 µm, average 16.9 µm, in diameter, wall smooth, thickness 1.7 µm.

The dimensions given here agree with those of Middleton (1943).

Pythium echinulatum (Matthews)

Hyphae measuring 2 to 8 µm in diameter. Sporangia spherical to cylindrical terminal or intercalary 10 to 30 µm average about 20 µm in diameter. Oogonia spherical to cylindrical, terminal (Plate 1C arrow) or intercalary measuring 14 to 30 µm average about 24 µm, in diameter exclusive of the many spines 2 to 8 µm in length. Antheridia monoclinous typically hypogynal, 1 to 4 per oogonium, Oospores aplerotic, 14 to 24 µm, average about 20 µm in diameter, 1 to 2 per oogonium, enclosing a single reserve globule and refringent body. The identification was confirmed by the Commonwealth Mycological Institute No. 268536.

Pythium graminicolum (Subramaniam)

Hyphae measuring 2.5-6.7 µm in diameter average 5 µm, irregularly branched, appressoria irregular. Sporangia inflated filamentous (Plate 3C). Zoospores formed at 20°C, encysted zoospores 13-45 in number, 8-11 µm in diameter. Oogonia spherical, thin walled terminal, (Plates 3A,B) intercalary, measuring 17.3 to 36.0 µm mean 25.0 µm in diameter. Antheridia are of monoclinous origin, antheridial stalks originating at various distances from the oogonium, 1 to 5 per oogonium. Oospores plerotic, single, smooth 15.0 to 34.3 µm mean 23.0 µm in diameter.

The species is close to Pythium arrhenomanes and P. aristosporum but characteristic features, such as fewer antheridia per oogonium and smaller oogonia make P. graminicolum different from the other two. Confirmed by Commonwealth Mycological Institute No. 279307.

Pythium irregulare (Buisman)

Hyphae measuring 2.5–7.9 µm, average 5.1 µm in diameter. Sporangia of various shapes, mostly spherical to obovate, (Plate 2Da) terminal or intercalary measuring 10 to 26.5 µm average 18.0 µm in diameter. Zoospores seldom produced, about 8 µm in diameter upon encystement. Oogonia spherical to limoniform , measuring 9.5–28.2 µm average 17.8 µm in diameter, usually intercalary (Plate 2B) though also terminal, of irregular contour, fairly smooth or undulent to definitely irregularly eclinulate, (Plate 2Aa) the spines varying in shape but usually of broad base and acuminate apex. The ratio of ornamentated to smooth oogonia was 60% ornamented oogonia. Antheridia typically monoclinous 1–2 per oogonium. (Plate 2C) Oospores aplerotic, measuring 8.0–25.3 µm average 15.8 µm in diameter, wall 1.3 µm thick and containing a single reserve globule and refringent body. Confirmed by Commonwealth Mycological Institute No. 285717, 285720, 285713.

Pythium iwayamai (S. Ito)

Hyphae branched, measuring 2.7–8.5 µm average 6.6 µm in diameter. Sporangia spherical (Plate 1D) to prolate elipsoidal, ovoid or limoniform, thin-walled, 28–45 x 42 µm. Encysted zoospores 8–14 µm diameter. Hyphae swellings intercalary. Oogonia terminal or intercalary, globose, smooth, (Plate 1Ea) 21–27 µm, average 26 µm diameter. Antheridia single (Plate 1Eb) or occasionally 2 or 3 per oogonium, mono - or diclinous. Oospores plerotic or aplerotic, 19–23 µm, average 22 µm, diameter.

Pythium oligandrum (Drechsler)

Hyphae measuring 1.7-6.5 µm average 3.9 µm in diameter. Sporangia acragenous typically intercalary, subspherical, measuring 25-44.7 µm, average 33.3 µm in diameter, usually consisting of 1-4 subspherical bodies together with a contiguous portion of a filament of varying length, sometimes the filament irregularly swollen or branched. This kind of sporangium does not occur in other known species with ornamented oogonia. Oogonia subspherical, echinulate, (Plate 4C) terminal or subterminal, also laterally or tangentially intercalary 17-35 µm average 25.4 µm in diameter exclusive of the spines which are conical and acutely tipped 3-7 µm average 3.8 µm long and 1.5-3.5 µm average 2.3µm in basal diameter Antheridia lacking in many instances. Oospores aplerotic single 14-33 µm average 23.1 µm in diameter with a wall approximately 1.3 µm. Confirmed by the Commonwealth Mycological Institute No. 285718.

Pythium paroecandrum (Drechsler)

Hyphae measuring 2.5-9 µm, average 5.3 µm in diameter, appressoria often present, apices curved and clavate, measuring 8-11 µm wide. Sporangia subspherical to ellipsoidal, typically intercalary, when spherical measuring 12-33 µm, average 22.7 µm in diameter, zoospores 3-27 µm, biciliate, reniform, measuring 9 to 11 µm upon encystment. Oogonia subspherical, (Plate 1Fa) typically intercalary, measuring 11-28 µm average 20.9 µm in diameter, smooth and thin walled. Antheridia mono and diclinous 1-2 (5) per oogonium, (Plate 1F arrows) when monoclinous often undifferentiated and adjacent to oogonium. Oospores aplerotic, with a moderately thin wall, 1.1 to 1.4 µm, average 1.3 µm thick, containing a

single reserve globule, and refringent body, oospores measuring 10-21 µm average 18.3 µm in diameter. Confirmed by Commonwealth Mycological Institute No. 285719.

Pythium rostratum (Butler)

Hyphae large measuring 6.5-8 µm, average 7.3 µm in diameter. Sporangia are spherical to oval, terminal or intercalary, measuring 23.9-34 µm average 27.8 µm in diameter, appearing before sexual reproductive bodies; zoospores formed at 25°C, dicharge tubes about 5 µm wide. Oogonia spherical to subspherical, smooth, typically intercalary, (Plate 2Ea) measuring 13 to 29.0 µm average 21 µm in diamter. Antheridia typically monoclinous, 1-2 per oogonium mostly sessile and arising immediately below the oogonium or hypoginous. (Plate 2E) Oospores plerotic, single, wall about 2 µm thick. Characteristic of this species are the intercalary oogonia and monoclinous, sessile or hypogynous antheridia. P. rostratum was easily distinguished from P. pulchrum and P. ultimum by its plerotic oospores. P. hypogynum is distinguished from P. rostratum by the mainly hypogenous antheridia and mostly terminal oogonia.

Pythium ultimum (Trwo)

Hyphae 2.8-6.9 µm, average 3.8 µm, in diameter, branched, septate only in old cultures. Sporangia spherical, usually terminal, 8.6-27.2 µm, average 18.8 µm in diameter, wall thickness 0.7 µm. Oogonia smooth, spherical, (Plate 4Aa) terminal, occasionally intercalary 12.4-22.0 µm, average 20.8 µm in diameter. Antheridia, one per ogonium, monoclinous, occasionally diclinous. Oospores aplerotic, single, spherical, 12.2-18.4

µm average 16.4 in diameter, with the smooth, thick wall, containing a single central reverse globule and refringent body. The measurements agree well with those of Middleton. The predominance of monoclinous, sharply curved, and swollen antheridia which, according to Middleton, is the chief characteristic that serves to distinguish P.ultimum from P.debaryanum. Confirmed by the Commonwealth Mycological Institute No. 285716.

Pythium vexans (de Bary)

Mycelium fine, branched, up to 5 µm wide. Sporangia terminal or intercalary, pyriform, ovoid, 17.3-23.7 µm average 21 µm in diameter, usually germinating by germ tubes. Oogonia spherical, smooth, (Plate 4Ba), terminal on short lateral branches from 15.0 to 27.5 µm average 21.7 µm in diameter. Antheridia 1 per oogonium, monoclinous arising below the oogonium or from the main hyphae. Antheridial cells large typically bell-shaped. Oospores aplerotic, smooth measuring 11-23 µm average 18.1 µm in diameter, wall 1.2 µm thick.

The monoclinous bell-shaped antheridia easily distinghish P. vexans from other species with spherical sporangia and aplerotic oospores.

Pythium volutum (Vanterpool and Truscott)

Pythium volutum has not been recorded in Australia before, isolates of this species have been obtained from Virginia, Strathalbyn and Mortlock Experiment Station soils, and from diseased roots of barley. The three strains from these respective regions showed no cultural differences, and were included in the same species.

Mycelium non-septate, lustrous, with an aerial tendency in culture, radial growth on cornmeal agar of approximately 10.0 mm in 24 hr at 25°C. Optimum temperature for growth is 25°C. Appressoria present, consisting of lateral falcate structures with rounded ends.

Sporangia inflated filamentous with small lobes, or toruloid outgrowths. Discharge tubes about 50 x 3-4 µm. Encysted zoospores biflagellate about 10 to 14 µm in diameter. Oogonia smooth, subspherical, dark brown terminal on short side stalks, (Plate 5a) formed copiously in culture, but a large percentage remain sterile, measuring 19.2 to 40.0 µm average 3C µm in diameter. Antheridia typically diclinous, (Plate 5b) rarely monoclinous, producing 3 to 10 antheridia per oogonium, the stalks entwining the oogonial stalk (Plate 5c). A single stalk producing 1-4 antheridial cells which are straight or crook-necked, clavate, making narrow apical contact with the oogonium. Oospores aplerotic, smooth spherical to oblong on average diameter 27.5 µm, oospores wall 2.0 µm containing 1 reserve globule and a single refringent body. This species may be distinguished from others on the antheridial character alone. The antheridial branch is usually diclinous in origin and is helicoidally disposed about the oogonial stalk, usually only a single antheridial branch coils about the supporting branch. P. volutum is a moderately low growing species. According to Middleton (1943) the macroscopic aspect of mycelial development of P. volutum resembles that of many strains of P aerhenomanes, though P. volutum is quite distinct morphologically from P. arrhenomanes. The difference between the two species is further borne out by their temperature growth responses as reported by Vanterpool (1938). According to Van der Plaats-Niterink (1981), among the species

with filamentous inflated sporangia, only two other described species, P. helicum and P. zingiberis, show similar characters. These species have about equally large oogonia and oospores. They may differ in that P. helicum has mainly monoclinous antheridia and P. volutum and P. zingiberis have mainly diclinous antheridia. The description of P. zingiberis is somewhat confusing, and does not agree with the figures. P. helicum and P. zingiberis are possibly synonymous with P. volutum. Confirmed by Commonwealth Mycological Institute No. 279308.

PLATE 1

A. Pythium achanticum. Oogonium (a) with short monoclinous antheridium (b).
Scale bar = 16 µm

B. Pythium debaryanum. Oogonia (arrows).
Scale bar = 20 µm.

C. Pythium echinulatum. Oogonium (arrow).
Scale bar = 21 µm.

D. Pythium iwayamai. Spherical sporangia.
Scale bar = 14 µm.

E. Pythium iwayamai. Slightly aplerotic oogonium (a) and antheridium (b).
Scale bar = 15 µm.

F. Pythium paroecandrum. Oogonium (a) and antheridia (arrows).
Scale bar = 14 µm.

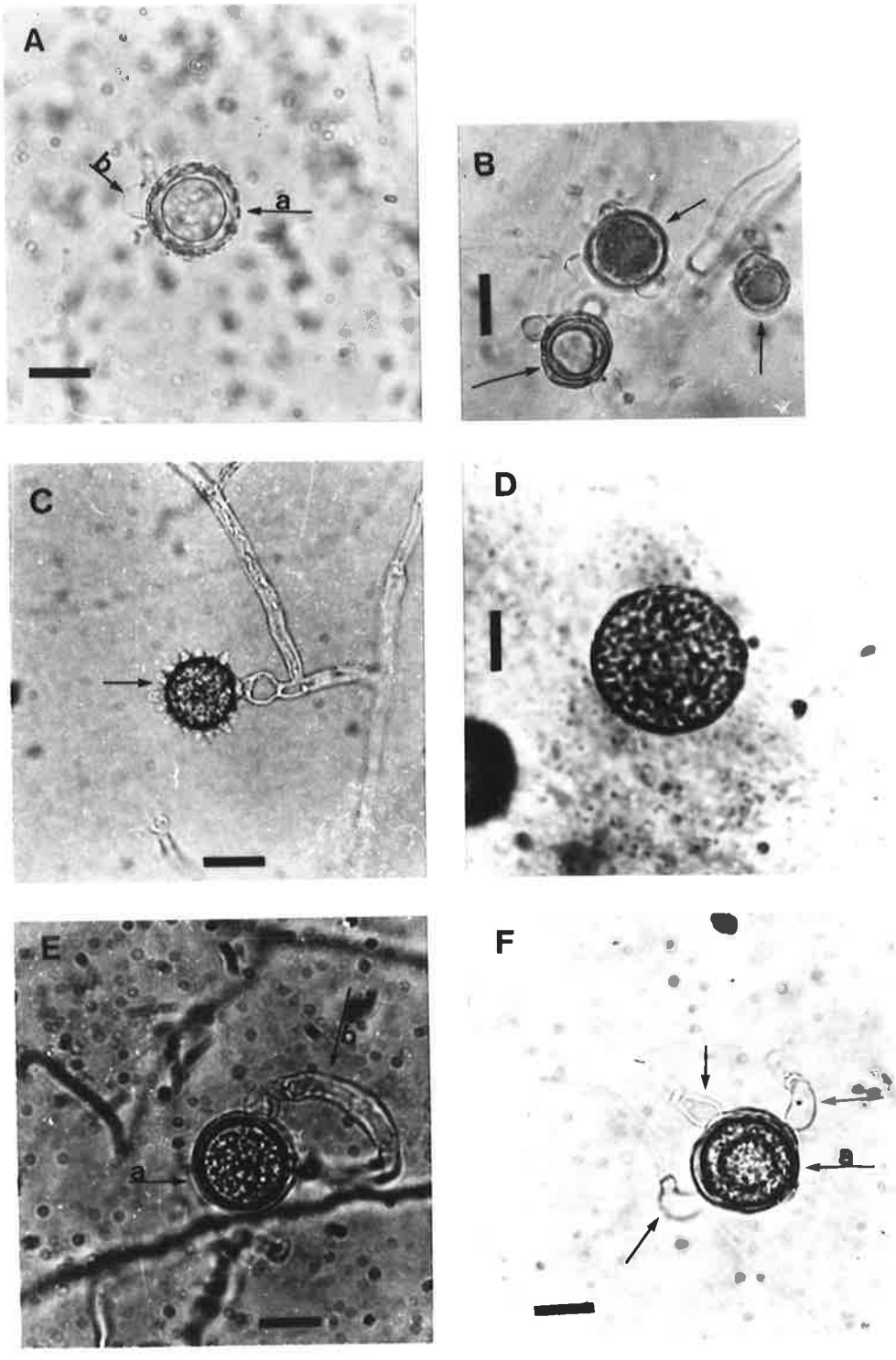


PLATE 1

PLATE 2

Pythium irregulare

A. Typical spiny oogonium (a) with monoclinous antheridium (b).

B. Typical intercalary oogonium (a).

C. Oogonium (a) with falcate antheridium (b).
Scale bar for A, B, C = 15 µm.

D. Germinating sporangium.
Scale bar = 10 µm.

Pythium rostratum

E. Oogonium (a) and hypogenous antheridium.
Scale bar = 13 µm.

F. Vesicle (a) tube (b) and sporangium (c).
Scale bar = 15 µm.

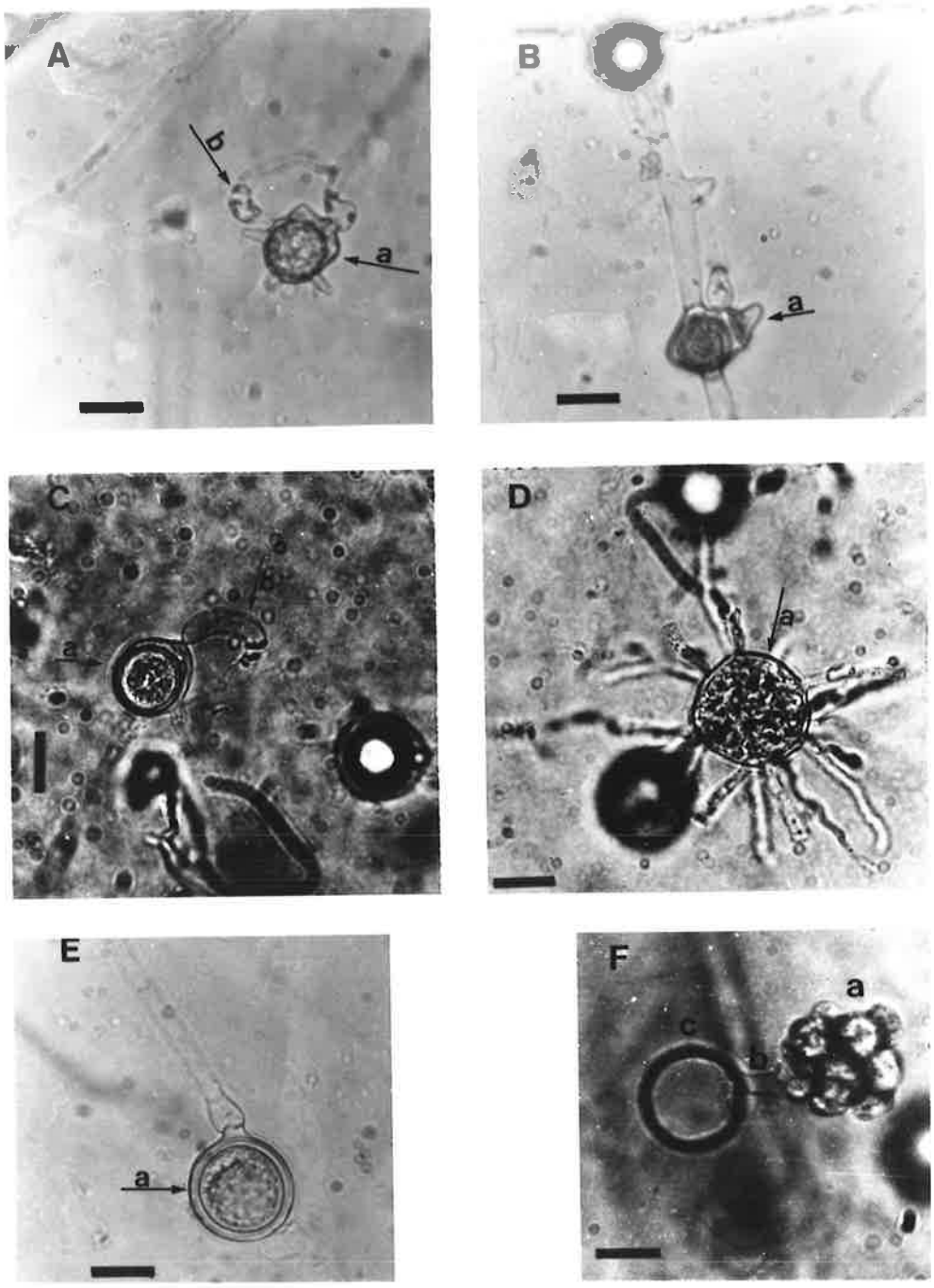


PLATE 2

PLATE 3

Pythium graminicolum

A,B. Diagram showing terminal, smooth oogonia (a) antheridia with antheridial cells crook-necked clavate (c).
Scale bar = 5.77 µm.

C. Inflated sporangia.

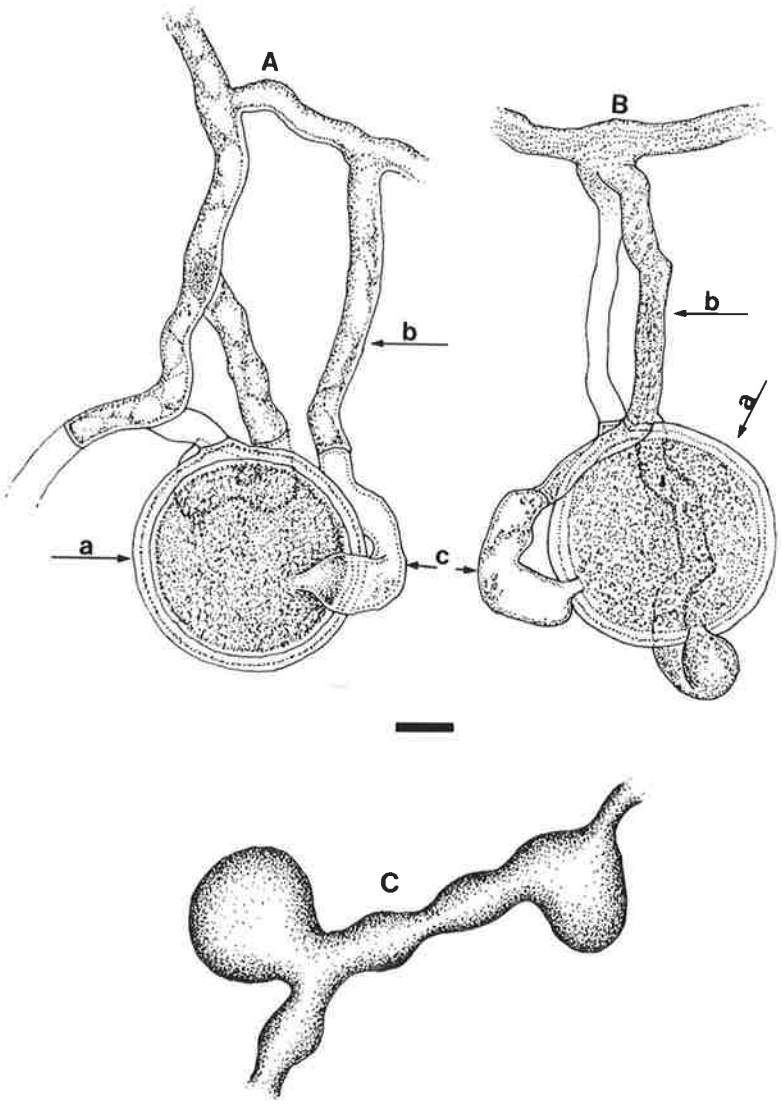


PLATE 3

PLATE 4

A. *Pythium ultimum*. Oogonium (a).
Scale bar = 11 µm.

B. *Pythium vexans*. Oogonium (a).
Scale bar = 14 µm.

C. *Pythium oligandrum* (a). Echinulate oogonium.
Scale bar = 15 µm.

D. *Pythium oligandrum*. Sporangium.
Scale bar = 17 µm.

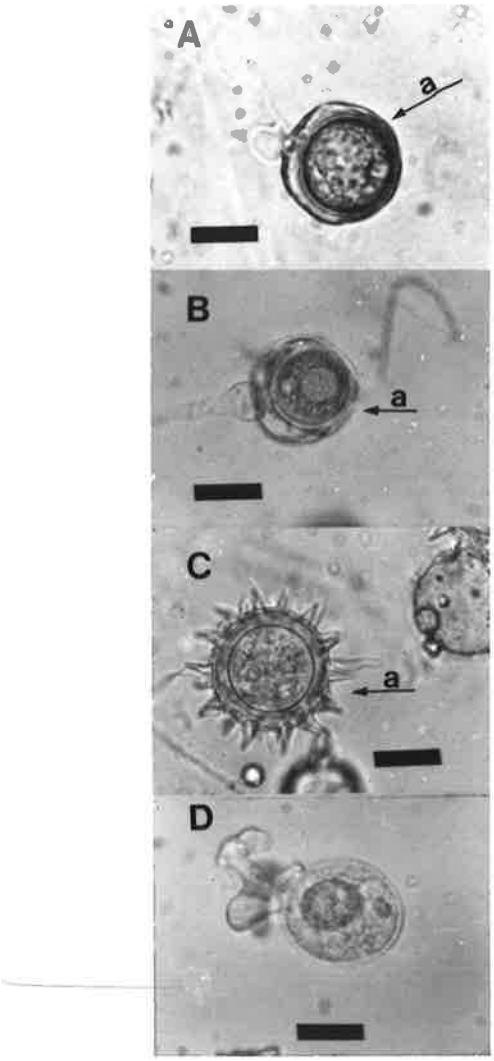


PLATE 4

PLATE 5

Pythium volutum

Terminal, smooth oogonium (a), diclinous antheridia (b), the antheridial stalks entwining the oogonial stalk (c).
Scale bar = 29 µm.

