Cylindrocarpon Dieback of *Eucalyptus obliqua* regrowth forests in Tasmania

by

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This thesis contains no material which has been accepted for the award of any other degree or diploma in any University, and to the best of my knowledge contains no copy or paraphrase of material previously published or written by any other person except where due reference is made in the text of this thesis.

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SUMMARY

Many of the natural Eucalyptus obliqua, and E. regnans regrowth forests throughout Tasmania have recently been affected by crown dieback which frequently results in the death of scattered former dominant and co-dominant trees. The unexplained dieback occurs in some of the best natural eucalypt forests and was of major concern to the future, productivity and stability of these forests. The investigations described in this thesis, were aimed at studying the aetiology of the dieback complex and evaluating the consequences of regrowth dieback in the natural development of these forests.

The relationship between dieback and the natural transition of regrowth forests to cool temperate rainforest was examined. These initial studies indicated that the dieback may be a natural factor in the development of these forests that has occurred previously and is unlikely to result in tree deaths beyond those associated with normal forest development. Furthermore the dieback of former dominant trees may also be natural during the transition of regrowth forests to mature forests and be related to relatively higher stress in dominant trees than in the former subdominant trees which survive to form natural oldgrowth forests. While these studies should substantially reduce concern about the unexplained dieback of regrowth forests they also provide a new perspective of forest growth within which factors likely to contribute to regrowth dieback could be investigated and interpreted.

A wide range of factors which may have contributed to the drought stress and dieback in the regrowth forests were investigated. Shallow soils with low capacities for holding moisture, periodic

waterlogging and drought stress, and the restriction of the fine root systems of regrowth trees relative to those of understorey plants were all associated with dieback. However, these factors could not explain the high level of fine root decay and the progressive dieback of trees even during periods and on sites favourable for plant growth. Therefore the involvement of potential root pathogens was examined. Nematodes and Pythium spp. were inconsistently isolated from rhizosphere soil and eucalypt roots and may contribute to a root rot complex. Phytophthora cinnamomi which was isolated infrequently from forest soils could not be isolated from the fine roots of regrowth trees and is unlikely to be a major factor in the dieback. However, a previously undescribed Cylindrocarpon sp. was consistently isolated from diseased fine roots of regrowth trees and was confirmed to be pathogenic on E. obliqua seedlings. Consequently detailed studies were conducted on the role of the Cylindrocarpon sp. in the infection and decay of E. obliqua roots and in the causation of the dieback complex.

The population and activity of Cylindrocarpon sp. increased in soils from E. obliqua forest from low levels following burning to a maximum in 70-90 year old forests. This closely parallels the intensification of dieback in these forests. The Cylindrocarpon sp. appeared to be natural in these E. obliqua forests and was well adapted for growth and survival in the conditions existing in the regrowth forests.

The Cylindrocarpon sp. was capable of infecting and killing E. obliqua seedlings which had been grown under the soil, environmental, host stress and inoculum conditions representative of those occurring in regrowth forests. Sublethal levels of root

infection but no deaths occurred when Cylindrocarpon sp. was inoculated under conditions favourable for seedling growth. Waterlogging, defoliation, high inoculum concentrations, lower soil temperatures, pre-inoculation drought stress and changes in host physiology with the transition of growth stages all contributed to the susceptibility of the E. obliqua seedlings to infection by Cylindrocarpon sp.

Detailed studies of Cylindrocarpon sp. in the rhizosphere of E. obliqua indicated that root infection was largely governed by the interactions between Cylindrocarpon sp. , other micro-organisms in the rhizosphere and changes in root exudates. A hypothetical model of fungistasis, lysis and survival of Cylindrocarpon sp. inoculum in the E. obliqua rhizosphere was developed which could account for these interactions.

While Cylindrocarpon sp. infection of the root generally caused little damage in the cortex, infection could also induce extensive discoloration and blockage of xylem vessels by tyloses and gummosis without the fungus colonising and subsequently being recoverable from affected stelar tissues. This dysfunction of the roots of E. obliqua occurred in pathogenic Cylindrocarpon sp. infections and was consistent with the high level of staining and blockage observed in the stele of fine roots of E. obliqua regrowth trees affected by dieback. A phytotoxin was isolated from Cylindrocarpon sp. cultures on natural as well as artificial substrates which accounted for the observed dysfunction of fine roots and the death of E. obliqua seedlings.

