A COMPARISON OF THE OCCURRENCE, SPORULATION AND SURVIVAL OF *Phytophthora cinnamomi* RANDS IN SOILS SUPPORTING NATIVE FOREST IN SOUTH-EASTERN NEW SOUTH WALES AND SOUTH-WESTERN WESTERN AUSTRALIA

by

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This thesis is my original work and contains no material previously published or written by another person except where acknowledged in the text.

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ABSTRACT

Four aspects of the behaviour of *Phytophthora cinnamomi* in soil collected from healthy and diseased sites at Durras in south-eastern New South Wales and Jarrahdale in south-western Western Australia were examined.

The occurrence and distribution of *P.cinnamomi* in these two areas was compared using two different sampling and isolation techniques over a 16 month period. Samples of soil and fine roots collected from along a transect from the New South Wales site yielded *P.cinnamomi* when baited using the lupin seedling technique while similar baiting of comparable samples from Western Australia failed to yield the organism. Direct plating of samples of upper roots and root collars of recently dead *Banksia grandis* from Western Australian sites yielded *P.cinnamomi* while the organism was not isolated from comparable samples of chloratic *Macrozamia communis* collected at the New South Wales site. These results suggested that the form of occurrence of *P.cinnamomi* and its association with disease in Australia varies in different situations.

A comparison was made of the microbial populations in soil from healthy and diseased forest at both sites using the soil dilution plate technique. There was a general association between low microbial populations and the occurrence of disease. However, it could not be ascertained whether disease occurred in areas where lower

microbial populations existed or whether microbial populations dropped as a result of disease incidence. An inverse association was demonstrated between the frequency of isolation of *P.cinnamomi* and the population of the *Aspergillus* + *Penicillium* group of microorganisms.

The survival of chlamydospores and mycelium of *P.cinnamomi* in the field and in soil subjected to three moisture regimes was investigated using soils from diseased and healthy forest at both sites. The moisture regime appeared to have a greater effect on the survival of the fungus than the particular soil employed. *P.cinnamomi* survived for three to four months in the field when the soil was moist and for a comparable period in the laboratory in soil maintained at -5 kPa. *P.cinnamomi* may have survived even longer in waterlogged soils but the fungus could not persist for three weeks in soils which were allowed to air dry. These results provided an explanation for the observed pattern of ready isolation of *P.cinnamomi* from soil samples collected from Durras in New South Wales in contrast with those from Jarrahdale in Western Australia.

The sporangial inducing ability of soils from healthy and diseased forest at Durras and Jarrahdale were examined. It was concluded that there was probably no major difference in the sporangial inducing ability of these soils because soils as distinct as washed river sand and garden loam induced comparable numbers of sporangia on mycelial mats of *P.cinnamomi* inserted directly in soil.

It was suggested that the sporangial inducing principle *per se* had little potential epidemiological significance in *P.cinnamomi* induced disease except in exceptional circumstances. Using three different techniques it was demonstrated that the results of experiments studying sporangial induction performed on separate occasions were not comparable because of variation in the sporangial producing capacity of different cultures of a single *P.cinnamomi* isolate. Hence, experiments were not reproducible.

The ecological and management implications of the differences in the behaviour of *P.cinnamomi* in the two areas studied were discussed and some future research priorities were briefly outlined.

CONTENTS

CHAPTER 1

THE EXTENT OF THE *Phytophthora cinnamomi* PROBLEM IN AUSTRALIA AND THE SCOPE AND AIMS OF THE INVESTIGATION UNDERTAKEN

1.1 THE *P.cinnamomi* PROBLEM IN AUSTRALIA

Fifteen years ago, Podger *et al* (1965) associated the root rotting fungus *Phytophthora cinnamomi* Rands with dieback disease occurring in the *Eucalyptus marginata* Donn. ex Sm. forest of Western Australia. Later, this fungus was recognised as the causal agent of jarrah dieback disease in Western Australia (Podger, 1968) and eucalypt dieback on the Brisbane Ranges of Victoria (Weste and Taylor, 1971; Weste *et al*, 1973). Several other eucalypt forest diseases, referred to as eucalypt diebacks, have been recognised and in most instances, *P.cinnamomi* has been implicated at some stage during the investigation of their cause (Podger, 1975).

No national inventory exists of the total area affected by *P.cinnamomi* induced diseases. However, by 1975, 10% or 172,000 ha of the government controlled jarrah forest was estimated to be infected (Shea and Malajczuk, 1977). *P.cinnamomi* induced disease also causes severe losses in horticultural crops e.g. avocado (Broadbent, 1977) and pineapple (Pegg, 1977) and in a wide range of nursery plants.

In addition to the loss of productive timber and horticultural trees, *P.cinnamomi* induced diseases have eroded the aesthetic and recreational appeal of a significant portion of Australia's forested area. In Western Australia the greatest potential threat is the effect that deforestation consequent to root rotting may have on the salinity of the water supply of the most densely populated area of that State (Shea *et al*, 1975).

These problems have stimulated a sustained research effort by more than thirty research workers throughout the country. Knowledge of the disease in Australia and relevant contributions from overseas researchers can be categorised as follows:

(i) the biology of *P.cinnamomi*, i.e. the taxonomy, genetics and morphology of the fungus and the effect of physical and nutritional factors on the organisms' functional organisation;

(ii) the behaviour of *P.cinnamomi* in the soil. This includes the occurrence, survival and sporulation of the fungus in soil and non-living organic matter and its interactions with the physical and biotic environment of the soil;

(iii) *P.cinnamomi* and the host plant; the infection, penetration and establishment of the fungus and the resistance mechanisms or mode of disease escape by the host.

This thesis is concerned with certain aspects of the biology and behaviour of *P.cinnamomi* in soil. The following review summarises the current knowledge of the behaviour of *P.cinnamomi* in the soil environment. This account attempts to highlight the areas deemed important to our understanding of the aetiology and epidemiology of *P.cinnamomi* induced disease and to expose the gaps in our knowledge. Very few of the multitude of interactions between *P.cinnamomi* and the soil environment have been investigated, hence, the picture is fragmented and different points of view exist. However, this format elucidates the reasons for undertaking the study far better than a straight forward review of the literature of which there are many excellent examples covering various aspects of the topic (Waterhouse, 1963; Hickman, 1970; Gallegly, 1970; Zentmyer and Erwin, 1970; Newhook and Podger, 1972; Podger, 1975; Old, 1979a).

1.2 *P.cinnamomi* IN THE SOIL

Knowledge of the occurrence and distribution of *P.cinnamomi* in soil is hampered by the lack of adequate techniques to detect and especially to quantify the pathogen population. The most successful detection techniques are based on live baits (Kuhlman, 1964; Chee and Newhook, 1965; Pratt and Heather, 1972; Marks and Kassaby, 1974). Plant baits used together with serial end-point dilution of soil samples, have been employed to quantify the *P.cinnamomi* population in soil (Marks *et al*, 1973; Weste and Ruppin, 1977). However, plant baits are

not necessarily infected from soil samples containing the fungus, hence, there is an unknown margin of error. A variety of other techniques have been employed; direct plating of soil particles and soil dilution plating (Hendrix and Kuhlman, 1965; Tsao, 1960), soil sieving in conjunction with direct plating (McCain *et al*, 1967) and sieving and direct plating in conjunction with the use of fluorescent stains (Malajczuk *et al*, 1978).

Even when the most convenient techniques are employed only a small percentage of the soil from an area under investigation can be tested for *P.cinnamomi*. In addition, none of the techniques measure the activity of the fungus at the time of sampling. Hence, studies examining the occurrence of *P.cinnamomi* in field soil generally only determine the presence or putative absence of the organism. Isolation of the fungus from soil suggests that the pathogen was operative in the past and possibly at the time of sampling. However, in some situations these techniques do not even register the past activity of *P.cinnamomi* because the temporal distribution of the fungus may vary greatly e.g. *P.cinnamomi* is believed to be an ephemeral organism in the jarrah forest soils of Western Australia (Shea, 1979b), and is discontinuous in space and time in areas of the south coast of New South Wales (Arentz, 1974).

Baiting techniques do not reveal the position of *P.cinnamomi* in the soil matrix* (whether it is in the soil itself, decaying organic matter or living roots) or the dominant form (propagule type) of the fungus. Hence, knowledge about the behaviour of *P.cinnamomi* in the soil matrix (whether colonising living tissue, surviving as resting propagules or living saprophytically) is derived mainly from laboratory studies.

The protected niche of the plant root is possibly the best starting point from which to view the behaviour of *P.cinnamomi* in the soil matrix. This habitat provides a buffer to the soil physical, chemical and microbiological environment so the behaviour of *P.cinnamomi*, its penetration and establishment in living root tissue depends predominantly on host-parasite interactions which are presumably partly environmentally determined. Tippett (1978) reviewed related work in this field and instigated important studies using Australian native species (Tippett and O'Brien, 1976; Tippett *et al*, 1976; Tippett *et al*, 1977). The topic is not directly related to this study and will not be pursued except to say that host-parasite interactions, by definition, must influence the quantity and type of *P.cinnamomi* propagules formed and thus the type and quantity of inoculum in the soil matrix.

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*soil matrix is defined as the material generally included in soil samples collected for baiting *P.cinnamomi* i.e. mineral soil, dead and decaying organic matter and living fine root systems.

The level of inoculum and the type of host response i.e. whether the fungus attacks only the fine feeder roots or colonises the secondary tissue as well e.g. *B.grandis* (Shea, 1979b), will have epidemiological significance. However, when the host root dies, the fungus will be subjected to similar soil environmental pressures and will face the same behavioural options irregardless of the mode of root colonisation.

Sporangia, hyphae and chlamydospores of *P.cinnamomi* are commonly observed in or on infected roots (Tippett, 1978). The subsequent behaviour of the fungus depends on the type of propagule examined. Sporangia provide the potential means of continuing the parasitic growth habit because they produce the major infective agents (zoospores) in soil (Byrt and Holland, 1978). Many factors affect the production of sporangia e.g. temperature, pH, light and nutrition (Zentmyer and Marshall, 1959; Chee and Newhook, 1965), bacteria (Zentmyer, 1965; Chee and Newhook, 1966; Ayers, 1971; Ayers and Zentmyer, 1971; Broadbent and Baker, 1974a), soil moisture and aeration (Reeves, 1975; Sterne *et al*, 1977). However, to stimulate sporangia most studies employed axenically produced cultures of *P.cinnamomi* and non-sterile soil extracts or fungal mats placed in soil. Hence, factors affecting the production of sporangia at the root surface are unknown although the studies cited suggest that high soil moisture potential, adequate aeration and a bacterial stimulus are necessary prerequisites.

Sporangia produced at the root surface are subject to the effects of the soil microflora. Malajczuk *et al* (1977) reported the production of abortive sporangia in soils suppressive to the development of *P.cinnamomi* induced disease (Broadbent and Baker, 1974b; Malajczuk, 1976). In addition, breakdown of sporangia and lysis by bacteria has been observed in these soils (Broadbent and Baker, 1974a; Malajczuk *et al*, 1977). Healthy sporangia may germinate directly by germ tube or indirectly by zoospores (Zentmyer and Erwin, 1970). Direct germination has received little attention but may be significant when conditions are unsuitable for indirect germination e.g. in the absence of free water (Zentmyer and Erwin, 1970).

The production of zoospores and their subsequent behaviour has been closely studied because in the short term they provide the means of increasing the disease-producing capacity of the fungus. Cytoplasmic differentiation of the sporangium produces a large number of biflagellate zoospores which, after a brief chilling period, are released into the soil solution (Podger, 1979). Zoospore production, release and activity is directly affected by the matric potential of the soil and, hence, soil texture and structure (Duniway, 1976). Griffin (1979) described the ideal site for zoospore activity as shallow, light textured soils or structured heavier soil overlying impermeable layers. Given such suitable conditions, zoospore release and migration may occur.

A number of tactic phenonema have been implicated in the attraction of zoospores to plant roots; commonly to the unsuberised zone above the root tip (Zentmyer, 1961; Hickman and Ho, 1966). Chemotaxis (Zentmyer, 1961) of zoospores to roots of susceptible and field resistant plant species (Tippett, 1978; Halsall, 1979) has been demonstrated. Electrotaxis (Khew and Zentmyer, 1974) may also contribute to zoospores accumulation on plant roots. Zoospores encyst in the vicinity of the source of the stimulus (Hickman, 1970) and may produce a germ tube if the source of attraction is a plant root. The subsequent behaviour of the fungus then depends predominantly on host-parasite interactions. Zoospores encysting in the soil, remote from a plant root may germinate to produce microsporangia and another generation of motile zoospores (Palzer, 1976 as cited by Podger, 1979) or they may remain dormant in which case they lose viability in a short time (Hwang and Ko, 1978).

When infected plant roots die, *P.cinnamomi* in the decomposing root tissue is exposed to the effects of the soil environment. The fungus may persist as dormant resting propagules or survive saprophytically by colonising dead organic matter in competition with the soil microflora. Saprophytic colonisation of dead organic material has been demonstrated under controlled conditions by Zentmyer and Mircetich (1966), Reeves (1975) and Hwang and Ko (1978). These studies employed soils amended with large amounts of fresh organic matter and high rates of *P.cinnamomi*

inoculum, hence, it would be presumptive to speculate on the significance of saprophytic colonisation in the field situation. However, at least in newly killed roots *P. cinnamomi* probably survives saprophytically because micro-organisms which invade living host roots frequently occupy the killed tissue until readily available nutrients are depleted (Baker and Cook, 1974) i.e. the declining saprophytic phase of a typical root-inhabiting fungus.

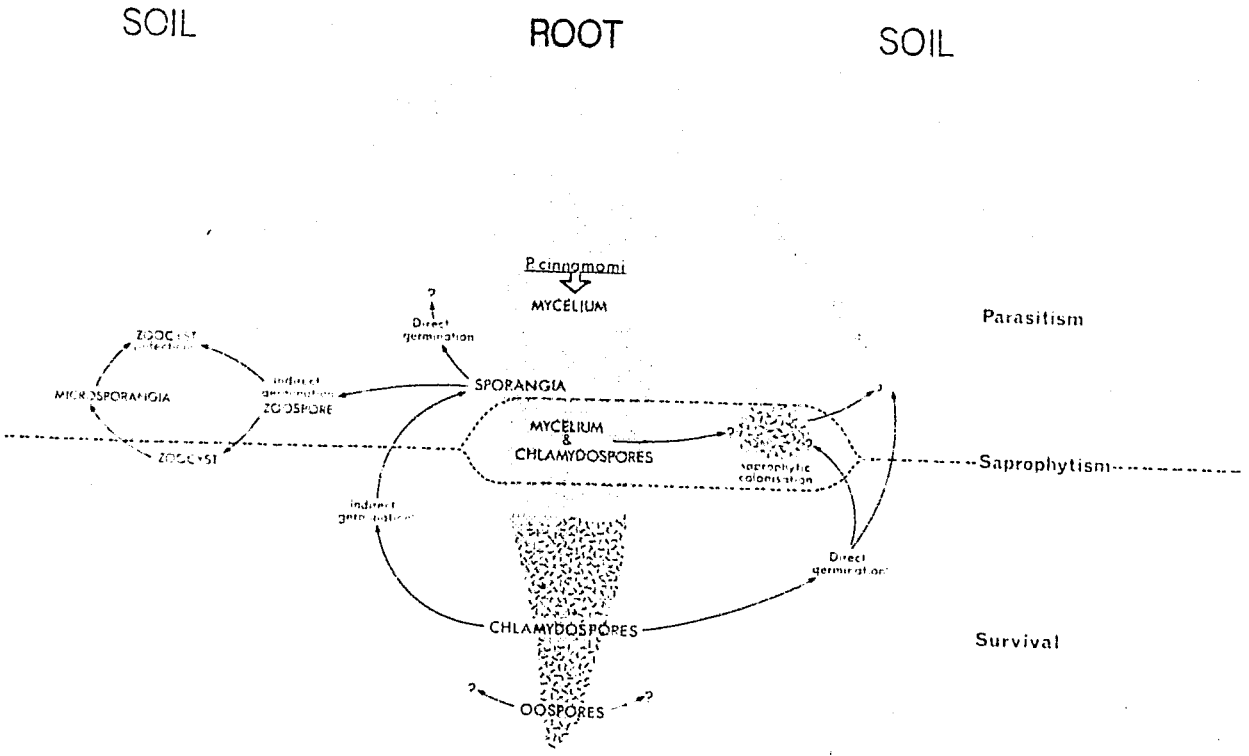
Chlamydospores are probably the principle organs of survival during the non-parasitic or declining saprophytic phase of the life cycle of *P. cinnamomi* (Zentmyer and Erwin, 1970; Podger, 1979). Mycelium, zoospores and sporangia have a brief life-span in soil (Reeves, 1975; Malajczuk *et al*, 1977; Hwang and Ko, 1978) but chlamydospores may survive for several months or more (Hwang and Ko, 1978; Weste and Vithanage, 1979). Chlamydospores germinate by forming many germ tubes or in some instances by producing a sporangium (Mircetich *et al*, 1968; Zentmyer and Erwin, 1970). Viable chlamydospores have been recovered directly from soil (Kuhlman, 1964; Hendrix and Kuhlman, 1965; McCain *et al*, 1967) but the epidemiological significance of this propagule and its importance to the survival of *P. cinnamomi* in different soil types is still poorly understood.

Cothler and Griffin (1973) and Kassaby (1977, as cited by Tippett, 1978) concluded that *P. cinnamomi* produced much smaller resting spores or microchlamydospores about 8-10 microns in diameter. The existence of these

propagules was questioned by Tippett (1978) and Blowes (unpublished) who were unable to induce microchlamydospore production in uncontaminated experimental systems.

The thick walled sexual spores of *P.cinnamomi* - oospores - may be significant long term survival propagules in soil (Zentmyer and Erwin, 1970; Podger, 1979). However, little is known about their behaviour because there are no recorded observations of these spores in naturally infested Australian soil or plant material and in experimental systems their germination is usually slow and irregular. In addition, one of the sexual compatability types, the A1 type, is uncommon in southern Australia (Pratt *et al*, 1972; Arentz, 1974). This has caused doubt as to the significance of oospores as survival structures in this part of the country. However, recent reports suggest that *P.cinnamomi* may behave homothallically (Chang *et al*, 1974) or produce oospores in response to mechanical damage (Reeves and Jackson, 1974) or volatiles of *Trichoderma* spp (Pratt *et al*, 1972; Brasier, 1975). Hence, oospore survival in soils of southern Australia may be more common than was previously believed and the behaviour of these propagules requires attention *albeit* they are difficult spores to germinate.

A diagram summarising the behavioural options of *P.cinnamomi* in the soil matrix is presented in Figure 1.1. The course adopted depends on the host-pathogen-environment interactions of the site in question. Research conducted in different parts of southern Australia suggests



1 HYPOTHETICAL REPRESENTATION OF THE BEHAVIOURAL OPTIONS OF *P.cinnamomi* IN THE SOIL MATRIX.

that the behaviour of *P.cinnamomi* in the soil matrix varies considerably between sites.

1.3 *P.cinnamomi* IN NEW SOUTH WALES AND WESTERN AUSTRALIA

The identification of *P.cinnamomi* as the causal agent of jarrah dieback disease (Podger, 1968) in the south-west of Australia stimulated investigations in other parts of the country into the occurrence, distribution and behaviour of the fungus in soil supporting native eucalypt forest.

In New South Wales no significant areas of eucalypt forest were known to be affected by *P.cinnamomi* induced disease and the cause of some of the eucalypt diebacks investigated could not be attributed principally to the effect of the fungus. In addition, research conducted in New South Wales and Western Australia into the behaviour of the fungus in soil and the disease it induced, yielded some conflicting results. These are summarised in Table 1.1.

The conflicting nature of the evidence and the assumption by most of the parties involved that the fungus would behave similarly in both situations, polarised the Australian *Phytophthora* research community and two opposing hypotheses developed.

Western Australian workers concluded that *P.cinnamomi* was introduced into the State and that the disease induced was a host-pathogen dominated situation. New South Wales investigations suggested that the fungus was an endemic organism and that factors other than the

1.1 Contrast in the behaviour of *P.cinnamomi* and the disease induced in south-western Western Australia and south-eastern New South Wales.

WESTERN AUSTRALIA	NEW SOUTH WALES
P.cinnamomi was apparently absent (never isolated) from unaffected forest sites but could be isolated from within diseased areas (Podger, 1968; 1972).	*P.cinnamomi* occurrence was common in some areas of native forest where no disease symptoms were obvious although known susceptible host species were abundant (Pratt and Heather; pers. comm., Arentz, 1974). *P.cinnamomi* could be isolated from remote and relatively undisturbed areas of native forest (Pratt *et al*, 1973).
The incidence of jarrah dieback was associated with logging and roadmaking (Podger, 1972).	No association was found between *P.cinnamomi* occurrence and road gravel or logging practices (Gerrettson-Cornell *et al*, 1977).
The flora of the jarrah forest was both very susceptible and vulnerable to *P.cinnamomi* induced disease (Podger, 1975).	While some species were susceptible to *P.cinnamomi* the community was apparently not very vulnerable (Arentz, 1974).
It was difficult to isolate *P.cinnamomi* from soil and fine root systems of diseased trees (Shea, 1979b).	*P.cinnamomi* was readily isolated from the soil matrix even from soil from apparently unaffected forest soils (Pratt and Heather, 1973a; Arentz, 1974).
The soil physical environment was only marginally favourable to *P.cinnamomi* survival although disease caused by the fungus was severe (Shea, 1975).	The soil physical environment was more conducive to the survival of the fungus but *P.cinnamomi* induced disease was not apparent (see Chapter 2).

occurrence of a susceptible plant community and *P.cinnamomi* were required to induce disease. This led to an argument about the origin of *P.cinnamomi* in Australia (whether introduced or endemic) which remains unreconciled today.

In this thesis some aspects of the behaviour of *P.cinnamomi* in soils in Western Australia and New South Wales are compared using identical techniques. Four fairly discrete aspects were chosen for investigation to encompass the range of behavioural options open to *P.cinnamomi* in soil (see Figure 1.1) and to accentuate the differences, if any, between the two situations. The topics investigated were:

(a) the occurrence and distribution of *P.cinnamomi* in the Durras area of New South Wales and Jarrahdale area of Western Australia;

(b) microbial populations in soils from both sites and their relationship to the isolation of *P.cinnamomi*;

(c) the survival of *P.cinnamomi* in these soils;

(d) sporangial production of *P.cinnamomi* in these soils.

It was hoped that this investigation would allow a better comparison of the behaviour of *P.cinnamomi* in the soils of New South Wales and Western Australia and elucidate the differences between the two situations.

CHAPTER 2

THE OCCURRENCE AND DISTRIBUTION OF *Phytophthora cinnamomi* IN THE DURRAS AREA OF NEW SOUTH WALES AND JARRAHDALE AREA OF WESTERN AUSTRALIA

2.1 INTRODUCTION

Phytophthora cinnamomi was recovered from forest soils on the south coast of New South Wales during surveys to determine the distribution of the fungus in this area (Pratt *et al*, 1973; Arentz, 1974; Arentz *et al*, 1974). No comparable studies on the occurrence and distribution of *P.cinnamomi* from forest soils in Western Australia have been published.

In this study, sites from near Durras in New South Wales and Jarrahdale in Western Australia were surveyed to determine:

(a) the distribution of *P.cinnamomi* within and between sites;

(b) the pattern of *P.cinnamomi* recovery throughout the year;

(c) the relationship between disease expression in the vegetation and ability to isolate the fungus.

2.2 DESCRIPTION OF THE NEW SOUTH WALES STUDY SITE

2.2.1 Location

The site was located near Durras, 15 km north-east of Batemans Bay on the boundary of the Murramorong National Park and Benanderah State Forest (Map Ref. 35°39'S, 150°17'E). It extended from a major ridge on the southern perimeter into an adjacent valley and covered an area of approximately 1.5 ha. The site was bounded by an unsealed road to the east and a change in understorey vegetation 120 m to the west (Figure 2.1).

2.2.2 Climate

Climatic data (Anon. 1975) recorded at Ulladulla and Moruya Heads (Appendix 1), the nearest recording stations north and south of Durras, were examined. The district receives a mean annual rainfall of approximately 1000 mm spread over 100 rain days. This is evenly distributed throughout the year except for a drier period in July, August and September.

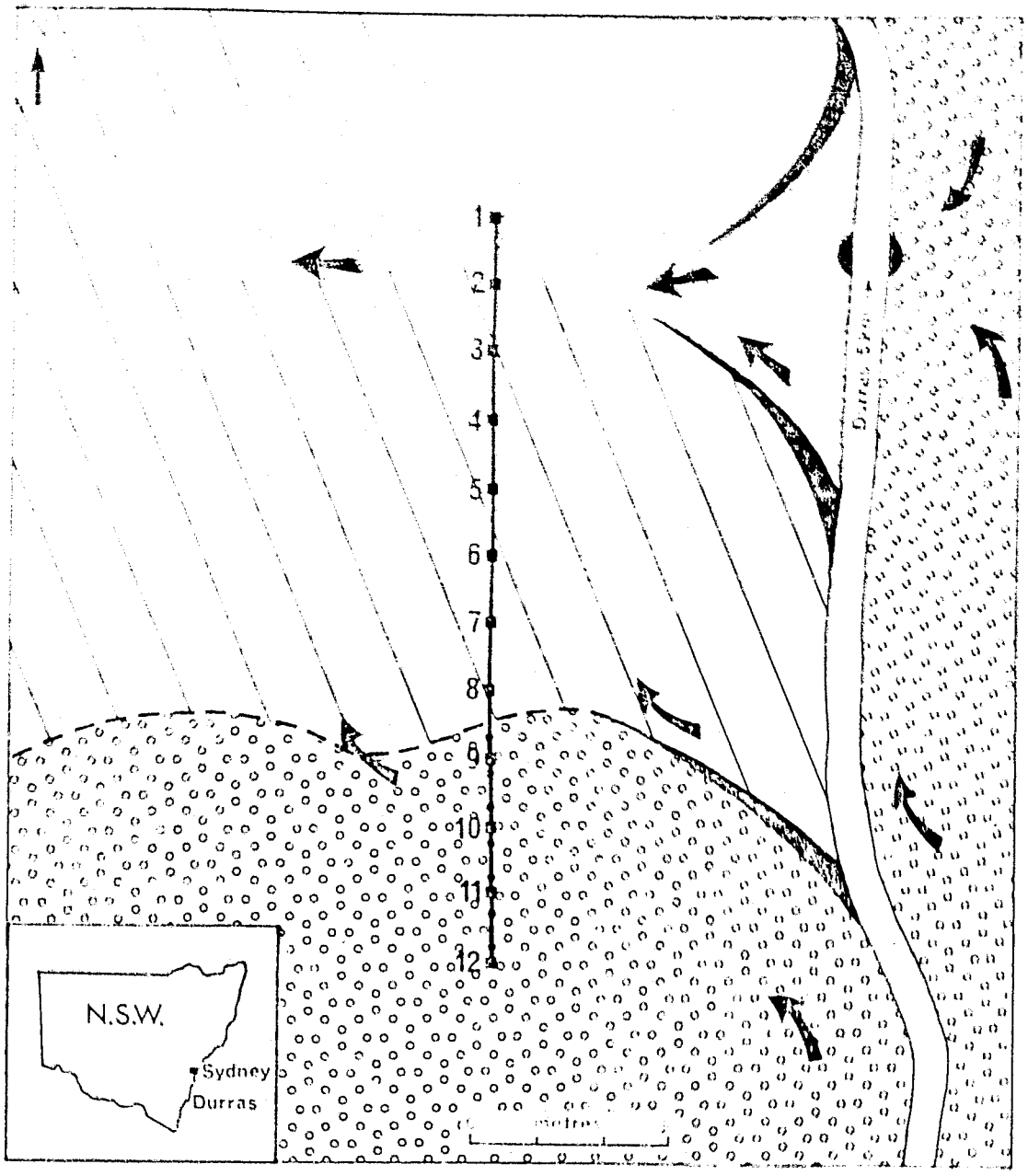
Mean monthly maximum and minimum temperatures at Ulladulla and Moruya Heads range from 23°C and 15°C in summer to 17°C and 7°C in winter.

2.2.3 Vegetation

The vegetation was a *Eucalyptus maculata* Hook - *E.pilularis* Sm. dry schlerophyll association (McColl and Humphreys, 1967) with a dense understorey in which *Macrozamia communis* L. Johnson, was the predominant species.

M.communis were abundant and healthy on the southern ridge (Figure 2.2) but were chlorotic and less

FIGURE 2.1 THE DURRAS SURVEY STUDY SITE



common in the depression (Figure 2.3). In this study, *M.communis* with yellowing foliage were recorded as diseased. Herbacious species such as *Lomandra longifolia* Labill. dominated the valley understorey but were absent from the ridge.

Overstorey eucalypts which included the co-dominant species *E.botryoides* Sm. and *E.paniculata* Sm. in addition to the dominant *E.maculata* and *E.pilularis* exhibited no apparent symptoms of disease in any part of the site but were sparcer in the valley area.

Lists of species and their distribution are given in Appendix 2.

2.2.4 Edaphic Factors

The Conjola formation of Permian age forms a thick sequence of quartz, sandstone, siltstone and conglomerate in this area (McElroy and Rose, 1962).

Soils on the southern ridge of the trial site were described as orange podsols. These were composed of 15-20 cm of light brownish-grey sandy loam above a medium density orange clay which contained fine grained sedimentary gravels. Soil in the valley region was deeper and was described as yellow podsol consisting of 60-70 cm of light sandy clay loam (grey in colour) above yellow-grey mottled clay. This soil was gleyed which indicated slow movement of soil moisture and poor aeration.

Drainage in the undulating landscape flowed towards the west via the northern valley which received runoff from road drainage culverts and the southern

ridge (Figure 2.1). Details of the moisture characteristics and chemical analyses of soils from the healthy and diseased zones of this site are given in Appendix 3.

2.3 SURVEY OF THE DURRAS STUDY SITE

2.3.1 Materials and Methods

A North-South line transect was located through the centre of the site traversing the *M.communis* disease front i.e. the boundary of the healthy (green) *M.communis*, sometimes referred to as the greenline* (Figure 2.1). This transect traversed from diseased *M.communis* at the northern end to apparently healthy specimens at the southern extremity. Sampling points were located at 10 m intervals along the transect line.

On ten occasions during 1978-79, four replicate 150 g samples of soil and fine root material from the surface 15 cm of soil were taken from around each transect point, packed in airtight containers, and returned to the laboratory. Within 48 hr of collection each sample was tested individually for the presence of *P.cinnamomi* using a modified lupin baiting technique.

Three newly germinated New Zealand blue lupins (*Lupinus angustifolius* L.) were suspended from a cork float mat in individual 1 litre vessels each containing

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* Greenline : a term adopted originally by Western Australian researchers to describe the boundary between vegetation exhibiting disease symptoms and apparently healthy vegetation.

FIGURE 2.2 HEALTHY *M.communis* ON SOUTHERN RIDGE OF THE DURRAS TRANSECT

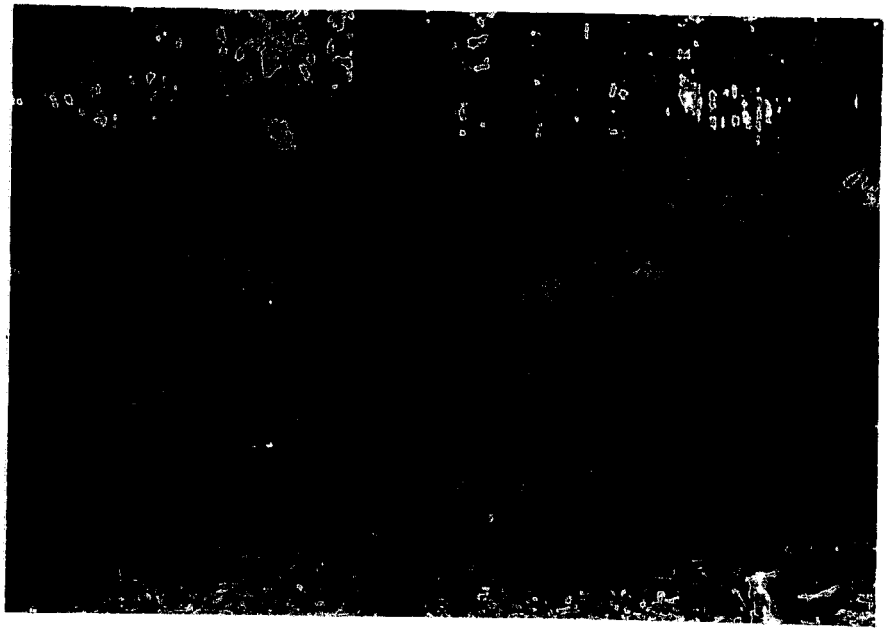


FIGURE 2.3 DISEASED *M.communis* LOOKING TOWARDS THE VALLEY FROM GREENLINE

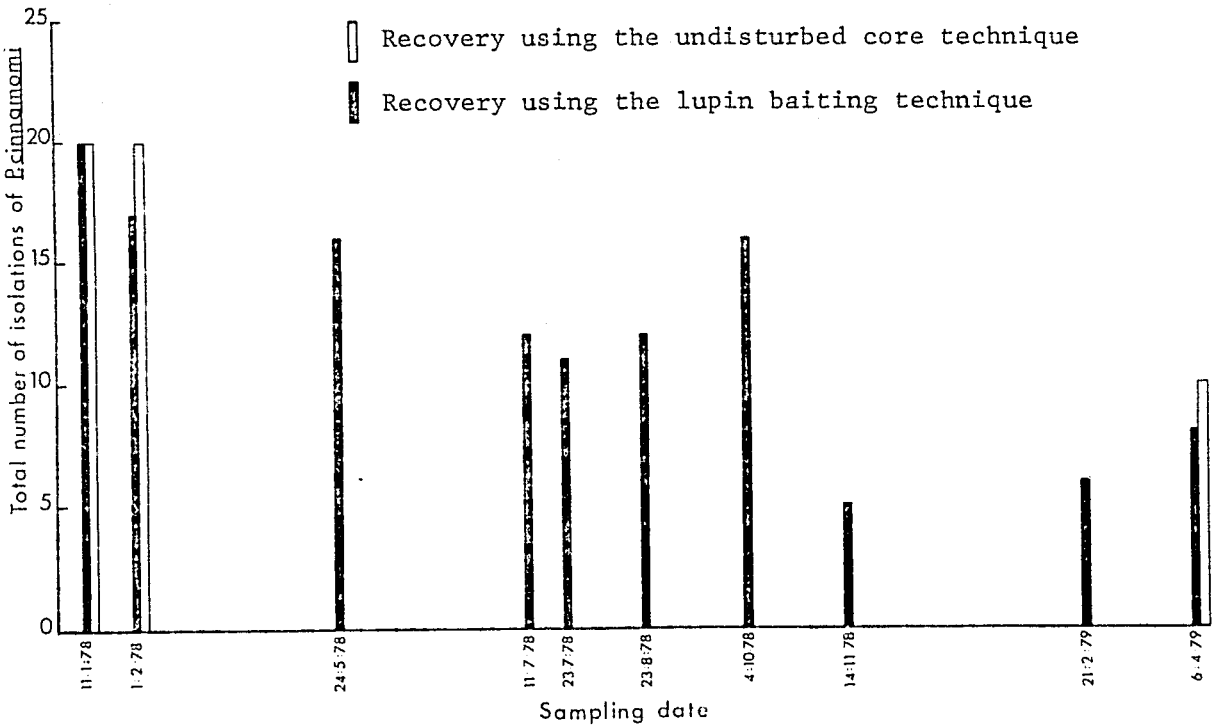
a 150 g soil/fine root sample and 750 ml of tap water. These were incubated in alternating light (180 micro-einsteins $M^{-2}sec^{-1}$) and dark at 17°C - 24°C. Lupins were examined closely and lesioned roots were excised, surface sterilised in 70% ethanol, and plated on anti-biotic cornmeal agar; A.C.A., (Appendix 4). Nine days after the commencement of baiting, all remaining roots were plated on A.C.A.

In most instances, *P. cinnamomi* was identified on A.C.A. by its morphology characteristics. Isolates not positively identified in this manner were checked by observing the hyphal morphology on lupin root agar, chlamydospore production on full strength V8 agar, and sporangial production on discs of $\frac{1}{10}$ strength V8 agar immersed in non-sterile soil extract. Details of the media used are given in Appendix 4.