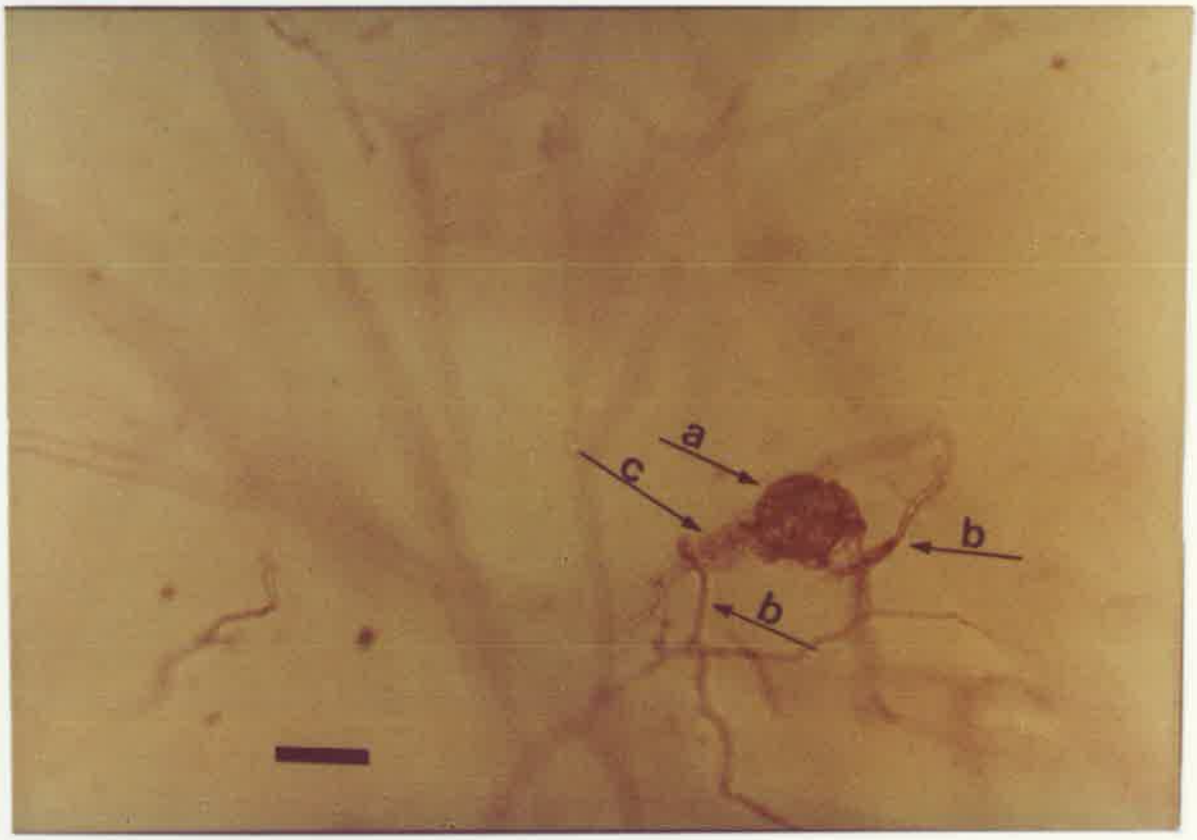


PLATE 5

CHAPTER 4

THE INFLUENCE OF ENVIRONMENTAL FACTORS INFLUENCING *PYTHIUM* SPECIES ON BARLEY

A. INTRODUCTION

In recent years the importance of the role played by environmental factors in controlling the activities of soil microorganisms has been widely realized. In the field of plant pathology an example is provided by studies on the influence of soil temperature and pH on the incidence of damping-off disease of seedlings (Roth and Riker, 1943 ; Leach, 1947 ; Wright, 1957 ; Griffin, 1958). In the zoological literature a number of papers deal with the effect of the soil regime on soil organisms (Parry, 1954 ; Wallace, 1954, 1955a,b, 1956a,b,c, 1958a,b,c, 1959,a,b ; Collis-George and Blake, 1959). An understanding of the importance of soil water is available in the standard text-books and various papers (Childs and Collis-George, 1950a ; Richards and Wadleigh, 1952 ; Baver, 1956 ; Black, 1957 ; Marshall, 1959 ; Russell, 1961 ; Griffin, 1963, 1969, 1972, 1977 ; Cook and Papendick, 1970a, 1972, 1978 ; Duniway, 1973, 1976b, 1979 ; Cook, 1973, 1980 ; Papendick and Campbell 1975 ; Schoeneweiss, 1975 ; Baker and Cook, 1974 ; Kozlowski, 1978).

Little research has been done on the influence of environmental factors of *Pythium* in cereals in Australia. The results of my field survey (Chapter 2) raise questions concerning the influence of environmental factors on *Pythium* spp. that might be answered by experiments.

Barley is grown in the southern part of South Australia where rainfall is higher and temperatures are lower than in the arid north (Figure 1). Within the barley growing area annual rainfall increases and temperature decreases from north to south. Such climatic differences plus differences in soil pH are likely to influence the soil environment, particularly the level of soil water and so, in turn, influence the abundance of Pythium and the amount of disease it causes in the barley crop. The aims of the following experiments described in this chapter are to determine:

1. the relationship between soil water and different species of Pythium on the growth of barley,
2. the relationship between soil water and inoculum density of P. irregulare on the growth of barley,
3. the relationship between pH of the medium and growth rate and reproduction of Pythium,
4. to determine the influence of temperature on the rate of growth of Pythium spp. and on root disease in barley seedlings.

B. THE RELATIONSHIP BETWEEN SOIL WATER AND NINE SPECIES OF PYTHIUM ON THE GROWTH OF BARLEY

B.1. Materials and Methods

The Pythium spp. used in the pot experiments and their origins are as follows: P. acantichum, P. echinulatum, P. oligandrum, P. rostratum, P. paroecandrum were isolated from barley soil, and P. debaryanum, P. graminicolum, P. irregulare, P. volutum were isolated from diseased roots of barley seedlings. Pythium spp. were grown separately on Difco cornmeal agar at 25°C for 48 h. Two plugs of medium were taken from the

margin of the growing cultures and inoculated into sand cornmeal culture medium (5 g cornmeal, 95 g sand, 15 ml sterile distilled water). Inoculation was achieved by mixing 4-week old sand cornmeal medium of each Pythium species with potting soil at a rate of 1% w/w prior to sowing barley seed. All barley seed used originated from a single seed lot of the cultivar Clipper. Damaged seeds were removed and planting tests showed the seed to be free from fungal infection. Seeds were surface sterilized in 1% sodium hypochlorite-absolute alcohol 2:1 (v/v) then rinsed in sterile distilled water immediately prior to use. There were 3 seeds per pot. A sandy loam field soil was sterilized and 300 g were placed in each pot. Pots, 11 cm high and 8 cm in diameter allowed uninhibited root growth of seedlings. All experiments were done in a growth chamber at 15° ± 1°C with 12 hr light per day. Soil in pots was maintained at the required level of soil water 25, 50, 75, 90% field capacity, by daily weighing of pots. There were five replicates per treatment. Experiments lasted four weeks and at the conclusion seedlings were removed from soil, washed and measurements of total length and dry weight of shoots and roots were made. There was a close correlation between length and dry weight for both shoots and roots. Data were analysed by analysis of variance, regression and Duncan's multiple range test using appropriate transformation to normalize data.

The moisture characteristic curve of the soil used in experiments was determined as follows: three samples of the soil passed through a 2 mm sieve were moistened and placed on moist filter paper inside sintered glass funnels and suctions of -0.98 to -19.6 KPa (10 to 200 cm of water) were applied for two weeks before their soil moisture contents were estimated. Suctions of -100, -500 and -1500 KPa were obtained using a pressure chamber apparatus. Plastic conduits (2.5 cm long, 3.5 cm

internal diameter) were filled with the moist soil and placed on top of a high pressure ceramic plate in the chamber. Pressure was applied for 2 weeks using a compressed air cylinder. The moisture characteristic curve of the sandy loam soil used in the experiments is shown in Figure 5.

B.2. Results

The influence of nine species of Pythium and a control treatment with no Pythium on the dry weights and lengths of shoots and roots of barley seedlings indicated that some species were significantly ($p < 0.01$) different from others in their effect on the seedlings. There were close correlations between dry weights and lengths and between roots and shoot measurements. Hence, results in Figure 6 show only the influence of Pythium species on mean length of shoots. A statistically significant interaction ($p < 0.01$) was obtained between Pythium species and soil water on length of shoots, hence it is necessary to discuss relative pathogenicities of the nine Pythium species in terms of the response to soil water. The interaction is explained by the markedly high inhibition to plant growth caused by P. irregulare, P. volutum, and P. graminicolum at the higher levels of soil water. In the controls and most of the other Pythium species shoot growth increased as soil water increased. In practical terms it is possible that the most serious reductions in barley growth due to Pythium occur when soils are close to field capacity and where population densities of such species as P. irregulare are high.

Figure 5 : Moisture characteristic curve of experimental soil.

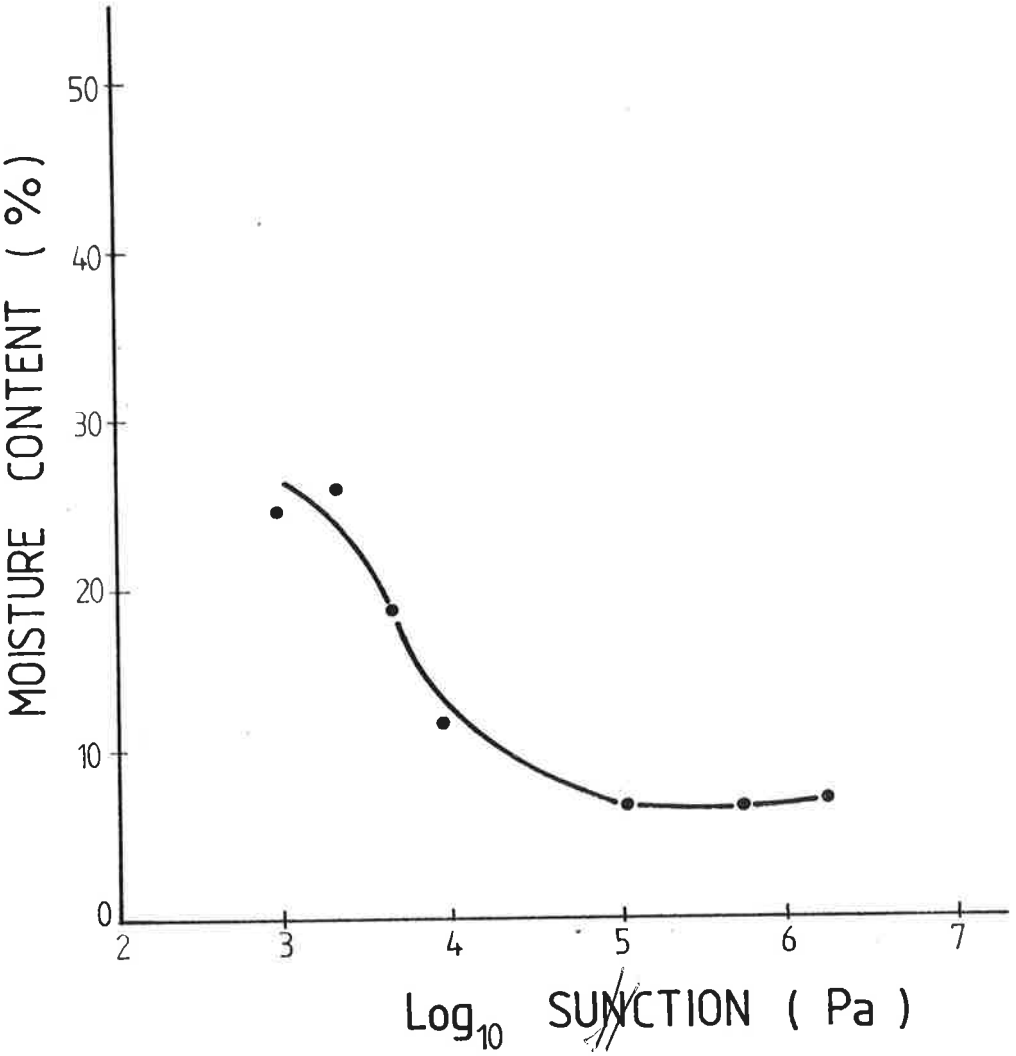
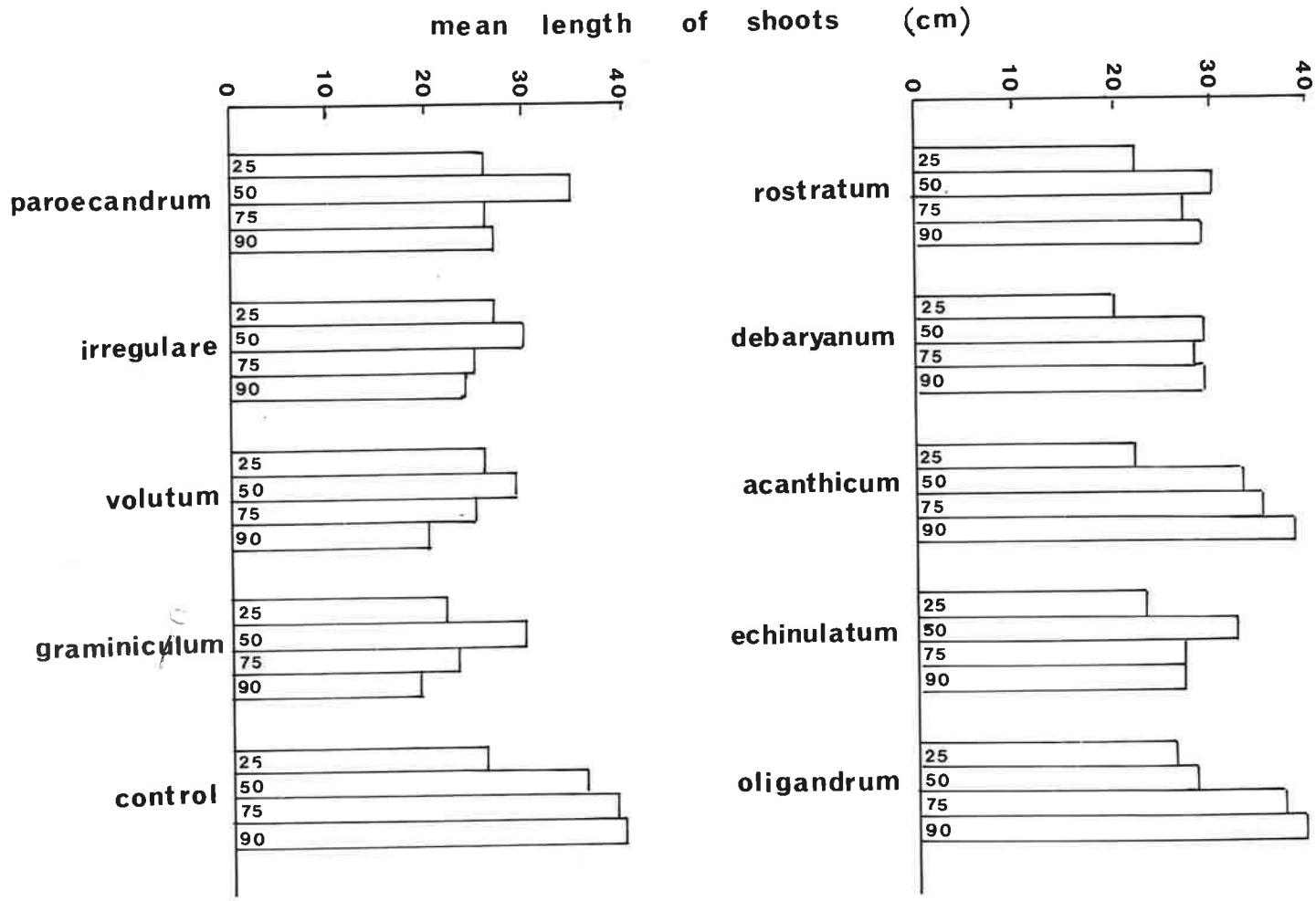

MOISTURE CONTENT (%)
50
40
30
20
10
0
2
3
4
5
6
7
Log10 SUNCTION (Pa)

Figure 6 : The influence of nine species of Pythium on the mean height of shoots of barley seedlings 4 weeks after sowing at four levels of soil water 25, 50, 75 and 90% field capacity. There were five replicates per treatment.


mean length of shoots (cm)
0
10
20
30
40
paroecandrum
irregulare
volutum
graminicylum
control
rostratum
debaryanum
acanthicum
echinulatum
oligandrum
25
50
75
90

B.3. Discussion

There is a high positive correlation between soil moisture and the incidence of disease on barley seedlings caused by P. irregulare, P. graminicolum and P. volutum. There are many references to the effect of changes in soil moisture content on the incidence of various diseases. Garrett (1938, 1939, 1944) concludes that diseases caused by phycomycete fungi are notably favoured by high soil moisture levels. Roth and Riker (1943) observed that damping-off of red pine seedlings caused by P. irregulare increased linearly as soil moisture was increased from 15% to 90% M.H.C. (moisture holding capacity). On the other hand Kerr (1964) showed that the importance of soil moisture is in its influence on the amount of sugar exuded from pea seeds, which determines disease incidence. My results may be interpreted as indicating an indirect effect of soil moisture ; that is, soil moisture may affect soil aeration, this in turn playing a major role in the development of physiological malfunction (disease) caused by Pythium. Pythium spp. as phycomycetes, are probably well adapted to a semi-aquatic environment, with resultant low oxygen tensions. Also, since the results indicated that normal root growth of barley plants (control) was not affected to any great extent by soil moisture over the range employed, the influence of soil moisture upon disease development, may be more closely associated with the pathogens than the host. The preceeding results demonstrate that soil moisture has a significant effect upon the development of barley root rot, and that damage by Pythium can probably be greatly reduced by manipulation of soil moisture.

C. THE RELATIONSHIP BETWEEN SOIL WATER AND INOCULUM DENSITY OF *P. IRREGULARE* ON THE GROWTH OF BARLEY

C.1. Materials and Methods

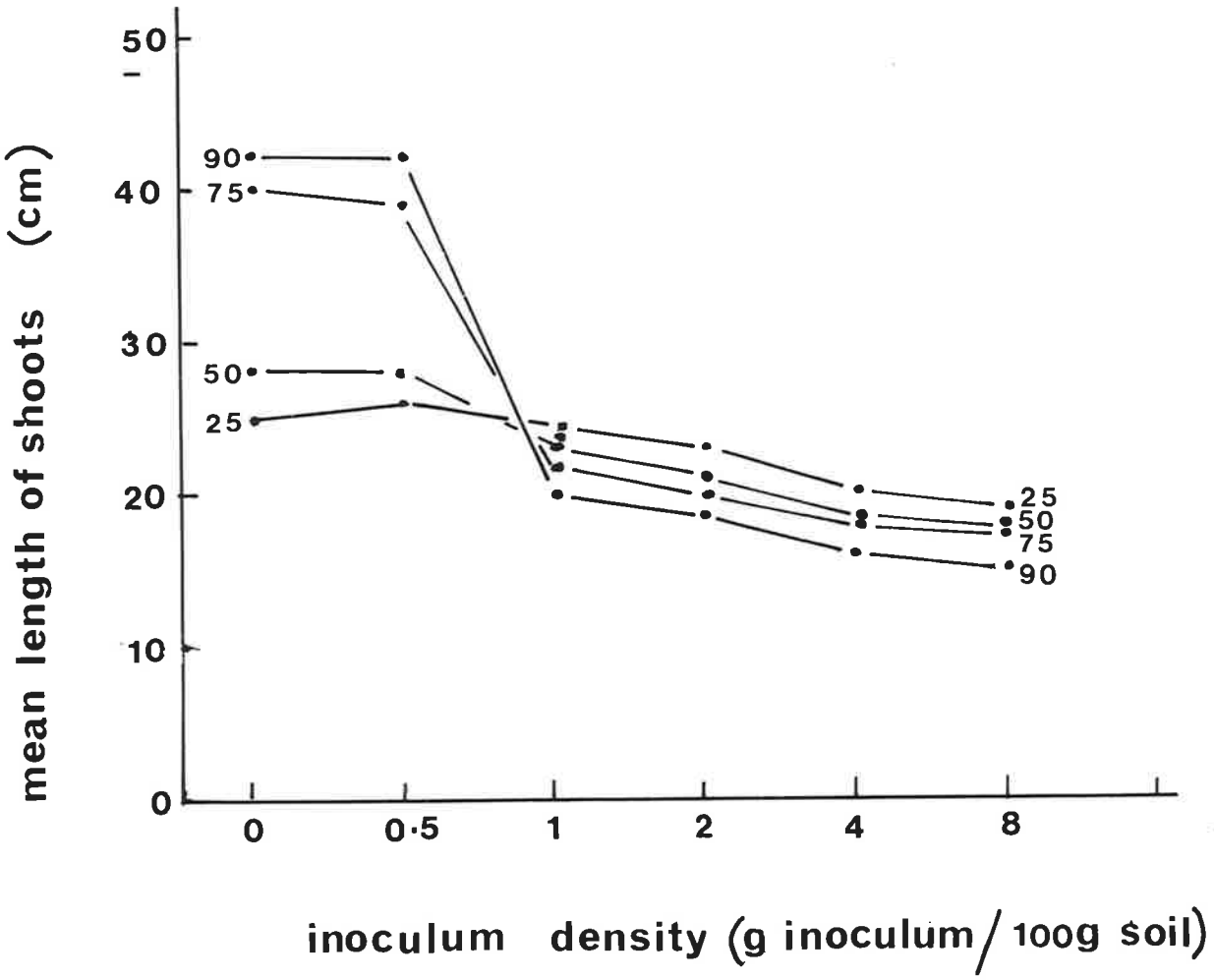
Six inoculum levels (0, 0.5, 1, 2, 4, 8 g/100 g of soil) of *P. irregulare* similar to those found under field conditions, and four levels of soil water (25, 50, 75, 90% field capacity) with five-fold replication were used in a factorial experiment. The material and method used were similar to those described in Section B.1.