Two stages appear to be involved in the association of Cylindrocarpon sp. with the fine roots of E. obliqua. The first stage involves the infection of the cortex, is nonpathogenic and is consistent with the common association of Cylindrocarpon spp. with healthy roots as companion fungi (Kurbis 1937, Garrett 1970). The second stage which may follow involves the production of phytotoxins by Cylindrocarpon sp. in the cortex of infected roots which result in the blockage of the xylem vessels by tyloses and the subsequent drought stress and death of the affected plant. Recognition of these two stages in the assocation of Cylindrocarpon sp. with E. obliqua fine roots can account for many of the responses observed in seedling and in regrowth forests affected by dieback. These stages may also help to explain much of the uncertainty about the pathogenicity of Cylindrocarpon spp. in other root diseases.

Consequently Cylindrocarpon sp. appears to be a natural and primary factor in the aetiology of E. obliqua regrowth dieback. However, the involvement of Cylindrocarpon sp. in dieback must always be considered in the context of the contributing complex of interacting site, physiological and pathological factors, and their effect on the susceptibility of E. obliqua to infection and root decay.

The implications of these results are discussed in relation to the assocation of Cylindrocarpon spp. with the development of similar eucalypt forests in eastern Australia. The implications of these studies are also discussed in relationship to forest diebacks elsewhere in the world and the future development of other forests.

CHAPTER 1

INTRODUCTION

The tall open forests of Eucalyptus obliqua L'Herit and E. regnans F. Muell comprise over two thirds of the high quality forests of Tasmania and are the resource base for much of Tasmania's forest industry and economy. The forests are among the tallest and most productive natural hardwood forests in the world and may serve as a model for the growth and future development of many eucalypt plantations throughout the world. Aspects of the floristics, structure, management and development of these forests in Tasmania have been described by Gilbert (1958, 1959), Cunningham (1960), Jackson (1968), and Mount (1969) whereas Ashton (1975, 1976) has described similar E. regnans forests in Victoria. The forests regenerate naturally from seed following hot fires and form dense stands of even aged eucalypt regrowth which may reach a height of 50 metres in 50 years (Figure 1). In the absence of further fires the regrowth forests mature to form a tall emergent canopy above cool temperate rainforest (Figure 1).

Recently extensive areas of regrowth forests in southern Tasmania have been observed to be affected by a crown dieback (Bowling and McLeod 1968). The dieback appears to be intensifying both in frequency and degree and often results in the death of former dominant and co-dominant trees (Figure 1). The unexplained dieback of former dominant trees in these high quality forests has raised concern about the future productivity and stability of these and similar forests. Consequently research has been undertaken to determine the cause of regrowth dieback, and its likely impact on the future development of these forests.

Figure 1. Stages in the development of Eucalyptus obliqua forests in Southern Tasmania.

a. Seven year old sapling stands of E. obliqua regenerating on previously logged and burnt oldgrowth forest sites. Tree heights 7-10 m.

b. Healthy E. obliqua regrowth forest at 70 years of age. Tree heights 35-50 m

c. Dieback affected E. obliqua regrowth forests at 70 years of age with developing rainforest understories and various intensities of crown dieback.

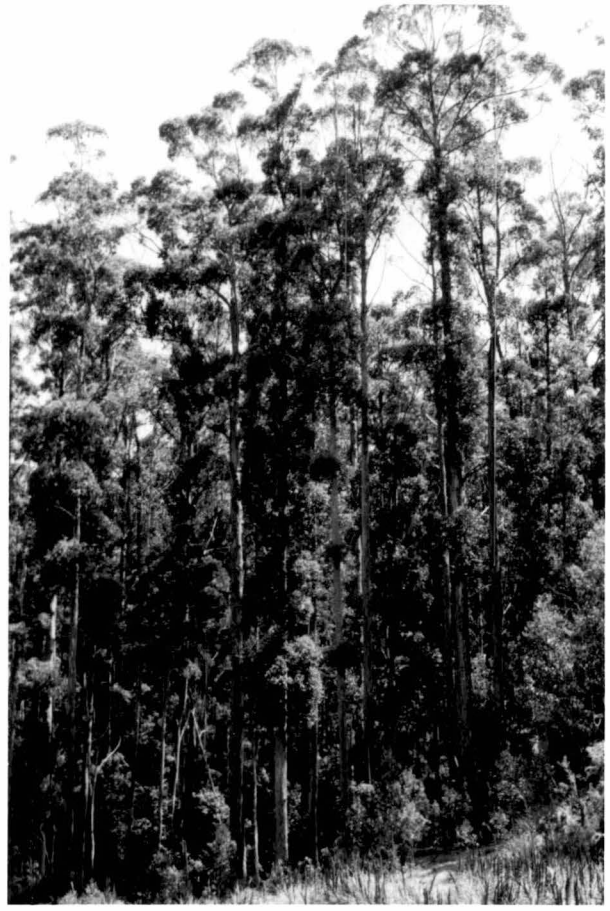
d. Dieback affected E. obliqua regrowth forests at 70 years of age with developing rainforest understories.