The efficiency of the lupin baiting method was checked by employing an undisturbed core technique. This technique sampled a greater soil mass and subjected more lupin roots to a range of moisture regimes for a longer period than lupin baiting. Details of this method are outlined in Appendix 5.

On three separate occasions, undisturbed cores were collected adjacent to samples taken for conventional lupin baiting and these cores were baited for one month to determine the comparability of results obtained by the two methods.

FIGURE 2.4 RECOVERY OF *P. cinnamomi* FROM THE DURRAS TRANSECT



2.3.2 Results

P.cinnamomi was recovered from 173 of the 624 soil/fine root samples collected at the Durras transect during 1978/79. The number of occasions in which the fungus was isolated on each sampling date using the lupin baiting and undisturbed core techniques is illustrated in Figure 2.4.

The significance of the difference in the total number of isolations using the two techniques was tested using the GLIM* computer programme. This programme analysed the deviance due to the techniques on the three occasions they were employed concurrently at each position in the transect where *P.cinnamomi* was isolated (Table 2.3.1).

TABLE 2.3.1 Comparison of the lupin baiting and undisturbed core techniques for the isolation of *P.cinnamomi* from Durras soil.

Source	df	Deviance
Position.Time	9	66.6 ***
Techniques	1	1.5
Residual	37	37.0

*** Deviance $> x^2$ (0.005)

* The GLIM computer programme employs the analysis of deviance which provides an extension of a standard statistical technique, the analysis of variance, to data which are not normally distributed.

While there was no significant difference between the two techniques, the time of sampling and transect position were significant at the P = 0.005% level.

All data collected in 1978/79 using the lupin baiting technique was then analysed to test for significance of the major factors: transect position, sampling time and the position.time interaction. Again the GLIM programme to assess deviance was employed (Table 2.3.2).

TABLE 2.3.2 The effect of time, position and the time.position interaction on the recovery of *P.cinnamomi* from the Durras transect.

Source	df	Deviance
Time	9	44.7 ***
Position	7	36.4 ***
Time.Position	63	68.1

*** Deviance $> x^2$ (0.005)

Time and position of sampling were highly significant (P = 0.005) but the interaction term was not. Hence, the ability to recover *P.cinnamomi* from the site depended on the time of sampling and the position sampled and their combined effect was additive. The total number of isolations of *P.cinnamomi* recovered from each transect position during 1978/79 is illustrated in

FIGURE 2.5 TOTAL NUMBER OF ISOLATIONS OF *P. cinnamomi* FROM EACH TRANSECT POINT DURING 1978/79

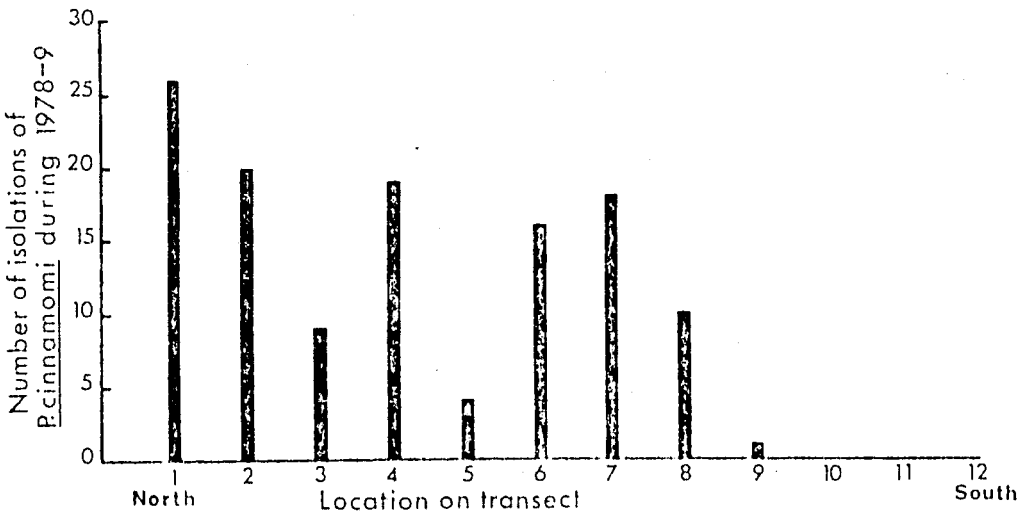


Figure 2.5. *P.cinnamomi* was readily isolated from the northern valley zone but the number of isolations of the organism decreased with increased elevation along the transect. The fungus was never isolated from sampling positions on the southern ridge.

Sampling time was then treated as a quantitative variable and it was found that the actual time effect (i.e. number of days after the date of the first sampling) accounted for almost two thirds of the deviance due to time in Table 2.3.2 above (Table 2.3.3). The number of successful isolations from the site decreased steadily with time during 1978 (Figure 2.4).

TABLE 2.3.3 The effect of actual time (i.e. number of days after 1.1.78) on the recovery of *P.cinnamomi* from Durras transect soil.

Source	df	Deviance
Position	7	43.6***
Day	1	26.5***
Day 2	1	0.02
Day 3	1	0.2
Position.Day	7	7.1
Residual	62	70.8

*** Deviance $> x^2$ (0.005)

Time was then treated seasonally so that the time variable represented a position within a calendar year e.g. 21.2.79 became day 52 of the year as would 21.2.78 had an observation been made on that date (Table 2.3.4).

TABLE 2.3.4 The effect of time of the year on the recovery of *P.cinnamomi* from Durras transect soil.

Source	df	Deviance
Position	7	40.7***
Day	1	3.8*
Day 2	1	0.0
Day 3	1	16.4***
Position.Day	7	6.8
Residual	62	77.2

*** Deviance $> x^2$ (0.005)

* $x^2(0.05) \leq$ deviance $\leq x^2$ (0.01)

It was found that the residual deviance increased from 70.8 using actual time to 77.2 using seasonal time indicating that time was not well represented by a fixed seasonal effect.

2.3.3 Discussion

Arentz (1974) showed that the distribution of *P.cinnamomi* on the south coast of New South Wales was discontinuous in space and time. Under these circumstances, the volume of soil sampled for *P.cinnamomi* analysis by lupin baiting was very important. Large samples

containing numerous *P.cinnamomi* propagules would not demonstrate changes in spatial or temporal distribution unless soil populations were assessed. Conversely, samples less than a certain size would show a fragmented distribution pattern and no clear distinction between infested and uninfested areas would emerge.

During this survey, two isolation techniques were tested. The lupin baiting technique was a modification of that used previously on the south coast by Pratt *et al* (1973) and Arentz (1974). The efficiency of this procedure was tested by employing an undisturbed core technique (Appendix 5). The latter baited four times the volume of soil, using three times as many host lupins to probe the soil mass for three times as long as the lupin baiting technique. In addition, a soil matric potential ranging from 0 kPa to -1 kPa was maintained in the cores. Thus, some of the physical shortcomings of the lupin baiting procedure, e.g. the necessity for total waterlogging, the exclusion of the host root from the soil and the transient nature of the system in general, were overcome by using the undisturbed core technique.

A statistical comparison of the techniques, when used concurrently on three occasions, showed that there was no significant difference in the ability of either technique to isolate *P.cinnamomi* (Table 2.3.1). This suggested that the efficiency of the lupin baiting technique for isolating *P.cinnamomi* could not be improved

by increasing the size of the soil samples collected for baiting and that the above mentioned 'shortcomings' of the technique were not adversely affecting the isolation of *P.cinnamomi* from soil. Alternatively, some limiting factor may have rendered the undisturbed core technique less efficient (per unit volume of soil) than the lupin baiting technique for isolating *P.cinnamomi*. Further investigation of the relative efficiency of the two techniques required baiting undisturbed cores and lupin baiting samples of different sizes. This would have been desirable, had the assessment of fungal populations been envisaged. However, such refinement was not required for the determination of fungal distribution where only the presence or apparent absence (inability to isolate) of the fungus was measured. Consequently, the lupin baiting technique was used to isolate *P.cinnamomi* during the course of this study in preference to the undisturbed core technique which was more time consuming.

The spatial distribution of *P.cinnamomi* (Figure 2.5) showed a close association with the distribution of chlorotic *M.communis* (Figure 2.1). Some samples collected from the latter zone always yielded isolates of *P.cinnamomi* while those from the area carrying healthy *M.communis* never yielded *P.cinnamomi*. While a causal relationship between *P.cinnamomi* occurrence and *M.communis* chlorosis was not established, it appeared that site conditions favourable to *P.cinnamomi* occurrence were deleterious to the health of *M.communis*.

It was decided that a detailed investigation of the site conditions responsible for this association, in terms of isolating individual environmental parameters, would have been futile (see Section 4.2.3). However, from evidence presented by Pratt and Heather (1973b) and Arentz (1974) and from the results of this study, it seemed probable that soil moisture and its associated effects were major contributory factors.

Road making and construction of road drainage culverts certainly realigned the drainage pattern of the site (Figure 2.1). Water was channelled into the northern valley zone i.e. the area from which *P. cinnamomi* was isolated most frequently and of pronounced *M. communis* decline. Soil from this zone was gleyed which indicated restricted movement of water through the profile and prolonged periods of waterlogging. In contrast, soil from the healthy *M. communis* zone was not gleyed. In addition, the moisture characteristic curve (Appendix 3) for soil collected from the zone of chlorotic *M. communis* was steep compared to that for soil taken from the healthy *M. communis* zone. Thus, a smaller percentage soil moisture content increase was required to waterlog soil from the diseased *M. communis* zone than that from the healthy zone.

The concentration of drainage into the northern zone and the presence of healthy *M. communis* east of the road and in the southern zone suggest that road making practices have caused an environmental modification which was deleterious to the survival of *M. communis* but favourable

to *P.cinnamomi*. This has resulted in an observable alteration in the understorey vegetation. The overstorey species are apparently unaffected by this modification or alternatively there has been insufficient time for them to react to it.

Clarification of the relationship between *M.communis* chlorosis and *P.cinnamomi* would have required extensive investigation of a number of sites and *P.cinnamomi* inoculation of uninfested areas.

During the course of this study, there was a gradual decline in the number of *P.cinnamomi* isolations with time (Figure 2.4). This was not closely associated with mean monthly rainfall (Appendix 1) or season (Table 2.3.4). This suggested that the temporal distribution of *P.cinnamomi* was not controlled by soil moisture as seemed to be the case with spatial distribution. Thus, while the soil water relations of the site probably depicted the broad boundaries of the spatial distribution of the fungus, its occurrence within these boundaries and with time was controlled by other factors.

The short length of the survey period made it impossible to determine whether the decrease in *P.cinnamomi* isolation with time was a real and continuing effect or transient in nature. However, a decline in *P.cinnamomi* occurrence would eventually be expected as susceptible host species died and substrate for the fungus became scarce.

2.4 DESCRIPTION OF THE WESTERN AUSTRALIAN STUDY SITE

2.4.1 Location

The Western Australian site was located within a more extensive area used by the C.S.I.R.O. for research on the ecology of the jarrah forest. This area encompassed approximately 500 ha of recently logged jarrah forest 50 km south-east of Perth and 13 km east of Jarrahdale (Map ref. $32^{o}18'S$, $116^{o}12'E$). Although the area was effected by *P.cinnamomi* induced disease, approximately half was apparently healthy forest situated on the uplands.

The particular site chosen for the North to South transect (Figure 2.8) included healthy, dieback and dead forest (Figure 2.6, Figure 2.7).

2.4.2 Climate

The district has a mean annual rainfall of approximately 800 mm which is distinctly seasonal, the winter (April to October) to summer (November to March) ratio being 6:1. This rainfall is brought by moist westerly winds which precipitate as they rise over the coast and Darling scarp (Havel, 1975).

Mean maximum and minimum temperatures range from $28^{o}C$ and $13^{o}C$ in summer to $15^{o}C$ and $6^{o}C$ in winter.

2.4.3 Vegetation

Eucalyptus marginata Donn.(Jarrah) and *E.calophylla* Lindl. (Marri) were the dominant tree species. *Banksia grandis* Willd, *Persoonia longifolia* R.Br., *P.elliptica* R.Br. and saplings of the dominant eucalypts formed a middle strata in the healthy forest zone which supported a generically diverse understorey.

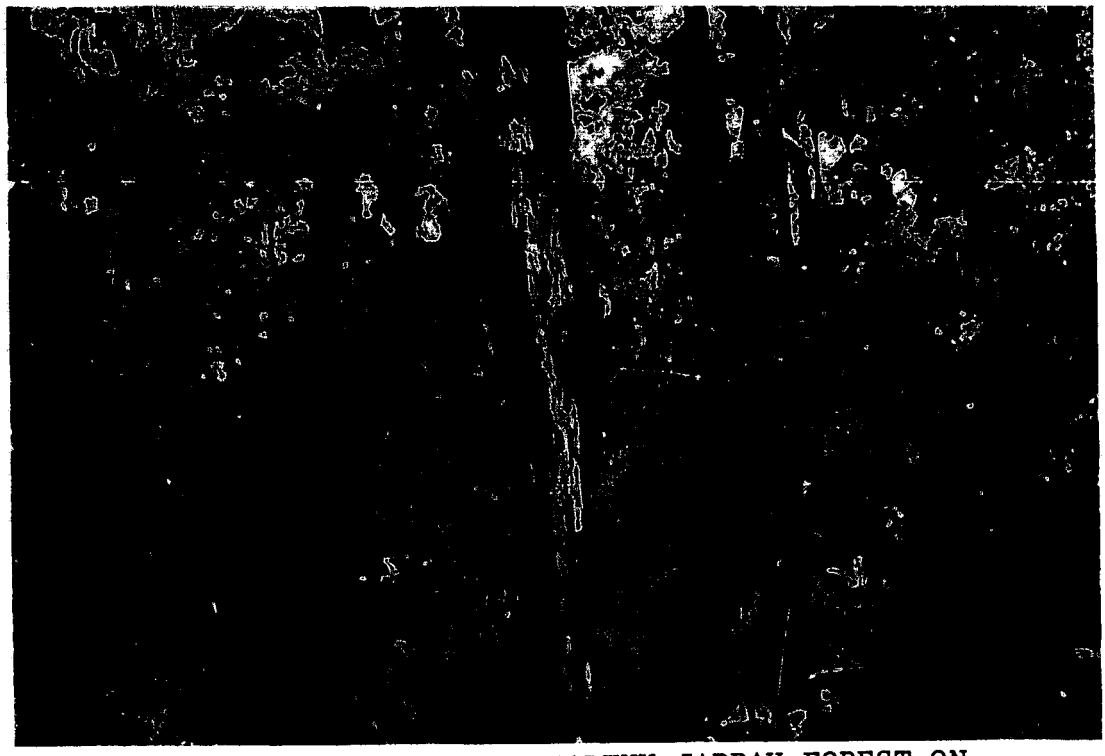


FIGURE 2.6 APPARENTLY HEALTHY JARRAH FOREST ON THE UPLAND AREA OF THE JARRAHDALE TRANSECT

FIGURE 2.7 DEAD AND DIEBACK JARRAH FOREST - JARRAHDALE TRANSECT

The zone of dead forest on the lower slopes was characterised by dead dominant and middle storey trees except for occasional *E.calophylla* regrowth saplings. Less than half the number of species of understorey plants recorded in the healthy zone were present in the diseased area.

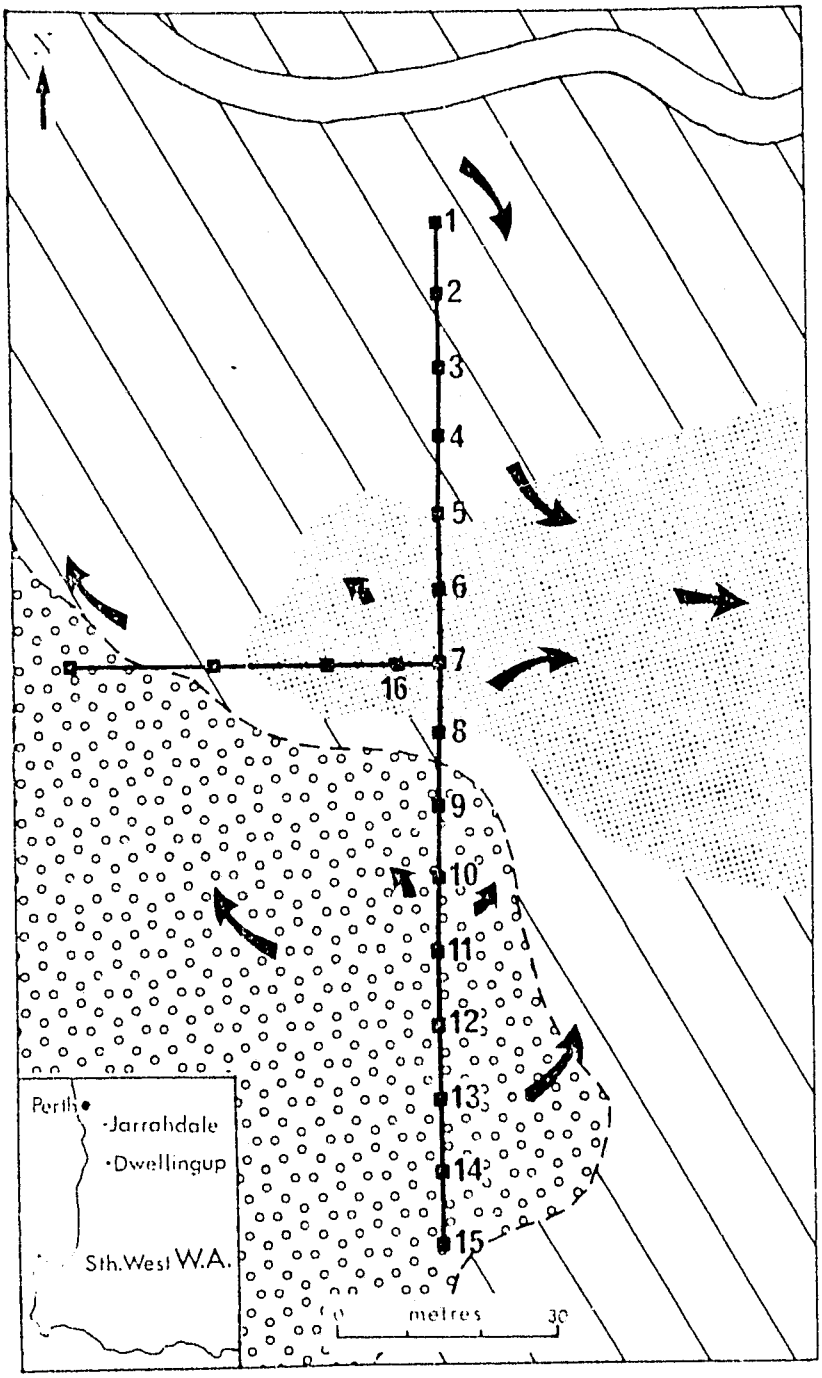
The dieback zone was intermediate. Dominant trees exhibited progressive dying of the crowns, sometimes associated with epicormic shoot development. Jarrah and marri regrowth saplings were living but the understorey was generically less diverse than in the healthy forest zone. Detailed species lists for the three zones are given in Appendix 6.

2.4.4 Edaphic Factors

The site included a lateritic mantled upland (Dwellingup soil association) and the upper slopes of a broad valley (Yarrigal soil association). Soil throughout the site was described as a 'yellow sandy gravel' akin to the upland laterites.

The healthy forest and dieback areas were situated on gentle slopes, the healthy zone lying upslope of the dieback zone. Surface soils were dark-brown sandy loams with abundant ferruginous gravel. These became strong brown sandy, clay loams at 60-70 cm depth. The dead forest area was located on a flat midslope shoulder and soils in this zone were dark brown sandy loams with few gravels in the surface horizons. Drainage flowed from the slopes into this area (Figure 2.8).

FIGURE 2.8 THE JARRAHDALE STUDY SITE



LEGEND

Green line

Transect sample point

Drainage direction

Jarrah forest

Healthy

Dieback

Dead

Details of the moisture characteristics and chemical analyses of soils from the healthy and diseased zones of this site are given in Appendix 7.

2.5 SURVEY OF THE WESTERN AUSTRALIAN STUDY SITE

2.5.1 Materials and Methods

A line transect was set up traversing the tongue of dead forest vegetation and penetrating the dieback and healthy zones. Sampling points were located at regular intervals along the transect (Figure 2.8).

On six occasions during 1978 and once in 1979, four replicate 150 g samples of soil and fine roots were collected from around each transect point and baited as described in Section 2.3.1. For practical reasons, on five of these occasions, soil samples were sent to Canberra via road transport for baiting. This resulted in an additional 48 hr delay between sample collection and the commencement of baiting.

On two occasions during 1978 soil samples were sent from Durras to Western Australia for baiting to determine the effect of transport conditions and delay between sampling and baiting on *P. cinnamomi* recovery.

2.5.2 Results

P. cinnamomi was recovered from only one of the 532 soil samples baited during this survey. This isolate came from a sample collected in March 1978 from the dieback zone.

Infested soil sent from Durras to Western Australia via road transport yielded *P. cinnamomi* when lupin baited.

2.5.3 Discussion

The frequency of isolation of *P. cinnamomi* from soil/fine root samples collected at this site during 1978/79 was negligible compared with the Durras site. However, the fungus was isolated from infested soil samples that were collected at Durras and transported to Western Australia for lupin baiting. This indicated that the delay between sample collection and lupin baiting was not responsible for the low recovery of *P. cinnamomi* from Jarrahdale transect soils. Shea and Malajczuk (pers. comm.) said that this result was normal for randomised soil/fine root samples collected and baited from freely drained dieback sites.

This suggested that the fungus was present at very low propagule levels (per gram of soil baited) or that an artifact of the lupin baiting technique prevented isolation of *P. cinnamomi* from these soils. The latter seemed unlikely because *P. cinnamomi* could be isolated from jarrah forest soils inoculated with the fungus, provided the soil was kept moist prior to lupin baiting (Shea, pers. comm.) while the former possibility posed the question: "How could such low populations of *P. cinnamomi* cause a disease situation as evidenced in the jarrah forest?"

A hypothesis put forward by Dr S. Shea of the Western Australian Forests Department has proposed that *P. cinnamomi* is not found in the soil/fine root matrix for most of the year but survives in the large roots of an understorey species and occurs only as an ephemeral inhabitant in soil at very specific times (Shea, 1979b).

The following Section (Section 2.6) was based on this hypothesis and Experiment 3 formed a part of the work reported by Shea (1979b).

2.6 SAMPLING LARGE ROOTS OF *Banksia grandis* FOR THE PRESENCE OF *Phytophthora cinnamomi*

2.6.1 Introduction

Recently, Shea *et al* (1979) have shown that *P.cinnamomi* is an ephemeral organism in the soils of the Western Australian jarrah forest, occurring only at high population levels for short periods in spring when environmental conditions are suitable for sporangial production. Shea (pers. comm.) hypothesised that *P.cinnamomi* survived during the remainder of the year as an infection in the large roots of *Banksia grandis* trees; a species which constitutes a major component of the understorey of the jarrah forest.

2.6.2 Experimental

During a visit to the Western Australian Forests Department laboratories at Dwellingup in April 1979, three experiments were set up to test this hypothesis. In Experiments 1 and 3 described below, a site 500 m north of Dwellingup Research Station was used. This site exhibited an obvious greenline and was typical of many infested areas. For Experiment 2, the Jarrahdale site described in Section 2.4 was revisited.

Initially, transect sampling was used in conjunction with the lupin baiting isolation technique (Experiment 1). However, localised sampling of banksias, ranked according to their disease status, proved a more successful technique particularly when combined with direct root plating (Experiment 3). Banksia trees were classified

according to their disease expression into the following categories:

(i) Apparently healthy trees (leaves green)

(ii) Recently dead (leaves retained but dead)

(iii) Dead approximately 1 year (few leaves retained)

(iv) Dead and partially decayed (no leaves, stems partially decayed).

EXPERIMENT 1.

Materials and Methods

A line transect was set up traversing the greenline and including banksias of all disease categories. Nine transect points were located at regular intervals along the line and the banksia tree closest to each point sampled.

Four 150 g samples of stem and root material were chipped from around the collar of each tree. Additionally, four replicate 150 g soil and fine root samples were collected from around the base of each tree. Samples were returned to the laboratory at Dwellingup in Western Australia and lupin baited.

Results

P. cinnamomi was recovered from collar samples taken from two recently dead banksia trees situated close to the greenline . *P. cinnamomi* was not recovered from samples collected from healthy or dead and partially decayed trees. Soil and fine root material collected from around the base of banksias of all disease categories failed to yield *P. cinnamomi* (Table 2.6.1).

TABLE 2.6.1 Isolation of *P.cinnamomi* by lupin baiting from the root collar zone and soil surrounding *B.grandis* trees of various disease categories in the Dwellingup transect.

Transect position	Disease status of *B.grandis* trees	Isolation from the collar of *B.grandis* trees	Isolation from soil/fine roots from around trees
1	healthy	$^{0}/_{4}$*	$^{0}/_{4}$
2	"	$^{0}/_{4}$	$^{0}/_{4}$
3	"	$^{0}/_{4}$	$^{0}/_{4}$
4	"	$^{0}/_{4}$	$^{0}/_{4}$
5	"	$^{0}/_{4}$	$^{0}/_{4}$
6	Recently dead	$^{2}/_{4}$	$^{0}/_{4}$
7	" "	$^{2}/_{4}$	$^{0}/_{4}$
8	Dead and decaying	$^{0}/_{4}$	$^{0}/_{4}$
9	" " "	$^{0}/_{4}$	$^{0}/_{4}$

* Number of replicate samples yielding *P.cinnamomi* from four samples baited from each tree.

EXPERIMENT 2.

Materials and Methods

A banksia thicket, located 300 m east of the Jarrahdale site described in Section 2.4.1, was sampled.

Root and stem material (150 g) was collected from the collar region of five healthy, five recently dead and five dead and partially decayed banksias. Soil and fine root material was collected from around the base of each of these trees. Samples were returned to the laboratory in Western Australia in airtight plastic containers and lupin baited.

Results

TABLE 2.6.2 Isolation of *P.cinnamomi* by lupin baiting from the root collar zone and soil samples surrounding *B.grandis* trees of various disease categories in a localised sample at Jarrahdale.

Material sampled	Banksia disease status category		
	Healthy	Recently dead	Dead and decaying
Collar region	$^{0}/_{5}$*	$^{2}/_{5}$	$^{0}/_{5}$
Soil/fine roots	$^{0}/_{5}$	$^{0}/_{5}$	$^{o}/_{5}$

* Number of *P.cinnamomi* isolations from samples taken from five replicate trees.

P. cinnamomi was recovered from samples taken from two of the recently dead banksias. *P. cinnamomi* was not isolated from samples collected from healthy or dead and partially decayed trees or from soil and fine root material collected from around the base of banksias (Table 2.6.2).

EXPERIMENT 3.

Materials and Methods

The collar zones of six trees of *B. grandis* in each of the four disease categories were sampled at the Dwellingup site. Each tree was sampled just above the soil surface, immediately below the surface and 10 cm below the surface.

Four 3 cm diameter cores of bark and wood were taken with a metal punch which penetrated about 1 cm into the wood from around the tree at each level. Core samples were cut into twenty, 1.5 cm x 0.5 cm pieces and surface sterilised in 2% anti-bacterial solution (Milton). Bark and wood material was separated and plated independently on selective agar media (Tsao and Ocana, 1969; Masago *et al*, 1977) and incubated at 23°C for 48 hr.

Results

Samples taken from all recently dead banksias yielded *P. cinnamomi* from all positions on the collar. Trees categorised as dead for about 1 yr yielded the fungus from one third of the samples taken from immediately below the soil surface. Samples taken from healthy trees and from dead and decaying trees did not yield *P. cinnamomi* (Table 2.6.3).

TABLE 2.6.3 Isolation of *P.cinnamomi* by direct plating from the root collar zone of *B.grandis* trees of various disease categories from a localised sample at Dwellingup.

Sampling position on collar	Banksia disease status category							
	Healthy		Recently dead		Dead 1 year		Dead and decaying	
	Bark	Wood	Bark	Wood	Bark	Wood	Bark	Wood
Above soil surface	0/6 *	0/6	3/6	6/6	0/6	0/6	0/6	0/6
Below soil surface	0/6	0/6	6/6	5/6	2/6	2/6	0/6	0/6
10 cm below soil surface	0/6	0/6	6/6	5/6	0/6	0/6	0/6	0/6

* Number of *P.cinnamomi* isolations from six trees sampled at this level.

2.6.3 Discussion

P. cinnamomi was isolated from most replicates of samples taken from the root collar region of all recently dead *B. grandis* trees provided that specific methods of tree sampling and fungus isolation were used. Randomised tree sampling (Exp. 1) and lupin baiting (Exp. 1 and 2) yielded lower overall recovery of *P. cinnamomi* from banksias than localised tree sampling combined with direct plating, although the overall results of the experiments had the same implications.

In all experiments, *P. cinnamomi* was isolated from recently dead banksias but not from healthy or dead and partially decayed trees or from soil/fine root samples collected from around the base of the trees. This suggested that *P. cinnamomi* caused the death of *B. grandis* trees by invasion of their large root systems and that the fungus survived the death of the tree for only a relatively short time.

Shea (1979b) concluded that the death of banksias was caused by girdling at the collar region because excavation of a number of *B. grandis* root systems showed that only a portion of the horizontal root system was infected while 92% of the recently dead banksias he sampled had *P. cinnamomi* infection in the collar region. He suggested that fungal mycelium persisted in recently dead banksias but probably not in trees that were dead for more than a year. This explained the pattern of *P. cinnamomi* recovery from trees of different disease-age categories. He attributed the occasional isolation of

P.cinnamomi from trees that were dead and decayed to the survival of chlamydospores.

Some field observations support the supposition that *P.cinnamomi* behaves as a collar rot organism in *B.grandis* e.g. disease symptoms develop rapidly in banksias and death of the tree quickly follows suggesting that girdling is the cause of death rather than repeated root attack which generally causes progressive crown decline. In addition, the uphill spread of disease through banksia thickets in the jarrah forest of Western Australia is slow; less than 1 m/annum (Shea and Dillan, 1979). A sequence of dead trees generally occurs, ranging from the first killed, at the point of initial infection, to the most recently infected at the greenline .

This pattern of disease spread would be expected from a root-inhabiting fungus that can infect large roots and invade uninfected host tissue via root contact.

2.7 SAMPLING *Macrozamia communis* LARGE ROOTS FOR THE PRESENCE OF *Phytophthora cinnamomi*

2.7.1 Introduction

During the 1978/79 survey of the Durras area (Section 2.3), soil and fine root material was sampled from twelve points along a line transect to test for the presence of *P.cinnamomi*. Large roots of *M.communis* were not tested for the occurrence of the fungus.

Direct plating of the collar region of recently dead banksias (Section 2.6) indicated that *P.cinnamomi* infected *B.grandis* large roots and it appeared that the fungus behaved as a root-inhabiting fungus in the banksia component of the jarrah forest in Western Australia.

In this study, root and collar samples collected from the large roots of *M.communis* plants, were plated onto selective media to determine whether infection by *P.cinnamomi* occurred in *M.communis* large roots at the Durras site in New South Wales.

2.7.2 Materials and Methods

Six small *M.communis* plants, with large roots intact, were collected from each of the diseased, greenline and healthy zones of the Durras site. Large plants were not sampled because the root collars of mature specimens were buried about 1 m below the soil surface due to the formation of a bulbous corm in this species.

The root and collar region of each of the *M.communis* plants collected was cut into twenty, 1.5 x 0.5 cm pieces, surface sterilised and plated independently onto

selective agar media as described in Experiment 3, Section 2.6.2. Plates were incubated at 25°C for 48 hr.

2.7.3 Results and Discussion

P. cinnamomi was not isolated from any of the *M. communis* root or collar pieces sampled. This suggested that infection by *P. cinnamomi* did not occur in the large roots of *M. communis* at the Durras site.

This result supports the findings of Pratt and Heather (pers. comm.) who were unable to isolate *P. cinnamomi* from the corraloid roots or the large roots of chlorotic *M. communis* on the south coast of New South Wales by direct plating or lupin baiting.

2.8 GENERAL DISCUSSION

The role of *P.cinnamomi* in forest disease in Australia has been a contentious issue amongst research workers because of the contrast in nature of the Western Australian and New South Wales disease situations. In Western Australia it is difficult to isolate *P.cinnamomi* from soil supporting dead and dying vegetation but in many areas of New South Wales the fungus is readily isolated from soil in sites where no apparent disease is evident in the vegetation (Arentz, 1974). This situation has led to the development of different isolation techniques and sampling procedures in each State and comparable studies to determine the nature of the difference between the two disease syndromes have not been made.

The results of this study showed that at Durras in New South Wales *P.cinnamomi* inhabited the soil/fine root matrix of the moist part of the site for the duration of the sampling period. The fungus was not isolated from the large roots of *M.communis* which was the only species to exhibit obvious disease symptoms at this site. Using identical techniques, *P.cinnamomi* was not isolated from soil/fine root samples collected from the Jarrahdale site during 1978/79 but isolates were readily recovered from the large roots of recently dead *B.grandis* trees.

These results indicate that *P.cinnamomi* occupies a different ecological niche in the forest soils of Durras in New South Wales from that which it inhabits in the Western Australian jarrah forest. This makes it difficult

to categorise the fungus according to current ecological classifications of soil fungi. However, it is convenient to discuss the relationship of *P.cinnamomi* in these two situations using some existing classification system as a reference for comparison.

Garrett (1956) categorised soil fungi into root-inhabiting and soil-inhabiting types according to their competitive saprophytic ability in soil and parasitic behaviour on host species. Later Garrett (1970) referred to the parasitic members of these two groups as specialised and unspecialised root-infecting fungi. While conceding that some intermediate types must occur, e.g. the *Rosellinia* spp. of the tropics, he believed that most soil fungi could be assigned to one or other of these two classes. A comparison of the pathogen occurrence and disease syndromes in New South Wales and Western Australia using the criteria outlined by Garrett for classifying soil fungi suggests that *P.cinnamomi* overlaps both classes i.e. the fungus may behave as either a root-inhabiting or soil-inhabiting type depending on the particular host-parasite-environment conditions of the site.

Results from the Durras survey (Section 2.3) and the work of some other researchers suggest that *P.cinnamomi* may conform to the soil-inhabiting group of fungi. Garrett's criteria for classifying soil-inhabiting fungi specify that the fungus must be abundant and widely distributed in soil, have a wide host range and be able to exist as a competitive soil saprophyte in the absence of living host tissue. Arentz (1974) and Arentz *et al* (1974)

concluded that *P.cinnamomi* had a wide distribution on the south coast of New South Wales and that this distribution was discontinuous in space and time. The list of host species from which this fungus has been isolated is long and generically diverse (Titze and Palzer, 1969) and the work of Zentmyer and Mircetich (1966) and Reeves (1975) indicates that *P.cinnamomi* has some competitive saprophytic ability under certain conditions. In addition, soil and fine root samples collected in the moist zone of the Durras site consistently yielded *P.cinnamomi*, but samples taken from the large roots of *M.communis* did not. However, while the above factors are indicative of a soil-inhabiting fungus some of this evidence is of questionable significance. Despite the observations of Zentmyer and Mircetich (1966) and Reeves (1975) there is no evidence that *P.cinnamomi* survives as a competitive soil saprophyte in the field. Further, results from the Durras survey suggest that *P.cinnamomi* was not abundant or widely distributed in the drier part of the site and its competitive saprophytic ability in this situation was poor because it was not isolated from soil collected in that area although inoculation must have occurred on numerous occasions via infested soil carried on feral animals. Hence, while some of Garrett's requirements are satisfied for classifying the fungus in the Durras situation as a soil-inhabiting type there are some important contradictions.

In Western Australia, *P.cinnamomi* exhibited some of the features of a root-inhabiting fungus e.g. the

apparent absence of the fungus from the soil/fine root matrix, the infection of large roots of *B.grandis* and the mode of uphill extension of disease (see Section 2.6.3) are characteristics of a root-inhabiting fungus. However, the apparent absence of a specialised growth habit (e.g. mycelial aggregation), the rapid and total destruction of the *B.grandis* host, the ephemeral presence of the fungus in soil when conditions are suitable for sporangial production (Shea, 1979b) and the wide host range (Titze and Palzer, 1969) are uncharacteristic of a specialised parasite.