C.2. Results

Length and dry weight of shoots and roots were measured. As lengths and weights of roots and shoots were closely correlated only the results for shoot length are given (Figure 7). Analysis of variance indicated that for all measurements of shoots and roots, soil water and inoculum density had significant ($p < 0.01$) effects. There was also a significant interaction ($p < 0.01$) between soil water and inoculum density because *P. irregulare* appears to be particularly inhibitory at certain levels of soil water. A Duncan's multiple range test was used to help interpret the data and the following conclusions were drawn :

1. As inoculum density increased barley growth decreased.
2. As soil water increased from 25 to 90% of field capacity, barley growth decreased except in the uninoculated controls and the lowest inoculum level of 0.5 g of inoculum/100 g soil where growth markedly increased. This different response at low and high inoculum levels was responsible for the interaction. This result

Figure 7 : The influence of inoculum density of Pythium irregulare on the mean height of shoots of barley seedlings 4 weeks after sowing, at four levels of soil water 25, 50, 75 and 90% field capacity. There were five replicates per treatment. Numerals at the ends of the graphs denote % field capacity.


mean length of shoots (cm)
50
40
30
20
10
0
90
75
50
25
25
50
75
90
0
0·5
1
2
4
8
inoculum density (g inoculum/100g soil)

supports the hypothesis that at least for P. irregulare inhibition in growth of barley seedlings is associated with high population levels of the fungus and soils approaching field capacity. The biological explanation of the interaction is open to several interpretations which only further studies can elucidate. Thus high levels of soil water may reduce the resistance and tolerance of the plant to attack by P. irregulare, or infection by the fungus may reduce the tolerance of the plant to high soil water levels and consequent lack of aeration or soil water may influence the infectivity of P. irregulare through mobility of its zoospores if they are produced.

C. THE RELATIONSHIP BETWEEN pH OF THE MEDIUM AND GROWTH RATE OF PYTHIUM

The experiments were designed to test (a) linear growth and (b) weight of growth of mycelium of Pythium irregulare on substrates of varying pH.

D.1. Linear growth of P. irregulare on different pH substrates

D.1.a. Materials and Methods

A graduated pH series of Difco cornmeal agar plates was prepared. There were eleven treatments : pH 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 with four replicates for each treatment. The pH of the medium was adjusted by adding citric acid and/or potassium hydroxide. A disc cut with a No. 1 cork-borer of growing mycelium of P. irregulare from a three days Difco cornmeal agar culture was inoculated at the centre of each 9 cm Petri dish containing 10 ml of the Difco cornmeal agar medium,

and incubated at 25°C for 24 h. Linear growth of the mycelium was measured along four radii of each replicate. A randomized complete block design was used.

D.1.b. Results

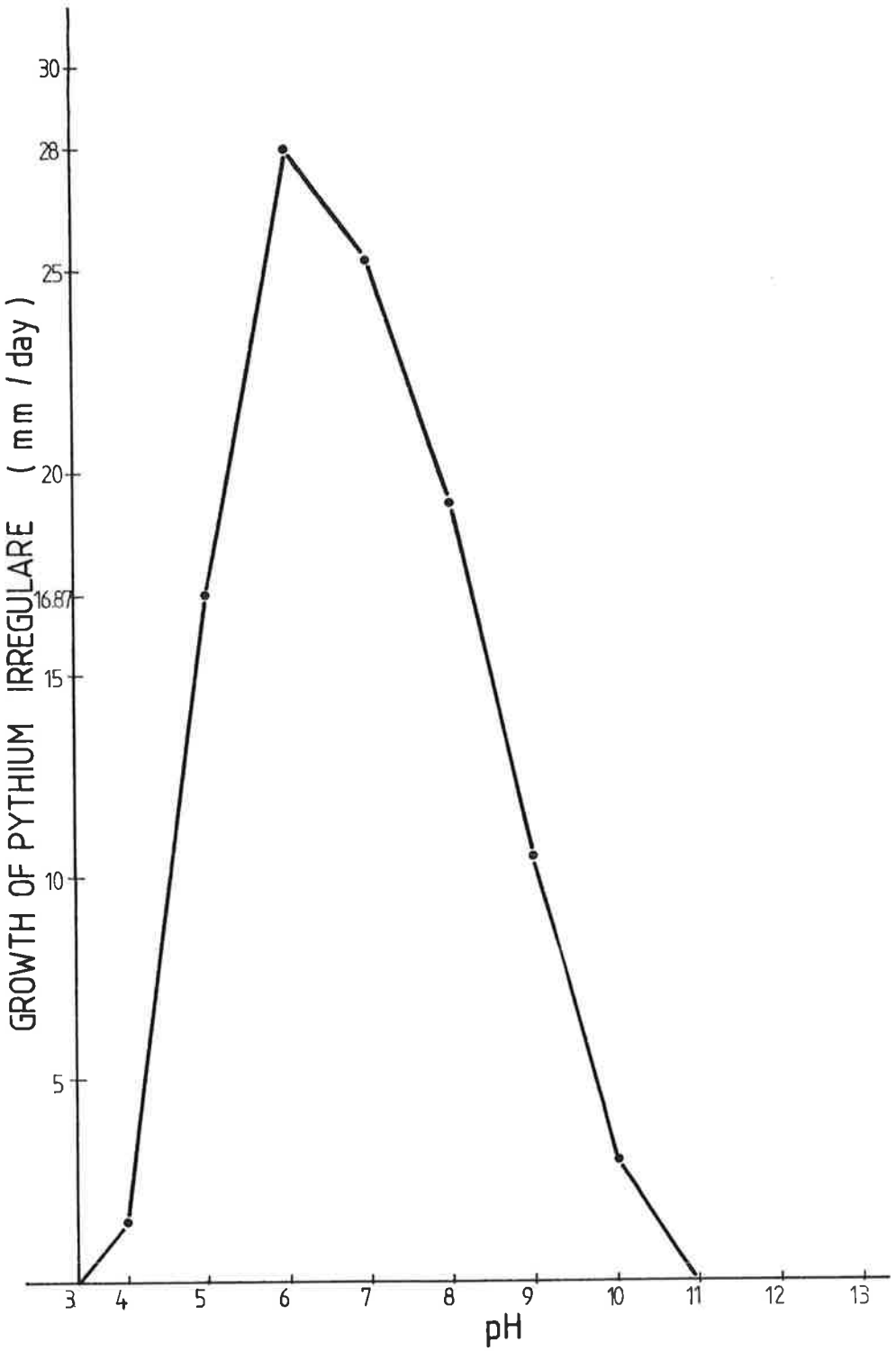
The relationship between pH of the culture medium and linear growth rate of P. irregulare (mean value of 4 replicates) is shown in Figure 8. The maximum, optimum and minimum pH values for growth are shown to be 11.0, 6.0 and 4.0. The analysis of variance showed that there are highly significant differences ($p = 0.01$) between linear growth of mycelium at different pH of the medium.

D.2. The growth of P. irregulare in different pH liquid cultures as determined by weight of mycelium

D.2.a. Materials and Methods

The liquid culture used was cornmeal 50 g/l distilled water. A graduated pH series of the liquid culture was prepared. There were eleven treatments as described in Section D.1.a., and four replicates. 75 ml of each solution were placed in 250 ml Erlenmeyer flasks, autoclaved, inoculated with two discs, cut with a No. 1 cork-borer, of growing mycelium of P. irregulare from a three days Difco cornmeal agar, and incubated at 25°C for two weeks. After two weeks the mycelium was harvested by filtering mycelium through Whatman No. 2 filter paper dried at 65°C and weighed. The final pH of the medium was measured.

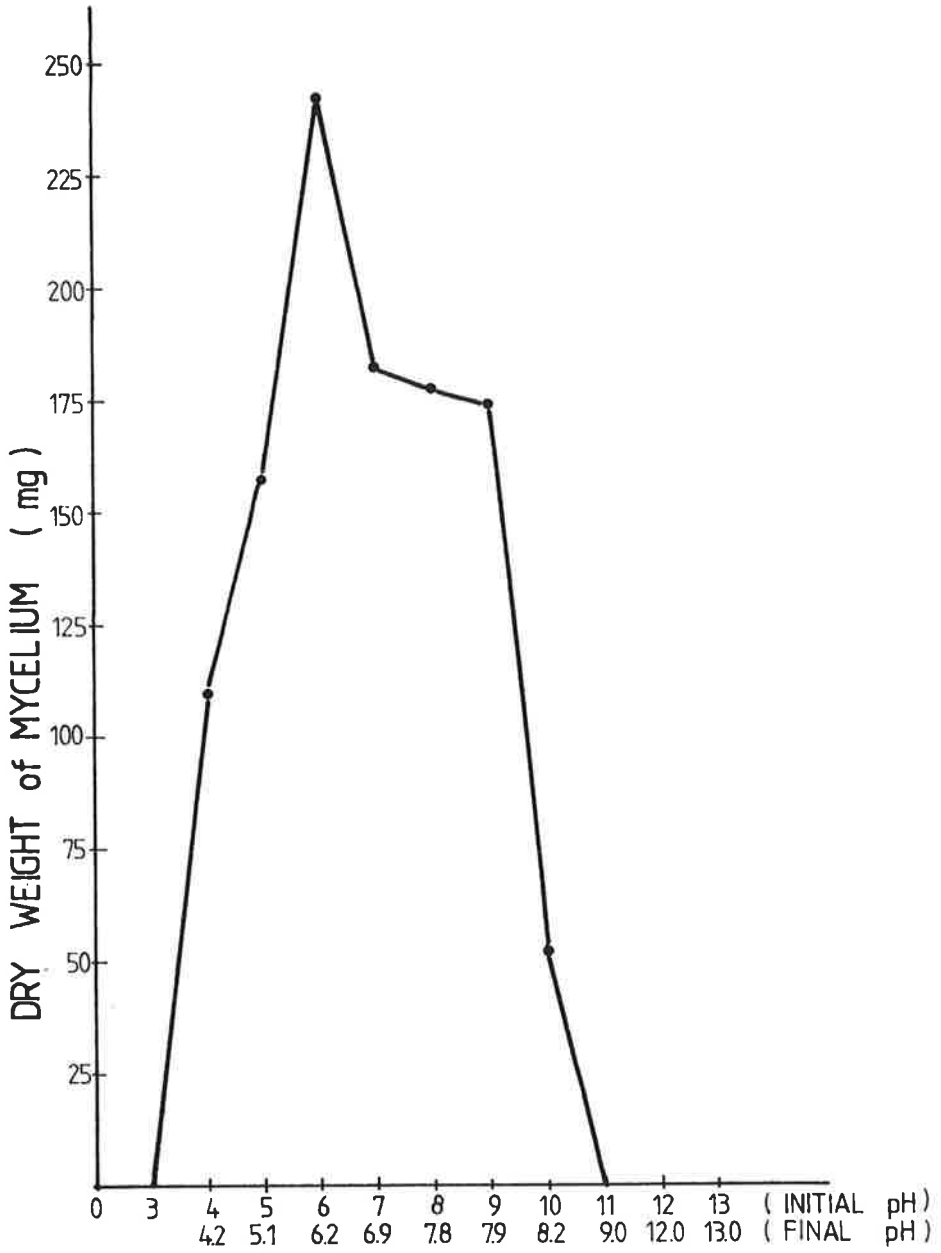
Figure 8 : Relation between pH of the medium and linear growth of Pythium irregulare. Mean values of four replicates L.S.D. = 2.1, p = 0.01.


GROWTH OF PYTHIUM IRREGULARE (mm / day)
30
28
25
20
16.87
15
10
5
3
4
5
6
7
8
9
10
11
12
13
pH

D.2.b. Results

The relationship between pH of medium and weight of mycelial growth of P. irregulare is shown in Figure 9. The optimum pH value for mycelial growth is 6.00 and the minimum is 4.0 (initial pH). There were significant differences ($p = 0.01$) between weight of mycelial growth at the different pH's of the medium. Over the range pH 4.0 - 9.0 the growth rate (linear and weight) of the fungus was high. A good correlation was found between radial growth on Difco cornmeal and weight of mycelial growth in liquid culture at pH 6.00. The data show that P. irregulare can grow (linearly and in weight) at relatively high and low extremes. It is likely that the mechanism whereby pH influences Pythium, differs according to the concentration of hydrogen ions. Because the pH of the medium is probably affected during growth by metabolic activities, a final reading of pH of the medium is required. It is likely that the fungus raised the pH of the medium which was initially unfavourable, by absorption of anions or production of ammonia from nitrogenous compounds and on the other hand lowered the pH of the medium which initially was high by formation of organic acids or absorption of cations, (Cochrane, 1958). The results of the experiments confirm the work of Roth (1935), Roth and Riker (1943), Carrera (1951) and Griffin (1958) that Pythium spp. grow over a wide range of pH with excellent growth from pH 4.5 - 7.0.

Figure 9 : Relation between pH of the medium and dry weight of Pythium irregulare mycelium after two weeks incubation at 25°C. Mean values of four replicates L.S.D. = 6.19, p = 0.01


DRY WEIGHT of MYCELIUM (mg)
250
225
200
175
150
125
100
75
50
25
0
3
4
4.2
5
5.1
6
6.2
7
6.9
8
7.8
9
7.9
10
8.2
11
9.0
12
12.0
13
13.0
(INITIAL pH)
(FINAL pH)

E. Production of oogonia and sporangia of *P. irregulare* at different pH levels of media

E.1. Materials and Methods

The material and method used were as described in Section D.1.a. There were eight pH treatments : 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0, and four replicates. After ten days incubation, the number of oogonia and sporangia within the microscopic field were counted at random in each plate using an ocular micrometer grid.

E.2. Results

The mean values of the number of oogonia or sporangia per cm^2 are given in Table 10. The analysis of variance showed a significant difference ($p = 0.01$) between numbers of oogonia or sporangia formed at different pH of the medium. A pH of about 9.0 appears to be optimum for the formation of sporangia. Also, there appears to be an inverse relationship between oogonia and sporangia formation i.e. as sporangia increase, oogonia decrease. It is possible that sporangia inhibit oogonia, thus oogonia have two optima at high and low pHs when sporangia are inhibited. This may indicate that sporangia are more sensitive to extremes of pH than oogonia. A regression between the two variable was made to see whether sporangial production is inversely related to oogonial production (Figure 10). However, the fact that the correlation between sporangial and oogonial production is insignificant throws doubt on this idea and suggests that further experiments are required.

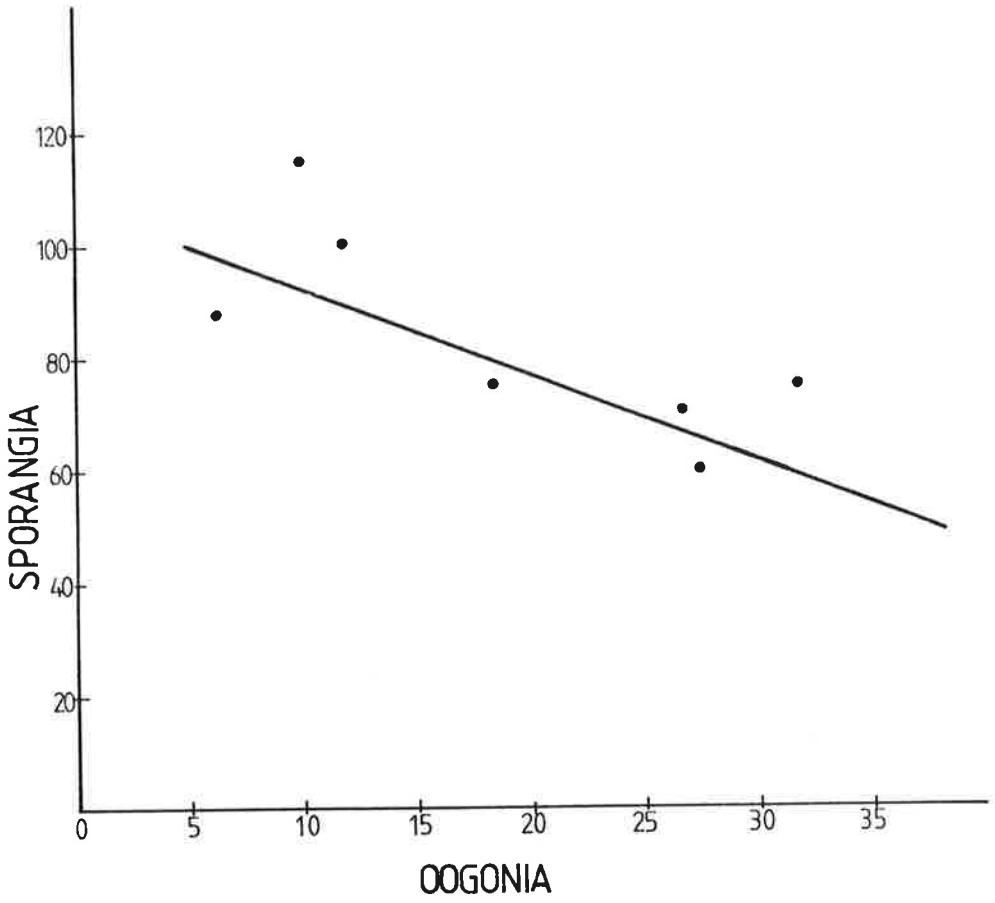
Table 10 : Influence of pH on the formation of oogonia and sporangia in Pythium irregulare grown on Difco cornmeal agar for ten days at 25°C.

pH of medium	*Number of sporangia/ cm^2	*Number of oogonia/ cm^2
4.0	60	28
5.0	72	27
6.0	75	18
7.0	100	12
8.0	88	6
9.0	116	10
10.0	76	33
11.0	0	0

LSD = 5.11 p = 0.01 LSD = 7.22 p = 0.01

* Mean value of four replicates.

Figure 10 : Regression of number of sporangia and oogonia grown in different pH medium. r = 0.47, p = n.s.


120
100
80
60
40
20
SPORANGIA
0
5
10
15
20
25
30
35
OOGONIA

F. INFLUENCE OF TEMPERATURE ON MYCELIAL GROWTH OF PYTHIUM SPECIES AND ON DISEASE SEVERITY

The purpose of this study was to evaluate the influence of temperature on mycelial growth of Pythium irregulare and to quantitatively, evaluate the severity of barley root disease induced by P. irregulare.

F.1. Materials and methods

Determination of the growth rate of the fungus

Some of the difficulties involved in measuring fungus growth rates have been reported by Fawcett (1921). The time factor is important but it is difficult to achieve comparable rates without selecting more or less arbitrary periods for the different temperatures. Measurements on Difco cornmeal agar were obtained by placing a 2 mm disk of agar from the periphery of a young fungus colony, in the centre of agar plates. At each temperature, radial growth was measured at intervals of 24 hours until the colony approached the edge of the 9 cm Petri dish and the average growth in mm per 24 hour period was calculated. There were five replicates per treatment. Near the optimum temperature, the maximum growth permitted by the dish was sometimes reached within 48 hours whereas at low temperatures several days were required. The growth rate of P. irregulare was also determined by dry weight yield in a liquid medium. 75 ml of cornmeal liquid medium were placed in 250 ml Erlenmeyer flasks, autoclaved, inoculated with 2 mm disk of agar from near the periphery of a young fungus colony. There were five replicates at each temperature. The length of the incubation period had to be adjusted according to the rapidity of growth. At the end of the incubation period

the fungus colony was separated from the medium in a filter and then washed, dried at 65°C and weighed. The growth rate was measured by the average weight of the dried colony per 24 hours after inoculation.

Pathogencity Test

Pythium irregulare was grown on Difco cornmeal agar at 25°C for 48 hours and then two discs of 2 mm each were cut from the margin of the growing culture and inoculated into sand cornmeal culture medium (Chapter 4.B.1.). After 4 weeks the inocula were incorporated into sterilized sandy loam potting soil (300 g soil per pot) at a rate of 1% w/w prior to sowing. Barley seed (cv. Clipper) used was previously surface sterilized (Chapter 4.B.1.). There were three barley seeds per pot (11 cm high x 8 cm diameter). Treatments consisted of barley seeds planted in sterilized, non infected soil (control), and barley seed planted in sterilized, artificially infested soil with P. irregulare. The experiment was done in growth chambers at 13°, 21°, 29°C, with 12 hours light per day. Soil in pots was maintained at a constant moisture of 75% field capacity by daily weighing of pots. There were five replicates for each treatment. After 4 weeks the measurements of length of roots of barley seedlings were made, by chopping the roots into about 1 cm pieces and using a Comair Root Length Scanner.