e. Crown condition of 70 year old E. obliqua regrowth trees at various stages of dieback.

f. Typical old growth E. obliqua emergent above cool temperature rainforest exhibiting characteristics of previous diebacks and successive epicormic crowns. Height approximately 50 m

a

b



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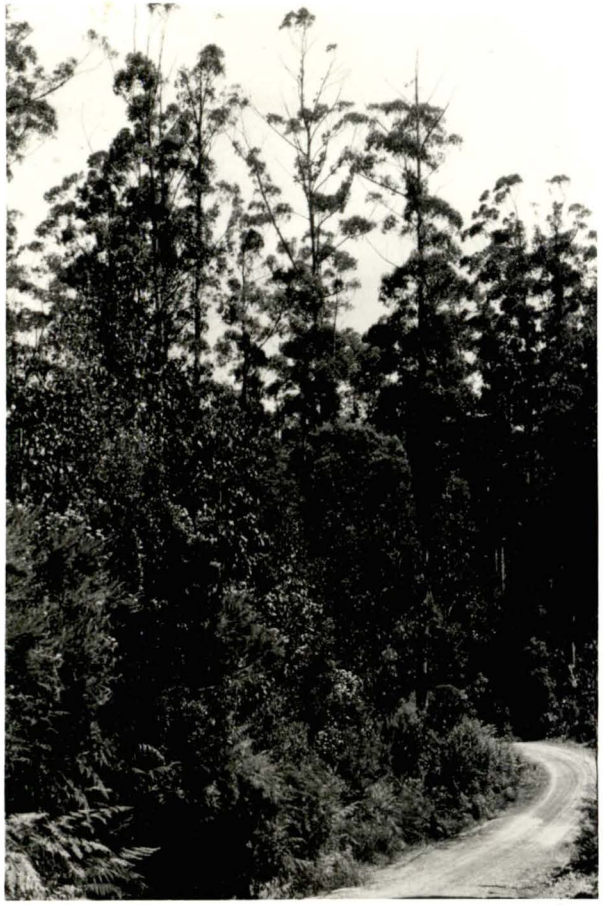

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This thesis describes part of that research. Initial studies were directed at defining regrowth dieback and investigating its relationship to the natural development of these forests. The factors which may be associated with the causation of regrowth dieback were also studied. Subsequent investigations examined the pathogenicity and microbiology of a Cylindrocarpon sp, a fungus which was isolated from the fine roots of E. obliqua, in order to define its role in dieback and the development of these forests.

1.2 REVIEW OF LITERATURE

1.2.1 The Dieback of Forests Throughout the World

The dieback of regrowth forests in Tasmania is not unique as similar often unexplained, diebacks have been reported in forests from throughout the world. The following review examines some of these diebacks and the factors with which they have been associated in an attempt to provide a basis for the subsequent studies on the aetiology of regrowth dieback in the E. obliqua forests of Tasmania.

The dieback of birch, Betula lutea and Betula papyrifera, in north-eastern North America occurs in maturing, logged and disturbed forests (Humboldt and Skolko 1948). Birch dieback has been associated with a complex of factors which include shallow soils and surface fine root systems (Pomerleau and Lortie 1962), high levels of fine root rot (Greenidge 1953, Redmond 1957) and periodic drought stress (Greenidge 1953, Hepting 1963). Greenidge (1953) found that:-

"The locus of action of the disease is, in the first instance, in the roots and excessive rootlet mortalities in apparently healthy trees constitutes the initial indication of a diseased condition in the species."

Although a wide range of insects and fungi were isolated from the crowns and roots of affected trees, Cylindrocarpon orthosporum (Sacc.) Wollenw. was the only fungus isolated with sufficient frequency from the roots of affected birch to be regarded as a possible pathogen (Hann and Eno 1956). The pathogenicity of this fungus was not confirmed as no disease symptoms were apparent when five year old birch seedlings, which were growing vigorously in a glasshouse, were inoculated with a 200ml suspension of Cylindrocarpon orthosporum culture. No attempts were made to recover the inoculated organism from the soil or roots (Hann and Eno 1956). The precise cause of the birch dieback does not appear to have been further elucidated although recent studies suggest the possible involvement of viruses and mycoplasmas in dieback complexes of birch (Cooper 1976).