Garrett (1956) suggested that the behaviour of a parasite on its wild (synonymous with natural?) host probably best signifies its present evolutionary status. If *P.cinnamomi* were indigenous to eastern Australia and only recently introduced to Western Australia, the New South Wales syndrome would be indicative of the 'natural' situation. While a classification of soil fungi such as that proposed by Garrett (1956) has undoubted value for the broad interpretation of the ecological status of soil fungi and serves as a useful basis for discussion in individual cases, it cannot be expected to be equally applicable to all soil fungi. Hence, it is probably preferable to study the saprophytic ability and parasitic behaviour of *P.cinnamomi* in different disease situations in this country, disregarding any rigid classification of the fungus because changed environmental conditions and the introduction of the fungus into uninfested areas has

created new host-parasite-environment relationships and, hence, entirely different disease syndromes. Thus prescriptions for disease avoidance/control will vary in different situations. Ecological classification of the fungus based on its behaviour in a specific situation can only create confusion and controversy and may lead to the adoption of inappropriate strategies for control/ avoidance of disease. This is particularly evident if the parasitic behaviour of *P.cinnamomi* on different host species in the jarrah forest of Western Australia is examined.

A hypothetical schema of the aetiology of jarrah dieback disease in the light of present day knowledge is as follows:

> Healthy forest areas become inoculated when infected root material is introduced by man or when fungal propagules are washed into an area from infested soil upslope. Large roots of *B.grandis* become infected providing the fungus with a large food base and a means of spread via root contact. Infective propagules of *P.cinnamomi* then occur at high population levels when environmental conditions are suitable for sporangial production; usually early spring. This results in the infection and destruction of fine feeder roots of many host species, including jarrah. Rapid distribution of the organism downslope may

occur as a consequence of the washing down of fungal propagules. The onset of hot-dry conditions (mid-summer to autumn) causes the death of fungal material in soil and fine roots and results in a contraction of its distribution to the large roots of *B.grandis*. Host species losing roots due to *P.cinnamomi* attack face added physiological stresses at this time and may die back. Repetition of this sequence is thought to culminate in a 'graveyard' forest situation (Figure 2.7) after several years.

This account describes *P.cinnamomi* as a "root-inhabiting" fungus in the large roots of *B.grandis* and a "soil-inhabiting" fungus parasitic on the fine roots of jarrah trees.

The parasitic behaviour of *P.cinnamomi* on host species in the jarrah forest is also important from an epidemiological viewpoint. For instance, provided that the infection of large roots by *P.cinnamomi* is confined to *B.grandis* (or a small number of species) the inoculum potential of the fungus could be decreased by the removal of that component from the forest. If several groups of host species harbour the fungus in their large roots or root collar regions, eradication of *B.grandis* as a means of decreasing inoculum potential, may be relatively ineffective.

Shea (1979a) proposed that a prescribed burning regime may reduce the impact of *P.cinnamomi* on freely drained *E.marginata* sites by altering the structure and composition of the forest and replacing the susceptible *B.grandis* understorey with more resistant species. The success of this proposed scheme will depend largely on its capacity to eradicate the means of root to root spread of the fungus and thus reduce its inoculum potential. The contraction of the distribution of *P.cinnamomi* into isolated pockets in the forest by reducing the number of understorey plants susceptible to large root and root collar infection may slow the spread of disease because the soil physical environment is generally unfavourable to the survival and spread of the fungus.

At the Durras site in New South Wales, increased spatial distribution of the fungus probably requires a major alteration of the soil environment (Section 2.3.3) so that the moisture status of the site surpasses some critical level for that soil type. There is no evidence to suggest that *P.cinnamomi* causes infection in the large roots of *M.communis* (the only species to exhibit obvious decline) or any other species in the site, Hence, in contrast to the Western Australian situation, the fungus is probably denied a large food base and means of root to root spread through the understorey. Host species may have developed resistance to attack of their large roots or susceptible species may have been eliminated during the evolution of the ecosystem.

The decline of *M. communis* at this site probably results from the combined effects of unsuitable soil conditions, physiological stresses and pathological microorganisms suggesting that it is an environmental disease rather than a host-pathogen dominated situation as appears to be the case in Western Australia.

In the Durras situation, disease prevention/ control can be best maintained by minimal disturbance of the environment.

The inherent differences in aetiology and epidemiology of *P. cinnamomi* disease in the two areas studied emphasise the need to treat each disease situation on its own merits so that appropriate control/ prevention strategy is adopted in each particular situation.

CHAPTER 3

MICROBIAL POPULATIONS IN THE DURRAS AND JARRAHDALE TRANSECT SOILS AND THEIR RELATIONSHIP TO THE ISOLATION OF *Phytophthora cinnamomi* AND TO DISEASE EXPRESSION IN THE VEGETATION

3.1 INTRODUCTION

It has been suggested (Weste and Vithanage, 1977) that differences in soil microbial populations may be correlated with the variation of disease expression seen in the field and that areas liable to severe disease contain small populations, particularly of actinomycetes.

Weste and Vithanage (1977) showed that microbial populations in a forest soil were significantly reduced following dieback disease and that soils from this site, which supported severely diseased vegetation, had small microbial populations compared to soils in less severely affected sites. They concluded that "this may be a factor in the severity of the disease due to *P.cinnamomi* and its rapid extension".

In this study, soil microbial populations from healthy, greenline and diseased vegetation zones of the Durras and Jarrahdale transects were assessed during 1978/79 to determine:

(i) differences associated with site, vegetation disease expression and sampling date; and

(ii) the association between the microbial populations of different soils and the isolation of *P.cinnamomi* from those soils.

3.2 MATERIALS AND METHODS

Transect points (which represented healthy, greenline and diseased vegetation zones) were selected at the Durras and Jarrahdale sites. On occasions when soil was sampled for lupin baiting analysis (Chapter 2), a 5 g subsample was taken from soil collected at each representative transect point. These samples were used to assay the soil microbial population of each vegetation zone by employing a soil dilution plate technique (Waksman, 1927 as modified by Malajczuk and McComb, 1979).

Three replicate dilution series were prepared from soil collected from each vegetation zone. Replicate 0.1 ml aloquots of each dilution were spread on duplicate plates of rose bengal - streptomycin agar (Martin, 1950) for the isolation of fungi, and on $\frac{1}{10}$ tripticase soy agar for bacterial and actinomycete isolation. Plates were incubated at 25°C. Fungal colonies were counted after four days incubation and the number of fungi belonging to the *Aspergillus* + *Penicillium* group were recorded as well as the total number of fungal colonies. Tripticase soy agar plates were incubated for 14 days before colonies belonging to the actinomycete and bacterial groups were counted. The number of viable units per gram of oven dry soil was calculated assuming that each colony arose from a single viable unit.

An analysis of variance was used to test for the significance of site, vegetation zone, sampling date and the interaction terms on the number of micro-organisms belonging to each microbial group counted. Initially, analysis of variance were applied to the untransformed data. Inspection of the residuals from these analyses showed that the variance of individual observations increased with the fitted values so a logarithmic transformation was applied and the data reanalysed.*

Homogeneity of variance of the data was tested using Bartlett's test (Bartlett, 1937) and the variances were found to be significantly different. However, Bartlett's test is sensitive to non-normal data and biological data often gave significant results. Hence Levene's test (Levene, 1960) was also applied. This generally gave similar significant effects to Bartlett's test, but at a lower level of significance. This means that the results of the analysis of variance must be interpreted with caution. Some improvement was obtained by discarding the data for particular dates.

The significance of the difference in the number of bacteria, actinomycetes and total fungi isolated from soil collected from the three vegetation zones at each site was tested using an analysis of deviance.

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* Analysis of variance was done by C.S.I.R.O., Division of Mathematics and Statistics, Canberra, A.C.T.

Analysis of deviance employing the GLIM computer programme (Nelder, 1975) was used also to test the significance of the association between the frequency of recovery of *P.cinnamomi* (from soil sampled from the *P.cinnamomi* infested zones of the Durras site) and the number of micro-organisms belonging to each microbial group isolated from subsamples of the same soil.

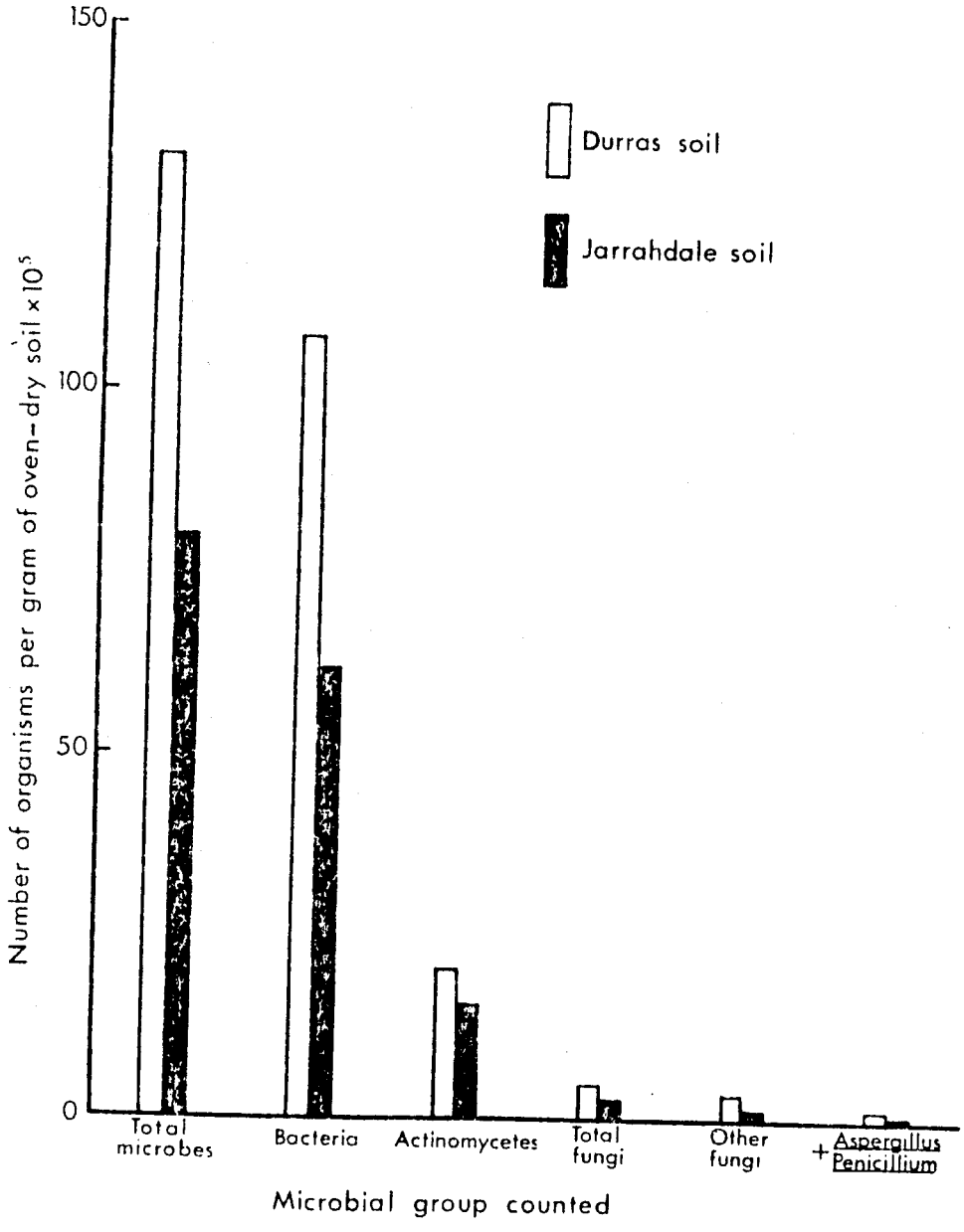
3.3 RESULTS

The mean number of viable units per gram of oven dry soil for each of the microbial groups counted during 1978/79 is illustrated in Figure 3.1. These values are the mean of eight counts made from Durras soil and five from Jarrahdale soil. They demonstrate the different magnitudes of the six microbial groups counted. The mean number counted for each of the microbial groups is similar in magnitude to those reported by Weste and Vithanage (1977) for some Victorian forest soils.

The analysis of variance (summarised in Table 3.1) suggests that differences existed between sites, vegetation zones and sampling dates for most of the microbial groups counted. The effect of vegetation zone (analysed using the analysis of deviance) on the number of bacteria, actinomycetes and fungi counted on some sampling dates at both sites is recorded in Table 3.2. This effect is illustrated in Figure 3.2.

The analysis of deviance, summarised in Table 3.3, records the association between the six microbial groups counted and the recovery of *P.cinnamomi* from soil collected from the infested zone of the Durras site.

FIGURE 3.1 MEAN NUMBER OF ORGANISMS OF SIX MICROBIAL GROUPS COUNTED IN SOIL COLLECTED FROM THE DURRAS AND JARRAHDALE SITES* DURING 1978/79



* Data from the healthy, greenline and diseased zones were combined.

TABLE 3.1 ANALYSIS OF VARIANCE FOR THE EFFECT OF SITE, VEGETATION ZONE, SAMPLING DATE AND THE INTERACTION TERMS ON THE NUMBER OF MICRO-ORGANISMS BELONGING TO SIX MICROBIAL GROUPS IN SOIL SAMPLED DURING 1978-79

Source	Total microbes	Bacteria	Actinomycetes	Total fungi	*Aspergillus* + *Penicillium*	Other fungi
Site	34.2***+	43.6***	7.0*	445.6***	127.6***	1752.7***
Vegetation zone	4.9*	3.5	23.7***	40.7***	4.8*	98.0***
Site.NSW date	2.9*	3.2*	3.7*	6.6***	15.9***	5.2**
Site.WA date	14.2***	18.4***	12.6***	73.8***	48.5***	143.8***
Site.Vegetation zone	0.0	0.0	4.4*	12.4***	4.3*	28.5***
Site.Vegetation zone.NSW date	0.5	0.4	2.1	2.4	2.0	3.7
Site.Vegetation zone.WA date	2.1	2.5	0.8	3.8*	4.2*	29.0***

+ Values represent the variance ratio followed by the level of significance

* Difference between treatments significant (P= 0.05)

** P = 0.01 *** P = 0.001

TABLE 3.2 THE DIFFERENCE IN THE NUMBER OF BACTERIA, ACTINOMYCETES AND FUNGI COUNTED IN SOIL SAMPLES COLLECTED FROM THE HEALTHY (H), GREENLINE (G) AND DISEASED (D) ZONES OF THE DURRAS AND JARRAHDALE SITES ON DIFFERENT OCCASIONS.

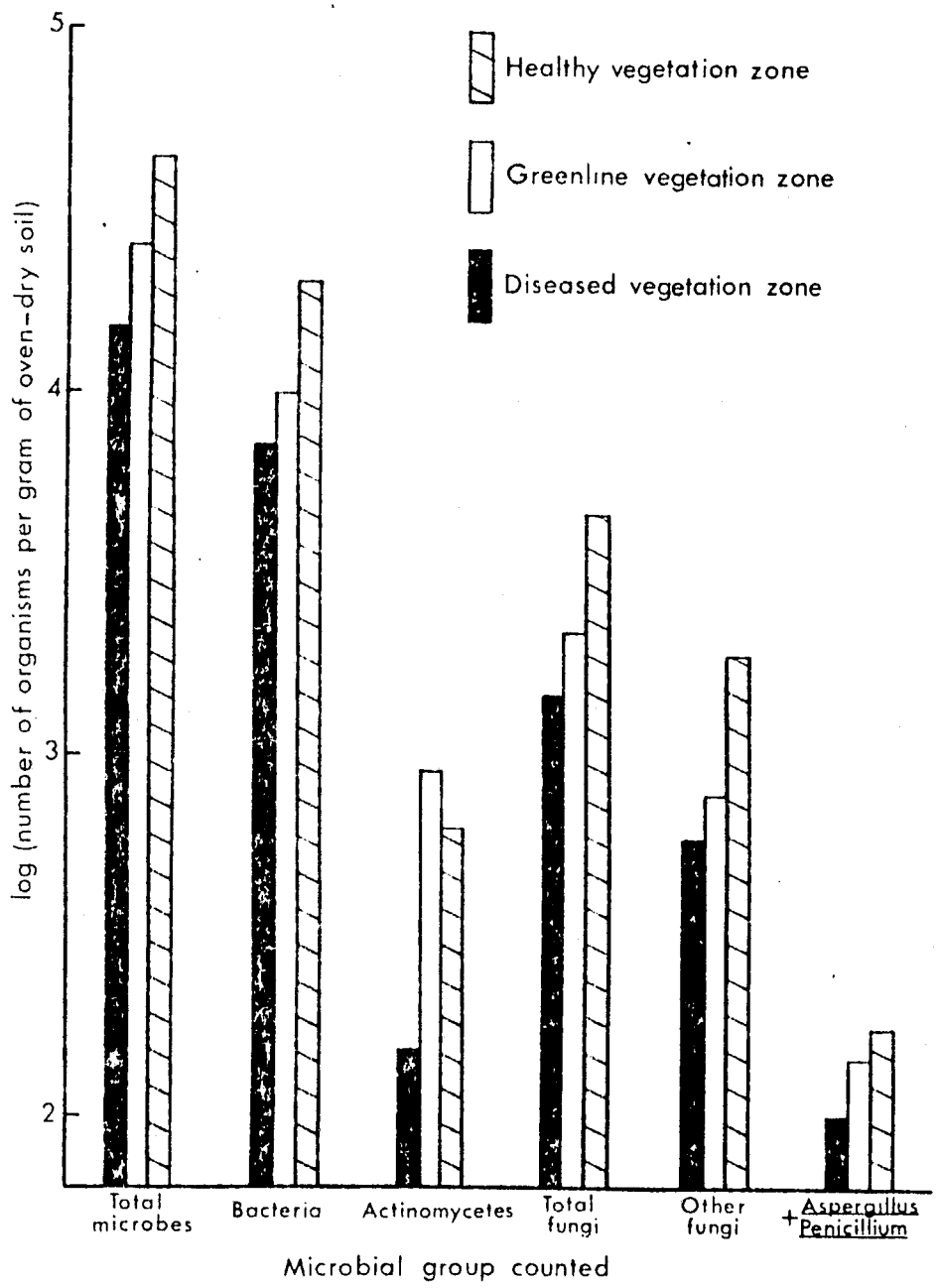
Site and sampling date	Bacteria	Actinomycetes	Total fungi
Durras			
1: 2:78	D < H < G*	D < G, H	D, G < H
11: 7:78	NS**	D < G, H	D, G < H
23: 7:78	G < D, H***	NS	H, G < D
14:11:78	NS	NS	D, G < H
21: 2:79	D, G < H	NS	D, G < H
6: 4:79	D < G, H	D, H < G	D, G < H
Jarrahdale			
29: 5:78	D, G < H	NS	D, G < H
15:11:78	NS	NS	D < G, H
12: 3:79	D, G < H	NS	NS

* D < G < T = the number of bacteria counted in soil collected from the diseased zone (D) was significantly less than the number counted in healthy zone soil (H) which was significantly less than the number in greenline zone soil (G).

**NS = no significant difference between the number in H, G or D.

*** G < D, H = the number of bacteria counted in soil collected from the greenline zone (G) was significantly less than the number counted in the diseased (D) and healthy (H) zones. There was no significant difference between the number counted in the diseased (D) and healthy (H) zones.

FIGURE 3.2 THE NUMBER OF ORGANISMS BELONGING TO EACH MICROBIAL GROUP IN SOIL COLLECTED FROM THE HEALTHY, GREENLINE AND DISEASED VEGETATION ZONES OF THE TWO SITES* SAMPLED



* Data used were the means of all determinations made from Durras and Jarrahdale soil for each vegetation zone.

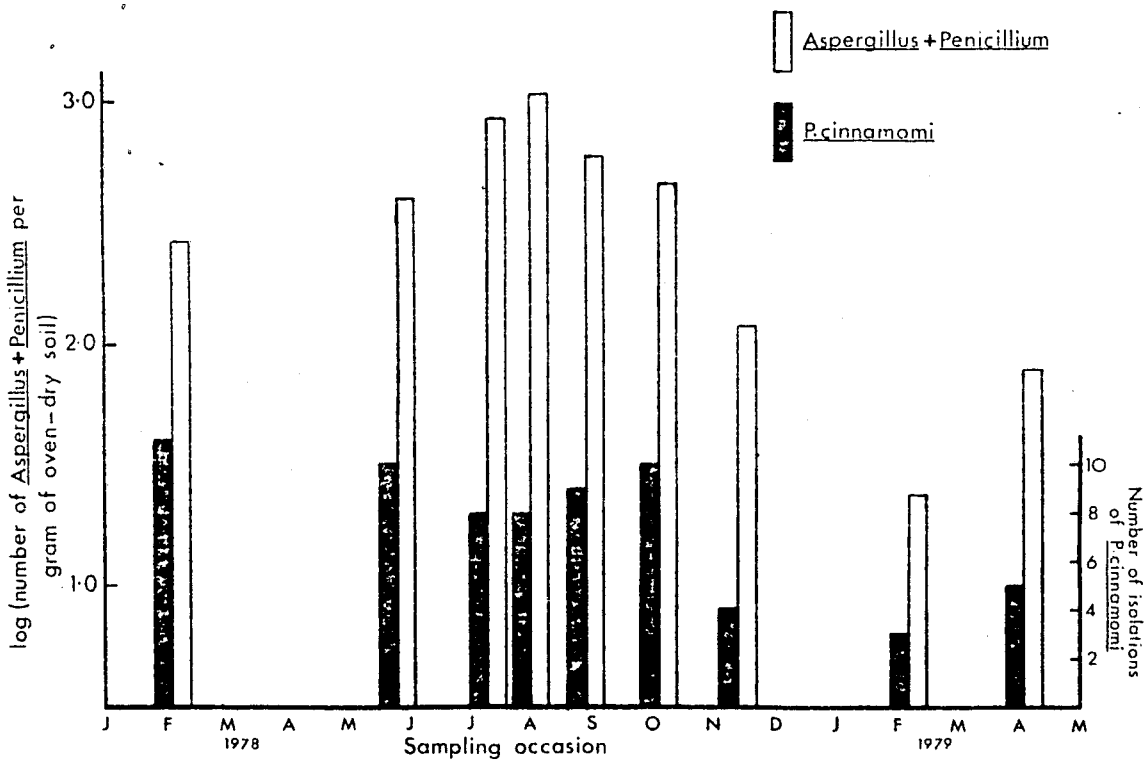
TABLE 3.3 The association between the population of six microbial groups and the recovery of *P.cinnamomi* from the infested zones of the Durras transect during 1978-79.

Source	df	Deviance
Total microbes	4	7.31
Bacteria	1	2.65
Actinomycetes	1	1.40
Total fungi	1	4.61*
Other fungi	1	0.16
Aspergillus + *Penicillium*	1	5.43*

* Deviance > x^2 (0.05)

The number of microbes belonging to the Total fungi and *Aspergillus* + *Penicillium* groups were significantly associated with the outcome of *P.cinnamomi* isolation from infested soil collected from the Durras site. However, the Total fungi group was not significant when the *Aspergillus* + *Penicillium* group was omitted from the count (see Other fungi; Table 3.3). This suggested that the *Aspergillus* + *Penicillium* group was the only group of microbes significantly associated with the isolation of *P.cinnamomi* from infested soil collected from the Durras site. This was an inverse association which is illustrated in Figure 3.3. To avoid complication, bulked data for the

FIGURE 3.3 · THE ASSOCIATION BETWEEN THE NUMBER OF *Aspergillus* + *Penicillium* COUNTED AND THE RECOVERY OF *P. cinnamomi* FROM SOIL COLLECTED FROM THE INFESTED ZONE OF THE DURRAS SITE.



two infested zones (diseased and greenline) was used to produce Figure 3.3 rather than data from individual zones which was used in the statistical analysis (Table 3.3).

3.4 DISCUSSION

The soil dilution plate technique (Waksman, 1927) has been criticised as biased towards heavily sporing organisms, particularly in soil fungi (Warcup, 1960; Garrett, 1963). While it is appreciated that the technique is selective toward some microbial groups, the aim of this study was to determine whether differences existed between the soils examined, rather than quantify precisely the microorganisms presented. The soil dilution plate technique seemed the most convenient way to compare the microfloras of different soils using identical methods.

In the past, the technique has been used by Australian researchers to compare the microbial populations of soils in various contrasting situations. Broadbent and Baker (1974b) compared the microbial populations of suppressive and conducive soils; Weste and Vithanage (1977) examined population levels in three forest soils, and Malajczuk and McComb (1979) used the technique to compare the microfloras on roots of two eucalypt species growing in two different soils. In general, the results suggested that soils conducive to *P.cinnamomi* disease or soils collected from areas carrying diseased vegetation had smaller microbial populations than suppressive soils or soil collected from healthy forest areas.

The results of the survey suggest that similar conclusions apply in this situation, e.g.:

(i) the microbial populations of soil collected from the diseased zone of the forest were smaller than those of soil collected from the healthy zone of the forest for all microbial groups (Figure 3.2). Often the difference reached statistically significant levels (Table 3.2).

(ii) irregardless of the disease status of the vegetation, the population level of all the microbial groups counted was generally smaller in soil collected from the Jarrahdale site than in soil collected from comparable vegetation zones of the Durras site (Figure 3.1).

While these results are consistent with results reported by other researchers, it should be pointed out that the microbial population levels of soil at each site were not calculated prior to disease incidence, hence, it is not known whether disease occurred in areas where lower microbial populations existed or whether microbial population levels dropped as a result of disease incidence. To examine this, a long term study monitoring microbial population levels before and after the incidence of *P.cinnamomi* induced disease would be required.

Weste and Vithanage (1977) reported a highly significant reduction in the microbial population of one of the soils they examined in spring and autumn when maximum populations of *P.cinnamomi* occurred. In this study the only statistically significant association

between the isolation of *P.cinnamomi* and the population level of any of the six microbial groups counted was the inverse association with the *Aspergillus* + *Penicillium* group (Table 3.3); *P.cinnamomi* isolation from soil was low on occasions when the *Aspergillus* + *Penicillium* population of that soil was high (Figure 3.3). This association is not surprising because soil conditions unfavourable to the growth and survival of *P.cinnamomi* (periods of low soil moisture) may still permit the activity of xerophytic fungi such as species of the *Aspergillus* + *Penicillium* group (Griffin, 1969). This group also includes species of fungal antagonists which may have some detrimental effect on the recovery of *P.cinnamomi* from soil on occasions when *Aspergillus* + *Penicillium* population levels are high. However, more research is required to verify this supposition.

CHAPTER 4

THE SURVIVAL OF *Phytophthora cinnamomi* IN THE FOREST SOILS OF DURRAS IN NEW SOUTH WALES AND JARRAHDALE IN WESTERN AUSTRALIA

4.1 INTRODUCTION

Phytophthora cinnamomi must survive periods of unfavourable environmental conditions in soil to establish itself as a pathogen in forest situations. Reeves (1975), Malajczuk *et al* (1977) and Hwang and Ko (1978) suggested that sporangia, zoospores and mycelium are relatively short-lived in forest soils and it is generally considered that chlamydospores are significant survival propagules of *P.cinnamomi* in soil.

In this chapter, the survival of the mycelium and chlamydospores of *P.cinnamomi* in soil collected from the healthy and diseased forest zones at Durras in New South Wales and Jarrahdale in Western Australia is reported. In retrospect the survival of chlamydospores of *P.cinnamomi* in forest soils in Western Australia may be superfluous because the fungus inhabits the large roots of *B.grandis* (Chapter 2), thus avoiding unfavourable soil environmental conditions. At Durras, however, there is no evidence of colonisation of the large roots of eucalypts and understorey species, hence, some propagules of *P.cinnamomi* must survive harsh soil conditions to maintain an inoculum source for periods favourable to root infection.

This study demonstrates the survival ability of mycelium and chlamydospores of *P.cinnamomi* under different soil conditions and provides an explanation for the observed pattern of *P.cinnamomi* isolation from soil samples collected at Durras and Jarrahdale during 1978/79 (Chapter 2).

4.2 EXPERIMENTAL

4.2.1 The Survival of *P.cinnamomi* in Undisturbed Soil Cores

Materials and Methods

Forty undisturbed soil cores (Appendix 5) were collected between positions 1 and 2 of the transect (Figure 2.1) in the depression of the Durras site in New South Wales. Soil cores were transported to the laboratory and placed in a controlled environment room which maintained the temperature at 10-13°C. Each core was placed in a watering saucer which saturated the lower 2 cm of soil and moistened the soil above to a matric potential ranging from 0 kPa to -1 kPa. Cores were kept in the dark to avoid weed growth, thus depriving the fungus of new host roots for possible infection.

On six occasions over a five month period, four soil cores were selected at random and planted with ten newly germinated, lupin seedlings. These were incubated in a controlled environment chamber at 17-24°C in alternating light (16 hrs at 180 microeinsteins M^{-2} sec^{-1}) and dark for one month. Twice per week lupin roots at the base of each core were checked for lesions caused by *P.cinnamomi* infection and the fungus was isolated as described in

Appendix 5 and Section 2.3.1. Results are presented in Table 4.1.

Results

TABLE 4.1 The isolation of *P.cinnamomi* from undisturbed soil cores collected at Durras and baited at intervals over a five month period.

Date cores were baited*	No. of cores yielding *P.cinnamomi* isolations
13. 7.77	$^4/_4$
28. 7.77	$^4/_4$
15. 8.77	$^2/_4$
8. 9.77	$^3/_4$
2.11.77	$^0/_4$
14.12.77	$^0/_4$

* Cores were collected from Durras on 11.7.77

Over a four month period the isolation of *P.cinnamomi* from undisturbed soil cores collected from the infested zone of the Durras site declined from 100% to 0% of the cores sampled. This decline occurred despite the high soil moisture potential and favourable temperature but in the absence of growing host roots.

Discussion

Inability to isolate *P.cinnamomi* from soil using this experimental technique after four months cannot be equated with propagule senescence. However, it is a useful initial experiment to determine whether a detailed study of

P.cinnamomi survival in soil is warranted; i.e. had the fungus been isolated over many months from soil cores subjected to harsh conditions in the laboratory, it would have indicated that once the organism is established, fungal inoculum is always abundant in soil and studies to determine the survival potential of propagules would not have been justified.

In this experiment, soil cores were maintained at conditions considered favourable to the survival of *P.cinnamomi*, hence, as the ability to isolate the fungus declined rapidly with time it is unlikely that the fungus would survive harsh soil conditions for long periods. Thus, the survival of *P.cinnamomi* in soil may be an important factor in determining the distribution of the fungus.

4.2.2 The Survival of *P.cinnamomi* Mats Placed in Field Soils

Materials and Methods

Isolate D.P.1 (A_2 compatability type) isolated from soil collected at Durras was used for the New South Wales section of this study while isolate W.A.1 (A_2 compatability type) isolated from roots of jarrah at Dwellingup was used in the Western Australian section of the experiment.

Mats of *P.cinnamomi* containing abundant chlamydospores were prepared as follows for insertion into soil:

(i) sheets of cellophane were cut into discs 6 cm in diam., boiled in water for 30 min to remove coating materials and placed individually between sheets of damp filter paper (Whatman No. 42, 9 cm diam.). Stacks of 30-40 discs of cellophane and filter paper were wrapped in aluminium foil and autoclaved (121°C for 20 min).

(ii) cooled, sterile cellophane discs were placed asceptically onto Petri plates containing V8 juice agar (20 ml/plate, Appendix 4) supplemented with cholesterol (20 ppm). This medium favoured abundant chlamydospore production.

(iii) each plate was inoculated at the centre of the cellophane with a 4 mm diam. disc of one of the *P.cinnamomi* isolates described above and incubated at 25°C in the dark.

(iv) after 25 days of growth, mycelium and chlamydospore mats of *P.cinnamomi* on cellophane were stripped from the V8 juice agar plates and a 7 cm diam. disc of clear plastic was stapled to the underside of each; this prevented the loss of chlamydospores into soil after the cellophane decomposed. The *P.cinnamomi* mycelium-chlamydospore mats on plastic (*P.c* mats + P) were placed in humidity chambers for transportation to the field.

Two hundred *P.c* mats + P were placed in the soil at Durras and Jarrahdale. Fifty, 10 cm long undisturbed soil cores and fifty, 5 cm long cores were removed in both the healthy and diseased zones of each site and a *P.c* mat + P placed at the base of each hole. The cores were then replaced. Twenty cellophane discs on plastic

but without *P. cinnamomi* mats were placed under 5 cm long cores in the diseased zone of the Durras site as a control treatment.

On six occasions at Durras and Jarrahdale, two replicate 10 cm and 5 cm cores were chosen at random and removed from the diseased and healthy zones. The *P. c* mats + P were carefully removed from the soil, placed in humidity chambers and returned to the laboratory. Using a steel punch each *P. c* mat + P was subdivided into twenty, 6 mm diam. discs. These were placed on Petri plates of antibiotic cornmeal agar (A.C.A.; Appendix 4) and incubated at 25°C for two days.

The number of 6 mm diam. discs yielding *P. cinnamomi* growth on A.C.A. collected from each site, zone and soil depth over a period of six months was recorded (Table 4.2 and 4.3).

Results

Mats of *P. cinnamomi* produced in the laboratory survived in the field at Durras and Jarrahdale for three to four months. There was no consistent difference in the survival of *P. cinnamomi* when mats were placed at different sites, zones within sites, or depths in the soil.

The cellophane discs buried at 5 cm depth in the diseased zone of the Durras site as a control (the zone of most frequent *P. cinnamomi* isolation) failed to yield *P. cinnamomi* on A.C.A.

TABLE 4.2 THE SURVIVAL OF *P.cinnamomi* BURIED IN SOIL AT TWO DEPTHS IN THE DISEASED AND HEALTHY FOREST ZONES OF THE DURRAS SITE.

Time (days) after insertion in soil*	Mean number of 6 mm diam. discs yielding *P.cinnamomi* on A.C.A.				
	Healthy forest zone		Diseased forest zone		
	Buried 10 cm	Buried 5 cm	Buried 10 cm	Buried 5 cm	Control
12	$^{20}/_{20}$	$^{20}/_{20}$	$^{20}/_{20}$	$^{20}/_{20}$	$^{0}/_{20}$
43	$^{16.5}/_{20}$	$^{17.5}/_{20}$	$^{9.5}/_{20}$	$^{15}/_{20}$	$^{0}/_{20}$
92	$^{17}/_{20}$	$^{14}/_{20}$	$^{13.5}/_{20}$	$^{7.5}/_{20}$	$^{0}/_{20}$
132	$^{12.5}/_{20}$	$^{5.5}/_{20}$	$^{5.5}/_{20}$	$^{2}/_{20}$	$^{0}/_{20}$
199	$^{0}/_{20}$	$^{0}/_{20}$	$^{0}/_{20}$	$^{0}/_{20}$	$^{0}/_{20}$
258	$^{0}/_{20}$	$^{0}/_{20}$	$^{0}/_{20}$	$^{0}/_{20}$	$^{0}/_{20}$

* *P.c* mats + P were inserted in soil on 11.7.78.

TABLE 4.3 THE SURVIVAL OF *P.cinnamomi* PLACED IN SOIL AT TWO DEPTHS IN THE DISEASED AND HEALTHY ZONES OF THE JARRAHDALE SITE.

Time (days) after insertion in soil*	Mean number of 6 mm diam. discs yielding *P.cinnamomi* on A.C.A.			
	Healthy forest zone		Diseased forest zone	
	Buried 10 cm	Buried 5 cm	Buried 10 cm	Buried 5 cm
28	$^{8}/_{20}$	$^{17}/_{20}$	$^{1}/_{20}$	$^{12}/_{20}$
58	$^{13.5}/_{20}$	$^{18.5}/_{20}$	$^{3.5}/_{20}$	$^{11}/_{20}$
85	$^{13.5}/_{20}$	$^{7.5}/_{20}$	$^{10}/_{20}$	$^{1}/_{20}$
128	$^{4.5}/_{20}$	$^{5.5}/_{20}$	$^{0}/_{20}$	$^{0.5}/_{20}$
170	$^{0.5}/_{20}$	$^{0}/_{20}$	$^{0.5}/_{20}$	$^{0}/_{20}$
186	$^{0}/_{20}$	$^{0}/_{20}$	$^{0}/_{20}$	$^{0}/_{20}$

* *P.c* mats + P were inserted in soil on 30.4.78.