F.2. Results

1. Determination of fungus growth rates.

As Table 11 shows the optimum temperature for linear growth of P. irregulare appeared to be between 25°C and 30°C. This agrees with

Table 11 : Mycelial growth rates of Pythium irregulare on solid and liquid medium at different temperatures.*

Temperature °C	Solid medium	Liquid medium
	mm/24 hours	mg/24 hours
4	0.7	1.0
8	4.3	3.9
12	12.6	13.0
16	18.7	18.3
20	23.1	22.8
25	32.4	31.7
30	29.5	14.8
35	8.0	9.5
40	0	0

* Mean value of five replicates.

the results obtained by Middleton (1943) who found the optimum growth rate at 28°C. The growth rate of P. irregulare on solid and liquid medium was similar over the range 4 to 40°C, but growth at 30°C was less in the liquid medium. Most writers have shown that measurement of fungal mass is more reliable than linear growth.

2. Pathogenicity Test.

Pythium irregulare was recovered from roots and soil of the various infestation treatments. Final soil infestation level was 358 colonies of fungus/g dry soil measured by the soil plate method (Warcup, 1950). Root lengths of barley seedlings grown in P. irregulare infested soil were significantly reduced ($p = 0.01$) below those in uninfested soil (Figure 11). A significant interaction (($p = 0.01$) between the severity of infection induced by P. irregulare and temperature was found. Root lengths of seedlings infected with P. irregulare at 13°C were about 52% lower than uninfected seedlings grown in the same environemntal conditions, whereas at 21°C and 29°C root length of infected seedlings were only reduced by about 15% and 5% respectively.

F.3. Discussion

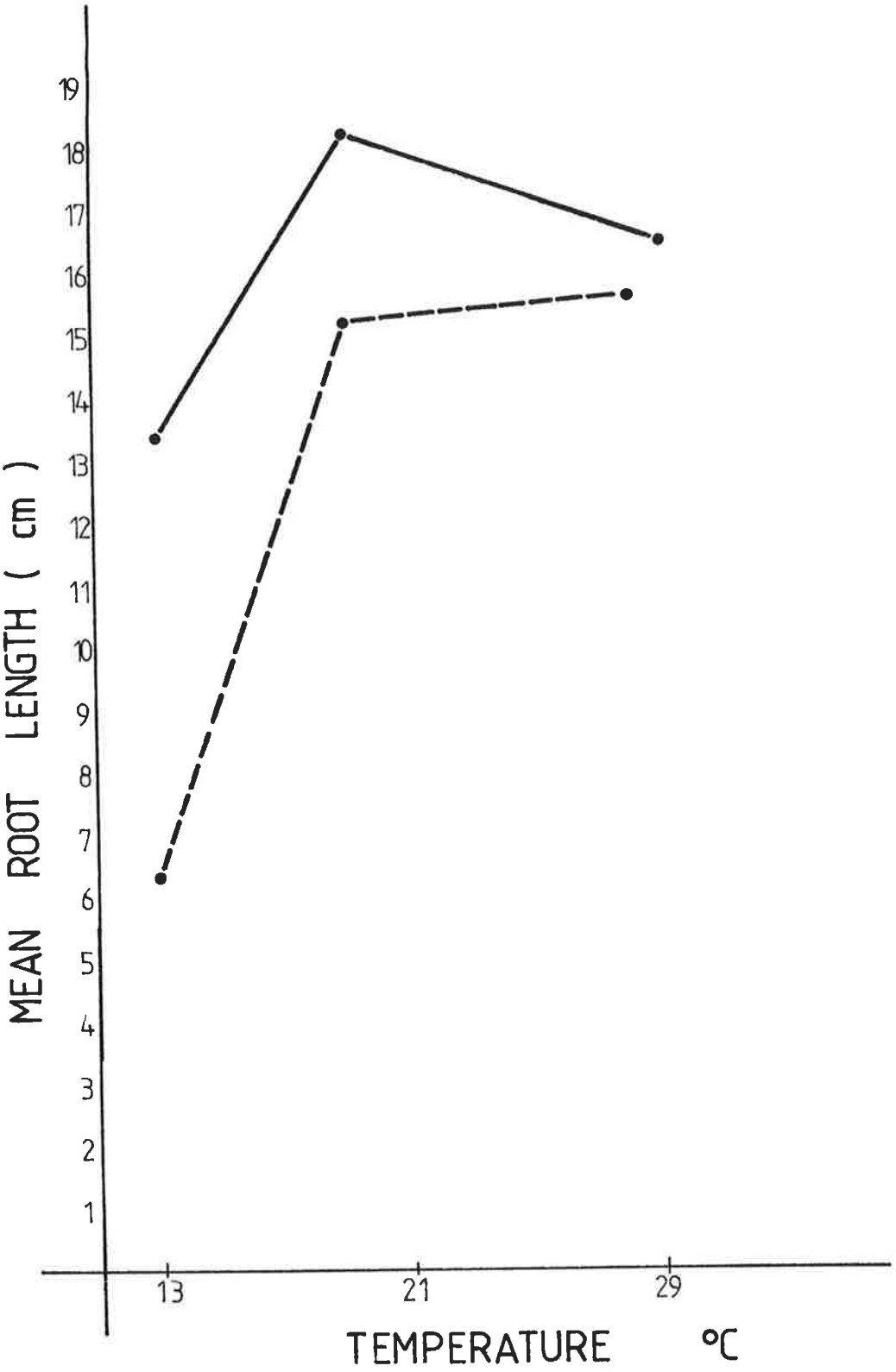
The influences of environment on disease severity of barley seedlings infected by Pythium is difficult to evaluate. Foliar symptoms of infected plants or even the root symptoms are not clearly specific. However, as shoot and root systems of barley plants infected by Pythium are restricted in growth, the influences of environment on disease severity can be objectively evaluated by measuring root or shoot length

Figure 11 : The influence of temperature on the severity of barley root disease induced by Pythium irregulare.

* Mean value of five replicates.

LSD = 0.67 p = 0.01

___ control --- infected roots


MEAN ROOT LENGTH (cm)
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
13
21
29
TEMPERATURE °C

of infected and uninfected plants. Diseases caused by Pythium spp. are reported to be most severe at temperatures unfavourable for growth of the host irrespective of the optimum temperature for the growth of the fungus (Lumsden and Haasis, 1964 ; Biesbrock and Hendrix, 1969). My studies support this conclusion. Growth reductions induced by this fungus were most severe at 13°C whereas the optimum for growth of barley roots is 20°C (Ranson and Parija, 1955) and the optimum for mycelial growth was between 25°C and 30°C. The results of my studies agree with the findings of prior temperature studies involving this pathogen (Hendrix et al. 1966 ; Biesbrock and Hendrix, 1969).

My results concerning relationships between Pythium spp. and soil water, inoculum density and soil water, as well as the relationship between Pythium spp. and temperature, indicate that winter and spring rains probably produce periodically saturated conditions in the field. Such conditions, together with low temperatures may favour the multiplication, liberation, dispersal and infection by Pythium inocula as well as lowering the tolerance of the host plant to infection.

CHAPTER 5

THE EFFECT OF *PYTHIUM IRREGULARE* AND *PRATYLENCHUS THORNEI* ON THE GROWTH OF BARLEY AND WHEAT

A. INTRODUCTION

The nematode *Pratylenchus thornei*, like *Pythium irregulare*, is common in South Australia and has a wide host range (Grandison and Wallace, 1974 ; O'Brien, 1982). Both are pathogenic on cereals (especially wheat and barley) in various regions of the world, including South Australia (Vanterpool and Truscott, 1932 ; Thorne, 1961 ; Baxter and Blake, 1968 ; Prasad, 1972 ; Van Gundy *et al.*, 1974 ; Orion *et al.*, 1979 ; Thompson *et al.*, 1980, ; Cook and Haglund, 1982). *Pratylenchus* species are often associated with pathogenic soil fungi such as *Rhizoctonia solani* on wheat and *Verticillium dahliae* on potatoes (Baxter and Blake, 1968) causing serious disease.

In this study the pathogenicity of *Pythium irregulare* was assessed and compared with that of the nematode *Pratylenchus thornei* in wheat and barley. The influence of the two pathogens singly and in combination was assessed to test the hypothesis that a statistical interaction occurred between the two in their effect on the host plants. It is possible that although *P. irregulare* is very pathogenic on its own, in association with a nematode such as *Pratylenchus thornei* the pathogenic effect might exceed the sum of the two separate effects.

B. Materials and Methods

A sandy-loam soil was steam-sterilized, adjusted to 80% field capacity by weighing and placed in pots 11 cm high and 8 cm diameter. Pythium irregulare was cultured on Difco cornmeal agar at 25°C for 48 hours. Two plugs of the medium were taken from the margin of the growing culture and introduced into a sand cornmeal medium (Chapter 4.B.1.). Inoculum was prepared by mixing 4 week old sand cornmeal agar medium containing P. irregulare with potting soil at rates of 0, 1 and 2% w/w, (P_o, P_1, P_2 in Figures 12-13). The wheat cultivar Festiguay and the barley cultivar Clipper were chosen as hosts as they are widely grown in South Australia. Seeds of wheat and barley were surface sterilized by dipping in 1% sodium hypochlorite for 1 min followed by three washes in sterile distilled water. Seeds were pregerminated and selected for uniformity ; three seeds were planted per pot. The pots were placed in a growth room at a temperature of 15°C with 12 hours light per day. Pratylenchus thornei was recovered from a wheat-field soil using a Seinhorst elutriator followed by a cleaning procedure in which the nematode suspension was washed on to a 300 mesh (50 µm aperture) sieve and then through a double layer of tissue paper. A bulk suspension of 380 P. thornei per ml was attained. The suspension contained 94% P. thornei, 2% Pratylenchus spp., and 4% free living nematodes. Inoculum levels of 1, 1900 and 3800 per pot (N_o, N_1, N_2 in Figure 12 and 13) P. thornei were used and inoculation occurred one day after planting the seedlings. Nematodes were introduced into the centre of the pot.

There were three replicates per treatment arranged as three blocks each of which contained all nine treatments (P_oN_o, P_oN_1, P_oN_2, P_1N_o,

P_1N_1, P_1N_2, P_2N_o, P_2N_1, P_2N_2). The treatments were completely randomised on the growth room bench in each block. Pots were weighed every other day, water being added to bring them to the required weight equivalent to 80% field capacity. Every third day heights of plants were recorded. 33 days after sowing, plants were removed from pots, numbers of *P. thornei* in the soil assessed and heights and dry weights of shoots determined. Total legnth of roots was assessed using a Comair root length scanner. Nematodes were extracted from roots which were then oven-dried and weighed.

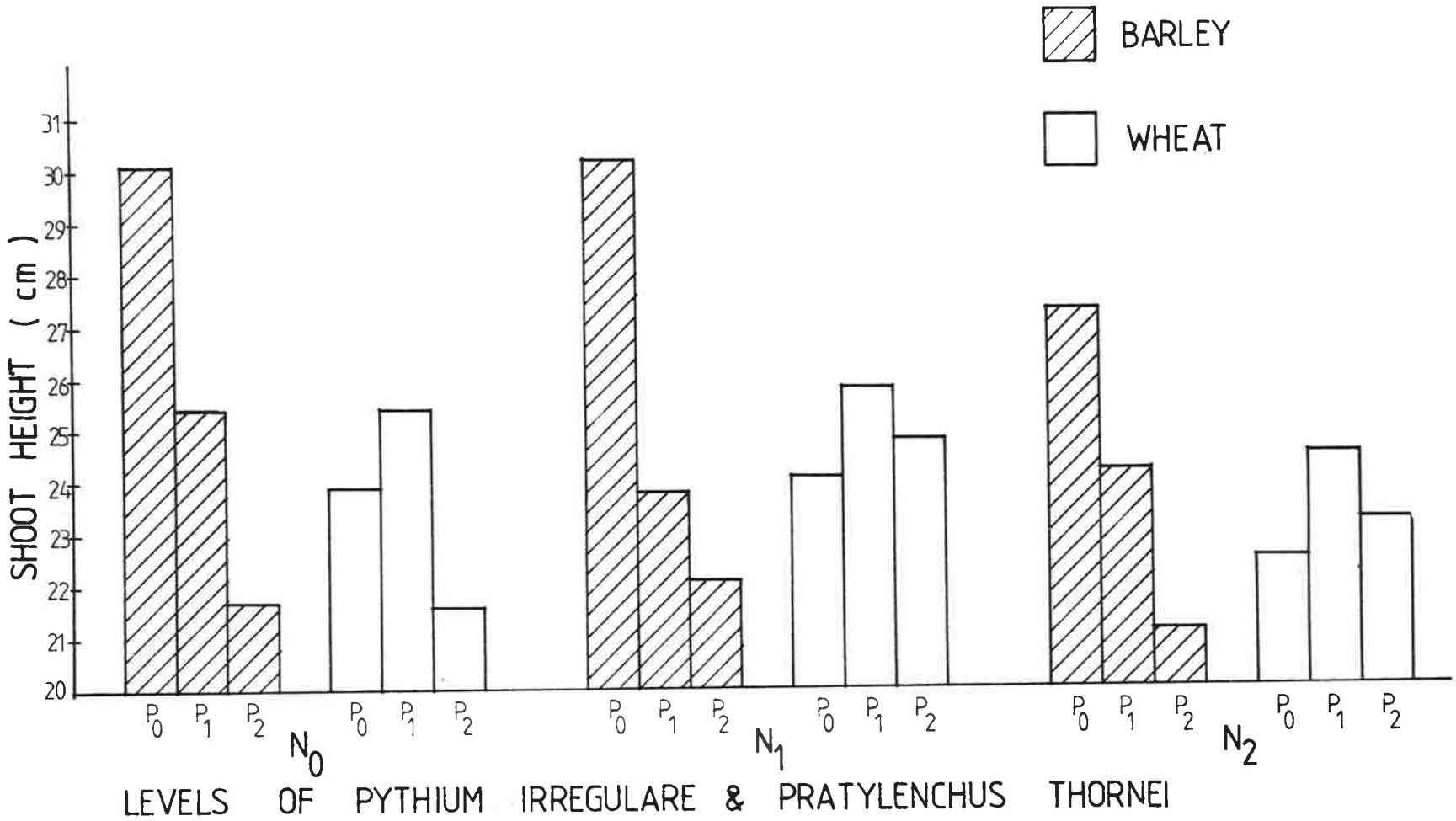
Data were analysed by analysis of variance and L.S.D.'s calculated.

C. Results

Pythium irregulare and the nematode *Pratylenchus thornei* each at three population levels were inoculated into pots containing either barley or wheat. The results are shown in Figure 12 and 13. Analysis of variance indicated that host (barley or wheat), *P. irregulare* and *P. thornei* all had significant ($p < 0.05$) influences on shoot height. An increase in the population levels of both pathogens was associated with a decrease in mean shoot height. *Pythium irregulare* had a greater inhibitory effect than *Pratylenchus thornei*. There was a significant statistical interaction ($p < 0.05$) between host and *P. irregulare*. Thus, although shoot height in barley decreases as the population level of *P. irregulare* increases, in wheat the picture is quite different. In wheat the mean shoot height of plants infected with *P. irregulare* (P_1, P_2) is significantly greater than that in uninfected plants (P_o). There was no statistical interaction between nematode and fungus on shoot height of

Figure 12 : The effect of Pythium irregulare and Pratylenchus thornei on shoot heights of barley and wheat after 33 days. LSD's (p = 0.05):

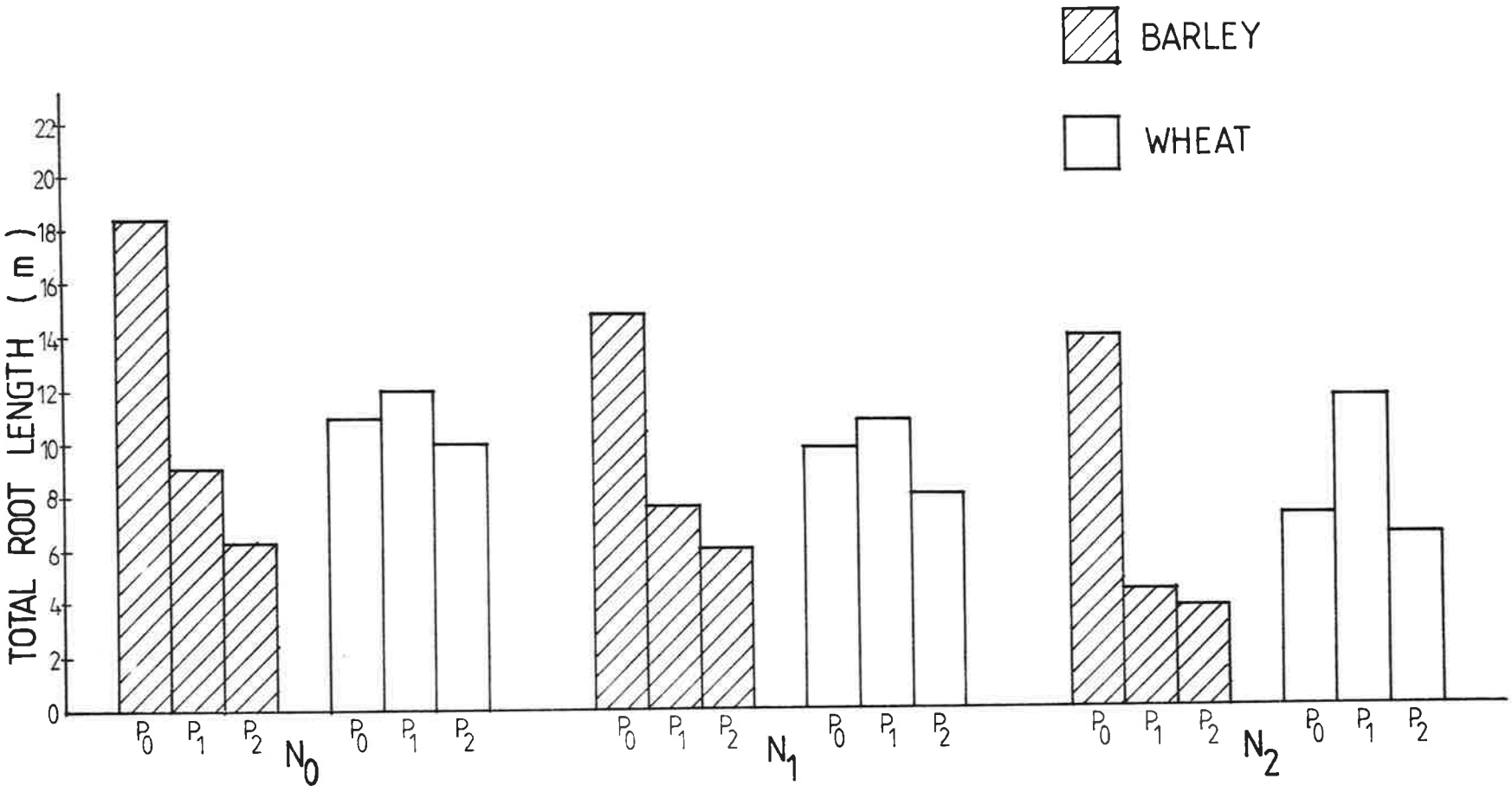
Host	= 3.04
Nematodes	= 1.086
Pythium	= 1.086



LEVELS OF PYTHIUM IRREGULARE & PRATYLENCHUS THORNEI

Figure 13 : The effect of Pythium irregulare and Pratylenchus thornei on total root lengths of barley and wheat after 33 days. LSD's (p = 0.05):

Host	= NS
Nematodes	= 1.21
Pythium	= 1.21



LEVELS OF PYTHIUM IRREGULARE & PRATYLENCHUS THORNEI

either barley or wheat ; the two pathogens seem to act independently. The results for shoot weight were similar to those for shoot height.

The results for root measurements are given in Figure 13. Analysis of variance indicated that P. thornei inhibited root growth in both barley and wheat. In barley, P. irregulare was also inhibitory but to a greater extent than P. thornei. A statistical interaction between host and P. irregulare again indicates the different response of the two hosts to this fungus. In wheat, increase in P. irregulare inoculum is not associated with decrease in root length. The data for dry root weights give the same picture as that for total root length. As with the shoots there was not a significant statistical interaction between fungus and nematode on the roots.

The results in Figures 12 and 13 suggest that barley and wheat respond similarly to infection by the nematode, P. thornei and the reduction in shoot and root growth is similar. P. irregulare on the other hand is more pathogenic in barley than in wheat. There was no evidence that the fungus and nematode acted synergistically or antagonistically in their influence on plant growth i.e. they appeared to act independently of each other. It is possible, however, that nematode and fungus influence each other in other ways.