Pole blight of Pinus monticola in the north-western United States of America also involves the dieback and death of former dominant trees and occurs in natural regrowth forests (Leaphart and Copeland 1957) and in maturing plantations (Leaphart and Johnson 1973). The stands form the primary site colonising stage of a succession to climax forests (Leaphart and Wicker 1966). Pole blight was found to be most severe on shallow soils with low available moisture and was associated with high levels of rootlet mortality (McMinn 1955, Leaphart and Copeland 1957). It has been suggested that seasonal drought stress, the relatively lower root to shoot ratios of P.

monticola seedlings (Leaphart and Wicker 1966) and long term climatic fluctuations (Leaphart and Stage 1961) contribute to this dieback. However, thinning of competing trees did not retard the development of pole blight, which would suggest that factors in addition to competition and drought stress are involved in this dieback (Leaphart and Foiles 1972). No pathogenic organism which could be regarded as a primary causal factor has been associated with affected trees even though a wide range of organisms were isolated (Leaphart and Gill 1955). In independent studies Brandsberg (1969) consistently isolated Cylindrocarpon cylindroides as a dominant coloniser of P. monticola litter in similar forests. No pathogenicity studies nor associations between pole blight and this fungus have been reported.

The decline of sugar maple Acer saccharum in the Eastern United States occurs in hardwood woodland and involves the death of scattered mature trees following crown dieback and reflushes of epicormic shoot growth (Giese et al. 1965). Westing (1964) and Hibben (1964) reviewed possible contributing factors. Although defoliating and woodboring insects and root infecting organisms were examined, no single organism was demonstrated to be the primary agent in sugar maple decline. Shallow root systems, root necrosis, periodic drought stress, soil temperatures, salt injury and tree age were all suggested to be involved in the development of this decline complex. Nitrogen deficiency (Mader and Thompson 1969) and root infections by the nematode Xiphinema americanum (Di Sanzo and Rohde 1969) have subsequently been associated with sugar maple decline.

The decline of red and scarlet oaks in the United States (Staley 1965, Nicholls 1968) have also been associated with drought stress,

frost, insect defoliation, shallow soils, restricted soil drainage and high rootlet mortalities. Similar oak diebacks have also been reported form Rumania (Marcu 1966) where they are associated with Ceratocystis, Armillaria, Thielaviopsis and Erwinia infection (Petresan 1974). The crown dieback of Quercus virginiana in Texas was associated with a Cephalosporium vascular wilt fungus (Van Arsdell and Halliwell 1970). Root rot of frost injured Quercus ellipsoidalis seedlings has been attributed to Cylindrocarpon destructans Zins Scholten (Hart 1965) (formerly called C. radicicola Wollenw.).

The dieback of Fraxinus americana is also associated with a complex of shallow soil, restricted root growth, drought and root decay factors (Ross 1966). Recently Hibben and Bozarth (1972) have identified a strain of tobacco ringspot virus associated with declining ash. The virus is transmitted by the nematode Xiphinema americanum.

Littleleaf disease of shortleaf and loblolly pines in the South Eastern United States (Campbell and Copeland 1954) is similarly associated with a complex of factors. These include shallow soils which are periodically waterlogged or droughted (Copeland and McAlpine 1962), plus decayed and restricted fine root systems (Jackson 1945, Copeland 1952). Jackson (1945) initially reported that a Torula sp. was consistently associated with stained and decayed roots and was able to confirm the pathogenicity of this fungus. He and subsequent investigators (Campbell 1951) also described the involvement of Phytophthora cinnamomi Rands in root rot of littleleaf affected pines which is now regarded as a major factor in the causation of root rot and dieback of these forests (Zak 1963).

The dieback of Liriodendron tulipifera forests in Georgia is of particular interest as it occurred in 27 year old trees which had previously been growing vigorously in healthy plantations. The dieback has been associated with a necrosis and staining of the fine roots, of the affected trees which is induced by Cylindrocarpon scoparium (Ross 1967).

In other studies Vincent and Kantor (1971) describe the dieback of Abies alba in Europe which was associated with fungal root rot, drought stress and limiting site conditions. The decline of Ohia, Metrosideros collina and Koa, Acacia koa forests in Hawaii has similarly been associated with impeded drainage, nutrient deficiency, root rots and drought stress. Phytophthora cinnamomi, Pythium spp., Fusarium spp, and Cylindrocladium spp. have all been isolated from the affected forests (Bega 1974, Kliejunas and Ko 1974). Dieback patches in Nothofagus forests in Papua New Guinea have been associated with root infections by Phytophthora cinnamomi and a Cylindrocarpon sp. (Cartledge, Shaw and Stamps 1975).