Discussion

This experiment suggests that some part of the laboratory grown *P. cinnamomi* mats placed in soil survived for up to four months; a period similar to that for the isolation of *P. cinnamomi* from undisturbed cores in the Section 4.2.1. Taken together, these experiments imply that mats of mycelium with many chlamydospores of *P. cinnamomi* are relatively short-lived in soil. However, this implication is based on indirect evidence. In Experiment 4.2.2, propagules of *P. cinnamomi* may have remained viable for longer than four months but were removed from the plastic discs by soil animals.

Microscopic examination of the 6 mm diam. discs showed that chlamydospores were often the origin of *P. cinnamomi* growth. However, soil particles on the mats prevented the identification of the source of cultures in many instances. Hence, the identity of the viable propagules and the cause of chlamydospore senescence, whether from lysis by soil organisms or by depletion of their energy reserves, could not be ascertained using this experimental technique.

The following experiment records the survival of *P. cinnamomi* mycelium-chlamydospore mats in soil at three moisture regimes to determine the life expectancy of *P. cinnamomi* propagules *per se* under different soil conditions and to study the possible cause of their senescence.

This experiment involved collaboration with Dr K.M. Old, Division of Forest Research, C.S.I.R.O., Canberra, whose expertise in scanning electron microscopy is gratefully acknowledged.

4.2.3 The Survival of *P.cinnamomi* Mats in Soil at Three Moisture Regimes

Materials and Methods

PREPARATION OF NUCLEOPORE FILTER PACKETS. *P.cinnamomi*-chlamydospore mats on cellophane (*P.c* mats + C) were prepared as described in Section 4.2.2 (Materials and Methods i, ii and iii) except that 4 cm diam. cellophane discs were used.

Twenty five days after inoculation with D.P.1 *P.c* mats + C were stripped from the V8 juice agar plates and trisected. Segments of *P.c* mat + C were placed between two Nucleopore membrane filters (Nucleopore, Pleasanton, New Jersey, U.S.A., pore size 5.0 μm diam.). Using chloroform and employing a fine brush, each pair of filters was fused together at the perimeter so that the *P.c* mats + C were encased in a membrane filter packet. This allowed access to the *P.c* mat + C by soil microbes less than 5 μm in diam. but prevented soiling of the mat. Nucleopore filter packets were placed in a humidity chamber prior to insertion in soil.

The control treatments in this experiment were prepared by asceptically trisecting fifteen *P.c* mats + C and placing half the segments on Petri plates containing

V8 juice agar and half onto plates with distilled water agar. These were incubated at 15°C in the dark and were sampled as described below.

INSERTION OF NUCLEOPORE FILTER PACKETS IN SOIL. Four soil types were used in this experiment. Soil samples were freshly collected from the healthy and diseased zones of the Durras and Jarrahdale sites. Nucleopore filter packets were placed in three sub-samples of each soil which were subjected to the following water regimes:

(a) waterlogged: five hundred grams of each soil was placed independently into four, 26 cm diam. plastic watering saucers. Twenty five nucleopore filter packets were inserted in each soil and flooded with water so that the water level was 3 mm above the soil's surface.

(b) ψm = -5 kPa: four tensiometers (Griffin, 1972) were constructed by cutting the base out of four 26 cm diam. plastic watering saucers and inverting them on four, 28 cm diam. 1 bar pressure plate cells (Soilcrete, Aust. Ltd.). A continuous column of water was established between each porous pressure plate and a reservoir of water. The pressure plates were situated 50 cm above the head of the water reservoir to maintain a matric potential of -5 kPa at the surface of the plate.

Individual, 500 g samples of each soil were placed in a tensiometer and twenty five nucleopore filter packets inserted in each. The soil was flooded, allowed to drain to -5 kPa and maintained at that potential.

(c) air dried: five holes were drilled in the base of four, 25 cm diam. plastic watering saucers. Each hole was covered with nylon mesh (Nybolt, 0.5 mm mesh size). Five hundred grams of each soil was placed independently into four saucers and twenty five nucleopore filter packets were inserted in each. The soils were flooded, allowed to drain and dry out.

All treatments were maintained at 13-15°C in the dark.

SAMPLING NUCLEOPORE FILTER PACKETS. Three replicate nucleopore filter packets were sampled from each treatment after 1, 3, 6 and 10 weeks in soil. Three control *P.c* mats+ C on V8 juice agar and distilled water agar were also sampled. The nucleopore packets were cut open and all the *P.c* mats + C disected and examined in the following ways:

(i) *P.cinnamomi* SURVIVAL STUDY: a 1 cm square was cut from the *P.c* mats + C, placed on a damp sheet of graph paper (GAF, 1 mm) and subdivided into twenty five, 2 mm squares with a single edge blade. Each 2 mm square was inverted, placed on A.C.A. and incubated at 25°C. The number of squares yielding *P.cinnamomi* growth after two days was recorded.

(ii) LIGHT MICROSCOPY: a wedge was cut from each *P.c* mat + C (1/4 - 1/3 of the segment) and placed on a glass microscope slide. The mats were flooded with 0.1% trypan blue in lactophenol for 1 hr, a coverslip was put in position and the wedge examined using bright field

light microscopy (Nikon x 100 magnification). Ten randomly chosen microscope fields were viewed per wedge and the number of stained and unstained chlamydospores counted and recorded for each replicate *P.c* mat + C sampled. Chlamydospores were considered viable if the entire protoplasm stained blue and non-viable if they were only partly stained or empty.

The additive and interactive effects of the four variables: site, soil, water regime and time (linear and quadratic), on the number of stained and unstained chlamydospores counted was analysed statistically using the GLIM computer programme.

Micrographs of each treatment were prepared on each sampling occasion using phase contrast light microscopy (Zeiss Universal Research Microscope fitted with a Leitz, Leica Camera).

(iii) SCANNING ELECTRON MICROSCOPY (S.E.M.): the remaining pieces of *P.c* mat + C were placed in glutaraldehyde. Specimens were critical point dried (Cohen *et al*, 1968) and coated with gold. Examination of the *P.c* mats + C was carried out in a JEOL J.S.M.U.3 scanning electron microscope. Representative examples of each treatment were micrographed on each sampling occasion.

Results

(i) *P.cinnamomi* SURVIVAL STUDY: The percentage of 2 mm squares yielding *P.cinnamomi* growth on A.C.A. is illustrated in Figures 4.1 to 4.5. These graphs show that *P.cinnamomi* survival was longest in all soils under

FIGURE 4.1 THE PERCENTAGE OF 2 MM SQUARES YIELDING *P.cinnamomi* ON A.C.A. AND THE MEAN NUMBER OF STAINED AND UNSTAINED CHLAMYDOSPORES COUNTED PER MICROSCOPE FIELD ON *P.c* MATS + C INSERTED IN SOIL COLLECTED FROM THE HEALTHY ZONE OF THE BURRAS SITE IN NEW SOUTH WALES.

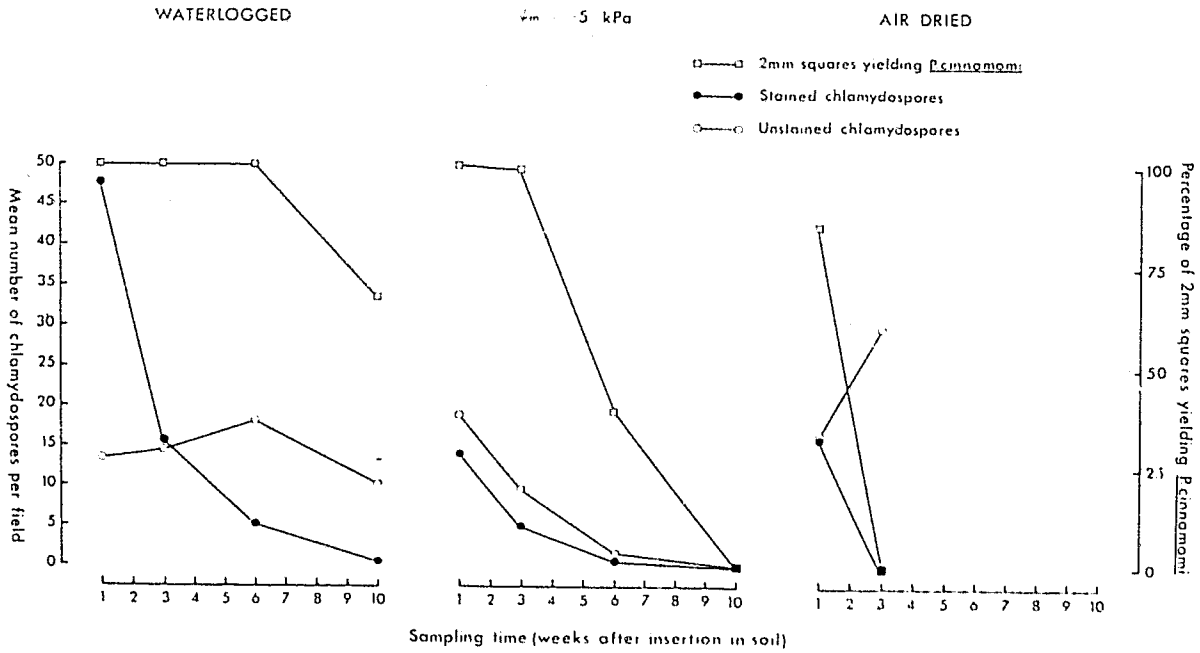


FIGURE 4.2 THE PERCENTAGE OF 2 MM SQUARES YIELDING *P.cinnamomi* ON A.C.A. AND THE MEAN NUMBER OF STAINED AND UNSTAINED CHLAMYDOSPORES COUNTED PER MICROSCOPE FIELD ON *P.c* MATS + C INSERTED IN SOIL COLLECTED FROM THE DISEASED ZONE OF THE DURRAS SITE IN NEW SOUTH WALES.

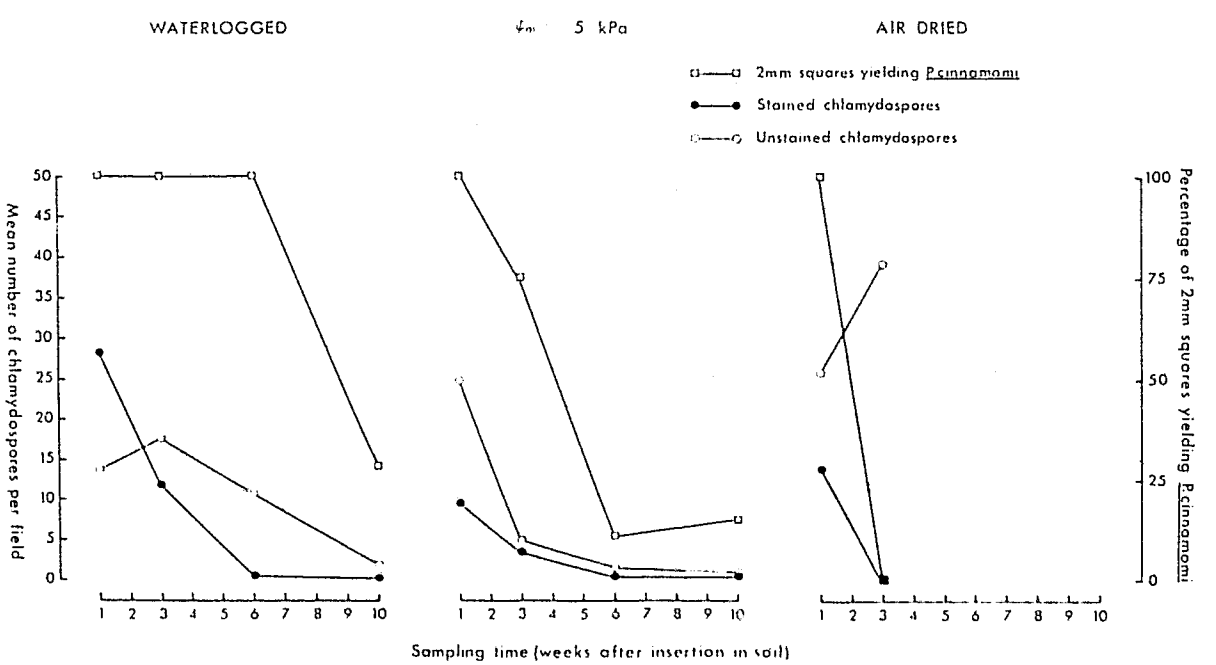


FIGURE 4.3 THE PERCENTAGE OF 2 MM SQUARES YIELDING *P.cinnamomi* ON A.C.A. AND THE MEAN NUMBER OF STAINED AND UNSTAINED CHLAMYDOSPORES COUNTED PER MICROSCOPE FIELD ON *P.c* MATS + C INSERTED IN SOIL COLLECTED FROM THE HEALTHY ZONE OF THE JARRAHDALE SITE IN WESTERN AUSTRALIA.

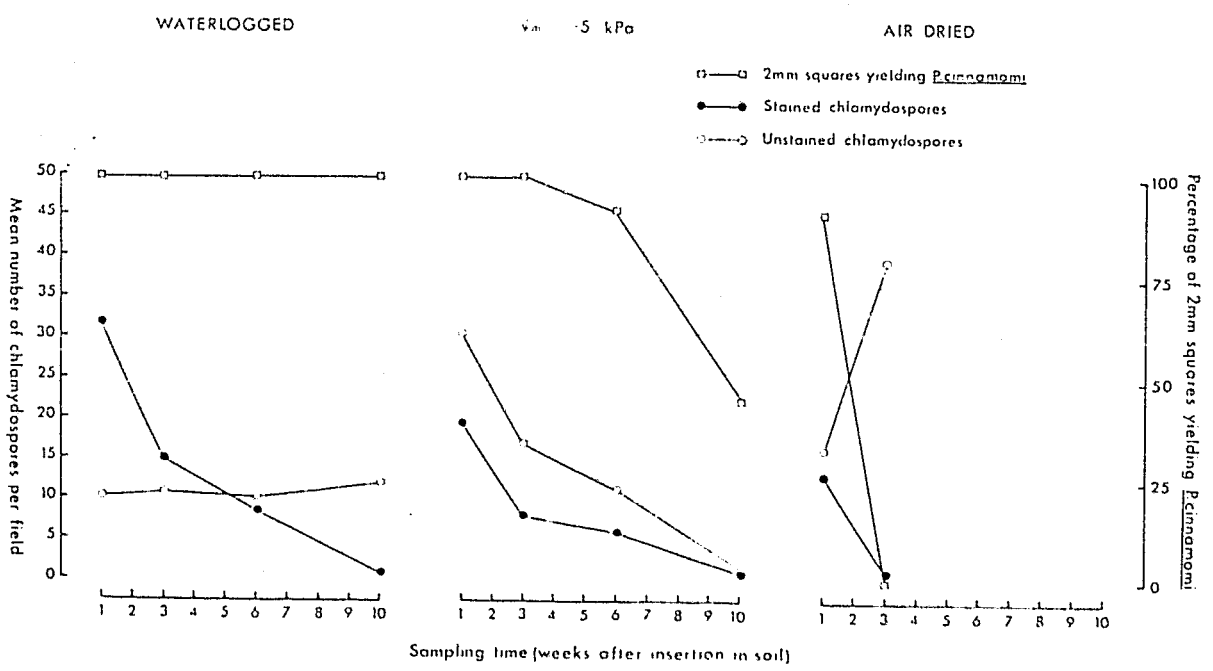


FIGURE 4.4 THE PERCENTAGE OF 2 MM SQUARES YIELDING *P.cinnamomi* ON A.C.A. AND THE MEAN NUMBER OF STAINED AND UNSTAINED CHLAMYDOSPORES COUNTED PER MICROSCOPE FIELD ON *P.c* MATS + C INSERTED IN SOIL COLLECTED FROM THE DISEASED ZONE OF THE JARRADHALE SITE IN WESTERN AUSTRALIA.

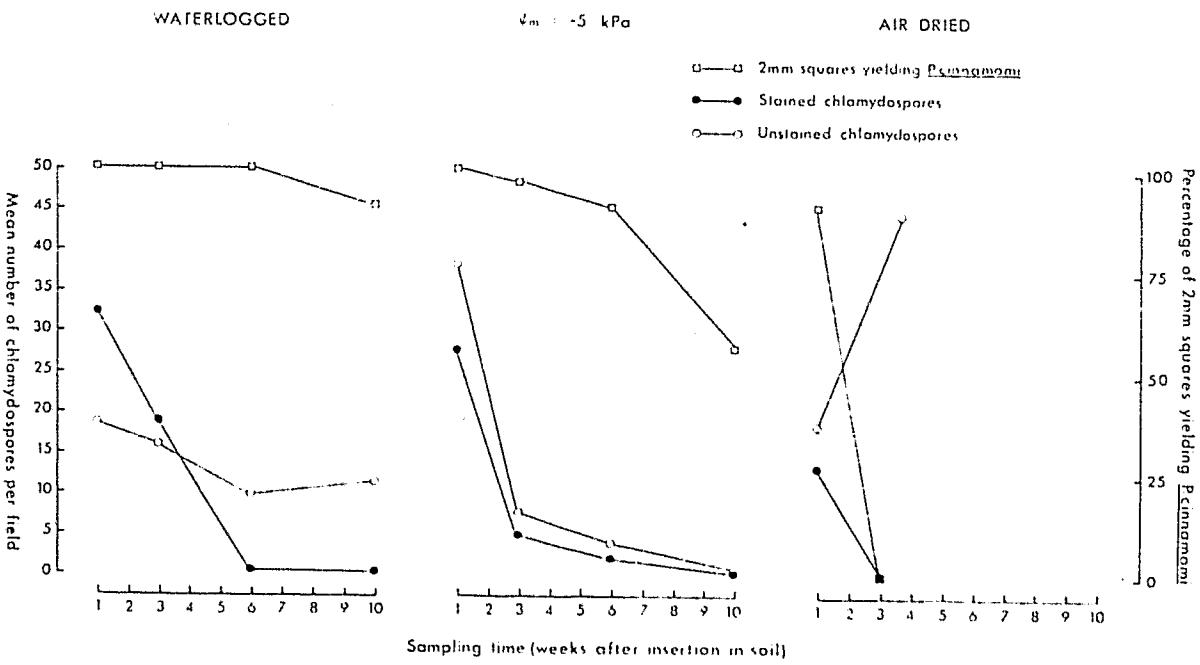
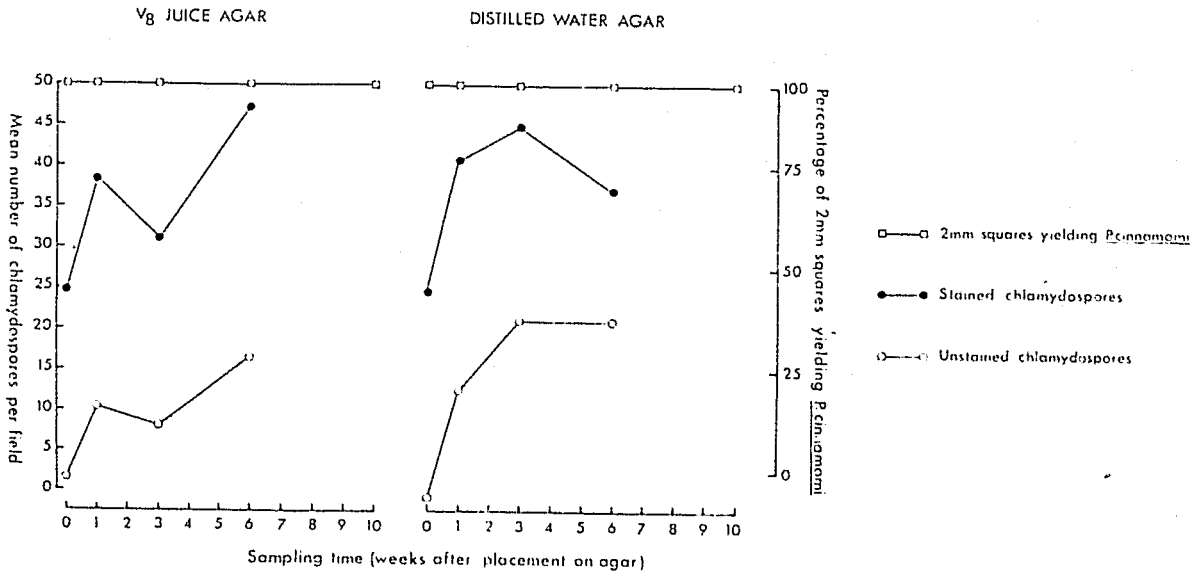


FIGURE 4.5 THE PERCENTAGE OF 2 MM SQUARES YIELDING *P.cinnamomi* ON A.C.A. AND THE MEAN NUMBER OF STAINED AND UNSTAINED CHLAMYDOSPORES COUNTED PER MICROSCOPE FIELD ON *P.c* MATS + C PLACED ON V8 JUICE AGAR AND DISTILLED WATER AGAR.



waterlogged conditions and shortest when the soils were allowed to dry out. The soil type appeared to have less effect on the survival of the fungus than the moisture regime. However, at -5 kPa, and under waterlogged conditions, *P.cinnamomi* always survived longer in the two Western Australian soils than in the New South Wales oils. There was no apparent difference in the survival of *P.cinnamomi* in soils that were allowed to dry out.

Despite contamination of the plates between the sixth and tenth weeks, one hundred percent of the 2 mm squares cut from segments placed on V8 juice agar and distilled water agar yielded *P.cinnamomi* after ten weeks incubation.

(ii) LIGHT MICROSCOPY: The mean number of stained and unstained chlamydospores per microscope field on *P.c* mats + C placed in four soils and subjected to three moisture regimes is illustrated in Figures 4.1 to 4.4.

The moisture status of the soils appeared to effect the number of chlamydospores counted more than the soil type *per se*. Under waterlogged conditions, the number of stained chlamydospores declined gradually to 0 per field at 10 weeks while the number of unstained chlamydospores remained reasonably constant at 10-15 per field. At -5 kPa the number of stained chlamydospores decreased markedly in the first three weeks and then declined gradually to 0 per field at 10 weeks. There was a similar trend in the decline of unstained chlamydospores. When soils were allowed to dry out there was a sharp

decline in the number of stained chlamydospores to 0 per field at three weeks while the number of unstained chlamydospores counted increased sharply over this period.

The number of stained chlamydospores counted on segments placed on V8 juice agar and distilled water agar (Figure 4.5) remained relatively constant over the six weeks of sampling. The number of unstained chlamydospores stabilised after an initial rise in the first week. Counting was discontinued after six weeks because the plates were contaminated between the sixth and tenth week.

Statistical analysis of the chlamydospore counts (Table 4.4) indicated that both of the fourth order interactions were highly significant.

TABLE 4.4 The effect of the interaction terms: Site.Soil.Water regime.$Time^2$ and Site.Soil.Water regime.Time, on the number of stained and unstained chlamydospores counted.

Chlamydospore	Source	df	Deviance
Stained	Site.Soil.Water regime.$Time^2$	1	9.4***
	Site.Soil.Water regime.Time	2	48.8***
Unstained	Site.Soil.Water regime.$Time^2$	1	39.0***
	Site.Soil.Water regime.time	2	41.7***

*** Deviance $> x^2$ (0.005)

This result indicates that no single variable (Site, Soil, Water regime or Time) describes the observed chlamydospore counts i.e. all the factors tend to interact with each other to generate the counts.

Light micrographs illustrating the condition of *P.cinnamomi* mats following exposure to various treatments are presented in Plates 1 to 12. The control treatments (Plates 1 and 2) show healthy chlamydospores and mycelium, prior to insertion into soil (Plate 1) and after six weeks on distilled water agar (Plate 2). In contrast, micrographs of *P.cinnamomi* mats inserted into soil which was allowed to dry out for one week (Plate 3) and three weeks (Plate 4) illustrate sensecent chlamydospores and mycelium. The condition of *P.cinnamomi* mycelium and chlamydospores after one, three, six and ten weeks in waterlogged soil is illustrated in Plates 5 to 8 respectively; chlamydospores lost more of their internal contents as time elapsed while much of the mycelium looked viable ten weeks after insertion in soil. *P.c* mats + C inserted in soil and subjected to -5 kPa for one, three, six and ten weeks are illustrated by Plates 9 to 12. At this moisture regime, intense microbial activity is evident. After one week (Plate 9) sporangia of *P.cinnamomi* were present and much of the mycelium was empty; some of the chlamydospores had deteriorated and there was no recognisable mycelium of *P.cinnamomi* after three weeks (Plate 10). After six weeks insertion in soil (Plate 11) the few remaining chlamydospores had germinated, and by ten weeks structures of *P.cinnamomi* could not be identified (Plate 12).

PLATE 1. *P.c* mat + C showing chlamydospores and mycelium prior to insertion into soil (x200).

PLATE 2. *P.c* mat + C showing chlamydospores and mycelium after six weeks on distilled water agar at 13°C (x400).

PLATE 3. *P.c* mat + C after one week in soil which was allowed to dry out at 13°C (x300). The cytoplasm of some chlamydospores has retracted from the chlamydospore wall. Some chlamydospores appear healthy.

PLATE 4. *P.c* mat + C after three weeks in soil which was allowed to dry out at 13°C (x500). The cytoplasm of all chlamydospores has retracted from the chlamydospore wall.

PLATE 1.

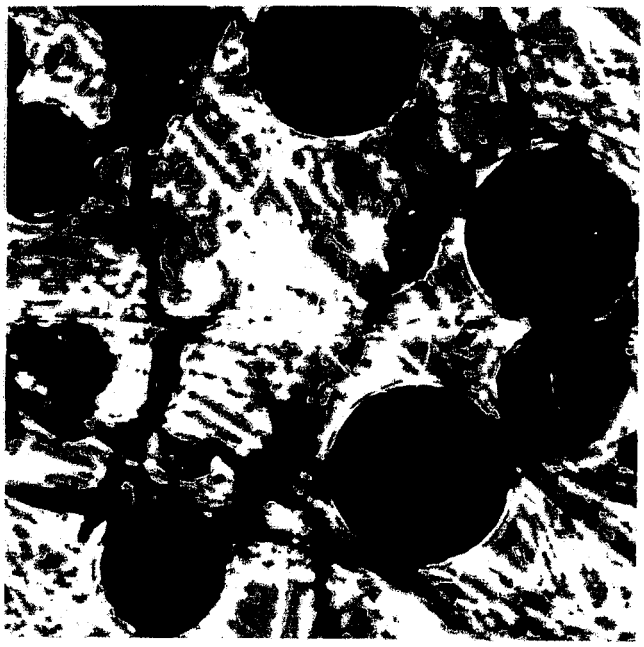


PLATE 2.

PLATE 3.

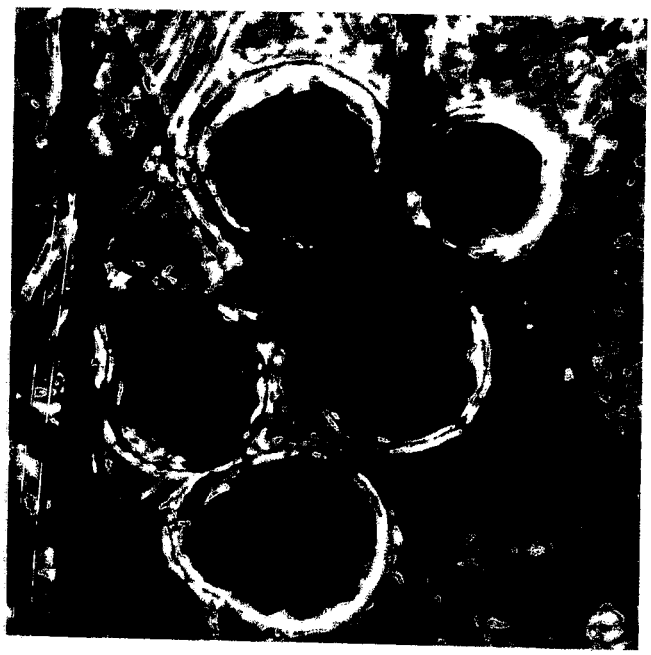


PLATE 4.

PLATE 5. *P.c* mat + C after one week in soil subjected to 0 kPa at 13°C (x300).

PLATE 6. *P.c* mat + C after three weeks in soil subjected to 0 kPa at 13°C (x300).

PLATE 7. *P.c* mat + C after six weeks in soil subjected to 0 kPa at 13°C (x300).

PLATE 8. *P.c* mat + C after ten weeks in soil subjected to 0 kPa at 13°C (x200). Note: the internal contents of the chlamydospores are drained, but much of the mycelium still look viable.

PLATE 5.

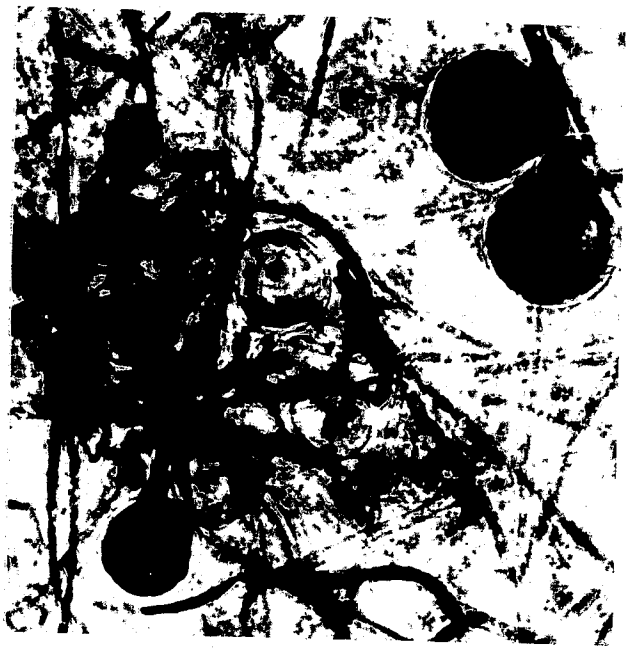


PLATE 6.

PLATE 7.

PLATE 8.

PLATE 9. *P.c* mat + C after one week in soil subjected to -5 kPa at 13°C (x250). Sporangia of *P.cinnamomi* are present and the majority of the mycelium look empty.

PLATE 10. *P.c* mat + C after three weeks in soil subjected to -5 kPa at 13°C (x200). Mycelium has lysed or is covered with bacterial slime. Some chlamydospores are starting to deteriorate.

PLATE 11. *P.c* mat + C after six weeks in soil subjected to -5 kPa at 13°C (x250). Most chlamydospores have lysed and those remaining are germinated.

PLATE 12. *P.c* mat + C after ten weeks in soil subjected to -5 kPa at 13°C (x300). No structures of *P.cinnamomi* can be seen. Mycrobial slime is very dense.

PLATE 9.

PLATE 10.

PLATE 11.

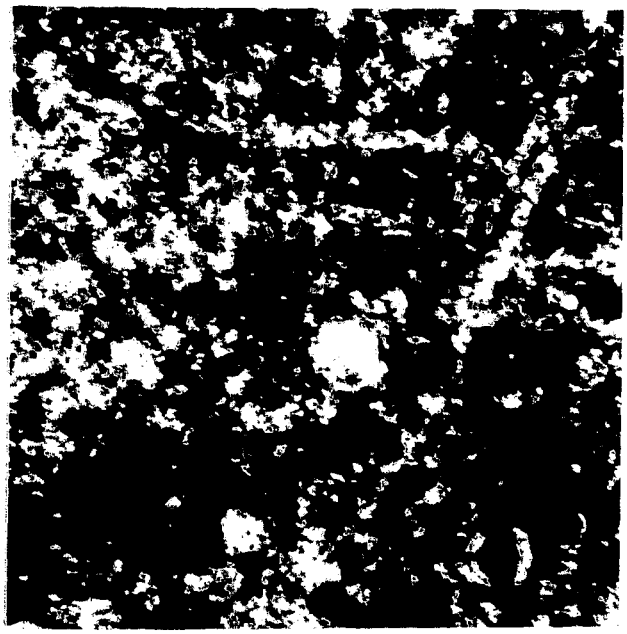


PLATE 12.

PLATE 13. *P.c* mat + C showing chlamydospores and mycelium prior to insertion into soil (x1,800).

PLATE 14. *P.c* mat + C after one week in soil subjected to 0 kPa at 13°C (x1,800). Very little microbial activity is evident.

PLATE 15. *P.c* mat + C after six weeks in soil subjected to 0 kPa at 13°C (x1,080). *P.cinnamomi* mycelium and chlamydospores look in good condition despite increased microbial activity.

PLATE 16. *P.c* mat + C after ten weeks in soil subjected to 0 kPa at 13°C (x6,000). Mycelium is perforated but intact.

PLATE 13.

PLATE 14.

PLATE 15.

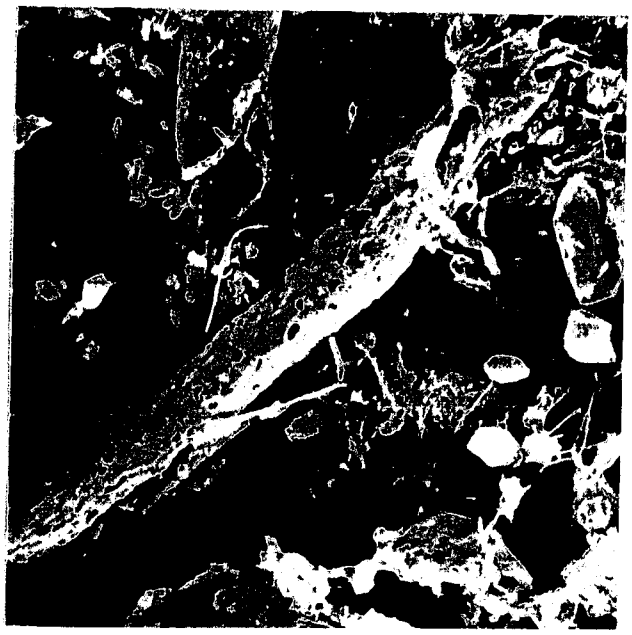


PLATE 16.

PLATE 17. *P.c* mat + C showing chlamydospores and mycelium after six weeks on distilled water agar (x1,800).

PLATE 18. *P.c* mat + C after one week in soil subjected to -5 kPa at 13°C (x1,800). Intense microbial activity is evident.

PLATE 19. *P.c* mat + C after six weeks in soil subjected to -5 kPa at 13°C (x6,200). Most of the mycelium is buried in microbial slime.

PLATE 20. *P.c* mat + C after ten weeks in soil subjected to -5 kPa at 13°C (x1,600). Only the remnants of fungal mycelium are evident.

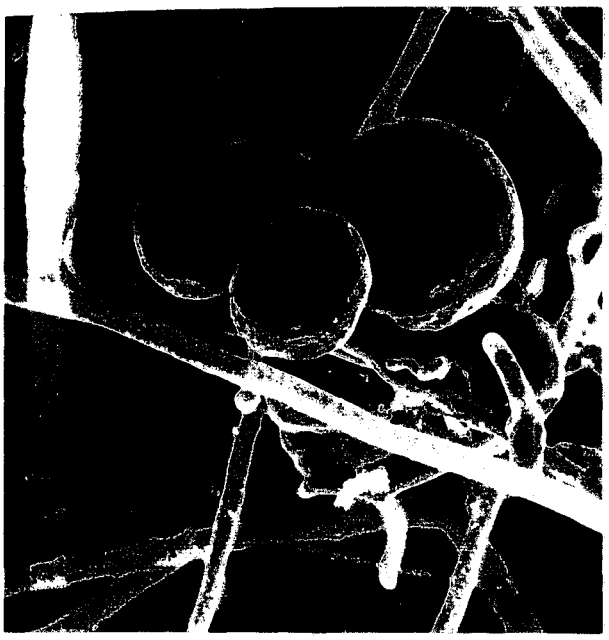


PLATE 17.