Microscopic examination revealed that P. thornei was attracted mainly to root hairs of wheat and produced "stubby root hairs" (Plate 6.A). Lesions on the main roots did not develop at this stage but some P. thornei were seen in the cortical parenchyma. Lesions normally take six weeks to develop (Baxter and Blake, 1968). Root hairs of barley were not affected by P. thornei as in wheat. The primary roots of barley were

Plate 6

A. Damage by Pratylenchus thornei on wheat root hairs (1000x).

B. Pythium irregulare initiating root rot of barley (50x).

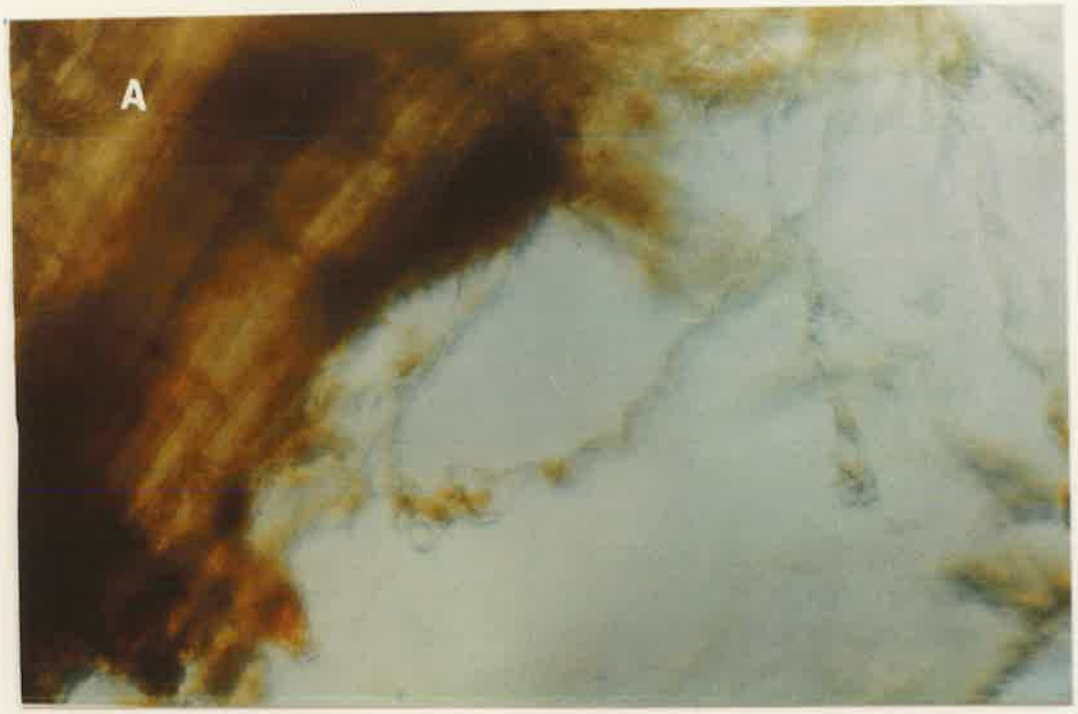


PLATE 6

Plate 7

1. Seminal root of a barley seedling infected by Pythium irregulare (50x).

2. Different stages of infection by Pythium irregulare on seminal barley roots (20x).

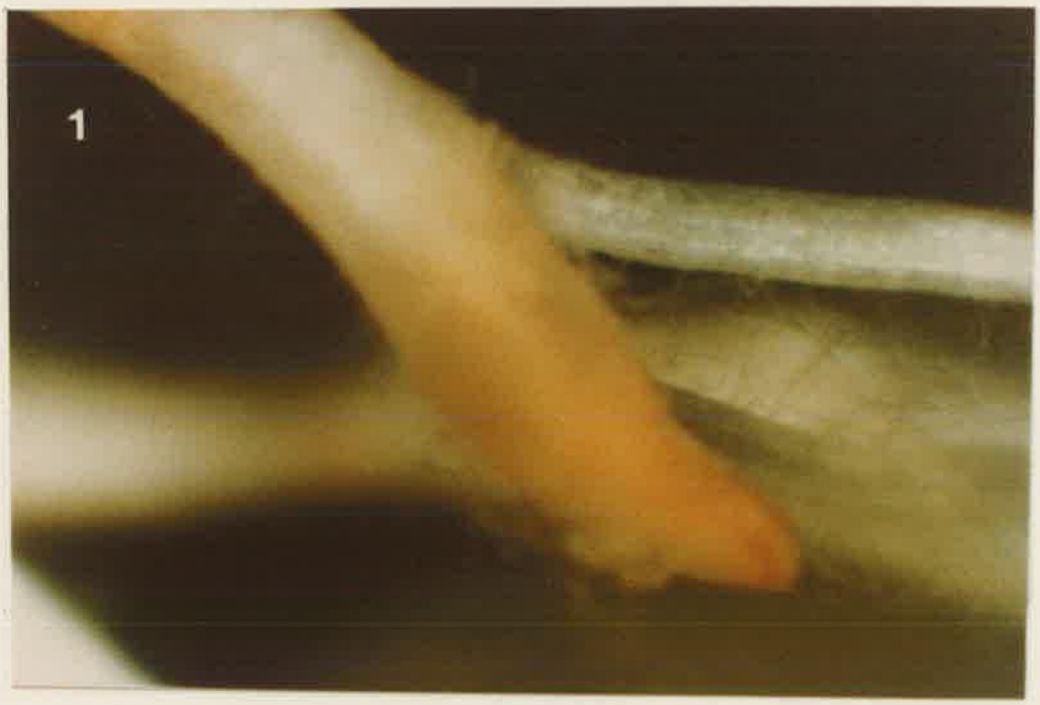


PLATE 7

Plate 8

A. Healthy root hairs of barley.

B. Collapsed barley root hairs due to Pythium irregulare infection (20x).

PLATE 8

the main sites of attack by P. thornei. Although the lesions did not develop, the invaded sites had many air bubbles in the cortical parenchyma, and were easily detected. When such root tissues were tested under a stereo microscope, P. thornei was detected. Barley roots were severely affected by P. irregulare. Main roots and root hairs showed heavy Pythium damage. Plate 6.B.; Plate 7.1,2 ; Plate 8.B, show Pythium root rot of barley. At the lower level of Pythium (P_1) the roots were rarely destroyed at this stage but showed signs of rotting (Plate 6.B). With the higher level of Pythium (P_2) seminal roots were often killed quickly after emergence, as is indicated by the length of dead root from the seed (Plate 7.2 arrow). By assessing the population of P. thornei on the hosts it was evident that wheat roots contained higher numbers of P. thornei than barley in all treatments in which P. thornei was added with the exception of N_2P_o. Barley had lower numbers of P. thornei in the roots but had more nematodes in soil than wheat (Table 12, 13). With increase in inoculum levels of P. irregulare final population of P. thornei decreased. Furthermore, P. irregulare had a greater inhibitory effect on the reproduction of P. thornei in barley than in wheat.

Table 12 : The effects of hosts and *Pythium* on the number of *P. thornei* in roots.

Treatment	Total number of Pratylenchus thornei per total root	
	Wheat	Barley
P_oN_o	0.00	0.00
P_1N_o	0.00	0.00
P_2N_o	0.00	0.00
P_oN_1	67.00	50.70
P_1N_1	56.70	12.30
P_2N_1	43.70	10.30
P_oN_2	89.30	111.00
P_1N_2	165.70	61.30
P_2N_2	114.70	54.00

LSD's ($p = 0.05$)

Host = 20.01
Nematodes = 7.78
Pythium = 7.78

Table 13 : The effects of hosts and Pythium on the number of P. thornei per pot.

Treatment	Total number of Pratylenchus thornei per pot after 33 days	
	Wheat	Barley
P_oN_o	0.00	0.00
P_1N_o	0.00	0.00
P_2N_o	0.00	0.00
P_oN_1	340.00	340.00
P_1N_1	350.00	280.00
P_2N_1	240.00	310.00
P_oN_2	600.00	760.00
P_1N_2	450.00	510.30
P_2N_2	396.00	543.00

LSD's (p = 0.05)

Host	= NS
Nematodes	= 36.82
Pythium	= 36.82

CHAPTER 6

STUDIES ON *PYTHIUM VOLUTUM*

A. Introduction

Pythium volutum was first reported as a parasite of wheat, oats and barley in Canada (Vanterpool and Truscott, 1932) and later of grasses in Holland (Van Luijk, 1934, 1938). In 1938, Vanterpool reported *P. volutum*, isolated from infected wheat seedlings in England, as severely parasitic. *P. volutum* has also been found in rye and maize (Bisby *et al.*, 1938). In Japan under the name *P. zingiberis* it has been recorded from ginger (Takahashi, 1954, 1973 ; Takahashi *et al.*, 1970) tomato, watermelon, *Hibiscus*, and morning glory seedlings (Takahashi and Morimoto, 1954, Takahashi *et al.*, 1970). This is the first report of *Pythium volutum* in Australia ; it was isolated among the other species of *Pythium* during studies to determine their occurrence in different barley soils of South Australia (Chapter 2). Although the fungus has been recorded in different parts of the world, as was mentioned earlier, little is known about it. Therefore, a series of experiments was initiated to determine whether *P. volutum* is widely distributed in South Australia, and to assess whether South Australia provides an environment in which it might multiply and reach damaging levels.

B. Occurrence of *Pythium volutum* in different soils of South Australia

B.1. Materials and Methods

A field survey of barley and wheat fields was conducted on twenty five sites in South Australia. The location of the sites is shown in Fig. 14, and the characteristics of the soils such as pH, clay content,

as well as crops and occurrence of P. volutum is presented in Table 14. The survey was conducted during July-August 1984.

Soil samples were collected from 0-10 cm deep as described in Chapter 2 C.1. For isolation and identification of the fungus the methods described in Chapter 2, C.2.1 and C.5 were used.

B.2. Results

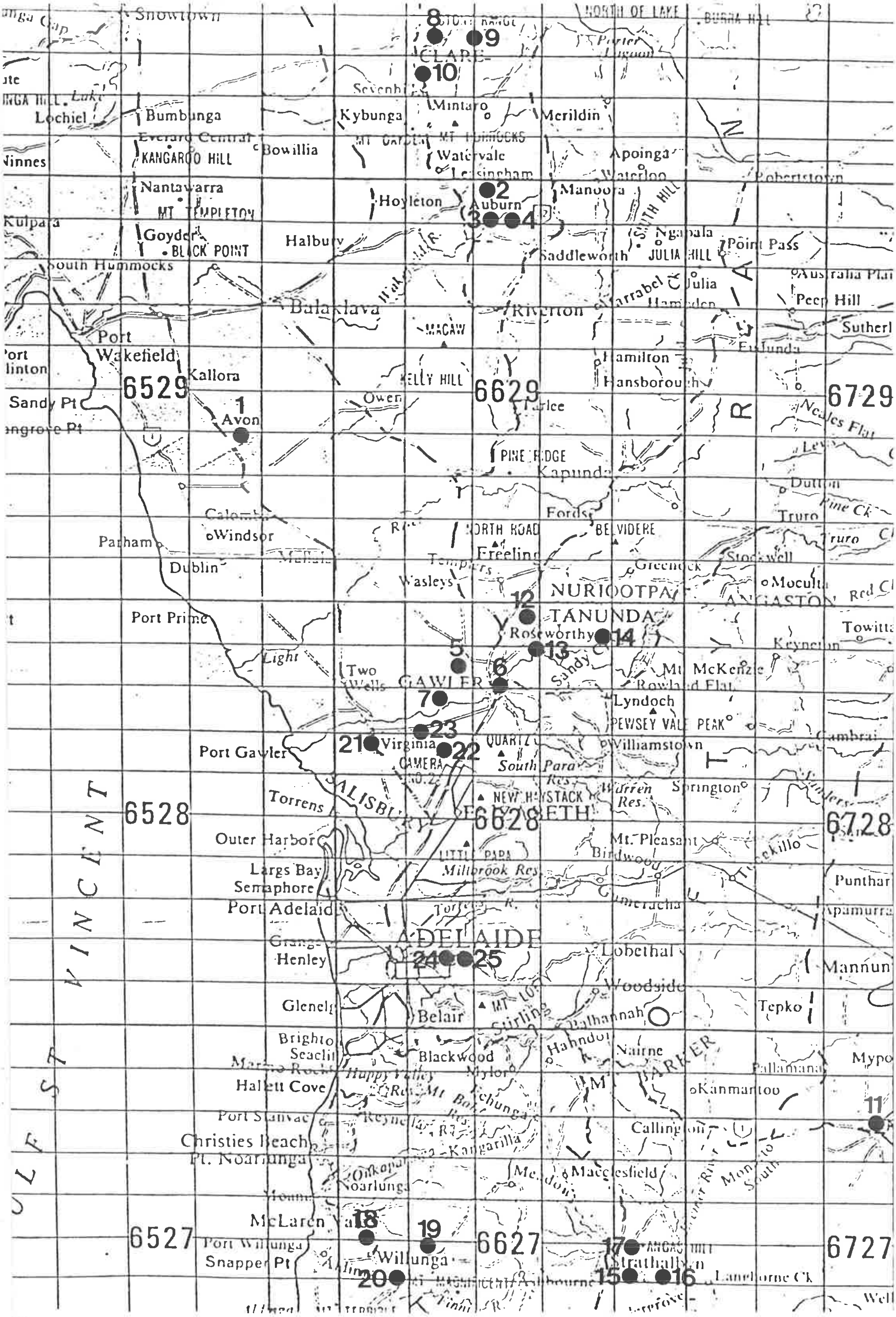
The data in Table 14 show that ten out of the twenty-five soils sampled contained Pythium volutum. Three out of eight wheat soils contained P. volutum. Of the soils investigated, P. volutum occurred in those within the pH range 5.5-7.5. Warcup (1952) found P. ultimum abundant in nursery soils with pH 6.8-7.2 and rare in soils with pH 5.3-5.5. Remy (1949) recorded Pythium in soils with pH values above 4.5. The presence of the parasite in soils is thus correlated with slightly acid or neutral conditions. Soils under crops generally have a pH range of 5.5-7.5, i.e. the optimum range for Pythium spp.. Therefore, in barley and wheat soils whose pH values ranged from 5.1 to 8.6 the absence of P. volutum in some of them can be ascribed to some other factors.

Soil texture

The soil examined ranged from 9.5% clay (Murray Bridge) to 29.1% (Mortlock Experiment Station) Table 14. There seems to be no correlation between the presence of Pythium volutum and soil texture. On the other hand, Remy (1949) found that clay soils contained more Pythium spp. than did sandy soils. The activity of Pythium is presumably favoured in such

Figure 14 : Location of the areas sampled for the occurrence of *Pythium volutum*.

Map provided by the Department of Lands, South Australia.



Snowtown
NORTH OF LAKE
BURRA HILL
CLARE
Porter Lagoon
Sevenhill
Mintaro
Kybunga
Bumbunga
Lochiel
Everard Central
KANGAROO HILL
Bowillia
MT HUMMOCKS
Watervale
Leasingham
Merildin
Apoinga
Waterloo
Robertstown
Nantawarra
MT TEMPLETON
Hoyleton
Auburn
Manoora
Kulpara
Goyder
BLACK POINT
Halbury
Saddleworth
Ngapala
JULIA HILL
Point Pass
South Hummocks
Balaklava
Riverton
Julia
Hamlyn
Australia Plain
Peep Hill
Port Wakefield
MACAW
Eudunda
Sutherlands
Port Clinton
Kallora
KELLY HILL
Hamilton
Hansborough
6529
6629
6729
Sandy Pt
Avon
Owen
Tarlee
Neales Flat
Mangrove Pt
PINE RIDGE
Kapunda
Dutton
Fords
Truro
Calomba
Windsor
Parham
NORTH ROAD
BELVIDERE
Freeling
Dublin
Mallala
Templers
Greenock
Stockwell
Moculta
Wasleys
NURIOOTPA
ANGASTON
Port Prime
TANUNDA
Roseworthy
Towitta
Keyneton
Light
Two Wells
GAWLER
Sandy Ck
Mt McKenzie
Rowland Flat
Lyndoch
PEWSEY VALE PEAK
Port Gawler
Virginia
QUARTZ
Williamstown
CAMERA
South Para Res.
SALISBURY
Torrens
NEW HAYSTACK
Warren Res.
Springton
6528
6628
6728
ELIZABETH
Outer Harbor
Mt. Pleasant
Tungkillo
Largs Bay
Semaphore
LITTLE PARA
Millbrook Res.
Birdwood
Punthari
Port Adelaide
Torrens R.
Gumeracha
Apamurra
ADELAIDE
Grange
Henley
Lobethal
Mannum
Woodside
Glenelg
Belair
MT LOFTY
Stirling
Balhannah
Tepko
Brighton
Seacliff
Blackwood
Hahndorf
Nairne
MT BARKER
Mypolonga
Marino Rocks
Happy Valley
Mylor
Hallett Cove
Kanmantoo
Pallamana
Port Stanvac
Reynella
Mt Bold Res.
Echunga
Callington
Christies Beach
Pt. Noarlunga
Kangarilla
Onkaparinga
Noarlunga
Meadows
Macclesfield
Mount Lofty
McLaren Vale
6527
6627
6727
Port Willunga
Snapper Pt
Willunga
ANGAS HILL
Strathalbyn
Langhorne Ck
Wellington
MT MAGNIFICENT
Ashbourne
MT TERRIBLE
Finniss R.
GULF ST VINCENT
MT LOFTY RANGES
1
2
3
4
5
6
7
8
9
10
11
12
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Table 14 : Occurrence of *Pythium volutum* in some South Australian soils.

Sample No.	Location and number of fields		pH	Clay %	Crop	Occurrence of P. volutum
1	Avon	1	8.6	16.9	B	–
2	Auburn	1	5.2	13.2	B	–
3		2	5.5	13.1	B	–
4		3	5.1	12.8	W	+
5	Gawler River	1	5.3	20.2	B	–
6		2	5.0	13.4	B	–
7		3	5.1	13.6	W	–
8	Mortlock Experiment Station	1	5.9	29.1	B	+
9		2	7.1	20.3	B	+
10		3	5.8	28.6	W	–
11	Murray Bridge	1	7.8	9.5	B	+
12	Roseworthy	1	7.9	12.35	B	+
13		2	7.6	12.30	B	–
14		3	7.8	12.10	W	–
15	Strathalbyn	1	7.5	19.28	B	+
16		2	7.1	19.20	B	–
17		3	7.0	19.16	W	+
18	Willunga	1	6.1	19.10	B	–
19		2	6.9	21.60	B	–
20		3	6.5	20.35	W	–
21	Virginia	1	7.4	11.52	B	+
22		2	6.9	13.14	W	+
23		3	7.2	11.60	B	+
24	Waite Institute	1	6.4	24.50	B	–
25		2	6.5	23.90	W	–

B = barley
W = wheat
\+ = P. volutum was isolated from soil

soils by their increased water holding capacity and resistance to desiccation.

C. Relationship between pH of the medium and growth rate of *Pythium volutum*

Experiments were done to determine to what extent pH of the medium influenced growth of *P. volutum*.

C.1. Linear growth of *P. volutum*

Materials and methods

A graduate pH series of Difco cornmeal agar plates was prepared. There were eight treatments : pH 3, 4, 5, 6, 7, 8, 9, 10 with four replicates for each treatment. Adjustment of the medium, inoculation and measurements of the fungus mycelium were done as described in Chapter 4.D.1.a. The experiment was designed in a randomized complete block.

Results

The relationship between pH of the medium and linear growth rate of *P. volutum* is shown in Table 15.

Optimum growth of *P. volutum* occurred at about pH 6.00, the maximum and minimum pH values for growth were 9.00 and 4.00.

An analysis of variance showed that there were significant differences ($p = 0.05$) in linear growth of mycelium between the different pH of the medium.

Table 15 : Relation between pH of the medium and linear growth of Pythium volutum

Treatment (pH)	*Linear growth of P. volutum (mm/24 h / at 25°C)
3.00	0.00
4.00	2.17
5.00	6.45
6.00	10.10
7.00	6.59
8.00	3.23
9.00	1.08
10.00	0.00

* Mean value of four replicates

L.S.D. = 1.42, p = 0.05

C.2. The growth reaction of P. volutum in different pH liquid cultures as determined by weight of mycelium

Material and methods

Cornmeal 50 g/l distilled water was used as a liquid medium, and a graduated pH series of cultures from pH 3.00 to pH 10.00 was prepared. The materials and methods used were as described in Chapter 4.D.2.a. The experiment was arranged in a randomized complete block.

Results

The relationship between pH of the medium and weight of mycelium of P. volutum is shown in Table 16. Maximum weight of mycelium occurred at pH 6.00, but the fungus grew well over a wide range of pH from 4.6 to 8.7 (final pH of the medium). There were significant differences ($p = 0.05$) between the weight of mycelium at different pH values of the medium. Although the fungus seems to grow well in a slightly acid pH, soils with pH < 4.5 may prevent the establishment and survival of P. volutum. Soils with neutral or slightly alkaline reaction probably provide the most favourable conditions for establishment and development of P. volutum.

D. The influence of temperature on mycelial growth of P. volutum

D.1. Material and Methods

The effect of temperature on the growth of P. volutum was measured by placing the fungus on soil at the centre of a Difco cornmeal agar plate. Growth was measured at temperatures ranging from 1° to 34°C. The

Table 16 : Relation between pH of the medium and dry weight of mycelium of Pythium volutum after two weeks incubation at 25°C.

Treatment (pH)		*Dry weight of mycelium of P. volutum (mg)
Initial	Final	
3.00	3.00	0.00
4.00	4.6	28.75
5.00	5.2	98.46
6.00	6.00	156.52
7.00	6.8	142.42
8.00	7.7	117.06
9.00	8.7	44.79
10.00	10.00	0.00

* Mean values of four replicates

L.S.D. = 4.63, p = 0.05

soil used in this study was taken from an infested barley field, air dried and placed in large test tubes, 1.5 cm diameter and 15 cm in length. Soil in the tubes was moistened and sterilized. After cooling, a 2 mm disc of cornmeal agar carrying P. volutum was cut from the margin of a growing culture and placed on the surface of the soil in each tube, and in the centre of the agar plates. There were five replicates per treatment. At each temperature the growth of the fungus was measured at intervals of 24 hours. The average growth of fungus on cornmeal agar and in soil, per 24 hour period was calculated.

D.2. Results

Data in Table 17 show that the optimum temperature was near 25°C and the minimum between 1° and 5°C when the organism is grown on Difco cornmeal agar or in soil. Data indicate that the mycelium did not grow at 1°C and that it grew only slowly at 5°C, moderately at 15°C, and rapidly at 20°C, 25°C.

E. Varietal resistance and susceptibility of barley to Pythium volutum

Experiments on varietal resistance and susceptibility of three barley cultivars to P. volutum infection were conducted in growth chambers.

E.1. Materials and Methods

The barley cultivars used in this experiment were Clipper, Forrest and Schooner. All barley seed used originated from a single seed lot of each cultivar. Damaged seeds were removed and planting tests showed the

Table 17 : The mycelial growth of *Pythium volutum* at various temperature conditions on Difco cornmeal agar and in soil.

Temperature °C	*Radial growth of mycelium (mm/24h) on Difco cornmeal agar	*Downward growth of mycelium (mm/24 h) in soil
1	0.0	0.0
5	1.5	1.2
10	4.3	2.3
15	5.8	3.1
20	8.1	7.6
25	10.3	8.8
30	1.4	0.9
35	0.0	0.0
	L.S.D. = 0.14, p = 0.01	L.S.D. = 0.12, p = 0.01

* Mean value of five replicates

seed to be free from fungal infection. The surface sterilization of seeds was done as described in Chapter 4.B.1. There were three seeds per pot. 300 g of sterilized sandy loam field soil were placed in each pot each of which measured 11 cm high and 8 cm in diameter. Inoculum used was obtained from infected barley roots and grown separately on Difco cornmeal agar at 25°C for 48 h. The inoculation of the soil was done as described in Chapter 4 at a rate of 1 g inoculum/100 g soil prior to sowing barley seeds. The experiment was done in a growth chamber at 15° ± 1°C with 12 h light per day. Soil in the pots was maintained at 80% of field capacity by daily weighing of pots. The experiment lasted 4 weeks at the conclusion of which seedlings were removed from the soil, washed and measurements of total length of roots and length of shoots were made. The root length measurements were done by chopping the roots into 1 cm pieces and using a Comair Root Length Scanner. There were four replicates per treatment randomized in a growth chamber. Data were analysed by analysis of variance.

E.2. Results

Table 18 presents the shoot length and total roots length of the barley infected seedlings together with a control (no fungus). All three cultivars show a considerable decrease of top growth of the seedlings and of the total roots length. The analysis of variance indicated that the lengths of tops and roots of seedlings in infested soil were significantly different ($p = 0.01$) from those in the uninfested soils. There was no difference between the three varieties to Pythium volutum infection. All three varieties showed a marked decrease of shoot growth as well as root length as a result of infection by the pathogen.

Table 18 : The infleunce of Pythium volutum on the mean length of shoots and roots of three barley cultivars, 4 weeks after sowing at 15°C.

Treatment	*Total root length of barley cultivars (m)			Shoot length of barley cultivars (cm)		
	Clipper	Forrest	Schooner	Clipper	Forrest	Schooner
Soil non infested	11.14	12.02	11.25	30.45	32.00	30.28
Soil infested	6.09	5.27	6.21	22.46	23.14	22.40
	L.S.D. = 1.49, p = 0.01			L.S.D. = 2.65, p = 0.01		

* Mean value of four replicates

Wen-Chun Ho and Meredith (1941) showed that the variation in percentage decrease of root length of barley seedlings infected by P. graminicolum was smaller than the percentage decrease of top growth. However, my results indicate the contrary ; the percentage decrease of root length of barley seedlings was higher (i.e. for Clipper cultivar 45.34%) than decrease of shoot growth (26.24%).

E.3. Discussion

As already stated by Vanterpool and Truscott (1931), the frequency of isolation of any particular parasitic species in a given season does not necessarily give a true representation of its distribution, because the duration of conditions favouring the active growth of these fungi, when they are readily isolated from soil, varies considerably from place to place. However, isolation made from barley seedling roots and soil samples as described in Chapter 2, D.3.1, as well as from another ten soils with different physical and environmental conditions, suggest that P. volutum is more abundant in northerly sites of South Australia characterized by higher temperatures, low rainfall and low clay content in the soil.

Cultural studies have shown that P. volutum grows on and in medium at low pH, but that the optimum growth occurs at about pH 6. Thus the presence of the parasite in soils could be correlated with slightly acid or neutral conditions as occurs in the northerly parts of South Australia. In southerly regions soils are more alkaline.

From the previous experiments with different levels of soil water already described in Chapter 2, it was clearly shown that the amount of

damage to barley seedlings caused by P. volutum increased with increasing moisture content of the inoculated soil. Thus, although P. volutum is more abundant in the north of South Australia it may be more damaging in the south where rainfall is higher. Under growth chamber conditions P. volutum injury did not manifest itself in reduced germination or as post-emergence death of the seedlings, but as a root rot reducing the length of shoots and roots of the growing plants, which could be further expressed in reduction of yields.

CHAPTER 7

STUDIES ON THE CONTROL OF PYTHIUM SPP.

A. Introduction

Control of soilborne pathogens is becoming increasingly important in agriculture because of awareness of the considerable losses they cause in a variety of crops. Most Pythium spp. occur in soil as parasites on plant roots or as saprophytes on organic material ; they produce oospores and chlamydospores that persist for many years. Once established in the soil the resting structures are virtually impossible to eliminate except with a wide spectrum drench hence direct control of Pythium on a field scale is difficult and expensive (Hendrix and Powell, 1970). Soil fumigation has been used by many workers over the past 30 years (McLaughlin and Melhus, 1943 ; Bruehl, 1951 ; Ebbels, 1969, 1970 ; Williams and Salt, 1970 ; Rovira, 1976 ; Cook and Haglund, 1982) as a means of controlling soilborne pathogens to protect wheat and barley. The increased crop growth following fumigation occurred partly because Pythium has been controlled (McLaughlin and Melhus, 1943). Fumigation with chloropicrin or methyl bromide or a combination of the two is now standard practice in many nursery and horticultural operations (Munnecke, 1971).

Such fumigants do not kill all soil organisms. During the first few weeks following fumigation, bacterial populations increase rapidly followed by other soil organisms (Hodges, 1959). Eventually, a biotic equilibrium is reached in which saprophytic fungi predominate, often providing effective competition for plant pathogens. After fumigation,

however, the soil flora is altered by the temporary removal of competitors to Pythium spp. and other pathogens, which multiply rapidly, and offer a greater threat to plants than before fumigation (Vaartaja, 1967 ; Kraft, 1969 ; Gill, 1970 ; Hendrix, 1970).

Soil amendaments such as sawdust, bark and crop residues as well as green manuring, have been used to control Pythium root-rots in nurseries and field crops (Vaartaja and Bumbieris, 1964). Such amendments are sometimes effective, mainly because they encourage soil flora antagonistic to Pythium spp. and other plant pathogens. On the other hand improvement in plant growth and survival may be due to a good host response, thus stimulating root formation that compensates for roots killed by pathogens.

Crop rotation is another method of reducing the populations of soil pathogens, but because the more common and destructive pathogenic Pythium spp. have wide host ranges, this procedure is not usually successful. However, crops differ in their moisture requirements as well as providing different degrees of shading, they accordingly affect the soil environment and hence influence the conditions that Pythium spp. require for optimum growth and parasitism. The standard recommendation for controlling diseases caused by pathogenic phycomycetes is the proper regulation of such environmental factors as site selection, soil moisture, fertilization, temperature and soil fungicides (Tompkins and Middleton, 1950 ; Littrell, Gay and Wells, 1969). While the environmental factors can sometimes be controlled to a reasonable extent in green-houses and nurseries, they generally cannot be regulated in a field planting, hence chemicals are used more extensively. There are many chemicals formulated to control Pythium such as Captan, Thiram,

Dowco 444, Previcur, Vitavax, Orthocide, Metalaxyl (Ridomil, Apron). Treatments with Apron as a seed dressing to control damping off caused by P. irregulare, increased seedling number and root weight of carrots (Morgan and MacGregor, 1982 ; MacGregor and Morgan, 1983). Ridomil and Gypsum to control pod disease (P. myriotilum) resulted in a significantly lower pod disease rating and significantly higher yields than the untreated (Boswell and Grichar, 1982). Abawi and Cobb, (1982) found that bean treatment with Ridomil, Dowco 444, or Previcur was highly effective for control of bean root- rot (P. ultimum). Ridomil plots planted with seeds treated with Captan + Demosan had the highest bean population and yield as well as the lowest rot-severity caused by P. ultimum (Abawi and Crosier, 1983). Apron 2E as a seed treatment of soybean produced significantly higher yields (Gessner, 1982). Seed treatment for control of seed and soilborne pathogens of wheat (Pythium spp.,Rhizoctonia sp.) with Vitavax 200F and Thiram and with Vitavax 200F alone, increased seedling emergence (Anzalone, 1982 ; Williams Jr., 1982). Ayers (1981) showed that Vitavax 200 and Mistomatic Mercury gave the best seedling emergence of wheat while 30F + Apron 2E gave the best grain yields. Control of Pythium blight (P. aphanidermatum) on ryegrass was obtained when the seeds were treated with Banol and Galben (Sanders et al., 1981).

Although metalaxyl [N-(2,6-dimethylphenyl)-N-(methoxyacetyl) alanine methylester] trade name Ridomil and Apron, proved to be an efficient fungicide to control Pythium spp. it is not commonly used on cereals therefore further studies were considered.

B. Efficacy of metalaxyl (Ridomil 25 W.P. CGA 48988) to control Pythium spp.

B.1. Introduction

Ridomil is a relatively new type of fungicide. It's active ingredient belongs to the group known as acylalanines. It shows very high but specific activity against fungal pathogens in the class Oomycetes. Ridomil is especially suited for preventive use but it can also be used curatively. It is rapidly absorbed by roots, stems, and leaves of plants and is transported acropetally in the xylem. Due to its swift penetration, the product is unaffected by rain falling several hours or more after application. Because of the systemic activity, new and existing growth is protected. (Technical Bulletin CiBA-GEIGY Australian Limited, 1979). The effect of metalaxyl on species of Pythium is not well known. Treatment of Pythium at 1-10 µg/ml produced reduction in longitudinal growth, increase in hyphal thickness, loss of apical dominance, thickening of the cell wall and changes in surface wall structure. Metalaxyl seems to cause a dimished protein synthesis, which may be connected with the decrease of ribosomal RNA in normally very active regions of hyphal tips. (Grohmann and Hoffmann, 1982). Studies on the antifungal mode of action of metalaxyl in the inhibition of nucleic acid systems in P. splendens indicated that RNA and DNA synthesis were already strongly inhibited 1-2 hr after addition of the fungicide. Protein synthesis was not inhibited but lipid synthesis, although almost unaffected at 0.1 µg/ml, was strongly affected at 0.3 µg/ml (Kerkenaar, 1981).

B.2. Bio-assay and soil test

B.2.1. Materials and Methods

To determine the efficacy of Ridomil 25 W.P. (C.G.A. 48988) to control Pythium spp. tests were done on cornmeal agar media and in soil, according to the method of Zentmyer (1955). For the bio-assay in cornmeal agar Ridomil 25 W.P. at concentrations of 250 ppm, 400 ppm, and 750 ppm active ingredient (a.i.) were used. Each concentration of Ridomil was mixed separately with cornmeal agar prior to pouring into 9 cm Petri dishes, each containing 20 ml medium. Pythium species used were : P. irregulare, P. graminicolum and P. volutum isolated from the infected barley seedlings. A plug, 5 mm in diameter of actively growing mycelium of each species of Pythium was individually placed at the centre of each Petri dishes, then incubated at 25°C for 3 days. Growth of mycelium was observed and measured. (Table 19). Treatments were replicated three times. For the soil drench test, field sandy loam soil was used, air dried, put through a sieve with an aperture of 1 mm and sterilized for 1 h at 120°C. Plastic vials, 25 mm in diameter and 80 mm deep, were used as containers. Ten ml of soil were placed in the vial and a plug of the required Pythium species, 7.5 mm in diameter, was placed on the soil and covered by a further 10 ml of the soil on top of which was placed a further plug of mycelium. A 5 ml solution of fungicide, enough to wet the soil, was poured into the vials. They were incubated at 25°C for 24 h. All plugs from the soil were taken and washed with distilled water, dried on Kleenex tissues, then placed on cornmeal agar and incubated for three days at 25°C to determine the growth of each Pythium sp. Results were assessed from the cornmeal agar as presence or absence of mycelial growth (Table 20). Treatments were replicated three times.

Table 19 : Efficacy of different concentrations of metalaxyl (Ridomil 25 W.P.CGA 48988) against Pythium spp. tested in cornmeal agar culture.

Ridomil 25 W.P. concentration (ppm a.i.)	*Mean value of Mycelium growth (mm/24 h)		
	P. irregulare	P. graminicolum	P. volutum
250	30	22	10
400	0	0	0
750	0	0	0
Control (Distilled water)	31	24	11

* Mean value of three replicates after three days incubation at 25°C.

Table 20 : Efficacy of different concentrations of metalaxyl (Ridomil 25 W.P. CGA 48988) against Pythium species in soil drenches.

Pythium spp.	*Mean growth of mycelium (mm/24h) at different concentration of Ridomil							
	a.i. 250ppm		a.i. 400ppm		a.i. 750ppm		Control (Distilled water)	
	20 mm deep	0 mm	20 mm deep	0 mm	20 mm deep	0 mmm	20 mm deep	0 mm
P.irregulare	30	31	18	19	0	0	31	32
P.graminicolum	22	23	14	5	0	0	23	23
P.volutum	9	9	4	4	0	0	9	10

* Mean value of three replicates after 3 days incubation at 25°C.

B.2.2. Results

With three different concentrations of metalaxyl (Ridomil 25 W.P.) (250 ppm, 400 ppm, 750 ppm a.i.) two were effective (400 ppm and 750 ppm a.i.) in preventing growth of P. irregulare, P. graminicolum, and P. volutum. There was no difference between the rates of growth of Pythium spp. on cornmeal agar containing metalaxyl (250 ppm a.i.) and rates of growth on fungicide-free cornmeal agar (Table 19). The colony morphology of all three species of Pythium tested was not affected by metalaxyl incorporated into the medium. However, the results in the cornmeal agar plates did not correspond to those found when the same level of Ridomil concentrations were applied in drenches in soil ; only the treatment with 750 ppm a.i. Ridomil appeared to give absolute prevention at 20 mm in the soil and surface (Table 20). The fungicidal effects decreased in the soil application, suggesting that the active ingredient (a.i.) at low concentration was inactivated or adsorbed by soil particles or chemicals. However, Klamparens and Vaughan (1952) reported good correlation between laboratory results of fungicides in most agar and field control of several turf pathogens. Zentmyer (1955) on the other hand, found that the fungitoxicity of many fungicides that are highly effective in other types of tests were reduced when they were tested as a soil drench. To elucidate the effects of metalaxyl (Ridomil 25 W.P.) on Pythium spp. further experiments were undertaken.

B.3. Assessment of metalaxyl (Ridomil 25 W.P. CGA 48988) effects on Pythium spp. under growth chamber conditions

B.3.1. Materials and Methods

The experiment was conducted under growth chamber conditions at 15°C ± 1°C with 12 h light per day. Pythium irregulare and P. volutum

isolated from barley soil, and previously tested to be pathogenic on barley were grown separately on cornmeal agar at 25°C for 48 h. Two plugs of medium were taken from the margin of the growing cultures and incubated on sand cornmeal culture medium. Inoculation was achieved as is described in Chapter 4.B.1. at a rate of 1% w/w prior to sowing barley seed. All barley seed originated from a single seed lot of the cv. Clipper. The seeds were dusted with metalaxyl (Ridomil 25 W.P.) to provide 0.250 g and 1.0 g a.i. per kg of seed. The soil was contained in 11 cm high, 8 cm diameter pots, and 5 seeds were sown per pot. Pots were watered daily. Treatments were replicated four times and randomised in a growth chamber. The experiment lasted 4 weeks and at the conclusion, seedlings were removed from the soil, washed, and measurements of shoot and root length were made. The root length measurements were done by chopping the roots into 1 cm pieces and using a Comair Root Length Scanner.

B.3.2. Results

Barley sown in the sterile sandy loam field soil artificially infected with Pythium irregulare or P. volutum, responded strongly to metalaxyl as a seed treatment at 1 g a.i./kg seed but it was ineffective at a rate of 0.250 g a.i./kg seed (Table 21). Metalaxyl applied as a seed treatment of 1 g a.i./kg seed protected barley roots against infection caused by Pythium spp. Root and shoot length of barley seedlings measured four weeks after sowing, were increased by metalaxyl treatment (Table 21). When the seeds were sown in artificially infected soil without treatment, or treated with 0.25 g a.i./kg seed of Ridomil 25 W.P., seedlings emerged but were severely stunted, exhibiting leaf distortions and with a shoot length about half of the healthy seedlings

Table 21 : Influence of Metalaxyl (Ridomil 25 W.P. CGA 48988) seed treatment, on root and shoot growth of barley seedlings, 4 weeks after sowing in artificially infected sandy loam soil, and incubated at 15° ± 1°C in a growth chamber.*

Ridomil(c) concentration g.a.i./kg seed	Total barley root length after 4 weeks (m)[a]		Barley shoot length after 4 weeks (cm)[b]	
	P.irregulare	P.volutum	P.irregulare	P.volutum
0	5.82	6.11	22.78	22.80
0.25	6.05	6.60	23.80	22.88
1.0	12.16	12.40	31.36	31.87

* Mean value of 20 measurements

a L.S.D. = 0.306 p = 0.01

b L.S.D. = 1.735 p = 0.01

c Seed treatment was applied as a dust.

which grew from seed treated with Ridomil 25 W.P. a.i./kg seed. The diseased plants had fewer and shorter roots. Root tips on plants not protected by metalaxyl were brown and contained Pythium oospores. A statistically significant interaction ($p = 0.01$) was obtained between Pythium species and Ridomil 1 g a.i./kg seed on length of roots, hence it is necessary to discuss relative inhibition of the two Pythium species in terms of the response to Ridomil treatment. Root growth of barley seedlings increased when concentration of Ridomil was increased to 1 g a.i./kg seed. In practical terms it is possible that the reduction in barley growth due to Pythium can be eliminated when the seed is treated with metalaxyl. Pythium causes damage to fine rootlets of plants (Bruehl, 1951 ; Anderson 1964) through which water may enter at rates of 0.07 to 0.44 $\mu m^3/\mu m^2$ root/min (Rosene, 1949). The elimination of Pythium damage could account for the greater plant vigour and probably greater yield. The increased growth response of barley to Ridomil treatment was detectable soon after sowing as shown by a significant improvement in shoot and root growth. As the number of tillers formed by a plant is influenced to a large extent by conditions early in the life of the plant (Cook and Haglund, 1982) it seems likely that elimination of Pythium damage by seed treatment with Ridomil might improve the leaf area and root length and penetration as the plant matures.

Increased growth of barley seedlings was achieved following seed treatment with Ridomil under the growth chamber conditions. However, Ridomil, like the majority of other chemicals is applied mostly to protect plants, or to control undesirable organisms in the field where environmental conditions are quite different from those in growth cabinets. Therefore, for a better understanding of the behaviour of

Ridomil in the natural environment, further experiments with Ridomil under field conditions, were done.

B.4. Field experiment

The aim of this experiment was to indirectly assess the importance of Pythium on barley by applying, Ridomil, a fungicide specific to pythiaceous fungi. The following aspects were investigated :

(1) influence of Ridomil 25 W.P. (applied as a seed treatment) on

(a) numbers of emerging plants
(b) growth of seedlings
(c) yield of grain

(2) To investigate whether there are any differences between barley cultivars in their response to application of Ridomil.

(3) To investigate whether the effect of Ridomil 25 W.P. on barley is influenced by soil type.

B.4.1. Materials and Methods

Design

A comparison was made between two barley cultivars at two levels of Ridomil 25 W.P., zero and 1 g active ingredient (a.i.) per kg of seed, in two types of soil in a field experiment carried out in 1984 at Mortlock Experiment Station, Mintaro, 130 km north of Adelaide, South

Australia (mean annual rainfall 603 mm ; May–October mean 474 mm). The two types of soil were a grey brown soil of heavy texture, pH 7.1 and a red brown soil, pH 5.9.

Crops in previous years were as follows:

Year	Grey brown soil	Red brown soil
1968 – 1978	lucerne	lucerne
1979	oats	oats
1980	barley	oats
1981	beans	oats
1982	oats	oats
1983	beans	beans

Barley cultivars used in the experiment were Clipper and Forrest. Both are used in South Australia, Clipper being sown on about 31 per cent of the barley area, and Forrest in about 5 per cent. (Barley variety recommendations for 1984, Department of Agriculture, South Australia). The seed of both cultivars was treated with metalaxyl (Ridomil 25 W.P.) to provide 1 g a.i. per kg seed prior to sowing.

Establishment and management

Plots were sown on July 17, 1984. Sowing rate was 70 kg/ha. All seed was sown with a basal application of superphosphate of 100 kg/ha. The area surrounding the plots was sown with wheat. A 16 row drill was used for both seed and fertilizer ; by dividing the seed box in the middle, seed of the two different cultivars (treated with Ridomil and

non treated) were sown at once. A light harrow was trailed behind the drill. Prior to sowing the experimental area was sampled (as described in chapter 2) and assayed for the natural occurrence of Pythium spp. using the Middleton Key. The soil plate method (as described in Chapter 2) was used.

Measurements

Seedling establishment was estimated by counting plants in situ, in 1 m of row in three positions of each replicate approximately six weeks after planting to determine whether treatment of seed with Ridomil improved establishment. Crop height was measured in situ 2 months after planting. There were 10 measurements in each replicate. At maturity (23 weeks) a grain yield harvest was made by cutting 1 m of row in three positions within each replicate. Subsequently, next day, the plots were machine harvested. Edge rows were avoided.

B.4.2. Results

(a) The level of Pythium populations and the identity of Pythium species in the experiment area

Soil samples, taken at 0-10 cm depth from the experimental area prior to sowing, were processed to determine the population levels of Pythium and the identity of Pythium species. Table 22 shows the number of Pythium colonies per g oven dry soil, and the occurrence of Pythium species found in each type of soil used in the experiment. The data show that a high density of Pythium spp. occurred in both soils.

Table 22 : The numbers of *Pythium* colonies per g oven dry soil, and the occurrence of *Pythium* species in both experimental areas

Grey brown soil			Red brown soil		
Nos. of Pythium colonies/g oven dry soil	Occurrence of Pythium spp.		Nos. of Pythium colonies/g oven dry soil	Occurrence of Pythium spp.	
	Pythium spp.	%		Pythium spp.	%
873	P.irregulare	28.73	657	P.irregulare	26.80
	P.ultimum	21.64		P.ultimum	24.15
	P.rostratum	18.30		P.rostratum	14.32
	P.debaryanum	11.28		P.debaryanum	9.60
	P.volutum	10.05		P.volutum	11.45
	P.echinulatum	5.40		P.echinulatum	3.95
	P.oligandrum	3.09		P.oligandrum	3.63
	Other Pythium spp.	1.51		Other Pythium spp.	6.11

Table 23 : Influence of metalaxyl (Ridomil 25 W.P.) seed treatment on emergence, height and yield of barley.

Treatment	Nos. of barley plants/ 1m*		Height of plants after 60 days (cm)**		Yield mechanical harvested kg/plot***		Yield mannual harvested kg/plot****	
	Grey Brown Soil	Red Brown Soil	Grey Brown Soil	Red Brown Soil	Grey Brown Soil	Red Brown Soil	Grey Brown Soil	Red Brown Soil
Clipper Non treated	28.22	27.38	26.92	24.46	10.65	7.80	11.92	11.00
Clipper Treated with Ridomil 25 W.P.	39.77	39.11	37.70	36.52	13.38	10.20	17.31	14.84
Forrest Non treated	31.00	31.16	33.53	31.41	10.56	7.34	14.25	10.23
Forrest Treated with Ridomil 25 W.P.	39.61	40.38	42.03	40.91	13.11	9.90	19.35	15.34

Table 23 continued

Standard Error								
Barley	0.529	1.317	0.459	0.3012	0.3435	0.7538	1.754	1.748
Barley Fung.	1.116	1.228	0.805	0.3747	0.3718	0.4487	3.048	3.319
Coefficient of Variation (%)								
Barley	1.5	3.8	1.3	0.9	2.9	8.5	1.8	2.2
Barley Fung.	3.2	3.5	2.3	1.1	3.1	5.1	3.1	4.1

* Mean value of 18 measurements
** Mean value of 60 measurements
*** Mean value of 6 measurements
**** Mean value of 18 measurements

(b) Effects of Ridomil 25 W.P. on emergence growth and yield of barley

Data summarized in Table 23 show that barley plants responded to treatment with metalaxyl. There was a visible and measurable effect of metalaxyl on emergence, growth and yield of the barley cultivars tested. Metalaxyl resulted in a significant increase ($p = 0.01$) in emergence of barley cultivars in both types of soil.