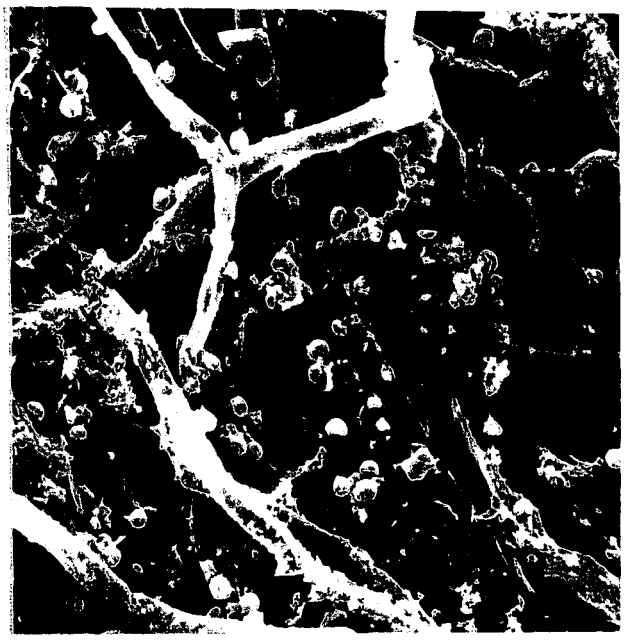


PLATE 18.

PLATE 19.

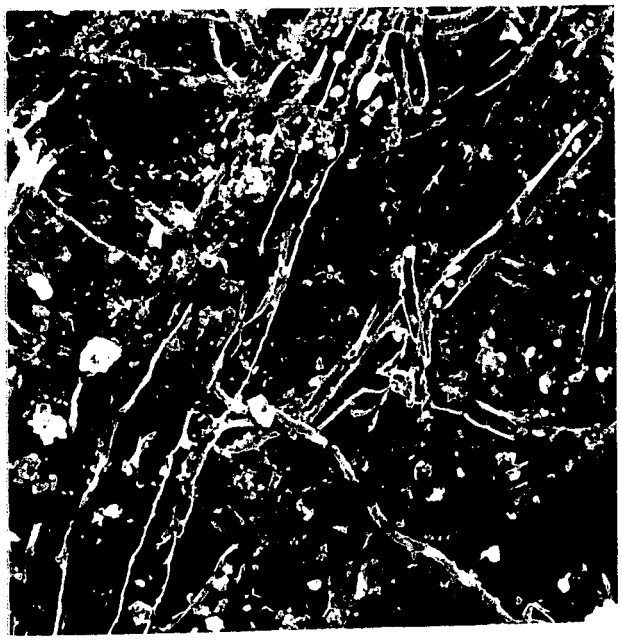


PLATE 20.

PLATE 21. *P.c* mat + C after three weeks in soil which was allowed to dry out (x2,000).

PLATE 22. *P.c* mat + C after one week in soil subjected to -5 kPa at 13°C (x10,000). Rod, coccal and spiral shaped bacteria are evident. Slime strands and sheets can be seen.

PLATE 23. *P.c* mat + C after six weeks in soil subjected to -5 kPa at 13°C (x10,000). Note: the testate amoeba attached to a perforated hyphal strand.

PLATE 24. *P.c* mat + C after ten weeks in soil subjected to 0 kPa at 13°C (x8,400).

PLATE 21.

PLATE 22.

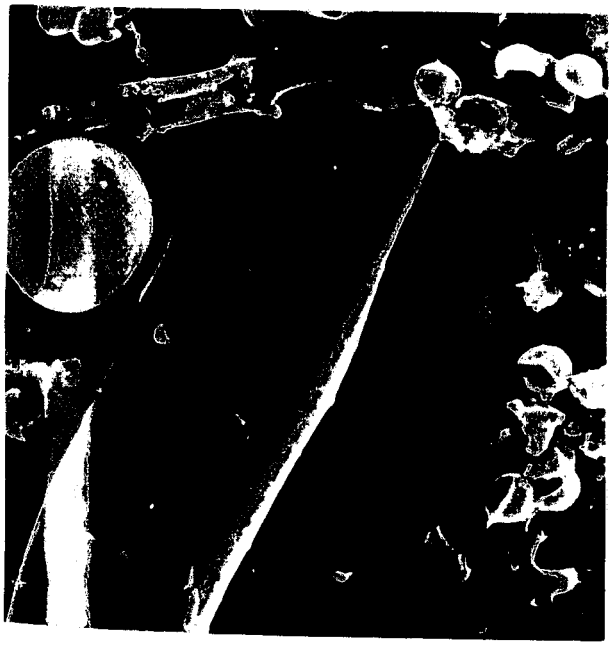


PLATE 23.

PLATE 24.

(iii) SCANNING ELECTRON MICROSCOPY: Representative scanning electron micrographs presented in Plates 13-24 support the results obtained by light microscopy. Plates 13-16 illustrate healthy chlamydospores and mycelium of *P.cinnamomi* prior to insertion into soil (Plate 13) and *P.c* mats + C after insertion in waterlogged soil for one, six and ten weeks respectively. Microbial activity was relatively low compared to that in the -5 kPa treatments (Plates 18-20). However, after ten weeks in soil (Plate 16) much of the mycelium looked perforated although intact (cf. Plate 8).

P.cinnamomi mycelium and chlamydospores appeared healthy after six weeks on distilled water agar (Plate 17). Plates 18, 19 and 20 illustrate the condition of *P.c* mats + C placed in soil at -5 kPa for one, six and ten weeks respectively. Intense microbial activity was apparent after one week (Plate 18) and by six weeks most of the mycelium had been lysed or buried in mycrobial slime (Plate 19). After ten weeks only the remnants of a few fungal hyphae could be identified.

P.c mats + C inserted in soil which was allowed to dry out for three weeks looked similar in external appearance to the control treatments (Plate 21 cf. Plates 13 and 17). Retraction of the cytoplasm from the chlamydospore wall (plasmolysis), as demonstrated by light microscopy (Plates 3 and 4) was the only obvious effect of this treatment. Plates 22 to 24 show some of the soil microorganisms present on *P.c* mats + C inserted into soil

and illustrates the damage they may cause to fungal hyphae. Plate 22 shows rod, coccal and spiral shaped bacteria and extensive slime development on mycelium of *P.cinnamomi*. Malajczuk *et al* (1977) described similar 'hyphosphere' microorganisms. Plate 23 illustrates a testate amoeba attached to a hyphal strand of *P.cinnamomi*; related soil organisms are commonly mycophagous (Old, 1979b) and these might be responsible for the hyphal perforations illustrated in Plates 23 and 24.

Discussion

P.cinnamomi survival was best when soil was water-logged and worst when the soil was allowed to dry out (Figure 4.1 to 4.4). In soil maintained at -5 kPa, some structures of *P.cinnamomi* survived for at least ten weeks after insertion into soil (Figure 4.1 to 4.4). However, apparently viable structures could not be found using the scanning electron or light microscope (Plates 12 and 20) after 10 weeks in soil. Viable structures may have been buried in the microbial slime deposited at this soil moisture regime.

Statistical analysis of the chlamydospore counts (Table 4.4) indicated that both fourth order interactions were highly significant. Thus, soil moisture was not the only variable responsible for the observed pattern of survival in this experiment (although it may have been a major influence). Soil type, site, the time factor and perhaps many other variables interacted to generate the observed pattern. Hence, in this and similar studies it

cannot be concluded that the observed pattern of survival was caused by the soil moisture potential *per se*. Rather, the survival pattern was the result of conditions prevailing when the soil was maintained at that particular moisture potential.

The microbial competition in moist soil was one variable that may have effected the survival of *P.cinnamomi* propagules. There appeared to be an inverse association between the microbial activity of the soil and the length of survival of *P.cinnamomi* mycelium and chlamydospores, e.g.:

(i) survival was better in waterlogged soils (Figures 4.1 to 4.4) which appeared to have lower microbial activity than soils maintained at -5 kPa (cf. Plates 5 and 14 with 9 and 18).

(ii) survival was better in soil collected from Jarrahdale than in soil from Durras (cf. Figures 4.3 and 4.4 with 4.1 and 4.2). Microbial population levels were lower in Jarrahdale soil than in Durras soil (Figure 3.1).

However, like all soil properties, microbial activity is dependent on a number of interrelated factors e.g. soil moisture, soil temperature, the organic matter content etc. Thus, in experiments employing field soil, it is probably not possible to isolate individual soil factors responsible for effects such as propagule survival. To gauge the potential importance of factors like moisture potential *per se* simplified experimental systems employing, for example, sterilised soil would have to be used.

However, such experiments have dubious significance in the field situation.

P.cinnamomi mats survived for less than three weeks in soils which were allowed to dry out. Due to the significant interactions in the statistical analysis it cannot be concluded that the poor survival was caused by the lack of moisture alone. However, it appeared likely that drying out was the integral cause e.g. while the external condition of the mycelium was good and the microbial competition was minimal (Plate 21), the cytoplasm of the chlamydospores had retracted from the chlamydospore wall (plasmolysed). Treatments employing sterilised soil may have been useful in this instance if they were set up and run concurrently with field soil treatments. The effect of drying out *per se* may have been more clearly demonstrated had similar results been obtained in simplified experimental systems (i.e. those employing sterilised soils). Logistically this was impractical.

The failure of *P.cinnamomi* propagules to survive in soil which was allowed to dry out helps to explain the inability to isolate *P.cinnamomi* from Western Australian soils (Chapter 2). Hot, dry summer months and low soil moisture contents (often below 5%) are a feature of the climate of the northern jarrah forest and Batini (1977) concluded that these conditions are unfavourable to the survival of *P.cinnamomi*. The results of this experiment suggest that *P.cinnamomi* chlamydospores and mycelium might be plasmolysed in very dry soils (Plate 21).

It is unlikely that Durras soils dry out to such an extent because the climate is relatively mild and the rainfall evenly distributed throughout the year (Appendix 1). Thus, *P.cinnamomi* might be able to survive periods of three or four months separation from a host root in Durras soil (at least in the valley zone) but not in Jarrahdale soil in summer. Mats of *P.cinnamomi* on cellophane survived for three to four months in the field at Jarrahdale (Section 4.2.2) but these were inserted in autumn (April 1978) and recovered throughout the winter months when the soil was moist. *P.cinnamomi* was not recovered later than spring of that year after the soil had dried out. The *P.cinnamomi* mycelium-chlamydospore mats placed in Durras soil (Section 4.2.2) were inserted in late winter and retrieved during spring and summer (Table 4.2).

The mechanism of *P.cinnamomi* survival might vary in soils subjected to contrasting moisture regimes. *P.cinnamomi* mycelium appeared viable after ten weeks (Plate 8) in soils kept waterlogged whereas mycelium in soils maintained at -5 kPa lysed after three weeks (Plate 10). In waterlogged soil, chlamydospores were intact but devoid of their internal contents by the tenth week (Plate 8); however, they appeared to stain darkly until lysis or germination (Plate 10 and 11) in soils maintained at -5 kPa. Perhaps in waterlogged soils, the integrity of the mycelium is maintained at the expense of chlamydospores. Abundant mycelium (instead of a few scattered chlamydospores) would

then be available for the production of sporangia immediately soil conditions became favourable. Such adaptations may be inherent because conditions suitable for sporangial production probably only occur for very short periods after rain in freely drained soils.

4.3 DISCUSSION

The results of these three experiments (4.2.1, 4.2.2 and 4.2.3) suggest that *P. cinnamomi* survived in moist soil in the absence of host roots for three to four months; perhaps longer if the soil was waterlogged. *P. cinnamomi* did not survive in soils which were allowed to dry out. The critical moisture level i.e. the matric potential at which plasmolysis occurred was not investigated.

Weste and Vithanage (1979) investigated chlamydospore survival in soils maintained at various moisture potentials. They also recorded poor survival in dry soil (-3000 kPa) but reported significant survival of chlamydospores for six to eight months in soils maintained at -300 kPa and -500 kPa. In addition, they suggested that the number of chlamydospores actually increased in most treatments four to six months after inoculation. The apparent disparity between their study and that reported in Section 4.2.3 might be explained by the fact that survival of *P. cinnamomi* was tested at different moisture potentials and also, different techniques were employed to maintain the potentials in the two studies.

Weste and Vithanage (1979) examined chlamydospore survival in soils maintained at -30, -300, -500, -1500 and -3000 kPa compared with 0 kPa, -5 kPa and in soil which was allowed to dry out (Section 4.2.3). Survival at -300 and -500 kPa may have been better because soils at those potentials were probably too dry to support bacterial growth, hence lysis (Broadbent and Baker, 1974a, Malajczuk *et al*, 1977) but not dry enough to cause chlamydospore plasmolysis. However, it should also be pointed out that they maintained the soil matric potential by enclosing soil, mixed with the requisite amount of water, in a double layer of polythene. Hence, the soil was poorly aerated and probably became anaerobic. This may have enhanced chlamydospore survival.

In another study, Hwang and Ko (1978) examined the persistence of *P. cinnamomi* propagules in soil subjected to two moisture regimes. In moist soil (soil adjusted to 60% moisture) inoculated with 1.5×10^2 chlamydospores per gram of soil, the population of viable chlamydospores decreased to very low levels after three months and to a non-detectable level after one year. This survival pattern (i.e. a steady decline in the population of viable chlamydospores) agrees essentially with the results obtained for moist soils (-5 kPa) in Section 4.2.3. In contrast to the results obtained in Section 4.2.3, Hwang and Ko (1978) reported that chlamydospore survival was better in moist soil than it was in submerged soil. However, survival by mycelium rather than chlamydospores

may have been responsible for the superior survival of *P.cinnamomi* observed in waterlogged soils in Section 4.2.3 (see Discussion 4.2.3).

Using the technique employed in Section 4.2.3, the particular propagules on the *P.cinnamomi* mycelium-chlamydospore mat which survived could not be ascertained. However, the microscopic studies usually gave a good indication. In the studies conducted by Hwang and Ko (1978) and Weste and Vithanage (1979) large numbers of individual chlamydospores were inoculated into soil and the ensuing results presumably gave an indication of chlamydospore survival *per se* under those conditions. Microscopic examination of the propagules was apparently not practicable, hence, the mode of chlamydospore senescence was not established i.e. whether by microbial lysis, autolysis, plasmolysis etc.

Hwang and Ko (1978) demonstrated saprophytic colonisation of dead stem segments by *P.cinnamomi* and suggested that *P.cinnamomi* was 'a good saprophyte'. Weste and Vithanage (1979) attempting to explain the multiplication of chlamydospores observed after two months in soil, implied that competitive saprophytic survival may have occurred. Hence, in both instances, the chlamydospores which were originally inoculated into the soil may not have been the propagules recovered at a later date. In Section 4.2.3, the survival of mycelium and chlamydospores which were initially inserted into the soil was studied; the nucleopore filters provided a

physical barrier between the soil and fungus. Hence, this technique which prevented saprophytic survival probably gauged the survival ability of particular propagules more accurately than the alternative techniques.

Zentmyer and Mircetich (1966) and Reeves (1975) also demonstrated competitive saprophytic survival in dead plant material under experimental conditions. However, there is no evidence to suggest that this is an important feature in the field situation. In infested undisturbed soil cores (Section 4.2.1), *P.cinnamomi* only survived for periods equivalent to the survival of the fungus in tensiometers (Section 4.2.3) where saprophytic survival was prevented. However, these results are not conclusive evidence against competitive saprophytic survival of *P.cinnamomi* in the field and this aspect requires further investigation.

CHAPTER 5

THE SPORANGIAL PRODUCTION OF *Phytophthora cinnamomi* IN SOIL AND IN SOIL SUSPENSIONS

5.1 INTRODUCTION

In the genus *Phytophthora*, sporangia are the major organ of vegetative reproduction. Zoospore production, via indirect sporangial germination, provides a means of increasing the population of infective propagules in a short time (Zentmyer and Erwin, 1970). Zoospores are regarded as the normal agents of root infection (Byrt and Holland, 1978).

The epidemiological significance of sporangial production depends on the consumation of each step in what I have termed the vegetative reproduction-infection process; a simplified schema of which is listed:

(i) the induction of sporangia on hyphae, or by germination of chlamydospores on infected roots or in soil;

(ii) the maturation and indirect germination of sporangia;

(iii) zoospore release, motility and encystment on a root or in soil;

(iv) zoospore germ tube production and infection of a plant root if available and susceptible.

Each step in this process is subject to the effects of soil environmental conditions and depends largely on the successful completion of the preceding step. Hence, to determine the epidemiological significance of sporangial production in different soils requires the examination of each step in the vegetative reproduction-infection process in each soil type.

In the past, the identification of factors affecting the initial step, the production of sporangia, using mycelial mats or mycelial-agar discs submerged in soil extract, has received much attention; see Zentmyer and Erwin (1970) for a review of this literature. The aim of this section of the work was to compare the production of sporangia of *P. cinnamomi* in soils collected from the Jarrahdale and Durras sites and determine whether the sporangial inducing principle *per se* had potential epidemiological significance in *P. cinnamomi* disease in the different soil types examined.

The following 'experimental' section is a summary of the procedures used and results obtained. Reference is made to particular experiments which are recorded in the experimental addendum (pp. 138). The development of the concepts and techniques used are presented in the addendum in their entirity and in chronological order. However, the salient points and their implications can be gleaned from this experimental section.

The data were presented in this manner to prevent encumbering the chapter with repetitious experimental technique.

5.2 EXPERIMENTAL

(i) In the initial investigation, a technique devised by Dr N. Malajczuk employing mycelial mats growing on cellophane was used to examine the sporangial production of *P.cinnamomi*. Mycelial mats were immersed in dilute, filtered soil suspensions prepared from soil collected at Jarrahdale and Durras (see EA.1. for details).

The results suggested that the soil type from which the soil suspension was prepared may have affected the sporangial production i.e. *P.cinnamomi* mycelial mats immersed in soil suspensions prepared from Durras soil always produced more sporangia than those immersed in comparable suspensions prepared from Jarrahdale soil (Figure EA.1.). However, this could not be demonstrated statistically because the intra-treatment variation (variation between replicate *P.cinnamomi* mycelial mats) was too large. Hence, modifications were made to the technique to reduce intra-treatment variation and demonstrate inter-treatment differences statistically.

(ii) Three different techniques for preparing *P.cinnamomi* mycelial mats were tested; Malajczuk's technique, the single inoculation technique, and the double inoculation technique (see EA.2. for descriptions). In addition, a mononucleate, single zoospore culture of *P.cinnamomi* isolate DP1 was used to reduce the intra-treatment variation.

A comparison of the techniques (EA.2.) suggested that variation in the number of sporangia produced on

replicate *P.cinnamomi* mycelial mats was least using the single inoculation technique. Hence, this technique was employed in subsequent experiments.

(iii) Preliminary experiments using the single inoculation technique suggested that a sand suspension amended with an inorganic source of nitrogen was suitable for use as a control treatment. Replicates of the amended sand suspension demonstrated reproducible sporangial inducing ability (EA.3. Exp. 1) and sensitivity to experimental treatment differences (EA.3. Exp. 2); two attributes deemed necessary for a suitable control treatment. However, significantly different numbers of sporangia were produced in identical treatments of experiments which were performed on separate occasions (EA.3. Exp. 3). Hence, it seemed probable that the sporangial producing capacity of *P.cinnamomi* cultures varied from occasion to occasion.

(iv) Different isolates of *P.cinnamomi* (recovered from widely separated geographical areas) demonstrated different sporangial producing ability (EA.4. Exp. 1). In addition, replicate subcultures of a single *P.cinnamomi* isolate grown in identical manner and subjected to identical immersion conditions yielded significantly different numbers of sporangia (EA.4. Exp. 2).

In an experiment set up and repeated eight times (EA.4. Exp. 3) it was demonstrated that sporangial production on mycelial mats of *P.cinnamomi* immersed in identical treatments in successive experiments was not comparable because of variation in the sporangial

producing capacity of the *P.cinnamomi* cultures used. In addition, no easily identifiable pattern of variation in sporangial production emerged over the eight 'replicate' experiments. Hence, it was not possible to predict occasions on which *P.cinnamomi* cultures may have had similar sporangial producing ability. Thus, this technique and similar techniques which employ mycelial mats immersed in soil suspensions, cannot be used with confidence to compare the sporangial inducing ability of soils if suspensions of the latter are tested on separate occasions.

(v) It was reasoned that the sporangial inducing principle in soil (as distinct from that in soil suspensions) may be strong enough to override the effect of cultural variation, hence, *P.cinnamomi* mats inserted directly into soil might not exhibit the variation between replicate cultures and isolates observed in soil suspensions.

Initial experiments examined the effect of soil moisture regime and soil type (EA.5. Exp. 1, 2 and 3) on the production of sporangia on mycelial mats of *P.cinnamomi* placed in soil. The results suggested that, at the moisture regime used, the sporangial inducing principle was present to a similar degree in soils as distinct as washed river sand and garden loam. Hence, it was unlikely that any major differences in the sporangial inducing ability of soils collected from the diseased and healthy zones of the Durras and Jarrahdale sites would be demonstrated using this technique. Probably the sporangial inducing principle *per se* has little epidemiological significance, since the

number of sporangia induced in very distinct soil types e.g. the washed river sand and the garden loam, was of the same order of magnitude.

When the experiment inserting mycelial mats of *P.cinnamomi* in soil was performed on eight successive occasions it was found that the number of sporangia produced on those occasions was not comparable (EA.5. Exp. 3). These results agree essentially with those for the soil suspension system (cf. EA.5. Exp. 3 and EA.4. Exp. 3). The high sporangial inducing ability of the soil system over that of the dilute soil suspension did not override the variation in sporangial production between different cultures of *P.cinnamomi*.

To recapitulate; - different cultures of *P.cinnamomi* may be used as replicate mycelial mats in different treatments of an experiment provided care is taken to ensure that each treatment contains the same number of mycelial mats from each culture. This generally results in large intra-treatment variation in the number of sporangia produced and inter-treatment differences must be very distinct to reach statistically significant levels. Consequently, while the same experiment performed on separate occasions may exhibit similar treatment trends, the absolute numbers of sporangia produced are unlikely to be comparable i.e. experiments are not reproducible using this technique.

Replicate mycelial mats which represent the entire range of variation in sporangial production of the

isolate used would have to be employed to attain comparable results in successive experiments.

(vi) Large groups of single zoospore cultures originating from successive cultures of a single *P.cinnamomi* isolate were employed and the number of sporangia produced by different groups was compared. There was a statistically significant difference in the number of sporangia produced by the different groups (EA.6.). Hence, experiments examining sporangial production are not reproducible even when large numbers of single zoospore cultures compose the replicate *P.cinnamomi* mycelial mats inserted in soil.

5.3 DISCUSSION

In this study, techniques which simulated soil conditions more closely than those techniques employed previously were developed. In experiments where *P.cinnamomi* mycelial mats were inserted directly into soil (rather than immersion into a soil suspension) it was demonstrated that there was probably no major difference in the sporangial inducing ability of different soils collected from the Durras and Jarrahdale sites. In addition, it was suggested that the sporangial inducing principle *per se* of different soil types had little potential epidemiological significance in *P.cinnamomi* induced disease except perhaps in exceptional instances (Broadbent and Baker, 1974b). This is in contrast to studies performed previously using soil suspensions, which suggest that soils collected from different areas (EA.1. and Zentmyer, 1965) and soils

collected at different times of the year (Chee and Newhook, 1965) varied in their ability to stimulate sporangia.

In the past, the assessment of sporangial production was often subjective and with few exceptions, experiments lacked control treatments. The application of the Malajczuk technique of mycelial mats grown on cellophane (EA. 1.) but subsequently modified (EA. 2.) enabled the scoring of sporangial production and statistical analysis of the results. Using an amended sand suspension as a control treatment, it was demonstrated that the results of experiments performed on separate occasions were not comparable because of variation in the sporangial producing ability (SPA) of different cultures of a single *P.cinnamomi* isolate. This occurred when mycelial mats were immersed in suspension and also when they were inserted directly into soil. Similarly, it applied when large groups of single zoospore cultures constituted the replicate mycelial mats used.

The variation between different mycelial cultures of a single *P.cinnamomi* isolate probably stems from variation in the sporangial producing ability of the mycelium of the mother culture i.e. sporangia are not produced randomly over a mycelial mat; generally some clumping of sporangia is evident. It is postulated that mycelium-agar discs taken from different areas of a mycelial mat yield cultures which have a SPA concurrent with the SPA of the region on the mycelial mat from which the discs originated. This would explain the variation

in SPA of successive cultures of a single *P.cinnamomi* isolate (EA.4. Exp. 3 and EA.5. Exp. 4).

To overcome this variation, an attempt was made to employ replicate mycelial mats which represented the entire range of variation in sporangial production of the isolate used. Large groups of single zoospore cultures were used because variation between single zoospore cultures was much greater than between replicate mycelial derived cultures. This suggested that the SPA of a sample of single zoospore cultures may represent the SPA of the isolate as a whole. However, groups of single zoospore cultures originating from successive cultures of a single *P.cinnamomi* isolate yielded significantly different numbers of sporangia. Possibly, the production of sporangia on single zoospore cultures is concurrent with the SPA of the region of mycelium from which agar-mycelium discs originated for the axenic production of sporangia.

Variation in sporangial production may have survival value in the field. In the experiments described, sporangial production was examined under a specific set of conditions e.g. the soil moisture regime, the incubation period and the growth conditions of the cultures used were closely controlled. Variation may ensure that sporangial production occurs over a range of soil environmental conditions i.e. cultures with low SPA under the experimental conditions used may exhibit different SPA under other conditions.

The results of this study suggest that the sporangial inducing principle is present in the majority of soil types to a similar degree and that the sporangial production step in the vegetative reproduction-infection process (see Section 5.1) has little (if any) epidemiological significance in *P. cinnamomi* induced disease. However, subsequent steps in the process may have a significant effect on the manifestation of disease and future studies should concentrate on these.

CHAPTER 6

CONCLUSIONS AND GENERAL DISCUSSION

During these investigations, some differences were demonstrated between the behaviour of *P.cinnamomi* in soils collected from Durras in New South Wales and Jarrahdale in Western Australia. In this chapter, the nature and some ecological and management implications of these differences are discussed.

The Occurrence of *P.cinnamomi*

At the Durras site in New South Wales *P.cinnamomi* occupied a different ecological niche from that which it inhabited in the northern jarrah forest of Western Australia. In the latter situation, *P.cinnamomi* colonised the large roots and collar regions of diseased *B.grandis*, a major component species of the understorey of the jarrah forest, and hence a large potential food base for the fungus. *P.cinnamomi* was not readily isolated from the soil matrix suggesting that the behaviour of the fungus was confined to those options illustrated in the root itself (see Figure 1.1) and that the disease predominated around the root collar region rather than at the root tip.

Shea *et al* (1979) suggested that *P.cinnamomi* occurred at high population levels in soil during a brief period in spring in the jarrah forest. Hence, *P.cinnamomi* activity on and beyond the root surface (i.e. the behavioural options responsible for the infection and

destruction of feeder roots of most species) probably only occurs in spring when suitable soil physical conditions prevail.

In the moist zone of the Durras site in New South Wales (the zone of chlorotic *M.communis*), the behaviour of *P.cinnamomi* on the roots of susceptible species may have been similar to that illustrated in Figure 1.1. A fairly balanced 'low key' cycle of *P.cinnamomi* infection, survival and senescence probably occurred since, although the majority of plant species at the site were apparently not adversely affected by the fungus, the latter was isolated by baiting from the soil matrix for the duration of the sampling period. Increased soil moisture caused by road making practices probably resulted in a soil environmental modification favourable to the occurrence of *P.cinnamomi* and unfavourable to the health of *M.communis* in this part of the site.

P.cinnamomi was not isolated from the soil matrix in the healthy vegetation zone at Durras although inoculation of the area must have occurred in the past. Possibly, in the absence of significant soil environmental modification in this zone, conditions may have been unfavourable for the development of a cycle of *P.cinnamomi* infection, survival and senescence in the soil matrix. The fungus was not isolated from the large roots of *M.communis*, the only species exhibiting obvious disease symptoms at Durras. Thus, in the zone of the healthy vegetation, *P.cinnamomi* possibly lacked a large food base and niche in which to harbour during unfavourable conditions.

Hence, the difference in the severity of *P.cinnamomi* induced disease in the two areas studied may essentially be an epidemiological difference rather than an aetiological one i.e. as a consequence of the colonisation of large roots of some species in the jarrah forest, the inoculum potential of *P.cinnamomi* may be much greater in the Western Australian situation than in the soil matrix at Durras. Thus, when suitable soil physical conditions occur in spring massive levels of *P.cinnamomi* inoculum might flood the soil. Assuming that the death of some susceptible species e.g. jarrah, is due to damage to numerous root tips, this flood of inoculum in the form of zoospores might kill many of the feeder roots in the vicinity. In the moist part of the Durras site, low levels of inoculum (due to the small food base i.e. fine roots) might be produced for most of the year because of the evenly distributed rainfall. This might maintain the fungus in that environment while causing only 'sub-clinical' damage to the root systems of most plant species.

Techniques for the direct isolation of *P.cinnamomi* propagules from soil are available (Shea, 1979a) and this hypothesis (that inoculum levels of different magnitude occur at different times of the year at Durras and in the jarrah forest) could now be tested.

However, inoculum levels are probably not the only factor contributing to the difference in disease severity in the two situations. For instance, due to severe summer drought, the damage caused by *P.cinnamomi*

may have a greater relative effect on the host in the Western Australian situation than at Durras e.g. hot, dry soils in summer and autumn following spring conditions suitable for large scale reproduction of *P.cinnamomi* and infection of fine roots, may be unsuitable for root regeneration; at Durras, no pronounced dry season occurs. Hence, the effect on the host of similar levels of root damage may be much greater in the Western Australian situation than at Durras.

The contrast in disease syndromes and behaviour of *P.cinnamomi* in each situation suggests that specific control/prevention measures are required for each.

At the Durras site, disease apparently affected only *M.communis* in the zone subjected to increased soil moisture and the spread of disease was probably dependent on an extension of this soil moisture modification. Hence, this may be considered an environmental disease and prevention/control can best be maintained by minimal disturbance to the environment. This could be achieved by avoiding or modifying road construction in areas susceptible to this type of disease (e.g. areas with a *M.communis* understorey) or by building roads in valleys instead of ridge tops. While these are available options, their implementation may seem unwarranted to the authorities in view of the initial number of plant species and area of forest affected. The significance of road location for other purposes such as fire control and logging, may outweigh disease prevention considerations.

In Western Australia, the options for prevention/control are different because the disease is probably a host-pathogen dominated situation and *P.cinnamomi* may pose a potential threat to the health of the northern jarrah forest wherever the following features coincide:

(a) the fungus, *P.cinnamomi*;

(b) a host species susceptible to large root colonisation;

(c) suitable soil physical conditions in spring.

Quarantine and hygiene practices have been implemented in the northern jarrah forest and probably have reduced the rate of expansion of disease. However, these practices must fail eventually *albeit* they have provided sufficient time to investigate alternative control measures.

Despite the obvious safety problems Shea (1979a) suggested employing a high to moderate intensity burning regime to reduce the *B.grandis* component of the understorey and, hence, the inoculum potential of *P.cinnamomi*. This burning regime may also favour the development of a resistant leguminous understorey (see Shea, 1979a) which may reduce the impact of *P.cinnamomi* on freely drained sites. However, the possibility that species other than *B.grandis* may be susceptible to large root colonisation is worthy of further investigation because these species (if any) may not be eradicated by the proposed burning regime.

Soil Microbial Populations

The microbial populations of soil collected from the Jarrahdale site were lower than those of soil collected from comparable vegetation zones at the Durras site. The populations of all the microbial groups counted at both sites were smaller in soil collected from the diseased vegetation zone than in that from the healthy vegetation zone. Hence, there was a general association between low microbial populations and the occurrence of disease. However, it is not known whether disease occurred in areas of lower microbial competition or whether microbial populations dropped after disease occurred. In addition, the effect of soil microbial populations on *P.cinnamomi* probably differs between states due to the different patterns of *P.cinnamomi* occurrence and behaviour in each situation.

In Western Australian soils, microbial competition probably directly affects the epidemiology of *P.cinnamomi* induced disease only in the brief period when soil physical conditions are favourable to sporangial production and when *B.grandis* roots die i.e. in the declining saprophytic phase of the fungus. Hence, to a large extent, *P.cinnamomi* probably escapes microbial competition by harbouring in the large root systems of some susceptible host species.

In the soil matrix (e.g. in the Durras situation) *P.cinnamomi* may face microbial competition during all behavioural phases with the possible exception of the active parasitic phase (e.g. in the fine feeder roots).

This phase in roots at Durras is probably short-lived compared with the infection of large roots of *B.grandis* in the Western Australian situation. A sustained level of microbial competition on all aspects of *P.cinnamomi* behaviour at Durras, might contribute to the balanced cycle of infection, survival and senescence which has been proposed for the Durras syndrome. Hence, while it is possible to demonstrate different microbial populations between sites and disease status zones, the results are only useful if the effect of the level and type of microbial competition is determined for each of the behavioural options of *P.cinnamomi* e.g. similar microbial populations may exert a greater epidemiological influence in Durras soils where *P.cinnamomi* is in the soil matrix than in Jarrahdale soil where the fungus harbours in large roots for most of the year.

An investigation of the microbial populations which by competition or antibiosis can more rapidly eliminate *P.cinnamomi* from recently dead *B.grandis* roots could be of value in the Western Australian situation. Similarly; the effect that soil microfloras (from soil in potentially disease susceptible and resistant sites) have on the consumation of the vegetative reproduction-infection process in spring may be worthy of research.

The Survival of *P.cinnamomi*

Inability to isolate *P.cinnamomi* from the soil matrix in Jarrahdale soil suggests that saprophytic colonisation of organic material and survival of *P.cinnamomi*

propagules in soil was insignificant in this area. High temperatures and low moisture levels in soil in summer following favourable spring conditions are probably responsible for this. Laboratory studies suggest that *P.cinnamomi* mycelium and chlamydospores were unable to survive for three weeks in soil which was allowed to dry out (see Section 4.2.3).

Shea (1979b) recovered *P.cinnamomi* from the collar region of *B.grandis* which had been dead for more than a year. He attributed this persistence to the survival of chlamydospores. Thus propagules of *P.cinnamomi* survive for significant periods in large dead root systems. In addition, the declining saprophytic phase of *P.cinnamomi* in infected large roots might extend for a considerable time because of low microbial competition and slow nutrient depletion. Hence, in Western Australia the saprophytic and survival phases of *P.cinnamomi* behaviour probably depend also on the niche provided by those species susceptible to large root colonisation.

P.cinnamomi survived for three to four months during spring and summer when mycelium-chlamydospore mats were placed in the soil at Durras. Laboratory studies indicated that *P.cinnamomi* survived for ten weeks in moist soil (-5 kPa) and probably even longer when the soil was waterlogged. This suggests that *P.cinnamomi* might have survived in soil in the absence of living host roots at Durras because the soil probably did not dry out to the level where *P.cinnamomi* desiccation occurred.

In addition, at least in the moist part of the site, the evenly distributed rainfall probably provided conditions suitable for root infection sufficiently often to maintain a cycle of *P.cinnamomi* infection, survival and senescence. In the healthy vegetation zone, conditions suitable for root infection may have been too infrequent to maintain a perpetuating cycle, although under experimental conditions *P.cinnamomi* propagules survived in soil from this zone (see 4.2.1 and 4.2.3) for three months and inoculation of the area probably occurred at times.

There was no evidence to suggest that *P.cinnamomi* survived as a competitive soil saprophyte at Durras. The fact that the fungus was not isolated from the healthy vegetation zone although inoculation of this area must have occurred, suggests that the competitive saprophytic ability of *P.cinnamomi* under these conditions was poor. In addition, *P.cinnamomi* infested undisturbed soil cores collected from the moist part of the Durras site survived for periods only equivalent to that of *P.cinnamomi* mats in tensiometers where competitive saprophytic survival was prevented. Hence, competitive saprophytic colonisation probably occurs in the field only under very specialised conditions.

Sporangial production of *P.cinnamomi*

When techniques which simulated soil conditions more closely than those techniques employed previously were used, it was demonstrated that the sporangial inducing principle was present to a similar degree in vastly different soil types. Hence, there was probably no major

difference in the sporangial inducing ability of different soils collected from the Durras and Jarrahdale sites. In addition, except in some exceptional circumstances e.g. in suppressive soils, the sporangial inducing principle probably has little or no epidemiological significance in *P.cinnamomi* induced disease. However, the sporangial production phase of the behaviour of *P.cinnamomi* in soil may still influence the epidemiology of *P.cinnamomi* induced disease e.g. soil physical conditions favourable to sporangial induction may vary in frequency (cf. the climate of Jarrahdale and Durras) and duration (see EA.5. Exp. 2) in different soils. In addition, under similar suitable soil physical conditions, but with the greater food base of the banksia roots, *P.cinnamomi* may produce more sporangia on these large roots in Western Australia than on the fine feeder root systems of susceptible species at Durras.