Plant height of Clipper and Forrest cultivars, measured 60 days after sowing, was also significantly increased ($p = 0.01$) in both types of soil, by the treatment with metalaxyl. The height of Clipper was increased by 40% in grey brown soil and 49% in red brown soil. Forrest responded to metalaxyl treatment by increasing the plant height by 25% in grey brown soils and 30% in red brown soil. Moreover, the root tips of plants without metalaxyl treatment had brown lesions containing Pythium oospores. The isolates from infected barley roots revealed the characteristics of P. irregulare, P. ultimum and P. volutum.

Grain yields estimated by two methods were different; as expected, the hand harvested data (Table 23) being higher. Fallen ears were picked up during hand harvesting from an area near to and corresponding to the plants harvested, but were not retrieved during the machine harvest. Clipper and Forrest barley cultivars showed a significant response ($p = 0.01$) to metalaxyl treatment in both types of soil, in both the hand and the machine harvest.

B.4.3. Discussion

It is known that Pythium spp. cause seed decay and preemergence seedling blight.

When populations of Pythium reach several hundred per g. of soil, the probability of soil infection appears likely. Thus, the growth response of barley to Ridomil 25 W.P. was observed from the beginning of plant development by a significant improvement in emergence and density. Furthermore, the greater plant height observed throughout the life of barley plants treated with Ridomil, is probably due to the protection of fine rootlets of plants by Ridomil against Pythium. According to Bruehl (1951) the most serious effect of Pythium on barley grown in pots was destruction of rootlets which absorb water and nutrients from soil. Cook and Haglund (1982) consider the effect of metalaxyl equivalent if not identical to the fumigation response in term of better emergence and vigour of plants.

Table 23 shows a significant yield increase of 45% in Clipper and 35% in Forrest (manual harvested), which leads to the conclusion that Pythium should not be considered as a "minor" pathogen as stated by Salt (1979) but as a potentially important pathogen.

C. Biological control of Pythium spp. pathogenic to barley seedlings by using the mycoparasite Pythium oligandrum

C.1. Introduction

There is increasing interest in the biological control of soilborne plant pathogenic fungi through the use of fungi which are either antagonistic towards them through antibiosis or directly mycoparasitic. Prospects seem particularly good for control of some seedling diseases by treating seeds, roots or soil with spores or mycelia of suitable species (Hoch and Abawi, 1979 ; Locke et al., 1979 ; Dutta, 1981 ; Harman et al. 1981 ; Hamdani et al., 1983).

Pythium oligandrum was first described as the cause of pea root rot in the U.S.A. (Drechsler, 1930) and has since been found on many other plants (Middleton, 1943 ; Drechsler, 1946). However it was weakly or not at all pathogenic to sugar beet (Vesely, 1977), barley, oat and wheat (Kilpatrick, 1968).

It is also a parasite on other Pythium spp. and is frequently found in association with the aggressive plant parasites, P. debaryanum and P. ultimum (Drechsler, 1946). This led Drechsler to suggest that it is perhaps not usually a primary parasite of plants, but is, instead, a secondary invader of diseased tissues and at least partly parasitic on these other fungi. In an extensive study of Pythium oligandrum ecology Tribe (1966) showed that it does not degrade cellulose and so makes only poor growth on cellophane. However, it grew well on these media in the presence of some, though not all, cellulotic fungi and formed abundant spiny oogonia. Deacon (1976) reported that P. oligandrum is an aggressive parasite of other fungi. P. oligandrum, as a vigorous necrotrophic mycoparasite, was shown to be active against P. debaryanum, P. ultimum, P. mamilatum and P. irregulare, by Drechsler (1943).

P. oligandrum, abundant in South Australian barley soils, (Table 8), is of special interest because of its aggressiveness towards Pythium irregulare, which seems to be a major incitant of root rot of barley seedlings (Chapter 4), and also the most abundant and widespread plant pathogenic Pythium species in South Australian barley soils (Table 8).

P. graminicolum and P. volutum occur in South Australian barley soils (Table 7, 8) and both species seem to be of economic importance because of their pathogenic activity towards barley seedlings. However,

so far little has been done to investigate the parasitic relationships between P. oligandrum and these two species. Considering that P. oligandrum is a strong hyperparasite on other species of Pythium mentioned before, experiments were done to see whether this hyperparasitism occurs also in P. graminicolum or P. volutum. The experiments were conducted in Petri dishes and in pots.

C.2. Parasitic relationships between Pythium oligandrum and some other species of Pythium, in vitro

C.2.1. Materials and Methods

Fungi

Hyperparasite : Pythium oligandrum, (C.M.I. No. 285718) was isolated from barley soil in South Australia.

Host : Pythium irregulare (C.M.I. No. 285717), Pythium volutum (C.M.I. No. 279307) Pythium graminicolum (C.M.I. No. 279307) isolated from diseased roots of barley seedlings.

Growth on agar

EXPERIMENT 1

Mycoparasitic relationships were investigated in vitro on agar plates at 25°C using Difco cornmeal agar (C.M.A.), 15 ml per 9 cm Petri dish. Plates were inoculated at the margin with the "host" fungi and incubated usually until plates were just completely covered. A 5 mm plug of P. oligandrum was then inoculated at the appropriate margin (on the

periphery of the host colony) and the plates re-incubated. The vegetative growth of Pythium oligandrum across colonies of the other fungi and microscopic examination of the mycelium were done at different times using the Deacon technique (1976). Three adjacent diametric strips of agar, 5 mm wide, were marked on the base of each plate such that the central one included both inoculum disks. One of these strips was cut and removed at each sampling time, the centre one being sampled last. They were cut into 5 mm segments, which were suspended in series but spaced 1 cm apart in a humid chamber. After 6 days incubation the segments were examined for the presence of spiny oogonia of P. oligandrum; each variant consisted of three replicates.

Results

Colonies of P. graminicolum covered the agar in 6 days after introducing the inoculum at the margin of the plate. P. irregulare took 4 days, and P. volutum 10 days. They were then inoculated at the margin a new growth with P. oligandrum and subsequent growth was assessed over the next 5 days. Results summarized in Table 24 indicate that P. oligandrum grew across host Pythium spp. almost at the same rate as it grew across fresh cornmeal agar plates (24.8 mm/24 h (Plate 9). Detection of an abundant number of P. oligandrum oogonia on precolonized plates but not on fresh cornmeal agar suggested that the host fungi provided nutrition for the parasite possibly due to sterols originating from lysed hyphae of the host. Furthermore, microscopic examination showed the hosts' mycelium to be enwrapped by the fine mycelial ramifications of the Pythium oligandrum penetrating in different places (Plate 10).

Plate 9 : Hyperparasite Pythium oligandrium (B) growing over six days old culture of Pythium graminicolum (A).

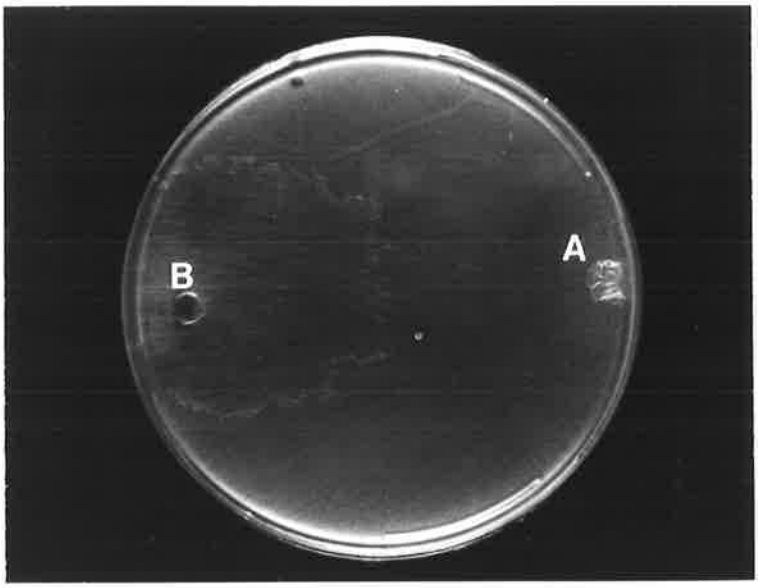

PLATE 9

Table 24 : Growth rates of *Pythium oligandrum* on plates of Difco cornmeal agar precolonized by *P. irregulare*, *P. graminicolum* and *P. volutum*.

Pythium species	Growth rate of P. oligandrum mm/24 h*
P. oligandrum alone (control)	24.8
P. oligandrum + P. irregulare	25.0
P. oligandrum + P. graminicolum	24.5
P. oligandrum + P. volutum	24.6

* means of 3 replicates plates at 25°C

PLATE 10 : Fine ramifications of the hyperparasite P. oligandrum enwrapping and penetrating the host mycelium of P. graminicolum (A) and P. volutum (B).

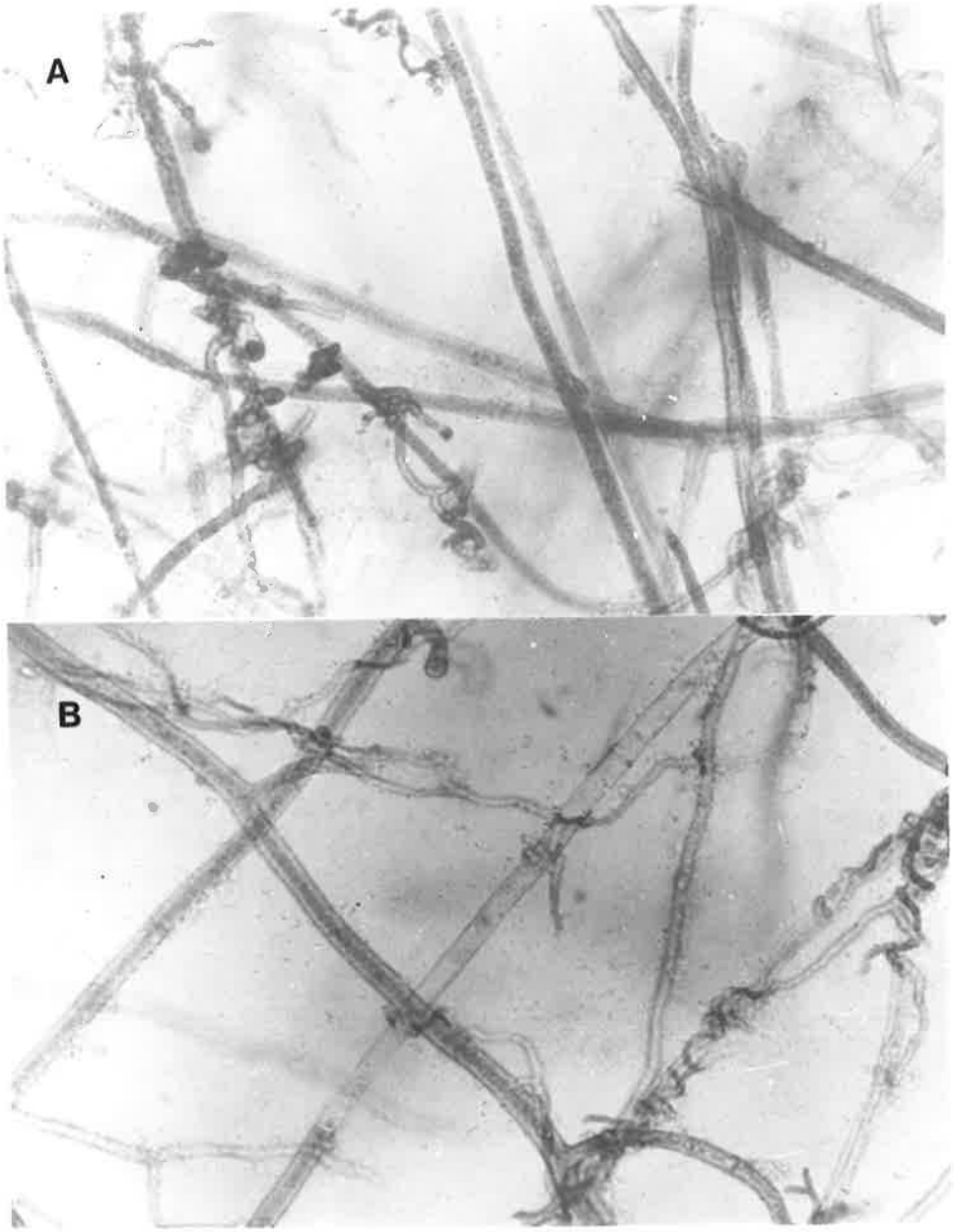

PLATE 10

EXPERIMENT 2

To investigate the different susceptibilities of the host fungi, P. oligandrum was added half-way across the fully colonized plates (on 3 day-old parts of the colonies of P. graminicolum, on 2 day-old parts of colonies of P. irregulare, and on 5 day-old parts of colonies of P. volutum, rather than at their margins). The growth of P. oligandrum into progressively older and younger regions was compared. The results (Table 25) show that the host colonies, became more resistant to parasitism (assessed by growth rate of P. oligandrum) as they matured.

EXPERIMENT 3

To study relationships between the various fungi, P. oligandrum was applied to the centre of agar plates and the other three species of Pythium were applied away from the centre on agar discs, 5 mm diameter and overgrown with the fungi. The experiment was conducted at 25°C ; there were three replicates. Biotic relationships of mycoparasitism were investigated in the following fungi : P. irregulare, P. graminicolum and P. volutum. Observations on colony growth and microscopical examination of the mycelium were done daily.

Results

In a culture containing all the three fungal species (P. irregulare, P. graminicolum and P. volutum) the same parasitic characters of P. oligandrum were found. P. oligandrum occupied the central disc and retarded the development of the other species as early as the second day after the culture was established. Three days later

when P. oligandrum was wide-spread, the growth of the other fungi had nearly stopped. There was no inhibition zone between the fungi. P. irregulare and P. graminicolum mycelia united to form a half-moon shape in regions where the mycelial growth of both species occurred in the absence of P. oligandrum. In the centre the hyphae of P. irregulare and P. graminicolum were constricted due to the parasite. The presence of P. oligandrum hyphae and the beginning of parasitism were detected microscopically seven days after the joint culture was established. The thicker hyphae of P. oligandrum began to enwrap the host hyphae and its parasitic threads were observed. Oogonia of P. oligandrum were extensively produced. Although hyphae and fructification organs of P. oligandrum occurred in all parts of the cultures of the host species, the parasitic threads of P. oligandrum were more abundant than hyphae of the host species in the regions of contact i.e. in younger parts of the colonies.

C.2.2. Discussion

The combined cultivation of different fungal species in the same culture on agar plates revealed relationships of these micro-organisms in vitro. The experimental results on relationships between P. irregulare, P. graminicolum and P. volutum, in vitro, showed that P. oligandrum was markedly destructive to these three fungal species whose colonies were susceptible when young to attack by the pathogen. However, they soon mature, and develop "mature plant resistance" (Garrett, 1970). On the other hand, Deacon (1976) showed that the ability of Pythium ultimum to support growth of the parasite P. oligandrum increased with age. The results of my experiments confirm Garrett's (1970) view that P. oligandrum is an unspecialized parasite of fungi, largely confined to

immature parts of its hosts. Its high growth rate, relatively unspecialized nutrition wide host range and necrotrophic infection habit are entirely consistent with this view. These characteristics contrast with those of specialized parasites of fungi (Barnett and Binder, 1973). This work and that of Haskins (1963) on Pythium acanthicum therefore supports conclusions on the biological status of Pythium spp. as unspecialized parasites (Garrett, 1970 ; Hendrix and Campbell, 1973).

Accordingly to Boosalis and Mankau (1970), destructive mycoparasites destroy the host or parts of it during their development. An internal encounter between the host and the parasite is established at the samc time. It may be concluded that this definition is applicable to P. oligandrum which produces numerous parasitic threads encircling the hyphae of host fungi which were entirely suppressed in a few days. Besides other species of Pythium such as P. irregulare, P. debaryamum, P. ultimum and P. mamillatum, already described by other authors to be parasitized by P. oligandrum, the results of my experiments showed that it was also parasitic and pathogenic on P. graminicolum and P. volutum.

C.3. Biological control of Pythium spp. that induced root disease in barley seedlings by treating the seeds with the myco-parasite, Pythium oligandrum.

The occurrence of Pythium oligandrum in South Australian barley soils (Table 8) led to the examination of the hyperparasitic characteristics of the fungus by means of dual culture in vitro. My previous observations on agar plates showed that P. oligandrum is a strong parasite of the main pathogens inciting root diseases in barley, P. irregulare, P. graminicolum and P. volutum. The next stage of the study involved pot experiments to ascertain whether an effective and

reliable protection of newly germinated barley plants against Pythium spp. could be achieved using P. oligandrum.

C.3.1. Materials and Methods

(a) Preparation of seed coated with P. oligandrum

An isolate of P. oligandrum used in previous experiments and maintained on cornmeal agar at 25°C, was used as a mycoparasite. The seeds were coated with P. oligandrum as follows : Mycelial mats bearing many oospores of P. oligadrum were obtained by growing the fungus in liquid culture on supplemented V8 tomato juice (Ayers and Lumsden, 1975). This comprised 200 ml V8 juice, 30 mg cholesterol added as a 1.5% solution in 95% ethanol, 2.5 g $CaCO_3$ solution (clarified by centrifugation at 13200 g) all made up to one litre with distilled water prior to autoclaving. Aliquots of 15 ml were dispersed into 9 cm - diameter Petri dishes which were then each inoculated using a single (0.5 cm - diameter) disc cut from a colony growing on cornmeal agar. The cultures were incubated for 10 days at 25°C in darkness. Mycelial mats were then removed, washed in sterile distilled water and fragmented in distilled water using a glass tissue homogenizer. The oospore load in seed coatings was between 180 - 350 per seed. This preparation of mycelial fragments and intact oospores was stored at 5°C until required for use. An equal volume of a 3% solution of carboxymethyl cellulose (CMC) was added to 100 ml of the preparation of mycelial fragments and oospores. After thorough mixing, the seeds of barley (Cv. Clipper) were added. These had previously been surface-sterilized in 1% sodium hypochlorite-absolute alcohol 2:1 (v/v) for 5 minutes then rinsed in sterile distilled water. After being allowed to soak for 2 minutes in the

preparation, the seeds were removed, spread in sterile open Petri dishes, and allowed to dry overnight in a laminar flow cabinet at 18-21°C. Batches of seed were also prepared using a fungus-free, carboxy-methyl cellulose coating. All seeds were then used immediately in experiments.

(b) Control of *Pythium* spp. inducing root diseases of barley seedlings in artificially infested soil.

Isolates of *Pythium irregulare*, *P. graminicolum* and *P. volutum* were obtained from diseased barley roots. All three species isolated were highly pathogenic towards barley (Chapter 4.B.2.). Inoculum was prepared using vermiculite (Chilvers, 1962). Samples of vermiculite (10 g each, grade DSF) were placed in 250 ml conical flasks and moistened with 35 ml V8 tomato juice diluted 1:3 with distilled water. After autoclaving, flasks were inoculated with either *P. irregulare* or *P. graminicolum* or *P. volutum*, using single, 0.5 cm-diameter discs cut from a 7 day-old stock colony growing on cornmeal agar. The cultures were incubated for 7 days at 25°C. The flasks were vigorously shaken by hand each day to ensure mixing of the contents. The trial was carried out using 11 cm high and 8 cm diameter pots, each containing 300 g of sterile sandy loam field soil (adjusted to 80% of field capacity with distilled water). Inoculation was achieved by mixing 7 day-old vermiculite medium of each *Pythium* species with potting soil at a rate of 1% w/w. The soil surface was made smooth and, each pot was sown with 5 barley seeds approximately 1 cm deep. There were four replicates per treatment, and all were inoculated in a controlled environment chamber at 15°C with alternating periods of 12 h light and dark. Water lost through evaporation and transpiration (as determined by weighing each pot) was replaced each day. The experiment lasted 4 weeks, and at the end of the experiment

measurements of root length were made by using a Comair Root Length Scanner. The treatments were as follows :

Seed coated with P. oligandrum sown in soil infected with :

1. P. irregulare
2. P. graminicolum
3. P. volutum

As controls seed coated only with carboxymethyl cellulose sown in soil infected with

1. P. irregulare
2. P. graminicolum
3. P. volutum

This factorial experiment was arranged in a randomised complete block design.

Results

The results of the experiment given in the Table 26 show that P. oligandrum applied to barley seed is associated with increased root growth probably because that fungus actively suppresses the pathogenic species of Pythium such as P. irregulare, P. volutum or P. graminicolum. The rhizosphere of emerging barley plants is probably colonized by a large number of micro-organisms, the presence of which may either inhibit or stimulate the activity of P. oligandrum and so its influence on populations of other fungi. Mycoparasites may also compete with other organisms for essential nutrients. Thus, the use of mycoparasites in biological control by its introduction into the soil may be unsuccessful

Table 26 : The influence of seed treatment of barley with Pythium oligandrum on root length of plants after 4 week.

Treatment	*Mean total root length of barley seedlings 4 weeks old (m)		
	P. irregulare	P. graminicolum	P. volutum
Seed coated with carboxymethyl cellulose (control)	6.03	5.09	6.56
Seed coated with Pythium oligandrum	9.62	8.06	9.35

L.S.D. = 2.14 p = 0.01

* Mean of 4 replicates

because other micro-organisms may inhibit their activity. Therefore, further experiments were done in pots using naturally infected soil.

(c) Control of *Pythium* spp. inducing root rot diseases in barley seedlings in naturally infected soil.

The aim of this experiment was to study the efficacy of the mycoparasite *P. oligandrum*, applied as a seed treatment in naturally infected soil, in controlling pathogenic species of *Pythium*. The experiment was done using sandy loam soil, pH 7.5, naturally infected with *P. irregulare* and *P. graminicolum*. The soil was used in previous studies and was known to contain pathogenic species of *Pythium* at levels sufficient to cause serious root rot in barley seedlings. Investigations on the occurrence of *Pythium* species in this soil failed to detect the presence of *P. oligandrum*, therefore, it was assumed to be absent.

The root and shoot lengths of barley seedlings were recorded. The materials and method used are as described in the previous experiment (section b). The study was conducted using a randomized complete block design with 2 treatments and four replicates.

A microscopic examination for the presence of *Pythium* oospores was done by taking 1 cm pieces of healthy and apparently diseased roots of each treatment. The pieces were cleared in a mixture of lactophenol and chloral hydrate stained in trypan blue, squashed and examined under the microscope.

Results

When the seedling roots were examined after 4 weeks, the typical pale lesions of Pythium root rot occurred within several cm of the root tip on the plants grown without seed treatment with P. oligandrum. Microscopic examination of cleared roots showed that the characteristic thick walled Pythium oospores were about 70% more frequent in roots of barley seedlings without P. oligandrum. The results of the experiment (Table 27) show the effectiveness of the protection of treated seeds with P. oligandrum when expressed as root and shoot length.

There were significant differences in root length ($p = 0.01$) and shoot length ($p = 0.05$) between plants from treated seeds and plants from seeds without P. oligandrum. The root and shoot length of plants from biologically treated seeds, was higher by 36% and 20% respectively.

C.3.2. Discussion

The parasitic ability and the aggressiveness of P. oligandrum towards the other species of Pythium tested were demonstrated in sterile sandy loam soil infected separately with P. irregulare, P. graminicolum and P. volutum, where the mycoparasite exerted a strong influence on active hyphae. The effectiveness of P. oligandrum in controlling other species of Pythium in non sterile soil indicates its ability to establish itself rapidly on its hosts in the face of possible microbial antagonism and so to provide protection for the barley seedlings against subsequent disease. According to Hamdani (1983) oospores are of major importance both for survival on seeds and for the subsequent establishment of hyphae around seed and newly formed roots. My trials

Table 27 : The influence of seed treatment in barley with *P. oligandrum* on root and shoot lengths of barley plants.

Seed Treatment	Total Root length of barley seedlings 4 weeks old grown in naturally infected soil (m)	Shoot length of barley seedlings 4 weeks old grown in naturally infected soil (cm)
Seed coated only with carboxymethyl cellulose	24.13	21.24
Seed coated with *P. oligandrum*	32.85	25.55
	L.S.D. = 1.50 p = 0.01	L.D.S. = 1.85 p = 0.05

support this view and suggest that biological protection of emerging barley seedlings with P. oligandrum against Pythium infection is indeed feasible.

CHAPTER 8

GENERAL DISCUSSION

As indicated in the Introduction the main aims of this study were to determine what species of Pythium occurred in South Australia ; to assess their relative pathogenicities on barley and the economic importance of the most abundant species ; to assess the influence of environment on Pythium spp. and to seek ways of controlling this fungus. Further aims arose during the course of the research e.g. to assess the pathogenic significance of P. volutum on barley.

The experiments using the different species of Pythium clearly indicate that it is necessary to determine what species are involved in a particular area where barley is grown as their pathogenicities are quite different ; some, like P. irregulare, are highly pathogenic whereas others, like P. oligandrum, are beneficial because of their mycoparasitic qualities. Furthermore, the relationships between the level of soil water and growth inhibition in barley varies with Pythium spp. The inhibitory effects of the most pathogenic species are associated with wet cool soils when the barley seedlings have reached the emergence stage.

The factorial experiment with the nematode Pratylenchus thornei, an abundant parasite of barley in South Australia, failed to show that it interacted with Pythium irregulare to cause serious damage. The two pathogens were independent in their effect on the host plant. The experiment did reveal, however, that P. irregulare was more pathogenic in barley (cv. Clipper) than in wheat (cv. Festiguay) and suggests that

it may be worthwhile to seek resistant or tolerant cvs. of barley. The fact that *P. irregulare* at low populations appeared to stimulate growth of wheat may indicate that the particular cv. used in the experiment (Festiguay) was able to compensate or even overcompensate for damaged roots by regenerating new roots.

The discovery of *P. volutum*, the first time it has been recorded in Australia, raised the question whether it had been recently introduced and whether because of its known pathogenicity, it posed a threat to the barley crop. Studies on this species confirmed its pathogenic properties as a root-rot fungus and its association with wet soils. However, further research is needed to determine its distribution and abundance in South Australia and its economic importance in the field.

Studies on *Pythium* populations in the field and subsequent experiments on the influence of various environmental factors gave no clear picture of the environmental components that influence the incidence of this fungus in barley fields. There was an indication that population levels increase from north to south of South Australia as annual rainfall increases and temperature decreases. This does not mean that *Pythium* spp. will be most damaging in the south, however, as the study on *P. volutum* indicated. More research is needed on the ecology of different *Pythium* spp. to answer these questions.

The studies on control of *Pythium* yielded clear-cut results. In the field, effectiveness of metalaxyl in increasing barley yields was clearly demonstrated. However, similar experiments need to be done in other years and in other areas of South Australia before it can be concluded that this fungicide is an effective and economic treatment for

barley crops infested with Pythium. Furthermore, the clear response of barley to metalaxyl in the field may not be entirely due to control of Pythium and this aspect needs to be clarified before conclusions are drawn on the economic importance of Pythium spp. in barley in South Australia. Further field experiments are currently under way.

A similar cautious view should be taken of the biological control experiments with P. oligandrum. The results in vitro and in pots were clear-cut but they only indicate a possible candidate for further research on a field scale.

There are many soil pathogens affecting the growth of yield of cereal crops and although Pythium has been the object of study by mycologists for a long time it has received relatively little attention in South Australia. The results of my studies suggest that Pythium spp. are probably one of the more important contributors to unthrifty barley growth in South Australia and deserves further study to assess their economic importance. In this respect my results support the views of Cook and Haglund (1982) that Pythium is an important pathogen and in some cases may even be the most important cause of reduced cereal yields.

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APPENDIX

Plant and Soil 00, 000–000 (1985). Ms. 5923

The influence of Pythium on the growth of barley seedlings as affected by soil water and inoculum density

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Received 16 May 1984. Revised November 1984

Key words Barley Inoculum density Interaction Pathogenicity Pythium Soil water

Summary *Pythium* spp. were more abundant in the southerly and more temperate regions of the barley growing region of South Australia than in the drier and hotter north. Populations were more abundant in the top 10 cm than in the 10 to 20 cm soil zone. Eleven species of Pythium were identified from barley crops. *P. irregulare* appeared to be the most abundant and was one of the most pathogenic species on barley. *P. volutum* was also highly pathogenic; it had not been recorded in South Australia before. A factorial experiment using nine *Pythium* spp. and four levels of soil water indicated that unlike other species, *P. irregulare*, *P. volutum* and *P. graminicolum* were most pathogenic in soils with a water content close to field capacity. A factorial experiment using *P. irregulare* at four levels of soil water and six inoculum levels showed that inhibition of growth in barley seedlings by *P. irregulare* increased as the level of water in the soil increased. The experiments support the hypothesis that inhibition of growth of barley seedlings by *Pythium* spp. is most severe in the southerly parts of the barley growing area of South Australia particularly where there is a combination of high soil water and high population densities.

Introduction

Many authors have suggested that *Pythium* spp. are of economic importance in cereal crops[1]. Moreover, barley may be more susceptible to Pythium root rot than wheat[8] especially after emergence[3]. The influence of soil water on *Pythium* spp. and the disease they cause is important. For example, *P. ultimum* has one of the highest soil water requirements for growth of soil-borne fungal pathogens[2]. Little research has been done on the influence of Pythium in cereals in Australia although fourteen species have been described from Queensland where *P. aphanidermatum* is pathogenic on barley[6]. Barley is grown in the southern part of South Australia where rainfall is higher and temperatures are lower than in the arid north. Within the barley growing area annual rainfall increases and temperatures decrease from north to south. Such climatic differences are likely to influence the soil environment, particularly the level of soil water and so, in turn, influence the abundance of Pythium and the amount of disease it causes in the barley crop. The aims of the research described in this paper are (1) to compare the abundance of Pythium at barley sites in different

climatic regions; (2) to identify the species of Pythium occurring in barley crops; (3) to determine the influence of soil water on the effects that different *Pythium* species and inoculum levels have on the growth of barley seedlings.

Materials and methods

Soil samples

Samples were collected monthly from September 1981 to August 1982 from three sites containing barley crops: Virginia and Roseworthy in the north and Stathalbyn in the south. Four areas 50 m square, were sampled at each site. Thirty subsamples were collected at random within each square and bulked to form one sample. Thus, four samples were obtained from each site. The soil in each sample was sieved through a 1.68 mm mesh screen and mixed thoroughly. Soil samples were stored at 4°C and processed to assess Pythium populations within 2 days of collection.

Assessment of Pythium populations

Two methods were compared, Warcup's soil plate method[9] and a soil dilution plate method. The soil plate method was chosen as it gave more consistent results. 10 mg of soil were transferred to petri dishes into each of which 10 ml of cornmeal agar, cooled to 40°C were poured. Soil particles were dispersed by gentle rotation before the agar solidified. Selective media[5,7] were used. Twenty five replicate places were used per sample. Plates were incubated in the dark at 25°C and numbers of colonies were counted 24 and 48 h later and recorded as the number of colonies per g of oven dried soil. Rainfall and temperature data for the three sites were obtained from the Bureau of Meterology.

Identification of Pythium species

Hyphal tips were transferred from all Pythium colonies to cornmeal agar (CMA). The plates were incubated for 24 to 48 h at 25°C and then a second hyphal tip, single sporangium or oogonium was transferred to a new CMA plate for identification. A key[4] was used for identification. Stock cultures of *Pythium* spp. were maintained by periodic transfers.

Pot experiments

All barley seed used originated from a single seed lot of the cv Clipper. Damaged seeds were removed and planting tests showed the seed to be free from fungal infection. Seeds were surface sterilized in 1% sodium hypochlorite – absolute alcohol 2:1 (v/v) then rinsed in sterile distilled water immediately prior to use. There were 3 seeds per pot. A sandy loam field soil was sterilized and 300 g were placed in each pot. Pots, 11 cm high and 8 cm in diameter allowed uninhibited root growth of seedlings.

Inoculum

The nine species of Pythium used in the pot experiments were grown separately on Difco cornmeal agar at 25°C for 48 h. Two plugs of medium were taken from the margin of the growing cultures and inoculated into sand cornmeal culture medium (5 g cornmeal, 95 g sand, 15 ml sterile distilled water). Inoculation was achieved by mixing 4 week old sand cornmeal medium of each *Pythium* species with potting soil at a rate of 1% w/w prior to sowing barley seed. All experiments were done in a growth chamber at 15° ± 1°C with 12 h light per day. Soil in pots was maintained at the required level of soil water (% field capacity) by daily weighing of pots. Experiments lasted 4 weeks and at the conclusion seedlings were removed from the soil, washed and measurements of length and dry weight of shoots and roots were made. There was a close correlation between length and dry weight for both shoots and roots.

Statistics

Data were analysed by analysis of variance, regression and Duncan's multiple range test using appropriate transformations to normalize data.

Results and discussion

Preliminary survey of Pythium populations at three sites

Soil samples, taken at 2 depths from 3 barley sites from September 1981 to August 1982 were processed to determine population levels of *Pythium* spp. The sites were chosen for their different climates. Virginia and Roseworthy have a lower mean annual rainfall and higher temperatures than the more southerly site in Strathalbyn. Barley crops were sown in May 1981 and harvested in December 1981. As indicated in Fig. 1, populations in the top layer of soil 0 to 10 cm deep were consistently higher than those at 10 to 20 cm. Populations were also higher in Strathalbyn than at Roseworthy or Virginia. These observations suggest that Pythium probably has its greatest inhibitory effect on the growth of barley at germination and emergence when roots of seedlings are growing through the upper soil layers that contain the highest densities of the fungus. The results also suggest that the degree of inhibition of plant growth might be associated with the cooler and wetter conditions at Strathalbyn.

Regression analysis of population levels of Pythium against time indicated that at Roseworthy and Strathalbyn population levels increased from September 1981 to August 1982 in the 0 to 10 cm soil zone but decreased at 10–20 cm. No statistically significant regressions with time were evident at Virginia. Superimposed on such long term seasonal trends were monthly fluctuations. To try and account for such variations, regression analyses of population levels of Pythium against mean monthly temperature and mean monthly rainfall at each site were done. There were no statistically significant regressions at Strathalbyn. At Virginia population levels in the 10 to 20 cm zone increased with temperature whereas at Roseworthy, population levels at 0 to 10 cm decreased with increasing temperature and rainfall. In the 10–20 cm layer populations increased with temperature as at Virginia. Apart from indicating that temperature in the surface layers of soil in the more northerly regions may reduce Pythium populations and that population increase may occur at greater depths, the data did not support the hypothesis that mean monthly temperature and rainfall accounted for monthly fluctuations in Pythium populations. It is possible that recordings of soil water and soil temperature rather than meterological data are required and that the influence of other factors such as pH and salinity should be assessed. Furthermore,

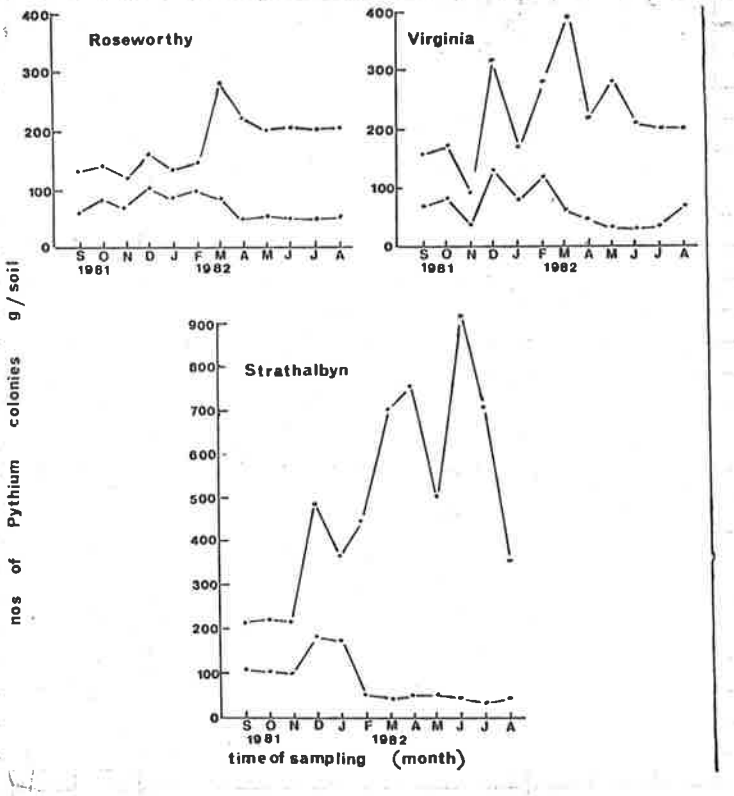

Fig. 1. Population densities of Pythium at three sites, Roseworthy and Virginia in the north and Strathalbyn in the south of the barley growing region of South Australia. Populations were assessed monthly at two soil depths, 0–10 cm (*upper graph*) and 10–20 cm (*lower graph*). Letters on the *abscissa* denote the first letter of the month.

populations of the genus Pythium at the three sites may contain several species that respond to environment in different ways.

Species of Pythium found in barley growing areas

Pythium species were isolated from soil containing barley plants and from barley roots. Eleven species were identified: *P. rostratum, P. debaryanum, P. acanthicum, P. echinulatum, P. oligandrum, P. paroecandrum, P. irregulare, P. volutum, P. graminicolum, P. iwayamai,* and *P. vexans.* All species were isolated from both soil and roots except *P. graminicolum* which was found only in roots. The most common species were *P. irregulare, P. debaryanum* and *P. rostratum. P. volutum* has not been recorded in South Australia before and because it appears to be one of the most pathogenic species, further studies are required to ascertain whether it is a recent arrival that might pose a threat to

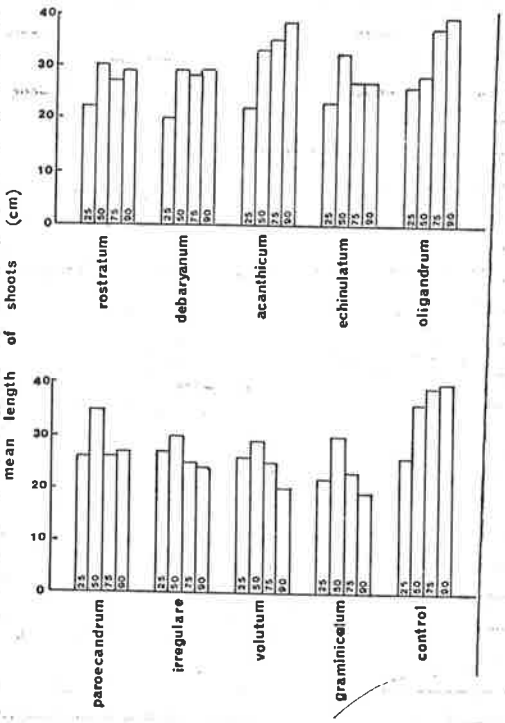

Fig. 2. The influence of nine species of Pythium on the mean height of shoots of barley seedlings 4 weeks after sowing at four levels of soil water, 25, 50, 75 and 90% field capacity. There were five replicates per treatment.

barley crops or an old inhabitant which is less abundant than other species.

The relationship between soil water and nine species of Pythium on the growth of barley

The influence of nine species of Pythium and a control treatment with no Pythium on the dry weights and lengths of shoots and roots of barley seedlings indicated that some species were significantly ($p < 0.01$) different from others in their effect on the seedlings. There were close correlations between dry weights and lengths and between roots and shoot measurements, hence, results in Fig. 2 show only the influence of *Pythium* species on mean length of shoots. A statistically significant interaction ($p < 0.01$) was obtained between *Pythium* species and soil water on length of shoots, hence it is necessary to discuss relative pathogenicities of the nine *Pythium* species in terms of the response to soil water. The interaction is explained by the markedly high inhibition to plant growth caused by *P. irregulare*, *P. volutum* and *P. graminicolum* at the higher levels of soil water.

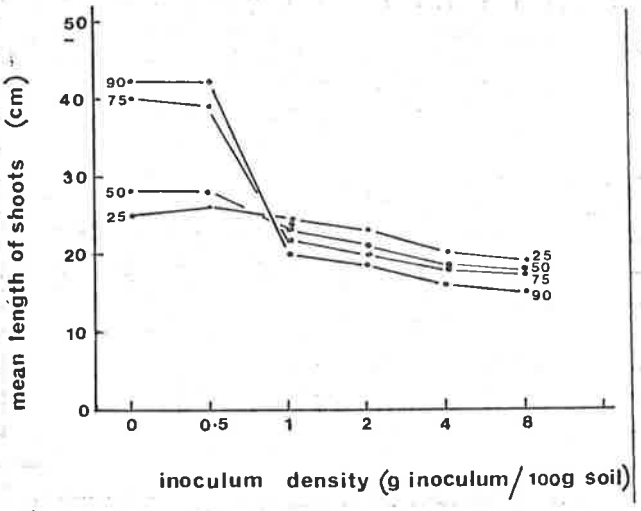

Fig. 3. The influence of inoculum density of *Pythium irregulare* on the mean height of shoots of barley seedlings 4 weeks after sowing, at four levels of soil water 25, 30, 75 and 90% field capacity. There were five replicates per treatment. Numerals at the ends of the graphs denote % field capacity.

In the controls and most of the other *Pythium* species shoot growth increased as soil water increased. In practical terms it is possible that the most serious reductions in barley growth due to Pythium occur when soils are close to field capacity, and where population densities of such species as *P. irregulare* are high.

The relationship between soil water and inoculum density of Pythium irregulare *on the growth of barley*

Six inoculum levels, similar to those found under field conditions, and four levels of soil water with five-fold replication were used in a factorial experiment. Length and dry weight of shoots and roots were measured. As lengths and weights and roots and shoots were closely correlated only the results for shoot length are given (Fig. 3). Analyses of variance indicated that for all measurements of shoots and roots, soil water and inoculum density had significant ($p < 0.01$) effects. There was also a significant interaction ($p < 0.01$) between soil water and inoculum density because *P. irregulare* appears to be particularly inhibitory at certain levels of soil water. A Duncan's multiple range test was used to help interpret the data and the following conclusions were drawn: (1) As inoculum density increased barley growth decreased. (2) As soil water increased from 25 to 90% of field capacity barley growth decreased except in the uninoculated controls and the

lowest inoculum level of 0.5 g of inoculum/100 g soil where growth markedly increased. This different response at the low and high inoculum levels was responsible for the interaction. This result supports the hypothesis that at least for *P. irregulare* inhibition in growth of barley seedlings is associated with high population levels of the fungus and soils approaching field capacity. The biological explanation of the interaction is open to several interpretations which only further studies can elucidate. Thus, high levels of soil water may reduce the resistance and tolerance of the plant to attach by *P. irregulare*, or infection by the fungus may reduce the tolerance of the plant to high soil water levels and consequent lack of aeration or soil water may influence the infectivity of *P. irregulare* through the motility of its zoospores if they are produced.

Acknowledgement The authors thank Dr J H Warcup and Mr M J Bumbieris for advice on the identification of *Pythium* spp. and Miss S Wayte for assistance with statistical analyses.

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APPENDIX

Explanatory Note

Percent Field Capacity

The procedure adopted to obtain four levels of soil water in the experiment described on Page 53 was to determine the moisture characteristic of the soil i.e. the relationship between the matric potential and soil water content, to select a matric potential (or suction) at which most of the water had drained from the soil (designated as field capacity by Bratoloveanu) and then to select levels of matric potential that give an adequate coverage of the release curve. Such a procedure enables soils to be controlled over a range of water contents. No attempt was made to relate the actual value of matric potential to the pathogenicity of the nine species of *Pythium*; the hypothesis was less precise than this and was mainly concerned with the question whether the different *Pythium* species behaved similarly at the different soil water levels and whether there were any statistical interactions between soil water content and *Pythium* species on shoot growth. The results of this experiment were accepted by the referees of "Plant and Soil" where this work was published.

Metalaxyl Treatments

Concentrations of 250, 400 and 750 ppm a.i. of metalaxyl were used and similar concentrations have been used by other workers in agar assays for *Sclerotinia sclerotiorum*, a fungal pathogen of several vegetable crops. Dr. Cook is correct in saying that the published ED50 value for *Pythium* is about 1 ppm. I can only assume that the ED50 value is questionable or the strains of *Pythium* used in the experiments are more resistant to the fungicide than those used to determine the ED50.