While most soils probably possess a similar potential to induce sporangia, the subsequent steps in the vegetative reproduction-infection process (see Section 5.1) deserve further consideration. Some evidence suggests that soil environmental conditions affect these processes and, hence, disease epidemiology. For instance, Broadbent and Baker (1974a) and Malajczuk *et al* (1977) demonstrated that soil bacteria may cause lysis and abortion of sporangia in certain soils. In addition, the practical implications of zoospore movement in soils of different type and the significance of zoospore migration in runoff water have

received little attention although the soil physical conditions required for zoospore release and movement in soil have been investigated (Duniway, 1976; MacDonald and Duniway, 1977).

Conclusions

These investigations have demonstrated pronounced differences in the behaviour of *P.cinnamomi* in the two areas studied. It was suggested that entirely different disease syndromes exist in New South Wales and Western Australia and that differences in the aetiology and epidemiology of the disease call for different control/prevention strategies in each situation.

The features which I consider distinguish the two disease situations with respect to the behaviour of *P.cinnamomi* are:

(a) the colonisation of large roots of at least one understorey species in the northern jarrah forest provides a large food base and niche for *P.cinnamomi* to inhabit during unfavourable soil physical conditions. This niche probably does not exist in the Durras situation.

(b) the slow rate of decline of *P.cinnamomi* in large dead root systems ensures a plentiful supply of inoculum for at least a year after the end of the active parasitic phase in the Western Australian situation. In the absence of root infection *P.cinnamomi* might not survive for more than three or four months in soil at Durras.

(c) the sustained microbial competition on most phases of *P.cinnamomi* occurrence in soil at Durras compared with the probable limited competition experienced in the Western Australian situation.

(d) the likely capacity of *P.cinnamomi* to sporulate profusely (due to a large food base) around the collar region of *B.grandis* and possibly significant subsequent migration of zoospores in runoff water compared with less prolific sporangial production and limited zoospore movement in the soil at Durras.

(e) the probable fast turnover of the parasitic, saprophytic and survival phases in the soil matrix at Durras compared with a slower cycle of behavioural phases of greater magnitude in the Western Australian situation. In the soil matrix of the jarrah forest, a perpetuating cycle of *P.cinnamomi* infection, survival and senescence probably could not operate due to unfavourable soil physical conditions in summer and autumn. Hence, plants susceptible to fine root colonisation only, are probably infected consequentially to the annual cycle of parasitic spread from the large roots of a few understorey species.

Assuming that these conclusions are correct, then future research priorities could possibly establish whether:

(a) *B.grandis* is the only host species in the jarrah forest susceptible to large root colonisation;

(b) jarrah roots are susceptible only to distal root infection;

(c) the levels of infective propagules of *P.cinnamomi* in soil in the jarrah forest in spring are sufficiently high to infest the majority of fine roots of jarrah. How do these levels compare with those recorded in the eastern states?

(d) the impact on the vegetation, of the root damage caused by these inoculum levels is capable of causing the disease observed. What is the significance of climatic stress in such disease development?

If it is established that (a) and (b) are correct, then any technique that reduces the level of primary inoculum of *P.cinnamomi* by removing the food base of *B.grandis* e.g. fire (Shea, 1979a) should lessen the occurrence and/or effect of the disease.

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EXPERIMENTAL ADDENDUM

EA.1. INITIAL INVESTIGATION OF THE SPORANGIAL PRODUCTION OF *P.cinnamomi* IN SOIL SUSPENSIONS PREPARED FROM SOIL COLLECTED AT THE DURRAS AND JARRAHDALE SITES

Materials and Methods

This experiment involved collaboration with Dr N. Malajczuk at the C.S.I.R.O., Division of Land Resources Management, Wembly, Western Australia. The experimental procedure described was the technique used in that laboratory for examining the sporangial production of *P.cinnamomi*. The technique employed the use of mycelial mats of specific size which facilitated the enumeration of sporangia per unit area of mat; an improvement on the techniques used previously.

PREPARATION OF *P.cinnamomi* SQUARES: Cellophane discs (7.0 cm diam.) were prepared as described in Section 4.2.2 (i) and (ii) and placed asceptically on Petri plates containing $\frac{1}{10}$ strength potato dextrose agar ($\frac{1}{10}$ P.D.A.). Five inoculum plugs (2 mm diam.) of *P.cinnamomi* (isolate W.A.1) growing on full strength P.D.A. were placed in a line across the centre of the cellophane disc. After five days growth at 25°C, 1 cm squares of similar age (Figure EA.2. (i)) were cut from the disc for immersion in the soil suspension.

PREPARATION OF THE SOIL SUSPENSION: Soil collected from the greenline zone of the Durras and Jarrahdale sites was

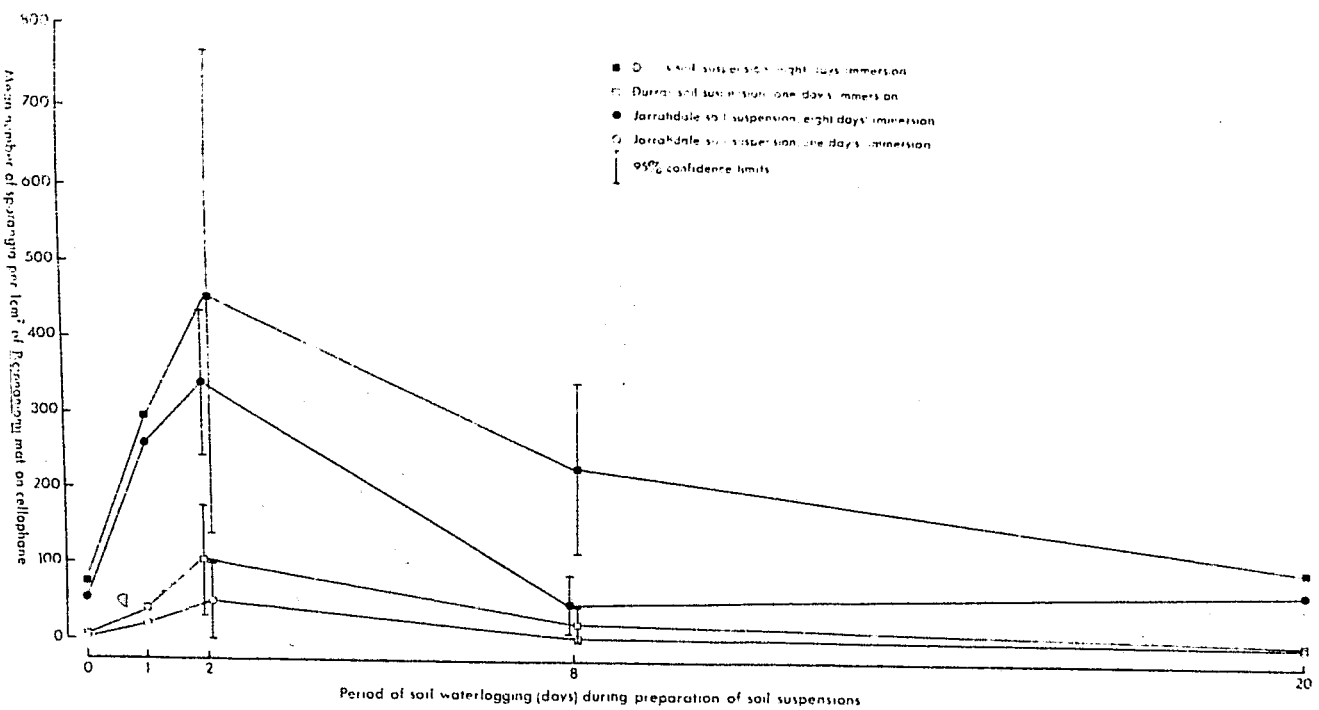
used in this experiment. Five, 10 g samples of each soil type were placed in individual, 100 ml capacity measuring cylinders. Ninety millilitres of distilled water was added and the soil stirred with a glass rod. One suspension of each soil type was waterlogged for 0, 1, 2, 8 and 20 days at 25°C. After the prescribed period of waterlogging, soil suspensions were decanted, passed through Whatman No.1 filter papers and 25 ml of each suspension placed in glass Petri dishes (90 cm diam.).

IMMERSION AND SAMPLING OF *P.cinnamomi* SQUARES IN THE SOIL SUSPENSION: Twenty five, 1 cm squares of *P.cinnamomi* on cellophane were immersed in each filtered soil suspension and incubated at 25°C. After one, two, four and eight days of immersion, five squares were removed from each soil suspension and placed on glass microscope slides. Squares were stained and mounted in 0.1% trypan blue in lactophenol and the number of sporangia produced per cm^2 counted using a Zeiss 14 microscope (x125 magnification).

Results and Discussion

The mean number of sporangia produced per cm^2 of *P.cinnamomi* mat immersed for one and eight days in five different waterlogging treatments of Durras and Jarrahdale soil suspension is illustrated in Figure EA.1. Graphs for the number of sporangia produced on mats immersed for two and four days were omitted to avoid confusion; they were similar shaped curves situated between the lines shown for one and eight days immersion.

FIGURE EA.1. THE MEAN NUMBER OF SPORANGIA PRODUCED ON *P.cinnamomi* MATS IMMERSED IN FIVE WATERLOGGING TREATMENTS OF SOIL SUSPENSIONS PREPARED FROM SOIL COLLECTED AT THE DURRAS AND JARRAHDALE SITES.



The 95% confidence limits calculated for each curve on the 2 and 8 days waterlogging treatments (Figure EA.1.) suggest that the variation between replicate *P. cinnamomi* mats was too large to demonstrate statistically significant differences between soils or treatments. However, the results illustrated in Figure EA.1. suggest the following trends:

(i) the period of soil waterlogging employed for preparing the soil suspension may effect the number of sporangia produced;

(ii) the period of immersion of *P. cinnamomi* squares in the soil suspension may effect the number of sporangia produced; and

(iii) the soil type may effect the number of sporangia produced. *P. cinnamomi* squares immersed in Durras soil suspension always produced more sporangia than those immersed in comparable treatments prepared from Jarrahdale soil.

To demonstrate these trends statistically it would be necessary to reduce the level of intra-treatment variation (i.e. variation between replicate *P. cinnamomi* mats).

To reduce the intra-treatment variation, two modifications of the above technique were tested; in addition, a single zoospore, mononucleate culture of *P. cinnamomi* was used. The following experiment records the results of these investigations.

EA.2. A COMPARISON OF THE VARIATION BETWEEN REPLICATE *P.cinnamomi* MATS PREPARED USING THREE DIFFERENT TECHNIQUES

Materials and Methods

Three techniques for preparing *P.cinnamomi* mats (Figure EA.2) for immersion into soil suspension were tested and the intra-treatment variation examined. The culture used in all instances was SZDP1, a single zoospore, mononucleate culture of the D.P.1 isolate recovered from the Durras site.

The soil suspension used was a 1:10 river sand: distilled water suspension amended with 10^{-4} g/l ammonium nitrate. This suspension had the potential to induce numerous sporangia per unit area of *P.cinnamomi* mat and replicate suspensions had comparable sporangial inducing ability (see Section EA.3. Exp. 1).

The techniques tested were:

(i) MALAJCZUK'S TECHNIQUE: Thirty, 1 cm squares of *P.cinnamomi* (SZDP1) on cellophane were prepared as described in EA.1. These were immersed in 25 ml of amended sand suspension (see EA.3. Exp. 1) and incubated at 25°C. After three days, twenty five 1 cm squares were removed from the suspension, stained and mounted in 0.1% trypan blue in lactophenol and the number of sporangia of *P.cinnamomi* per cm^2 counted.

(ii) SINGLE INOCULATION TECHNIQUE: Cellophane discs on $\frac{1}{10}$ P.D.A. were prepared as described in EA.1. The

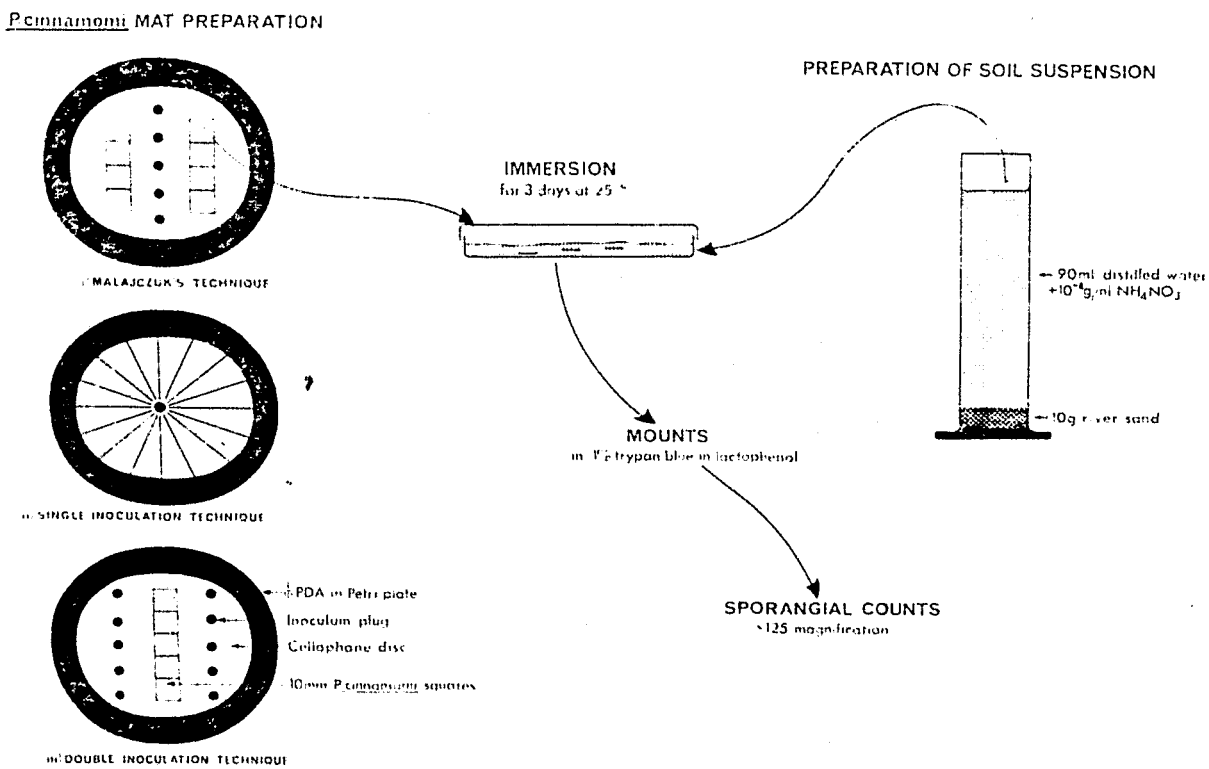


FIGURE EA.2. THREE TECHNIQUES OF PREPARING *P. cinnamomi* MATS FOR IMMERSION IN SAND SUSPENSION.

centre of each disc was inoculated with one, 2 mm diam. plug of *P.cinnamomi* (SZDP1). After four days growth at 25°C, each *P.cinnamomi* mat on cellophane was stripped from the agar and cut into sixteen equal sized wedges (Figure EA.2. (ii)). Thirty wedges were immersed in 25 ml of amended sand suspension and incubated at 25°C. Twenty five wedges were removed from the sand suspension after three days, stained and mounted and the number of sporangia of *P.cinnamomi* per wedge counted.

(iii) DOUBLE INOCULATION TECHNIQUE: Cellophane discs on $\frac{1}{10}$ P.D.A. were prepared and each disc inoculated with two rows of 2 mm diam. plugs of *P.cinnamomi* (SZDP1) as shown in Figure EA.2. (iii). After eight days growth at 25°C, five, 1 cm squares were cut from between the rows on each disc. Thirty squares were immersed in 25 ml of amended sand suspension for three days at 25°C. Twenty five squares were sampled, stained and mounted and the number of sporangia of *P.cinnamomi* per cm^2 counted.

Results and Discussion

A comparison of the means and their respective standard deviations suggest that the variation between the number of sporangia produced on replicate *P.cinnamomi* mats using different techniques of preparation was least in the single inoculation technique. Hence, the single inoculation technique might demonstrate significant inter-treatment differences provided a sufficient number of replicate *P.cinnamomi* wedges were used per treatment.

TABLE EA.2a. The number of sporangia produced on twenty five *P.cinnamomi* mats, prepared using three different techniques and immersed in an amended sand suspension.

Technique of preparation	Number* of sporangia produced per wedge or 1 cm square *P.cinnamomi* mat immersed
MALAJCZUK'S TECHNIQUE	21.1± 24.2
SINGLE INOCULATION TECHNIQUE	206.3± 64.5
DOUBLE INOCULATION TECHNIQUE	86.4± 174.9

* Mean ± the standard deviation of the mean of twenty five *P.cinnamomi* mats immersed.

It was not practically feasible to use twenty five wedges per treatment as in the above experiment. A t-test was used (Sokal and Rohlf, 1966: Test of equality of the means of two samples whose variances are assumed to be unequal) to compare the mean of five randomly selected *P.cinnamomi* wedges to the mean of twenty five wedges, which was taken to represent the population mean. This calculation was repeated for four different groups of five randomly selected wedges and in all instances there was no significant difference between these means at the P = 0.05% level. Hence, it was assumed that five replicate *P.cinnamomi* wedges per treatment were a sufficient number to give a reliable estimate of the population mean at this level of significance.

EA.3. EXPERIMENTS ON THE SPORANGIAL PRODUCTION OF *P.cinnamomi* USING THE SINGLE INOCULATION TECHNIQUE

EXPERIMENT 1. The Preparation of Amended Sand Suspension for Use as a Control Treatment.

In the past, experiments employing soil suspensions to induce sporangial formation have lacked suitable control treatments because the sporangial inducing ability (S.I.A.) of suspensions prepared from soil collected in different areas (Zentmyer, 1965) and at different times of the year (Chee and Newhook, 1965) vary considerably.

This experiment describes the preparation of an amended sand suspension for use as a control treatment in future experiments.

Materials and Methods

Six replicate suspensions were prepared as described in Section EA.1. using washed river sand instead of soil. Individual replicates were amended with 1M ammonium nitrate solution to give a concentration of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 0 gm of NH_4NO_3 per ml of suspension. Suspensions were allowed to settle for 24 hr.

P.cinnamomi wedges, prepared as described in Section EA.2 (ii) were immersed in 25 ml of each sand suspension in individual Petri plates and incubated at 25°C. After three days, *P.cinnamomi* wedges were removed

from each sand suspension, stained and mounted as described previously and the number of sporangia per *P.cinnamomi* wedge counted.

The experiment was repeated four weeks later; on this occasion the 10^{-2} g/ml NH_4NO_3 sand suspension treatment was omitted and a 10^{-7} g/ml NH_4NO_3 sand suspension treatment included.

Results and Discussion

TABLE EA.3a. The mean number of sporangia per *P.cinnamomi* wedge immersed in washed river sand suspension amended with different levels of ammonium nitrate.

NH_4NO_3 g/ml of sand suspension	Number* of sporangia per *P.cinnamomi* wedge	
	Initial experiment	Repeated experiment
10^{-2}	0^a	
10^{-3}	0.2 ± 0.2^a	0.2 ± 0.2^a
10^{-4}	90.2 ± 19.2^c	87.6 ± 13.8^c
10^{-5}	105.4 ± 19.1^c	100.4 ± 19.2^c
10^{-6}	36.2 ± 7.3^b	37.0 ± 4.6^b
10^{-7}		38.8 ± 6.3^b
0	41.2 ± 8.4^b	32.4 ± 4.5^b

* Values represent means ± S.E. of five *P.cinnamomi* wedges.

a Means followed by the same letter are not significantly different, (P = 0.05).

The difference in the S.I.A. of suspensions amended with low levels of ammonium nitrate (10^{-6} and 10^{-7} g/ml) and that of the non-amended treatment was not significant. Levels of 10^{-4} and 10^{-5} g/ml NH_4NO_3 stimulated the S.I.A. threefold, while higher concentrations inhibited sporangial production. The difference in the number of sporangia produced in comparable treatments of the initial and repeated experiment was not significant.

These results suggested that replicate river sand suspensions, prepared in an identical manner, and amended with ammonium nitrate, had reproducible S.I.A. This seemed to overcome the problem of variation in the S.I.A. of suspensions prepared from soils and implied that one of these treatments could be used as a control treatment in future work.

River sand suspension amended with 10^{-4} g/ml ammonium nitrate (RS + N^{-4}) was chosen as the control for future studies. This treatment had an elevated S.I.A. which was considered an advantage because, as well as being reproducible (under the same treatment conditions on different occasions), a control should be sensitive to treatment differences within an experiment. It was thought that a control which induced abundant sporangia would be more sensitive to treatment differences.

The following experiment examines the effect of dilution of the RS + N^{-4} on its S.I.A. Chee and Newhook (1965) found that the S.I.A. of soil suspensions was unchanged below dilutions of 1:30. The relative

sensitivity of the RS + N^{-4} control to treatment differences should be demonstrated by the effect of dilution of the suspension on its S.I.A.

EXPERIMENT 2. The Effect of Dilution of the RS + N^{-4} Treatment on its Sporangial Inducing Ability

Materials and Methods

P. cinnamomi wedges were prepared as described in EA.2. (ii). Ten wedges were immersed in each of the following RS + N^{-4} dilutions:

(a) FULL STRENGTH : 100% RS + N^{-4}

(b) $\frac{1}{2}$ STRENGTH : 50% RS + N^{-4}
50% distilled water

(c) $\frac{1}{5}$ STRENGTH : 20% RS + N^{-4}
80% distilled water

(d) $\frac{1}{10}$ STRENGTH : 10% RS + N^{-4}
90% distilled water

(e) $\frac{1}{100}$ STRENGTH : 1% RS + N^{-4}
99% distilled water

Results and Discussion

Dilution of the RS + N^{-4} significantly reduced the S.I.A. A 1:1 dilution reduced the S.I.A. of the RS + N^{-4} control by 70%. This suggested that the RS + N^{-4} control would be sensitive to experimental treatment differences imposed on soil suspensions in future experiments.

The number of sporangia produced on wedges immersed in the full strength treatment (which was prepared in an identical manner to the 10^{-4} g/ml NH_4NO_3 treatments

in Exp. 1) was three times greater than the number produced in comparable treatments in Experiment 1. This suggested that slight inaccuracies made during the preparation of the RS + N^{-4} may have had a large impact on the S.I.A. of the RS + N^{-4} or alternatively, replicate RS + N^{-4} prepared in identical manner do not have reproducible S.I.A. as was concluded from Experiment 1.

TABLE EA.3b. The effect of dilution of the RS + N^{-4} on its S.I.A.

Dilution	Number of sporangia/*P.cinnamomi* wedge Mean ± S.E.
FULL STRENGTH	321 ± 15.9[a]
$\frac{1}{2}$ STRENGTH	70.2 ± 14.7[b]
$\frac{1}{5}$ STRENGTH	19.0 ± 3.7[c]
$\frac{1}{10}$ STRENGTH	7.0 ± 1.8[d]
$\frac{1}{100}$ STRENGTH	2.8 ± 1.5[d]

[a] Means followed by the same letter are not significantly different, (P = 0.05).

The following experiment examines the S.I.A. of a number of replicate RS + N^{-4} to determine the source of the variation between comparable treatments in Experiments 1 and 2.

EXPERIMENT 3. The Sporangial Inducing Ability of Replicate RS + N^{-4}.

Materials and Methods

Three replicate RS + N^{-4} were prepared as described in Section EA.2. Two, 25 ml samples of one RS + N^{-4} and a single sample of the other two replicates were decanted and placed in Petri plates.

Ten, *P.cinnamomi* wedges (EA.2. (ii)), were immersed in each suspension and incubated at 25°C. After three days, five wedges were removed from each plate, stained and mounted and the number of sporangia per *P.cinnamomi* wedge counted.

The experiment was repeated a week later.

Results and Discussion

TABLE EA.3c. The S.I.A. of four replicate RS + N^{-4} prepared on two different occasions.

Replicate RS + N^{-4}	Number of sporangia produced/*P.cinnamomi* wedge	
	Initial experiment	Repeated experiment
1a	76.6 ± 11.3[a]	14.4 ± 2.84[b]
1b	58.8 ± 17.7[ac]	14.0 ± 3.11[b]
2	113.4 ± 23.8[a]	22.6 ± 0.87[b]
3	94.0 ± 7.4[a]	17.2 ± 6.8[bc]

* Values represent means ± S.E. of five *P.cinnamomi* wedges.

[a] Values followed by the same letter are not significantly different, (P = 0.05).

The results suggest that there was no significant difference in the number of sporangia produced on *P.cinnamomi* wedges immersed in replicate RS + N^{-4} that were prepared on the same day. However, the number of sporangia produced on *P.cinnamomi* wedges immersed in replicates of RS + N^{-4} in the initial experiment differed significantly from the number produced in replicates of suspensions in the repeated experiment.

It seemed inconceivable that the S.I.A. of replicate RS + N^{-4} could vary so much from occasion to occasion as a result of inaccurate preparation, particularly when replicate RS + N^{-4} prepared independently on the same day were relatively uniform in their S.I.A. Hence, some other component of the experimental system must have varied. A likely possibility was that *P.cinnamomi* cultures, grown on cellophane and cut into wedges for immersion in RS + N^{-4} varied in their sporangial producing ability on separate occasions.

Previously, *P.cinnamomi* cultures were assumed to have uniform sporangial producing ability, perhaps because great care was taken to produce uniform *P.cinnamomi* mats on cellophane e.g. a single zoospore, mononucleate culture was used, cultures of equal age were used to inoculate the cellophane discs and care was taken to ensure that the thickness and composition of the media did not vary.

The three experiments that follow, examine the variation in sporangial producing ability of different *P.cinnamomi* isolates and cultures.

EA.4. THE VARIATION IN SPORANGIAL PRODUCING ABILITY OF *P. cinnamomi* ISOLATES AND CULTURES

EXPERIMENT 1. Variation in the Sporangial Producing Ability of Different *P. cinnamomi* Isolates

Materials and Methods

Eight isolates of *P. cinnamomi* were selected from the culture collection at the Forestry Department, Australian National University. Isolates recovered from widely separated geographical areas were selected to enhance the chance of demonstrating variation.

The *P. cinnamomi* isolates selected, and the region from which they were recovered were:

(i) Pc 383 - Qld.
(ii) Pc 84 - Brisbane Ranges, Vic.
(iii) Pc 335 - Ravenshoe, Qld.
(iv) Pc 374 - United States
(v) Pc 89 - Pine Creek, N.S.W.
(vi) Pc 176 - Dwellingup, W.A.
(vii) Pc 43 - Ourimbah, N.S.W.
(viii) Pc 52 - Tasmania.

Cultures of each isolate were grown on full strength P.D.A. for one week. A 2 mm diam. plug of *P. cinnamomi* was taken from 5 mm inside the perimeter of each culture and placed on cellophane on $\frac{1}{10}$ P.D.A. *P. cinnamomi* wedges of each isolate were prepared as described previously.

Ten wedges of each isolate were immersed in individual 25 ml samples of RS + N^{-4} in Petri plates and incubated at 25°C. After three days, five *P.cinnamomi* wedges of each isolate were stained and mounted as described previously and the number of sporangia per *P.cinnamomi* wedge were counted.

The experiment was repeated at a later date using three isolates that showed marked differences in their sporangial producing ability in the initial experiment.

Results and Discussion

TABLE EA.4a The sporangial producing ability of eight isolates of *P.cinnamomi* in RS + N^{-4}.

Isolate	Mean number* of sporangia per *P.cinnamomi* wedge	
	Initial experiment	Repeated experiment
Pc 383	4.2 ± 1.2	
Pc 84	75.8 ± 12.5	102.4 ± 19.3
Pc 335	11.4 ± 4.8	
Pc 374	120 ± 1.48	
Pc 89	31.6 ± 6.9	199.6 ± 13.8
Pc 176	0.8 ± 0.49	130.2 ± 7.1
Pc 43	0.4 ± 0.55	
Pc 52	0	

* Values represent means ± S.E. of five *P.cinnamomi* wedges.

The results suggest that different isolates produce significantly different numbers of sporangia when

cultures of each isolate are grown in the manner described and immersed in RS + N^{-4}. Also, the same isolate may produce significantly different numbers of sporangia in successive experiments i.e. cultures of similar age originating from a single isolate on separate occasions may yield different numbers of sporangia.

EXPERIMENT 2. Variation in the Sporangial Producing Ability of Different Cultures of the Same *P. cinnamomi* Isolate.

Materials and Methods

Ten, 2 mm diam. plugs of *P. cinnamomi* were taken from 5 mm inside the perimeter of a seven day old culture of SZDP1 (mononucleate, single zoospore culture) growing on P.D.A. Each 2 mm diam plug was placed onto cellophane on $\frac{1}{10}$ strength P.D.A. and incubated at 25°C for four days. Four even sized cultures were selected and cut into wedges as described in Section EA.2 (ii).

A RS + N^{-4} was prepared and 100 ml placed in a 14 cm glass Petri dish fitted with two plastic dividers which disected the dish into four compartments. This allowed free movement of the RS + N^{-4} between compartments but prevented *P. cinnamomi* wedges, placed in different compartments, from intermingling.

Ten, *P. cinnamomi* wedges from each culture were immersed in RS + N^{-4} in individual compartments of the dish. After three days, five wedges from each compartment were stained and mounted and the number of sporangia per *P. cinnamomi* wedge counted.

Results

TABLE EA.4b. The sporangial production of four cultures of *P.cinnamomi* isolate SZDP1 in RS + N^{-4}

Culture	Number* of sporangia per *P.cinnamomi* wedge
SZDP1 (i)	14.8 ± 5.5
SZDP1 (ii)	25.8 ± 8.6
SZDP1 (iii)	23.2 ± 3.8 } s
SZDP1 (iv)	2.0 ± 0.8 } s

* Values represent means ± S.E. of five *P.cinnamomi* wedges

s Difference between the means is significant, (P = 0.05).

Cultures SZDP1 (iii) and SZDP1 (iv) taken from a single mother culture, grown in identical manner and subjected to identical immersion conditions yielded significantly different numbers of sporangia.

This would account for much of the intra-treatment variation (i.e. variation between replicate *P.cinnamomi* wedges) observed in previous experiments because only two or three wedges (of the ten used per treatment) immersed in each RS + N^{-4} treatment were taken from the same *P.cinnamomi* culture. Hence, each treatment contained wedges derived from three or more even sized *P.cinnamomi* cultures. While this ensured that a similar degree of variation occurred between *P.cinnamomi* wedges in each treatment, the intra-treatment variation was large which made it difficult to show inter-treatment differences.

EXPERIMENT 3. Variation in the Sporangial Production of *P.cinnamomi*, Isolate SZDP1, on Eight Separate Occasions.

Materials and Methods

This experiment was set up and repeated on eight separate occasions. This was done to demonstrate the variation in sporangial production of a single zoospore culture of *P.cinnamomi* and thereby possibly account for the variation observed in the numbers of sporangia produced in identical treatments in experiments set up on different occasions. In addition, it was hoped to determine whether there was any predictable pattern of variation from occasion to occasion.

P.cinnamomi wedges (EA.2. (ii)) of isolate SZDP1 were immersed in suspensions of RS + N^{-4} (EA.3. Exp. 1) and RS - N (RS + N^{-4} with no added ammonium nitrate). The latter suspension was included to demonstrate that the variation observed was due to variation in the *P.cinnamomi* cultures and not to variation in the RS + N^{-4} (see results and discussion). The procedure detailed below was followed and the same experiment was set up eight times in eight weeks using even-aged cultures on each occasion.

DAY: -7: Three full strength P.D.A. plates were inoculated with a 2 mm diam. plug of *P.cinnamomi* (SZDP1).

DAY: 0: Eight, 2 mm diam. plugs of SZDP1 were taken from 5 mm inside the perimeter of the DAY -7 culture. Five plugs were placed on cellophane on $\frac{1}{10}$ P.D.A. plates and three were used to inoculate three P.D.A. plates.

DAY: 3: Suspensions of RS + N^{-4} and RS - N were prepared and allowed to settle for 24 hr.

DAY: 4: Three even size cultures of SZDP1 on cellophane were stripped from the $\frac{1}{10}$ P.D.A. plates and cut into wedges. Ten wedges were immersed in 25 ml of each of the RS + N^{-4} and RS - N and incubated at 25°C.

DAY: 7: Five wedges were sampled from each suspension, stained and mounted and the number of sporangia per wedge counted. Eight, 2 mm diam. plugs taken from the SZDP1 culture inoculated on day 0 were used to inoculate five $\frac{1}{10}$ P.D.A. + cellophane plates and three full strength P.D.A. plates for use on day 14.

DAY: 10: As for day 3.

DAY: 11: As for day 4.

The experiment was repeated eight times and *P.cinnamomi* wedges sampled on each occasion were measured and the number of sporangia per cm^2 of wedge calculated. This was done to negate the effect of small differences in the size of the SZDP1 cultures used on each occasion. It was not necessary to do this for experiments set up on only one occasion because care was taken to select even sized cultures which each contributed *P.cinnamomi* wedges to all the treatments in the experiment.

Results and Discussion

The results of these experiments are illustrated in Figure EA.3. The number of sporangia per cm^2 of wedge,

produced in RS + N^{-4} was always greater than in RS - N suspension although the differences were not usually significant. However, the pattern of variation between replicate experiments was the same in both suspensions i.e. when large numbers of sporangia were produced per cm^2 of wedge in the RS + N^{-4} treatment, relatively large numbers of sporangia were also produced in the RS - N suspension. This suggested that the variation observed between 'replicates'* of the experiment was due to variation in the SZDP1 cultures rather than variations in the sand suspensions. The suspensions were prepared independently and had variation occurred in this component of the experimental system, different patterns of variation between the suspensions would have resulted.

The 95% confidence limits show that there were differences in the number of sporangia produced on separate occasions in both suspensions. This suggests that the numbers of sporangia produced by *P.cinnamomi* wedges in experiments set up on different occasions cannot be compared. In addition, no easily identifiable pattern of variation in sporangial production emerged over the eight 'replicate' experiments so it was not possible to predict occasions on which *P.cinnamomi* cultures may have had similar sporangial producing ability. Hence, this

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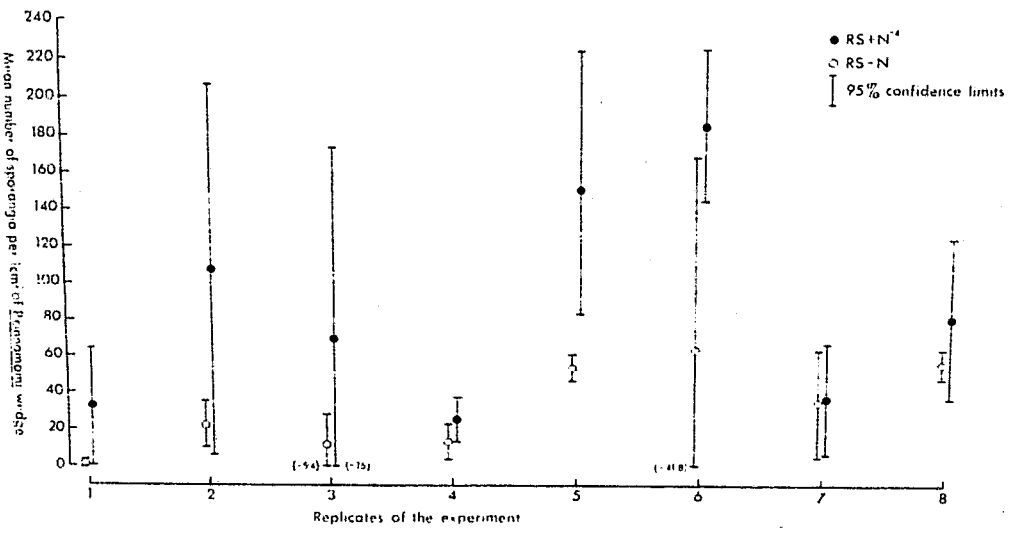
* It is appreciated that the eight experiments are not true replicates of one another because different even-aged cultures of *P.cinnamomi* were used in each. However, the eight experiments were prepared in an identical manner and are referred to as 'replicates' for simplicity.

FIGURE EA.3. SPORANGIAL PRODUCTION ON SZDP1 WEDGES IMMERSED IN SAND SUSPENSIONS ON EIGHT SEPARATE OCCASIONS.



technique and similar techniques which employ mycelial mats immersed in soil suspensions cannot be used to compare the sporangial inducing ability (S.I.A.) of soils if suspensions prepared from those soils are tested on different occasions.

Possibly the variation between 'replicate' experiments, originating apparently from the variation in successive, even-aged cultures of *P.cinnamomi*, may only be applicable in delicate artificial experimental systems such as that described. The following experiment examined the sporangial producing ability of robust *P.cinnamomi* mats inserted directly into soil.

EA.5. THE PRODUCTION OF SPORANGIA ON *P.cinnamomi* MATS INSERTED IN SOIL

Sporangia were observed on *P.cinnamomi* mats sampled after one and three weeks in soil at -5 kPa in the *P.cinnamomi* chlamydospore survival study (Section 4.2.3). This suggested that a similar system, employing robust *P.cinnamomi* mats and tensiometers to maintain the matric potential, could be used to study the production of sporangia in soil.

P.cinnamomi mats placed directly into soil may not exhibit the variation between cultures and isolates observed previously because the stimulatory principle inherent in soil may be strong enough to override the effect of cultural variation. In addition, a technique employing the use of soils instead of soil suspensions should give a more realistic indication of the sporangial inducing ability of soil.

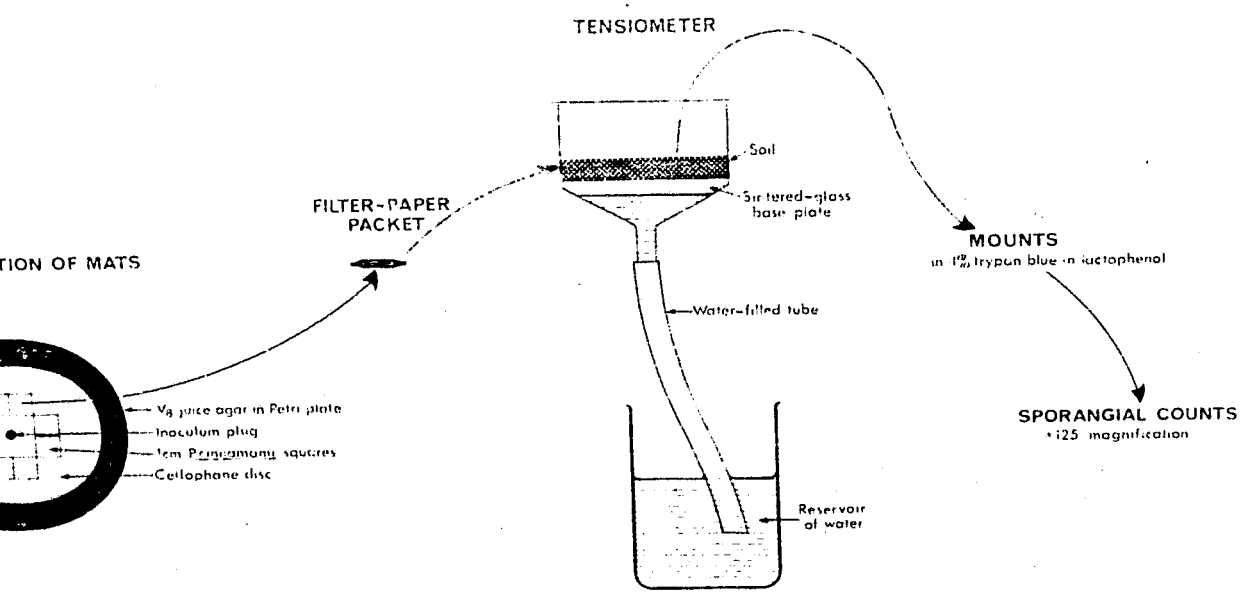
The following experiments examine the effect of soil moisture potential (Exp. 1 and 2), soil type (Exp. 2 and 3) and cultural variation (Exp. 4) on the production of sporangia on *P.cinnamomi* mats placed in soil.

EXPERIMENT 1. Initial Investigation on the Sporangial Production of *P.cinnamomi* Mats Placed in Soils Subjected to Different Moisture Regimes.

Materials and Methods

PREPARATION OF *P.cinnamomi* MATS: Cellophane discs (7 cm diam.) were prepared as described in Section 4.2.2 (i) and (ii) and placed asceptically on Petri plates containing V8 juice agar (Appendix 4). One, 2 mm diam. plug of *P.cinnamomi* taken from 5 mm inside the perimeter of a seven day old culture of SZDP1 growing on P.D.A., was placed in the centre of the cellophane and incubated at 25°C for seven days. *P.cinnamomi* mats on cellophane were stripped from the agar, placed on moist graph paper (GAF 1 mm) and eight, 1 cm squares of similar age cut from the disc using a single edge blade (see Figure EA.4.). Each 1 cm square was placed between two, 1.5 cm squares of moist Whatman No.42 filter paper. These filter paper packets prevented soiling of the *P.cinnamomi* mats but maintained the water potential around the mats at a matric potential value equal to that of the surrounding soil.

PREPARATION OF SOIL AND INSERTION OF *P.cinnamomi* FILTER PAPER PACKETS IN SOIL: Four tensiometers were constructed using Buchner funnels fitted with fritted glass base plates. Continuous columns of water were established between the fritted glass and a water reservoir and the matric potential of soil in the funnels controlled by adjusting the height of the funnel above the water reservoir.



URE EA.4. PROCEDURE FOR EXAMINING THE SPORANGIAL PRODUCTION OF *P. cinnamomi* MATS IN SOIL.

Air dried and sieved soil collected from the diseased zone of the Durras site was packed to a depth of 5 mm in each tensiometer. Six *P.cinnamomi* filter paper packets were placed on top and an additional 5 mm of soil used to cover the filter paper packets. The contents of each tensiometer were then saturated with distilled water. A 10 cm^2 sheet of plastic was placed across the opening of each Buchner funnel to retard evaporation.

The moisture potential of soil in one tensiometer was drained immediately to -5 kPa by lowering the water reservoir so that the head of the reservoir of water was 50 cm below the soil in the tensiometer. Soil in the other tensiometers was maintained in a waterlogged state (ψm = 0 kPa) for 10 hr, 2 days and 4 days before the soil moisture potential was altered to -5 kPa. Soil in each tensiometer was then maintained at -5 kPa for seven days before sampling.

SAMPLING THE *P.cinnamomi* FILTER PAPER PACKETS:

Five filter paper packets were sampled from each tensiometer. *P.cinnamomi* mats on cellophane were removed from between the filter paper, placed on glass microscope slides and stained and mounted as described previously.

Each *P.cinnamomi* mat was examined using bright field light microscopy (Nikon x 125 magnification). Ten random microscope fields were viewed per *P.cinnamomi* mat and the sporangia counted. The number of sporangia per cm^2 of *P.cinnamomi* mat was calculated for the five

replicate mats and the mean number of sporangia produced and its standard error recorded for each treatment. Treatment means were compared using a t-test (Sokal and Rohlf, 1966).

Results and Discussion

TABLE EA.5a. The number of sporangia produced per cm^2 of *P.cinnamomi* mat in soil subjected to four different moisture regimes.

Period of waterlogging	Mean number* of sporangia/cm^2 of *P.cinnamomi* mat, seven days after draining to -5 kPa
0	301 ± 150^{a}
10 hr	1296 ± 101^{b}
2 days	1156 ± 334^{ab}
4 days	2283 ± 259^{b}

* Values represent means $\pm$ S.E. of five *P.cinnamomi* mats.

[a] Means followed by the same letter are not significantly different, (P = 0.05).

The mean number of sporangia produced in treatments subjected to waterlogging was always greater than the treatment not waterlogged. No significant difference was demonstrated between the number of sporangia produced in the 0 and 2 days waterlogging treatments. However, this was probably a consequence of the low degree of replication and large intra-treatment variation in the number of sporangia produced on replicate *P.cinnamomi* mats.

As a result of this experiment, the moisture regime applied in all future experiments employing the use of *P.cinnamomi* filter paper packets and soil in tensiometers was; one day at 0 kPa followed by seven days at -5 kPa, unless stated otherwise.

EXPERIMENT 2. The Effect of the Water Regime and Soil Type on the Sporangial Production of *P.cinnamomi*.

Materials and Methods

Eight tensiometers were set up as described in EA.5. Exp. 1; half with Durras soil and half with washed river sand. Six *P.cinnamomi* filter paper packets (EA.5. Exp. 1) were inserted in each tensiometer. Water reservoirs were connected to each tensiometer and one sample of each soil type was subjected to each of the following water regimes:

(i) 0 kPa for 1 day : 0 kPa for 7 days
(ii) 0 kPa for 1 day : -1 kPa for 7 days
(iii) 0 kPa for 1 day : -5 kPa for 7 days
(iv) 0 kPa for 1 day : -10 kPa for 7 days.

Five *P.cinnamomi* filter paper packets were removed from each tensiometer after eight days, stained and mounted and the number of sporangia per cm^2 of *P.cinnamomi* mat calculated.

Results

Sporangial production was abundant in washed river sand and Durras soil at the lower matric potentials (-5 kPa and -10 kPa for seven days). At -1 kPa for seven

days, abundant sporangia were produced in the washed river sand but sporangial production was inhibited in the Durras soil. This suggested that aeration may have been inadequate for sporangial formation in the Durras soil but not in the washed river sand. Hence, light soils may have a greater potential to induce sporangia than heavier soils because the range of matric potential over which aeration is adequate for sporangial production may be greater.

TABLE EA.5b. The effect of four moisture regimes on sporangial production on *P.cinnamomi* mats inserted in two soil types.

Water regime after 0 kPa for 1 day	Number* of sporangia/cm^2 of *P.cinnamomi* mat	
	River Sand	Durras Soil
0 kPa for 7 days	0	0
-1 kPa for 7 days	1017.8 ± 328.3	0
-5 kPa for 7 days	377.3 ± 93.9	935.2 ± 224.0
-10 kPa for 7 days	928.9 ± 420.2	1482.6 ± 329.6

* Values represent the mean ± S.E. of five *P.cinnamomi* mats.

This is in contrast to the impression gained when *P.cinnamomi* mats are immersed in soil suspensions prepared from these two soil types; abundant sporangia are produced in the Durras soil suspension but scant production is evidenced in the washed river sand suspension. Hence, the difference in the sporangial inducing ability of different soils may not be as distinct as was suggested

in EA.1. where mycelial mats were immersed in soil suspensions prepared from soil collected from the Durras and Jarrahdale sites.

EXPERIMENT 3. The Sporangial Production on *P.cinnamomi* Mats Placed in Three Different Soils.

Materials and Methods

Six tensiometers were set up as described in EA.5. Exp. 1. Three soil types were used: washed river sand, garden loam and soil collected from the diseased zone of the Durras site. Four samples of the Durras soil were employed to test the variation between replicate soil samples.

Six *P.cinnamomi* filter paper packets (EA.5. Exp. 1) were inserted in the soil in each tensiometer. The soil was saturated, maintained at 0 kPa for one day and then drained to -5 kPa for seven days.

Five *P.cinnamomi* filter paper packets were removed from each tensiometer after eight days, stained and mounted and the number of sporangia per cm^2 of *P.cinnamomi* mat calculated.

Results

The only soils to show a significant difference ($P = 0.05$) in the number of sporangia produced per cm^2 of *P.cinnamomi* mat were the Durras B replicate and the washed river sand. The variation between replicate *P.cinnamomi* mats was large and this may have masked some differences. However, in all treatments relatively large numbers of

sporangia were produced suggesting that the sporangial inducing principle was present in all soil types. Numbers of sporangia produced even in soils as distinct as a washed river sand and a garden loam were of the same order of magnitude. Hence, it is unlikely that any major difference in the sporangial inducing ability of soils collected from the diseased and healthy zones of the Durras and Jarrahdale sites would be demonstrated using this technique.

TABLE EA.5c. The number* of sporangia produced per cm^2 of *P.cinnamomi* mat placed in soil samples maintained at 0 kPa for one day and -5 kPa for seven days.

Soil Sample	Mean number* of sporangia produced per cm^2 of *P.cinnamomi* mat	
Durras A	377.3 ± 93.9	
B	852.6 ± 236.3	s
C	445.1 ± 132.6	
D	353.0 ± 66.4	
Washed river sand	150.5 ± 82.2	s
Garden loam	490.0 ± 193.5	

* Values represent the mean ± S.E. of five *P.cinnamomi* mats.

s The two means joined by the arrow are significantly different, ($P = 0.05$).

Although the experiment was performed at only one moisture regime, the results suggest that the sporangial inducing principle is present to a similar degree in most soils, hence, it is unlikely that this principle *per se* has widespread epidemiological significance. Suppressive soils such as that reported by Broadbent and Baker (1974b) is the possible exception.

Although most soils may possess the potential to induce abundant sporangia under suitable conditions these conditions may eventuate only rarely in some soils. e.g. the range of matric potential in which sporangia are induced may differ in different soils; see EA.5. Exp 2. Hence, soil type may influence disease epidemiology through such factors as moisture retaining capacity, pore size etc rather than the sporangial inducing principle *per se*.

This experimental technique could be used to examine the effect of some of these soil factors on sporangial production in *P.cinnamomi* provided the problem of variation between *P.cinnamomi* cultures, observed in Section EA.4. Exp. 3 is not applicable. The following experiment examines the variation in sporangial production of *P.cinnamomi* mats on eight separate occasions.

EXPERIMENT 4. Variation in the Sporangial Production on *P.cinnamomi* Mats Placed in Soil on Eight Separate Occasions.

Materials and Methods

Two cultures of *P.cinnamomi* were used in this experiment; the culture used previously (SZDP1) and a slow growing culture (SZDP1s). The latter was subcultured from SZDP1 and produced few sporangia when *P.cinnamomi* SZDP1s wedges were immersed in RS + N^{-4}.

Six *P.cinnamomi* filter paper packets of SZDP1 and SZDP1s were placed in Durras soil in individual tensiometers as described in EA.5. Exp. 1. The experiment was repeated eight times to determine the difference in sporangial producing ability of the two cultures and examine the pattern of variation of each culture over the eight 'replicate' experiments.

The soil was air dried and sieved (2 mm mesh size) to minimise the variation between soil samples in 'replicate' experiments. In addition, replicates of each tensiometer were subjected to two different moisture regimes on each occasion; one tensiometer was subjected to the regime described in EA.5. Exp. 1 and the other received -5 kPa for eight days. This was done to examine the patterns of variation of the cultures at both moisture regimes on the eight separate occasions. Similar patterns of variation would suggest that the variation was mainly due to variation in the cultures while different patterns of variation between the two moisture regimes could mean that the soil component was responsible.

The procedure detailed below was followed to ensure that the same experiment was set up eight times in eight weeks using even-aged cultures on each occasion.

DAY: -7: Three Petri plates containing full strength P.D.A. were inoculated with a 2 mm diam. plug of *P.cinnamomi* SZDP1. Three plates were inoculated with SZDP1s.

DAY: 0: Eight, 2 mm diam. plugs of *P.cinnamomi* SZDP1 and eight SZDP1s were taken from 5 mm inside the perimeter of the cultures inoculated on Day -7. Five plugs of each culture were placed independently onto cellophane on V8 juice plates and three were used to inoculate three P.D.A. plates.

DAY: 7: *P.cinnamomi* filter paper packets were prepared using the *P.cinnamomi* mats on V8 juice agar inoculated on Day 0. Six *P.cinnamomi* filter paper packets of each culture were placed in independent tensiometers and subjected to 0 kPa for one day and -5 kPa for seven days. Another six filter paper packets of each culture were subjected to -5 kPa for eight days.

The procedure described for Day 0 was repeated using P.D.A. plates inoculated on Day 0 for use on Day 14.

DAY: 14: Procedure described for Day 7 repeated using Day 7 inoculated plates of P.D.A.

DAY: 15: Five *P.cinnamomi* filter paper packets were removed from each tensiometer, stained and mounted and the sporangia counted as described in EA.5. Exp. 1.

The experiment was repeated eight times and the number of sporangia per cm^2 of *P.cinnamomi* mat was calculated for both cultures and moisture regimes on each occasion that the experiment was done.

Results and Discussion

The results are illustrated in Figure EA.5. and EA.6.

P.cinnamomi SZDP1 filter paper packets inserted in soil and subjected to 0 kPa for one day and -5 kPa for seven days (Figure EA.5.) produced approximately twice the number of sporangia on each occasion (except in replicate 5) as were produced on each occasion when soil was maintained at -5 kPa for eight days (Figure EA.6.). Hence, the pattern of variation in the number of sporangia produced over the eight 'replicate' experiments was similar at both moisture regimes suggesting that most of the variation observed was due to variation in the *P.cinnamomi* cultures rather than variation between the soil samples used.

Significant differences occurred in the number of sporangia produced on *P.cinnamomi* SZDP1 mats on different occasions (cf. 'replicate' Experiments 1, 2 and 6; Figure EA.5.). In addition, there was almost always a significant difference between the number of sporangia produced on *P.cinnamomi* mats derived from cultures SZDP1 and SZDP1s. Hence, the number of sporangia produced on

FIGURE EA.5. THE SPORANGIAL PRODUCTION OF TWO CULTURES OF *P.cinnamomi* IN SOIL (MAINTAINED AT 0 kPa FOR 1 DAY AND -5 kPa FOR 7 DAYS) ON EIGHT SEPARATE OCCASIONS.

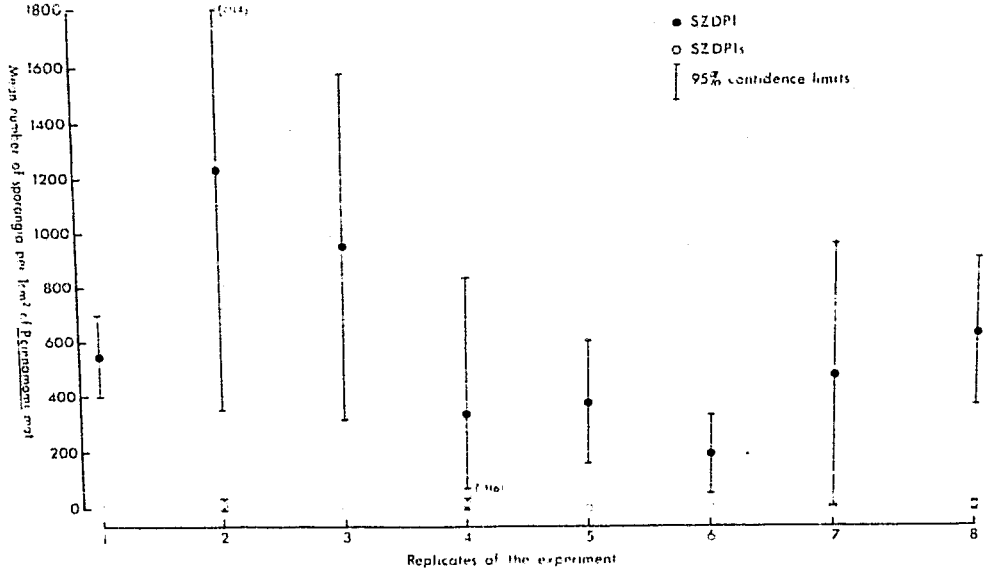
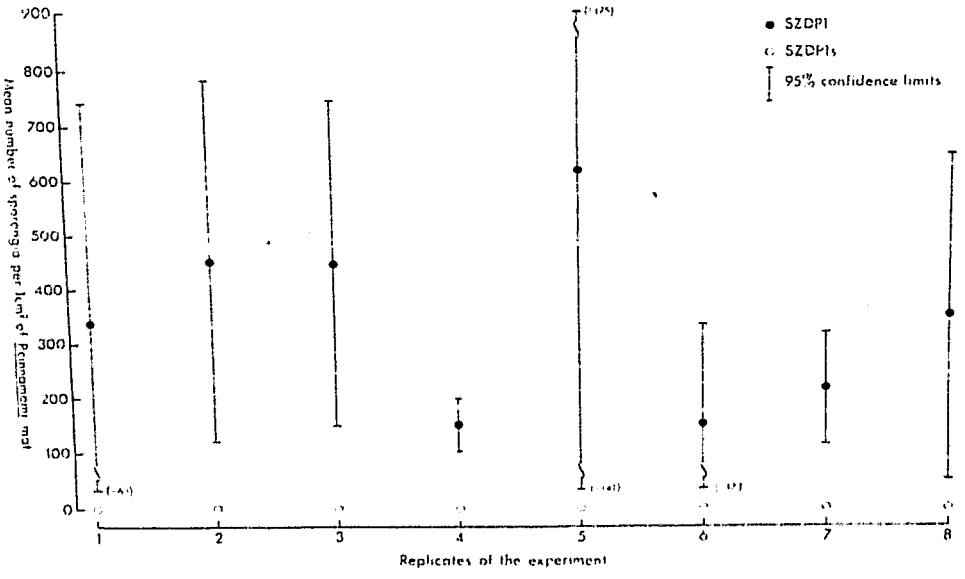


FIGURE EA.6. THE SPORANGIAL PRODUCTION OF TWO CULTURES OF *P.cinnamomi* IN SOIL (MAINTAINED AT -5 kPa FOR EIGHT DAYS) ON EIGHT SEPARATE OCCASIONS.



P. cinnamomi mats in experiments employing the use of different *P. cinnamomi* cultures, or in experiments set up on successive occasions, cannot be compared. These results agree essentially with those for the soil suspension system (EA.4. Exp. 3). The high sporangial inducing ability of the soil system over that of the dilute soil suspension did not override the variation in sporangial production between different cultures of *P. cinnamomi*.

This suggests that different cultures of *P. cinnamomi* may be used as replicate mats in different treatments of an experiment provided care is taken to ensure that each treatment contains the same constituent cultures. This generally results in large intra-treatment variation in the number of sporangia produced and inter-treatment differences must be very distinct to reach statistically significant levels. Consequently, while the same experiment performed on separate occasions may exhibit similar treatment trends, the absolute numbers of sporangia produced are unlikely to be comparable. Hence experiments are not reproducible using this technique.

EA.6. VARIATION IN THE SPORANGIAL PRODUCTION OF LARGE GROUPS OF SINGLE ZOOSPORE CULTURES ORIGINATING FROM DIFFERENT *P.cinnamomi* ISOLATES AND DIFFERENT CULTURES OF A SINGLE *P.cinnamomi* ISOLATE

A technique yielding comparable numbers of sporangia in identical treatments of repeated experiments is required to compare the treatments of experiments performed on separate occasions. This is essential for studies on sporangial production of *P.cinnamomi* where it may be desirable to examine the effect of a particular treatment on a number of separate occasions.

Results were not reproducible in previous experiments which employed different cultures of a single *P.cinnamomi* isolate i.e. the number of sporangia produced in identical treatments of separate successive experiments, varied from occasion to occasion. This suggested that a large number of replicate cultures (representing the total range of variation in sporangial production) would have to be used to attain comparable results in identical treatments of experiments performed on separate occasions.

In the following experiment, the variation in sporangial production of large groups of single zoospore cultures originating from different *P.cinnamomi* isolates and different cultures of a single isolate is compared. Experiments could be considered reproducible using this technique if comparable numbers of sporangia were produced by groups of single zoospore cultures originating from

different cultures of a single *P.cinnamomi* isolate. In addition, comparable numbers of sporangia produced by groups of single zoospore cultures originating from different *P.cinnamomi* isolates would indicate that any large group of single zoospore cultures could be used to examine the sporangial production in *P.cinnamomi*.

Materials and Methods

Tensiometers were constructed using 1 bar pressure plate cells as described in Section 4.2.3. Air dried and sieved soil (2 mm mesh size) collected from the diseased zone of the Durras site was employed in each instance.

Single zoospore cultures of the five *P.cinnamomi* isolates used were obtained in the following manner:

(i) Thirty to fifty mycelium-agar pieces (1-2 mm^2) cut from the perimeter of a seven day old *P.cinnamomi* culture growing on V8 juice agar were asceptically transferred to Petri plates containing a 6 cm diam. disc of sterile nylon mesh (0.5 mm mesh size) and 15 ml of 3% V8 juice broth (30 ml V8 juice, 1000 ml water).

(ii) Plates were incubated at 25°C for 24 hr then washed four successive times at 1 hr intervals with 20 ml of sterile mineral salts solution ($MgSO_4$, 0.25 g/l; $CaNO_3$, 0.24 g/l; KNO_3, 0.10 g/l; FeNa EDTA, 0.01 g/l). The final wash was left in the plates which were incubated in continuous fluorescent light (180 microeinsteins M^2 sec^{-1}) at 25°C.

(iii) Sporangia were produced within 12 hr and zoospores were released by chilling the plates (8°C) for 30 min and then returning them to 25°C for 1 hr.

(iv) Two to twenty drops of mineral salts solution containing zoospores were spread on a number of full strength P.D.A. plates and incubated at 25°C. After two days, clearly visible and separate single zoospore cultures were removed from the P.D.A. plates in a small quantity of agar and placed on cellophane on V8 juice agar. These were grown for seven days at 25°C.

A) VARIATION IN SPORANGIAL PRODUCTION ON GROUPS OF SINGLE ZOOSPORE CULTURES ORIGINATING FROM DIFFERENT ISOLATES OF *P. cinnamomi*.

Four *P. cinnamomi* isolates were used:

(i) Pc 335 recovered from Ravenshoe, Qld.

(ii) Pc 52 " " Tasmania.

(iii) Pc 24 " " Eden, N.S.W.

(iv) Pc 84 " " Brisbane Ranges, Vic.

Between fifteen and thirty single zoospore cultures originating from each *P. cinnamomi* isolate were obtained as described above. Filter paper packets (EA.5. Exp. 1) were prepared using a 1 cm square of each single zoospore culture. The origin (i.e. culture and isolate) of each filter paper packet was noted and the four groups were inserted in Durras soil in a single tensiometer.

Three groups of replicate filter paper packets using 1 cm squares cut from the same Pc 84 single zoospore cultures were placed in Durras soil in three other tensiometers. This was done to ensure that replicate samples of Durras soil had uniform sporangial inducing ability.

The contents of each tensiometer was subjected to the standard water regime (EA.5. Exp. 1). After eight days, filter paper packets were removed from the soil, stained and mounted, and the number of sporangia per cm^2 of *P. cinnamomi* mat calculated as described previously (EA.5. Exp. 1).

B) VARIATION IN SPORANGIAL PRODUCTION OF GROUPS OF SINGLE ZOOSPORE CULTURES ORIGINATING FROM DIFFERENT CULTURES OF A SINGLE *P. cinnamomi* ISOLATE.

On four separate occasions, groups of single zoospore cultures were obtained from seven day old cultures of *P. cinnamomi* isolate SZDP1 growing on V8 juice agar. On each occasion filter paper packets were prepared using a 1 cm square of each single zoospore culture.

Filter paper packets were inserted in Durras soil in tensiometers and subjected to the standard water regime (EA.5. Exp. 1). Eight days later the filter paper packets were removed and the number of sporangia per cm^2 of *P. cinnamomi* mat calculated as before.

Results and Discussion

TABLE EA.6a. The number of sporangia produced by groups of single zoospore cultures originating from different *P. cinnamomi* isolates.

Isolate tested and soil sample used	Number of single zoospore cultures in the group	Number* of sporangia per cm^2 of *P. cinnamomi* mat
Pc 335; Durras A+	24	85.4 ± 19.0[a]
Pc 52; " "	16	107.2 ± 22.2[a]
Pc 24; " "	14	238.3 ± 47.1[b]
Pc 84; " "	20	245.9 ± 57.0[b]
" " Durras B	20	234.1 ± 51.4[b]
" " Durras C	20	319.4 ± 45.8[b]
" " Durras D	20	288.8 ± 49.6[b]

+ Durras A = soil sample used.

* Values represent the mean ± S.E. of groups at single zoospore cultures.

[a] Means followed by the same letter are not significantly different, (P = 0.05).

Groups of single zoospore cultures originating from different isolates of *P. cinnamomi* produced significantly different numbers of sporangia (EA.6a). However, there was no significant difference in the number of sporangia produced when replicate mats from the same single zoospore cultures (originating from a single *P. cinnamomi* isolate) were placed in different samples of Durras soil. This suggested that the sporangial inducing

ability of different samples of the same soil type did not vary greatly.

TABLE EA.6b. The number of sporangia produced by groups of single zoospore cultures originating from different cultures of a single *P.cinnamomi* isolate.

Culture	Number of single zoospore cultures in the group	Number* of sporangia per cm^2 of *P.cinnamomi* mat
SZDP1a	45	93.4 ± 24.9^a
SZDP1b	30	0.7 ± 0.5^b
SZDP1c	30	26.4 ± 14.1^b
SZDP1d	27	15.2 ± 8.4^b

* Values represent the mean $\pm$ S.E. of groups of single zoospore cultures.

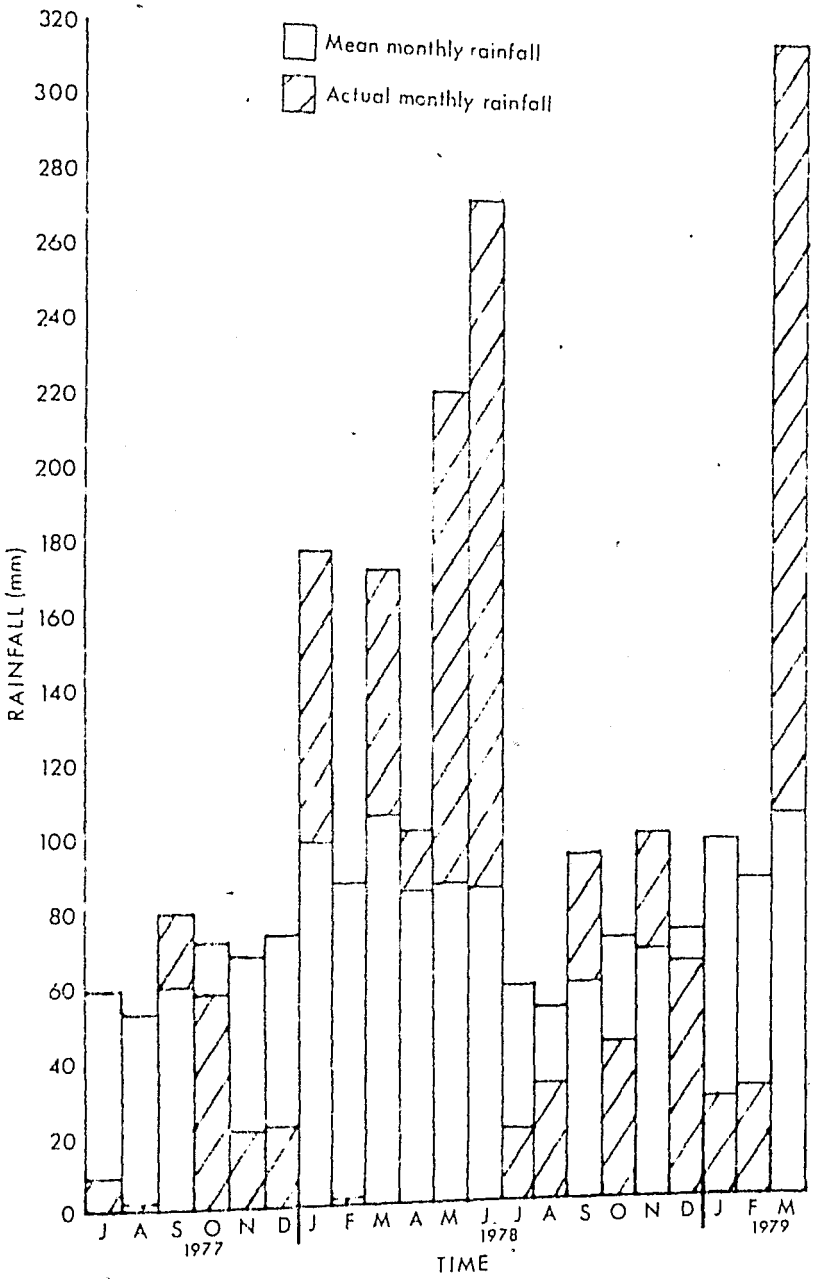
[a] Means followed by the same letter are not significantly different, (P = 0.05).

There was a significant difference in the number of sporangia produced by groups of single zoospore cultures which originated from *P.cinnamomi* isolate SZDP1 on four separate occasions i.e. from different cultures of a single isolate (EA.6b). This variation was probably not due to differences in the sporangial inducing ability of the soil samples employed on each occasion (see EA.6a). However, it may have been caused by variation in the sporangial producing ability of the different groups of single zoospore cultures used. Hence, experiments

examining sporangial production in *P.cinnamomi* are not reproducible even when large numbers of single zoospore cultures constitute the replicate *P.cinnamomi* mats inserted in soil.

APPENDIX 1

MEAN MONTHLY RAINFALL AND ACTUAL MONTHLY RAINFALL FOR MORUYA HEADS DURING THE SURVEY PERIOD



APPENDIX 2

LISTS OF PLANT SPECIES AND THEIR DISTRIBUTION AT THE DURRAS SITE

TABLE A. PLANT SPECIES IDENTIFIED AT THE HEALTHY AND DISEASED ZONES OF THE DURRAS TRANSECTS.

Vegetation layer	Healthy zone	Diseased zone
Overstorey species	*Eucalyptus maculata* Hook. " *pilularis* Sm. " *paniculata* Sm.	*Eucalyptus maculata* Hook. " *pilularis* Sm. " *botryoides* Sm.
Understorey species	*Macrozamia communis* L.A.S. Johnson *Pteridium esculentum* (Forst.f.) Cockayne *Acacia irrorata* Sieb. ex Spreng. *Gahnia melanocarpa* R.Br. *Hardenbergia violacea* (Schneev.) Stearn *Hibbertia diffusa* R.Br. ex D.C. *Hibbertia empetrifolia* (D.C.) Hoogl. *Notolaea longifolia* Vent. *Patersonia glabrata* R.Br *Pimelea linifolia* Sm. *Pultenaea daphnoides* Wendl. *Scaevola ramosissima* (Sm). Krause *Schelhammera undulata* R.Br.	*Macrozamia communis* L.A.S. Johnson *Pteridium esculentum* (Forst.f.) Cockaney *Acacia suavcolens* (Sm) Willd. *Adiantum aethiopicum* L. *Brachycome aculeata* (Labill.) Less. *Brachycome angustifolia* A.Cunn. ex D.C. *Breynia oblongifolia* J.Muell. *Casuarina littoralis* Salisb. *Dianella sp.* *Helichrysum scorpioides* (Labill.) *Hovea heterophylla* A. Cunn. *Imperata cylindrica* (L.) Beauv. *Kennedia rubicunda* Vent. *Lepidosperma laterale* R.Br. *Lomandra longifolia* Labill. *Lomandra multiflora* (R.Br.) J. Britt *Persoonia linearis* Andr. *Pratia purpurascens* (R.Br.) Berth. *Pultenaea villosa* Willd. *Veronia cinerea* Less.

APPENDIX 2 (Cont.)

TABLE B. DISTRIBUTION* OF REPRESENTATIVE PLANT SPECIES AT THE DURRAS SITE.

Species	Area of quadrats	Mean number of individuals per quadrat		
		Healthy zone	Green line	Diseased zone
Eucalyptus sp.	$4x20m^2$	13.3	10.8	6.0
M. communis	$4x5m^2$	15.3	12.3	0.8
L. longifolia	$4x1m^2$	0	11.5	87.0

* Distribution of representative species from each vegetation layer was assessed as the mean number of individuals in four quadrats of each size on vegetation adjacent to transect positions 1, 7 and 12.

APPENDIX 3

CHEMICAL ANALYSIS AND MOISTURE CHARACTERISTICS OF SOILS FROM THE DURRAS TRANSECT

TABLE A. CHEMICAL ANALYSIS* OF SOILS FROM THE HEALTHY, GREENLINE AND DISEASED ZONES OF THE DURRAS TRANSECT.

Factor	Transect position		
	Healthy zone	Greenline zone	Diseased zone
Mg (%)[t]	0.07	0.07	0.08
Ca (%)	0.30	0.25	0.25
K (%)	0.16	0.25	0.62
S (%)	0.024	0.027	0.033
P (%)	0.009	0.010	0.011
Fe (%)	1.9	2.5	2.2
T_1O_2 (%)	0.67	0.78	0.75
Zn (ppm)[o]	8	25	44
Cu (ppm)	2	0	0
Mn (ppm)	413	268	351
Na^+ (ppm)	47	30	25
K+ (ppm)	108	73	77
Mg^{++} (ppm)	155	120	110
Ca^{++} (ppm)	770	600	650
P (ppm)	63	52	56
C (%)	6.4	2.8	1.7
N (%)	0.143	0.051	0.043
C:N ratio	24:1	26:1	28:1
pH (20% W/V)	5.95	6.25	6.55

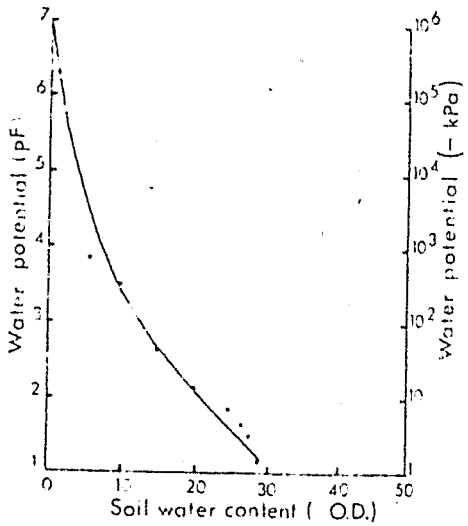
* Analyses done by C.S.I.R.O. Division of Land Resources Management, Wembly, W.A.

[t] (%) indicates per cent oven dry weight

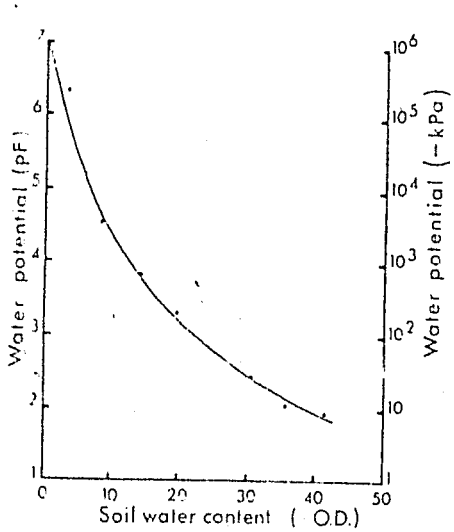
[o] (ppm) indicates parts per million of soil

APPENDIX 3 (cont.)

MOISTURE CHARACTERISTIC CURVES* FOR SOILS FROM THE HEALTHY AND DISEASED ZONES OF THE DURRAS TRANSECT



DISEASED ZONE SOIL



HEALTHY ZONE SOIL

* Filter-paper method (Fawcett and Collis-George, 1967)

APPENDIX 4

MEDIA USED FOR THE ISOLATION AND IDENTIFICATION OF *P. cinnamomi*

ANTIBIOTIC CORNMEAL AGAR (A.C.A.) - used for the isolation of *P. cinnamomi* from lupin roots.

Pimaricin ('Pimafucin') 2.5% sterile	4 ml
Penicillin G, potassium	50 mg
Polymixin B, sulphate	50 mg
Vancomycin HCl	50 mg
Cornmeal agar (BBL)*	20 g
Distilled water	1000 ml

Antibiotics added when agar had cooled to 50°C.

LUPIN ROOT AGAR - used for the production of corraloid hyphae in *P. cinnamomi*.

Lupin roots (3-4 days old)	100 g	Boiled for 30 min and filtered
Distilled water	1000 ml	
Agar (BBL)	20 g	

*(BBL) - Bacto Laboratories

APPENDIX 4 (cont.)

V8 AGAR (FULL STRENGTH) - for the production of chlamydospores

Cleared V8 broth **	200 ml
Distilled water	800 ml
Agar (BBL)	20 g

** Cleared V8 broth:

'Campbells' V8 juice	300 ml	Allowed to stand for 15 min. Centrifuged for 15 min at 5,000 R.P.M.
Calcium carbonate	3 g	

V8 AGAR (ONE TENTH STRENGTH) - used for the production of sporangia by *P. cinnamomi* in non-sterile soil extract.

Cleared V8 broth	20 ml
Distilled water	980 ml
Agar (BBL)	20 g

APPENDIX 5

AN UNDISTURBED CORE TECHNIQUE FOR THE ISOLATION OF *Phytophthora cinnamomi* FROM SOIL

Soil cores were collected in PVC sampling cylinders 9 cm in diameter x 9 cm long. A 4 kg drop hammer drove the sampling cylinder, which was sharpened at the base, down a brass guide tube into the ground. The cylinders and their soil sample were carefully removed from the ground and taken to the laboratory in airtight plastic containers and baited *in situ* to avoid further disturbance.

Each core was planted with ten, two day old lupin seedlings and placed in a watering saucer which saturated the lower 20 mm of soil. The cores were incubated at 17°C - 24°C in alternating light (180 micro-einsteins m^{-2} sec^{-1}) and dark for one month.

Lupin roots at the base of the core were checked twice per week for yellow-brown colouration near the root tip. Lesioned roots were excised, surface sterilised in 70% ethanol and placed on A.C.A. *P. cinnamomi* was identified in the same manner as described in Section 2.3.1.

APPENDIX 6

LIST OF PLANT SPECIES IDENTIFIED AT THE JARRAHDALE SITE

TABLE A. PLANT SPECIES IDENTIFIED IN THE HEALTHY, DIEBACK AND DEAD ZONES OF THE JARRAHDALE TRANSECT

Vegetation Layer	Healthy	Dieback	Dead
Overstorey species	*Eucalyptus marginata* Sm. (Dead) *Eucalyptus calophylla* R.Br.	*Eucalyptus marginata* Sm. *Eucalyptus calophylla* R.Br.	*Eucalyptus marginata* Sm. *Casuarina fraseriana* Miq.
Middle strata	*Banksia grandis* Willd. *Eucalyptus marginata* Sm. *Persoonia elliptica* R.Br. *Persoonia longifolia* R.Br.	*Banksia grandis* Willd. (Dead) *Eucalyptus marginata* Sm. *Eucalyptus calophylla* R.Br. (Regrowth)	*Eucalyptus marginata* Sm. (Dead) *Eucalyptus calophylla* R.Br. (Regrowth) *Persoonia longifolia* R.Br. (Dead)
Understorey	*Bossiaea ornata* (Lindl.) Benth. *Hakea lissocarpha* R.Br. *Hibbertia montana* Steud. *Macrozamia riedlei* (Gaud.) C.A. Gardn. *Xanthorrhoea gracilis* Endl. *Xanthorrhoea preissii* Endl. *Acacia urophylla* Benth. *Boronia spathulata* Lindl. *Eucalyptus marginata* Sm. (Seedlings) *Hibbertia perfoliata* Endl. *Adenanthos barbigera* Lindl. *Astroloma pallidum* R.Br. *Daviesia* sp. *Leptomeria cunninghamii* Miq. *Leucopogon capitellatus* DC. *Leucopogon propinquus* R.Br. *Leucopogon verticillatus* R.Br. *Lomandra sonderii* (F. Muell.) Ewart.	*Bossiaea ornata* (Lindl.) Benth. *Eucalyptus calophylla* R.Br. (Seedlings) *Hakea lissocarpha* R.Br. *Hibbertia montana* Steud. *Macrozamia riedlei* (Gaud.) C.A. Gardn. *Xanthorrhoea gracilis* Endl. *Xanthorrhoea preissii* Endl. *Acacia urophylla* Benth. *Boronia spathulata* Lindl. *Eucalyptus marginata* Sm. (Seedlings) *Hibbertia perfoliata* Endl. *Lasiopetalum floribundum* Benth.	*Bossiaea ornata* (Lindl.) Benth. *Dryandra nivea* R.Br. *Eucalyptus calophylla* R.Br. (Seedlings) *Hakea lissocarpha* R.Br. *Hibbertia montana* Steud. *Macrozamia riedlei* (Gaud.) C.A. Gardn. *Xanthorrhoea gracilis* Endl. *Xanthorrhoea preissii* Endl.

APPENDIX 7

CHEMICAL ANALYSIS AND MOISTURE CHARACTERISTICS OF SOILS FROM THE JARRAHDALE SITE

TABLE A. CHEMICAL ANALYSIS* OF SOILS FROM THE HEALTHY, DIEBACK AND DEAD ZONES OF THE JARRAHDALE TRANSECT.

FACTOR	Transect position		
	Healthy zone	Dieback zone	Dead zone
Mg (%)[t]	0.36	0.20	0.09
Ca (%)	0.16	0.23	0.07
K (%)	0.60	0.31	0.13
S (%)	0.018	0.029	0.011
P (%)	0.014	0.017	0.006
Fe (%)	2.6	1.3	0.9
T_1O_2 (%)	0.68	0.48	0.38
Zn (ppm)[o]	27	10	7
Cu (ppm)	28	12	9
Mn (ppm)	355	351	106
Na^+ (ppm)	61	72	46
K^+ (ppm)	113	117	69
Mg^{++} (ppm)	183	265	117
Ca^{++} (ppm)	515	709	265
P (ppm)	100	96	54
C (%)	3.3	3.7	2.0
N (%)	0.138	0.142	0.072
C:N ratio	45:1	55:1	40:1
pH (20% W/V)	6.05	6.35	6.1

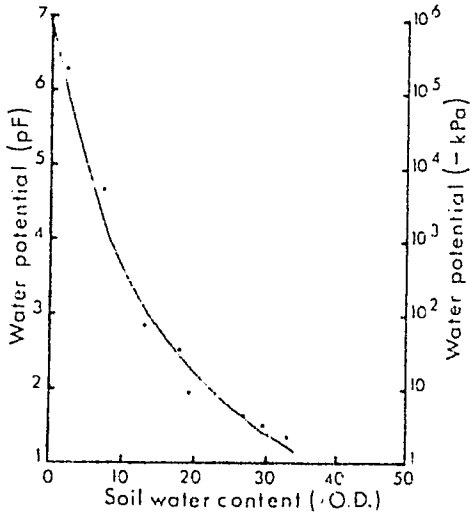
* Analyses done by C.S.I.R.O., Division of Land Resources Management, Wembly, W.A.

[t](%) indicates per cent oven dry weight

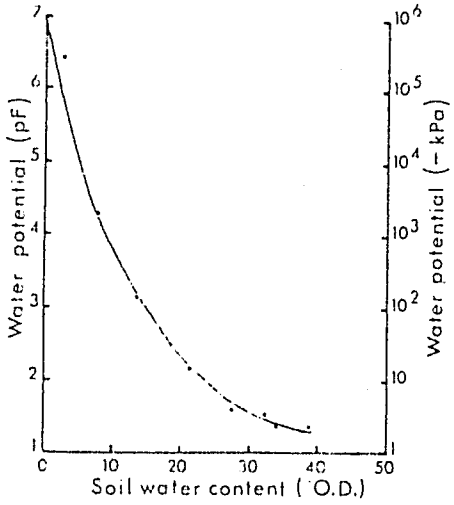
[o](ppm) indicates parts per million of soil

APPENDIX 7 (cont.)

MOISTURE CHARACTERISTIC CURVES* FOR SOILS FROM THE HEALTHY AND DEAD ZONES OF THE JARRAHDALE TRANSECT



DEAD ZONE SOIL



HEALTHY ZONE SOIL

* Filter-paper method (Fawcett and Collis-George, 1967)

Supplementary Review

1. The effect of soil moisture and aeration (soil moisture tension) on the behaviour of soil borne Phytophthora spp.

2. Overall status of *P. cinnamomi* as a forest pathogen in Australia.

3. Aspects of forest ecosystems relevant to the two study sites examined.

SUPPLEMENTARY REVIEW FOR THE THESIS

A COMPARISON OF THE OCCURRENCE, SPORULATION AND SURVIVAL OF *Phytophthora cinnamomi* RANDS IN SOILS SUPPORTING NATIVE FOREST IN SOUTH-EASTERN NEW SOUTH WALES AND SOUTH-WESTERN WESTERN AUSTRALIA

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1980

1. THE EFFECT OF SOIL MOISTURE AND AERATION (SOIL MOISTURE TENSION) ON THE BEHAVIOUR OF SOIL-BORNE *Phytophthora* spp.

Zentmyer and Erwin (1970) considered soil moisture and aeration important factors affecting the development and reproduction of species of the genus *Phytophthora*. However, until Griffin (1966, 1969, 1972) defined the terminology relating to soil moisture studies and described the mode of soil water potential control, few quantitative studies were conducted to determine the effect of this environmental parameter on the behaviour of *Phytophthora* species.

Initially, precise studies on the water relations of soil-borne *Phytophthoras* examined the influence of osmotic and matric potential on the growth of hyphae (Sommers *et al*, 1970; Adebayo and Harris, 1971). These studies indicated that the growth of hyphae was not very sensitive to reduced water potential and that fungi were more sensitive to matric than osmotic water stress in soil systems. Griffin (1979), discussed the sensitivity of *P.cinnamomi* hyphae to reduced water potential and suggested that this species had similar requirements to *Gaeumannomyces graminis* and *Rhizoctonia solani*; growth rate being half maximal in soil at matric potentials equivalent to -1 MPa to -1.5 MPa and negligible at -2.5 MPa.

Moore (1971) examined the effect of aeration on the growth of hyphae of three *Phytophthora* species. He showed that changes in the concentration of oxygen within the range likely to occur commonly in soil had virtually no effect on

the growth rate of hyphae and concluded that these species were unusually tolerant to conditions of reduced aeration at least in this phase of their development.

Sneh and McIntosh (1974) and Duniway (1975a, 1975b) recognised the epidemiological significance of the effect of soil moisture on the development of sporangia of *Phytophthora* species. Some studies indicated that few sporangia were formed in saturated soil (Duniway, 1975a and 1975b) but large numbers were produced in soils maintained at matric potentials between about -30 kPa and -300 kPa. These results suggested that aeration also had a major effect on the production of sporangia in soil and Duniway (1975b) speculated that sporangia might be formed in air filled spaces rather than water filled pores. Previously, Mitchell and Zentmyer (1971) found that the formation of sporangia by *Phytophthora* species was inhibited by low oxygen or high carbon dioxide concentrations and Pfender *et al* (1977) demonstrated that *P.megasperma* produced few sporangia in saturated soils at high temperatures, possibly because of reduced oxygen availability. Duniway (1979) noted that some studies yielded ambiguous data concerning the aeration and soil water requirements of *Phytophthora* species (cf Sneh and McIntosh, 1974 and Pfender *et al*, 1977 with Duniway, 1975b). He suggested that the variability inherent among different *Phytophthora* species and the use of different experimental techniques might explain such results. However, recent investigations (Gisi *et al*, 1980) suggested that variation

in sporangial production between different *Phytophthora* species might be greater than was previously thought.

Duniway (1976) and Pfender *et al* (1977) demonstrated that the production and release of zoospores was relatively insensitive to reduced oxygen concentrations and that *P.cryptogea* and *P.megasperma* required almost saturated, or saturated soils for successful indirect germination of sporangia. This supported statements by Stolzy *et al* (1965) and Zentmyer and Erwin (1970) that indirect germination of *Phytophthora* sporangia required free water. However, on rare occasions, indirect germination of sporangia in considerably dryer soils was observed (Duniway, 1975b and Reeves, 1975).

Direct germination by sporangia of *Phytophthora* species at matric potentials between -50 kPa and -30 kPa were observed by Duniway (1975a; 1975b), Sneh and McIntosh (1974) and Pfender *et al* (1977). However, the lower limits at which this occurred in soil were not determined.

Near saturated conditions were also required to facilitate zoospore movement in soil (Duniway, 1976; Pfender *et al*, 1977). In addition, Griffin (1979) suggested that zoospores needed water-filled pores of large diameter (50 - 150μ) through which to move, hence, pore spaces in most finer textured soils were generally too small to permit significant zoospore movement (Griffin, 1972; Hickman and Ho, 1966; Cook and Papendick, 1972). Filtration of zoospores through soil by water flow was also found only in very coarse textured soils (Duniway, 1976) while Pfender *et al* (1977)

showed zoospores of *P.megasperma* moved upward 65 mm in saturated sandy loam. These data suggested that zoospores moved small distances in coarse textured soil toward roots (Zentmyer, 1961; Khew and Zentmyer, 1974) or to the soil surface. Thomson and Allen (1976) demonstrated active and passive dispersal of zoospores in surface water.

Duniway (1979) reviewed the literature about the water requirements for zoospore germination. In the studies cited, only one soil-borne *Phytophthora* species was employed and the method of soil moisture determination was imprecise, so no conclusive results were reported on this facet of *Phytophthora* behaviour.

Reeves (1975) demonstrated that the formation of chlamydospores occurred in relatively dry soils (at least to -1.5 MPa; Griffin, 1979) but they were produced faster and in greater numbers in moister soils. Also Sterne *et al*, (1977) found that chlamydospore germination and germ tube growth were reduced at -2.5 kPa compared with potentials near saturation in sandy loam inoculated with *P.cinnamomi* chlamydospores. No such effect occurred in clay soil and amendment of the sandy loam with an energy source negated the effect. These data prompted the authors to echo an earlier warning by Sommers *et al* (1970) that the effect of soil moisture on fungal behaviour should not be interpreted only in terms of water potential

The effect of soil water potential on the survival of vegetative and sporing structures of *Phytophthora* species

in soil has also received some attention. Sporangia, zoospores and mycelium were generally considered short lived structures in soil (Reeves, 1975; Malajczuk *et al*, 1977; Hwang and Ko, 1978), hence, few quantitative studies have been performed to determine their persistence at different soil moistures. However, Turner (1965) reported that soils infested with zoospores of *P.palmivora* remained infective for 6 - 18 months at 50% moisture holding capacity and Sneh and McIntosh (1974) found that sporangia of *P.cactorum* persisted for 12 weeks at matric potentials of between -500 kPa and -20 kPa. In addition, Blowes (1980) suggested that mycelium of *P.cinnamomi* might survive for 10 weeks or more under waterlogged conditions. These studies suggest that additional quantitative work is required on these aspects of *Phytophthora* behaviour.

The survival of chlamydospores in soil maintained at different moisture potentials has been examined recently. Weste and Vithanage (1979) claimed chlamydospores survived for 8 - 10 months in soil at -300 to -500 kPa but did not persist in dry soil (-3000 kPa). In addition, viability was lost after 6 - 8 months at -30 kPa. Hwang and Ko (1978) also demonstrated longer survival of chlamydospores in moist soil (60% water by weight) than under submerged conditions. Blowes (1980) suggested that survival of chlamydospores was probably best in soils maintained at potentials too dry to support bacterial growth, hence lysis (Broadbent and Baker, 1974b; Malajczuk *et al*, 1977) but not dry enough to cause plasmolysis of the chlamydospores.

The availability of quantitative techniques for studying the moisture relations of *Phytophthora* species in soil has stimulated many studies in the past decade. While precise data were obtained for the soil water potential requirements of sporangial production, zoospore release and zoospore movement, much of the data obtained from studies using soil maintained at different moisture regimes yielded ambiguous results because the effects observed could not be attributed to water potential *per se* e.g. chlamydospore survival is probably dependent on bacterial lysis and, hence, soil organic matter level, aeration and many other interrelated factors as well as moisture potential. Also some studies which controlled water potential precisely overlooked other related parameters e.g. techniques employing sealed polythene bags to maintain moisture potential should not be compared with those using tensiometers to do the same because soil aeration differs in each system.

Until these problems are defined and the techniques standardised, reports of conflicting results will continue in the literature.

2. OVERALL STATUS OF *P.cinnamomi* AS A FOREST PATHOGEN IN AUSTRALIA

Fraser (1956) first implicated *Phytophthora cinnamomi* Rands with disease in Australian native vegetation when she associated the fungus with the death of some ground flora species near Sydney in New South Wales.

Subsequent studies in Western and Eastern Australia (Podger, 1968, 1972; Pratt and Heather, 1973a, Pratt *et al*, 1973) suggested that disease expression in forest vegetation caused by the effects of *P.cinnamomi* varied in different situations. Hence, in Australia, the status of *P.cinnamomi* as a forest pathogen must be assessed independently in each disease situation.

In Western Australia crown deterioration of *Eucalyptus marginata* Donn. ex Sm. trees in the northern jarrah forest was first noticed in 1921 (Wallace and Hatch, 1953 as cited by Newhook and Podger, 1972). This disease, known as jarrah dieback was not associated with *P.cinnamomi* until 1964 (Podger *et al*, 1965). Later, Podger (1968, 1972) reported that *P.cinnamomi* was constantly associated with diseased forest (involving both dominant and understorey species), could not be isolated from unaffected areas, and was pathogenic under field conditions following artificial inoculation. These points and the fact that the disease was associated with logging and road making in what appeared to be a very susceptible forest flora lead to the conclusion that *P.cinnamomi* caused jarrah dieback and that the fungus was an introduced organism to Western Australia (Podger, 1975).

By 1975, 172,000 ha or 10% of the state controlled jarrah forest was estimated to be infected by *P.cinnamomi* (Shea and Malajczuk, 1977). Some early estimates (Batini and Hopkins, 1972) suggested that the disease was spreading by as much as 4% per annum. However, Shea (as cited by Shepherd, 1979) recently reported that the rate of disease spread had slowed, possibly because the majority of very susceptible sites had already been infested and recorded as diseased.

Although best known for its effects in the jarrah forest, *P.cinnamomi* is thought to be responsible for severe damage in other dry schlerophyll woodland and heathland and in conifer shelter belts in Western Australia (Podger, 1972). However, the aetiology and epidemiology of disease in these ecosystems have been less closely studied although important preliminary research has been reported (Christensen, 1975; Schuster, 1978).

Victoria is also severely affected by forest dieback. Podger and Ashton (1970) isolated *P.cinnamomi* from dying ground vegetation in the Brisbane ranges in 1969 and since then the fungus has been recognised as the causal agent of dieback in East Gippsland, South Gippsland, Wilsons Promontory and the Otway and Grampian Ranges. The Brisbane Ranges syndrome is remarkably similar to jarrah dieback in that the soils and climate are similar (Podger, 1975), severe mortality occurs in the monocalyptus eucalypts but the symphyomyrtus species are apparently tolerant or immune to disease (Weste *et al*, 1972), *P.cinnamomi* is associated with diseased areas but

cannot be isolated from unaffected stands in the same district (Weste *et al*, 1972) and the pathogenicity of some local isolates of *P.cinnamomi* has been demonstrated under field conditions (Weste, 1974).

In the Gippsland and adjacent forests, *P.cinnamomi* has been isolated from extensive areas of apparently healthy forest (Marks *et al*, 1971; Marks *et al*, 1975) although the dominant tree species and some of the understorey vegetation are susceptible to the fungus. In these areas the range of disease expression varies from virtually none to almost complete destruction of the vegetation, hence, it is difficult to determine the relative impact of *P.cinnamomi*, other diseases, fire and logging history on the pattern of dieback in this forest (Anon, 1978). However, two reasonably distinct areas can be recognised i.e. an area of extensive and severe disease on the coastal plain associated with shallow soils and impeded drainage and an adjacent area on the lower slopes with good drainage and less extensive and severe disease expression in the vegetation.

Aerial and field surveys have indicated that 5,000 ha of East Gippsland forest is severely affected by dieback and the Victorian Forests Commission estimates that between 30,000 and 40,000 ha of coastal forest is situated on sites susceptible to the disease because of their soil and moisture relationships.

Weste and Law (1973) reported the occurrence of *P.cinnamomi* associated disease in the Wilsons Promontory National Park. They estimated that about 4,500 m^2 of forest

was affected and suggested that infested gravel carried into the area on tracked vehicles in 1961 might have caused the initial infection. *P.cinnamomi* was isolated from three types of plant community - swamps, heathlands and dry sclerophyll woodlands. By July 1972 the disease was spreading at a rate of 1.5% per month.

A forest disease in the lowland and foothill areas of Tasmania's east coast (east coast dieback) meets the classic criteria of a *P.cinnamomi* induced disease according to Podger (1975), i.e. monocalyptus eucalypts are killed but symphyomyrtus species show tolerance; *P.cinnamomi* has been isolated from diseased areas but not from adjacent unaffected areas, and field studies suggest that the local *P.cinnamomi* isolates are pathogenic. There have been no reported estimates of the area of forest affected. However, Felton (1980) suggested that in State forests damage is restricted to relatively small areas in lowland, water-gaining sites. The effect of this disease is of more concern in coastal heathland e.g. in the Greystone National Park, where *P.cinnamomi* may have been introduced with gravel used for roadmaking (Shepherd, 1979).

P.cinnamomi is not considered responsible for the more serious dieback diseases elsewhere in Tasmania (Anon, 1978) and the fungus is not generally considered a problem to production forestry.

P.cinnamomi has a widespread occurrence in coastal native forest in New South Wales (Pratt and Heather, 1973a;

1973b; Pratt *et al*, 1973) but there are no extensive areas of dieback that can be attributed solely to the effects of the fungus. A dieback disease occurring in scattered patches along the New South Wales coast, and best known in the Ourimbah State Forest, is almost certainly caused by a complex of factors including leaf sap-sucking psyllids, borers, and in some instances *Armillaria mellea* (Vahl ex Fr.) Kummer and *P.cinnamomi* (Podger, 1975). However, there is no clear or constant association between *P.cinnamomi* and disease, although Gerrettson-Cornell (1973) concluded that the fungus may contribute to the deterioration of trees first weakened by insects and water shortage.

The status of *P.cinnamomi* as a pathogen in Queensland's native forests was thought to be similar to the New South Wales situation i.e. *P.cinnamomi* was widespread throughout many forest communities (Pegg and Alcorn, 1972; Pratt and Heather, 1973a; Pratt, *et al*, 1972) but generally there was an absence of death in eucalypt and heathland communities (Shepherd, 1979). Recently however, Brown (1976) reported the occurrence of patch death in virgin and logged tropical rainforest in Queensland. He suggested that *P.cinnamomi* might have been spread or activated by the activities of logging, road construction or feral pigs because in each instance disease was associated with soil disturbance leading to raised water tables e.g. pig wallows. In both the areas he studied, Brown isolated *P.cinnamomi* from adjacent apparently healthy forest. This characteristic, the prerequisite for some type of soil disturbance and the

species of vegetation affected, differentiates this dieback from those reported in southern Australia by Podger (1968, 1972) and Podger and Ashton (1970).

In South Australia, *P.cinnamomi* is widely distributed in isolated pockets and Wallace (1977) suggested that the distribution of the fungus might be spreading at a significant rate. However, to date only the death of some understorey species, in a few very small wetter areas of the Adelaide Hills (Wicks, 1973; Shepherd, 1979) have been attributed to attack by *P.cinnamomi*.

Blowes (unpublished) was unable to isolate *P.cinnamomi* from soils supporting native vegetation in the north of the Northern Territory during a three month survey. In addition, *P.cinnamomi* was not isolated from the roots of known susceptible agricultural crops (notably avocados) imported from certified nurseries (mainly C.S.I.R.O., Merbein, Victoria) or from avocados propogated locally from such stock. However, the isolation of *P.cinnamomi* from the roots of several avocado trees imported from one nursery in Central Queensland suggested that the fungus could survive in the Northern Territory although it may not be a native soil inhabitant and does not appear to be widely distributed at this stage.

In Australia, the status of *P.cinnamomi* as a forest pathogen ranges from situations of no disease, as seen in the Northern Territory where the fungus appears to be absent from soils supporting native forest, to the

devastating host/pathogen syndrome evidenced when *P.cinnamomi* is introduced into a susceptible and vulnerable forest flora e.g. the northern Jarrah forest, Brisbane Ranges and perhaps East Coast dieback in Tasmania. On the east coast of mainland Australia *P.cinnamomi* appears to have been present for a long period and *P.cinnamomi* induced disease is relatively rare although the fungus is widely distributed. In instances where disease is associated with the fungus, other factors, such as soil disturbance in Queensland's rainforest, insects at Ourimbah and fire or logging history in East Gippsland, may contribute significantly to the disease, thus confusing the status of *P.cinnamomi* as a pathogen in such situations.

3. ASPECTS OF FOREST ECOSYSTEMS RELEVANT TO THE TWO STUDY SITES EXAMINED

The aspects considered in this summary for the two sites studied are: the physical environment, some soil characteristics and the vegetation types present.

There has been considerable discussion in relation to the influence of the physical environment on diseases associated with *P.cinnamomi*. Stahl and Jehne (1971) and Titze, (1971) suggested that the environment was the most significant aspect of the disease and that *P.cinnamomi* was of secondary importance. Pratt *et al* (1971) concluded that disturbance to the environment was a prerequisite for disease while others (Podger, 1972; and Newhook and Podger, 1972) suggested that *P.cinnamomi* was the most significant factor. Recent studies (Blowes, 1980) suggest that each theory may be applicable, but only in the particular area examined to formulate the proposal.

The site near Jarrahdale in Western Australia is typical of many in the northern jarrah forest (Malajczuk, pers. comm.). Rainfall averages approximately 800 mm per year but the bulk falls in winter between April and October (Havel, 1975). Mean maximum and minimum temperatures range from 28°C and 13°C in summer to 15°C and 6°C in winter. The Durras area in New South Wales receives about 1000 mm per year which is relatively evenly distributed (Anon, 1975). Mean monthly maximum and minimum temperatures range from 23°C and 15°C in summer to 17°C and 7°C in winter.

Shea (1975) conducted detailed studies to determine the length of time that infection can theoretically take place in some jarrah forest soils. Using moisture levels equivalent to field capacity and soil temperatures of 15°C as the critical limits below which infection was inhibited, he estimated that lowland sites had long periods in spring, summer and autumn during which soil moistures and temperatures were suitable for infection. Upland sites had relatively short periods (less than one month) of susceptibility; mainly during the spring. In addition, he demonstrated that the degree of canopy and litter cover affected the length of time that soil temperatures and soil moisture tensions were suitable for infection. Shea also estimated that there were long periods on upland sites during which moisture levels were unsuitable for fungal survival i.e. below wilting point (Batini, 1973).

Detailed environmental studies have not been reported for the area sampled in southern New South Wales, probably because no acute disease situation exists and estimates of the potential duration of fungal activity are of no practical interest. However, Blowes *et al* (1980) speculated on some aspects of the effect of the physical environment of soils when discussing the occurrence and distribution of *P.cinnamomi* at the Durras site. They concluded from their own studies and from evidence presented by Pratt and Heather (1973b) and Arentz (1974) that moisture levels in the lowland part of the site were adequate for continual survival of *P.cinnamomi* and that due to the evenly

distributed rainfall and mild temperatures (Anon, 1975) conditions were frequently suitable for fungal infection.

The effect of soil type on disease expression has been studied in Western Australia (Shea, 1975; Schuster, 1978) probably because not all forested areas show uniform disease expression after infection has occurred. While some differences between infection susceptibility and disease expression have been recorded on different soil types in the field (Schuster, 1978) it is unlikely that this can be attributed to soil type *per se* since many interrelated factors contribute to disease expression. In addition, Shea (1975) concluded from glasshouse experiments that soil type did not affect the pathogenicity of the fungus when high inoculum levels and adequate moisture tensions were applied. At low levels of inoculum, the mortality of a susceptible host species was high in lateritic silt but he attributed this to the more favourable moisture conditions in this soil which probably enhanced zoospore motility.

Equivalent studies have not been done for the soils of the south coast of New South Wales. Hence, to describe the two areas studied in relation to their edaphic factors, Blowes (1980) presented landscape and soil profile descriptions of the two sites. In addition, the soil moisture characterisitic curves (Fawcett and Collis-George, 1967) and the soil chemical analyses (C.S.I.R.O., Division of Land Resources Management, Wembly, W.A.) were reported. The former revealed that the moisture characteristics of

the lowland soil at Durras were similar to those at the Jarrahdale site whereas soil from the Durras upland zone was very different in that a larger percentage soil moisture content increase was required to waterlog soil in this zone. In addition, in all zones analysed, soils were generally more fertile at the Durras than the Jarrahdale site, (see Blowes, 1980; Appendices 3 and 7).

The microbial populations of the soils at these sites was possibly a reflection of the fertility and moisture characteristics of the zone from which they were collected (see Blowes, 1980). Pratt and Heather (pers. comm.) had previously found low microbial populations in Western Australian soils compared with those in soils collected from south eastern New South Wales. In addition, other Australian research workers (Broadbent and Baker, 1974a; Weste and Vithanage, 1977; and Malajczuk and McComb, 1979) had given attention previously to this ecological aspect. Their work suggested that microbial populations differed quantitatively in soils which were suppressive and conducive; in soils supporting healthy and diseased forest, and in the rhizospheres of resistant and susceptible host species.

Both sites studied were dry schlerophyll forests (McColl and Humphreys, 1967; Havel, 1975). Dry schlerophyll associations have been most severely affected by *P.cinnamomi* induced diseases in Australia (Podger, 1972; Weste and Taylor, 1971; and Marks and Kassaby, 1971) possibly

because they generally comprise a large proportion of susceptible plant species which are vulnerable to fungal infection and subject to physiological stresses at some period during the year (Weste, 1979).

Newhook and Podger (1972) reported a total of 404 recorded host species in Australia. They suggested that the families *Proteaceae, Myrtaceae, Papilionaceae* and *Epacridaceae* were particularly susceptible. Also, within the genus *Eucalyptus*, members of the subgenus Monocalyptus (M) were considered highly susceptible whereas members of the Symphyomyrtus (S) and Corymbia (C) were generally resistant (Podger and Batini, 1971; Newhook and Podger, 1972).

E.marginata (M) was the major dominant species on the Western Australian site. Titze and Palzer, (1969) considered this species as highly susceptible to *P.cinnamomi* attack. In terms of the dominant eucalypt present, the equivalent species on the Durras site was *E.maculata* (C), a field resistant species. The co-dominants, *E.pilularis* (M), *E.paniculata* (S), and *E.botryoides* (S) were ranked less susceptible than jarrah by Titze and Palzer (1969) but received the same ranking as *E.calophylla* (C), a co-dominant at the Jarrahdale site and generally considered field resistant (Malajczuk *et al*, 1977). A comparison of the reported susceptibility of the understorey species at these sites is prevented by the lack of data on species from southern New South Wales, however, *Banksia* spp is a common

component of the understorey in the south west area. In addition, the absence of a realistic and controlled method of testing pathogenicity and resistance confuses the concept of vegetation susceptibility. For example, Shea (1979a) suggested that *E.marginata* was not highly susceptible to *P.cinnamomi* although many jarrah trees were killed by it. He pointed out that it often took 10 years or more after the initial infection to kill a jarrah tree. In addition, Malajczuk *et al* (1977) noted that *E.marginata* survived on red-brown loams in river valleys that dissected the jarrah forest. They concluded that microorganisms of the rhizosphere might play an important role in the resistance of eucalypts to *P.cinnamomi* infection on such sites.

Shea (1979a) considered understorey species like *B.grandis* as highly susceptible because their large root systems were systemically infected by *P.cinnamomi* (Shea, 1979b). It is not known what number and to what degree species at each site are infected in this manner. Also, as Malajczuk *et al* (1977) implied, susceptibility may not be a consequence of an interaction between the host and pathogen only i.e. species and communities of comparable susceptibility may be more or less vulnerable under different environmental conditions.

Weste (1979) defined vulnerable communities as those in which:

(a) the pathogen is either present or likely to invade;

(b) the dominant species of understorey and tree are both susceptible;

(c) the environment at times is either unfavourable for host roots and/or favours the pathogen.

Pratt *et al* (1971) considered sites in south-eastern New South Wales vulnerable but only after environmental disturbance altered the ecological balance (i.e. (c) above) in favour of the pathogen e.g. the lowland zone at Durras. Weste (1979) and Newhook and Podger (1972) considered the northern jarrah forest community vulnerable when (a) occurred because the other factors (b) and (c) were inherent.

These differences in aetiology and epidemiology of *P.cinnamomi* disease in Australia emphasise the fact that disease is a consequence of the host-pathogen-environment interaction at each particular site. Hence, each disease situation should be considered individually so that the appropriate disease control measures are implemented in each instance.

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