Diebacks of unknown or complex causes have also been reported in natural and planted eucalypt forests. In Cyprus, Day (1959) described a dieback of eucalypts associated with restricted root development on shallow, waterlogged soils. The dieback of eucalypts in Tunisia was associated with impaired rooting efficiency, Armillaria root rot and shallow soils (Delatour 1969). Dieback of mature eucalypts in Brazil has been associated with root rot by Cylindrocladium scoparium cv Braziliensis (Batista 1951, Hodges and May 1972). Compact soils and periodic waterlogging were often associated with diseased trees.

In native eucalypt forests within Australia crown dieback has long been recognized as a normal characteristic of the crowns of maturing forest trees (Jacobs 1955). However, dieback has also been recorded in younger eucalypt forests and at intensities well above that generally expected in these stands. As these appear to be inconsistent with our expectations of "normal" forest growth they have been the subject of increasing concern and investigation as to their possible cause and consequences (Newhook and Podger 1972). Research on some of these diebacks has been reviewed by Hopkins (1973) and Old et al. (1981).

The dieback of E. delegatensis forests at higher altitudes in north-eastern Tasmania was associated with shallow clay soils and the development of the cool rainforest understorey in these forests in the absence of regular fires (Ellis 1964). The lowering of surface soil temperatures under the rainforest subcanopy has been suggested to limit regeneration of the fine roots of E delegatensis causing an imbalance in the metabolism rates of the roots and shoots (Ellis 1971). Investigations associated with the current study (Jehne, unpublished) reveal that the same Cylindrocarpon sp., later to be associated with E. obliqua regrowth dieback, is also associated with dieback in these forests.

Dieback has also been reported in the Jarrah, E. marginata, forests of Western Australia where it was associated with a complex of site deterioration and periodically waterlogged and droughted soils (Hamilton 1951). More recently fine root decay due to Phytophthora cinnamomi has been defined as the primary causal factor (Podger, Doepel and Zentmyer 1965, Batini and Hopkins 1972, Podger 1972). The importance of site conditions and environmental factors on P.

cinnamomi activity in these forest soils has been investigated by Shea (1975), and Shea, Malajczuk and Kitt (1976) have described the role of frequent mild fires in maintaining environmental and substrate conditions suitable for P. cinnamomi root infections.

Other eucalypt diebacks which have been associated with the isolation of P. cinnamomi from forest soils include reports of dieback in: the Brisbane ranges Victoria (Podger and Ashton 1970, Weste and Taylor 1971), the Australian Capital Territory (Jehne 1971), lowland forests in eastern Victoria (Marks, Kassaby and Reynolds 1972), Wilsons Promontory, Victoria (Weste and Law 1973), lowland forest on the east coast of Tasmania (Jehne, Palzer unpublished reports) and the central coast of New South Wales (Gerrettson-Cornell 1973). These diebacks were often associated with shallow, periodically waterlogged and droughted soils and forests of slow-growing, remnant trees left standing after the successive selective logging of these forests.

Eucalypt diebacks in which root rot fungi do not appear to be involved have also been described. These include: the dieback of E. delegatensis forests due to defoliation by the phasmid, Didymuria violescens (Readshaw and Mazanec 1974), other insect defoliations (Carne, Greaves, McInnes 1974) and dieback of E. dives associated with root parasitism by Exocarpus cuppressiformis (Jehne 1972).

Crown diebacks have also been reported in plantations of forest and horticultural tree crops (Jehne 1972, Broadbent and Baker 1974). The diebacks discussed emphasise the widespread occurrence of tree diebacks and the many factors with which they may be associated. However, as some factors appear to be common in so many of the

diebacks, a further review of these factors may be relevant to investigations of dieback in the E. obliqua regrowth forests.

1.2.2 Factors Commonly Associated With Forest Diebacks

Many different factors have been associated with particular forest diebacks. However several factors appear to be frequently involved and warrant investigation in the causation of diebacks. One of the conditions most frequently associated with the development and intensification of diebacks in forests was the imbalance in the water relations of the affected trees which lead to dieback symptoms very similar to those of drought stress. While droughts were implicated in some of the diebacks its initiation was often associated with the inability of the affected tree to obtain adequate moisture from the soil relative to its transpiration demand. Consequently shallow soils, soil with low capacities for storing moisture at tensions available to plants, waterlogging and competition were often found to contribute to an imbalance in water relations and dieback. Similarly the restriction and decay of the fine root system may limit water uptake by affected trees and contribute to many forest diebacks. Low soil temperatures, physical properties of soils adverse for root growth, physiological stress due to factors such as defoliation and root infections and dysfunction due to soil biota, principally fungi, were often involved in the restriction and decay of the fine root system. While the role of each of these factors needs to be studied in relation to root restriction, drought stress and dieback, dieback was often associated with a complex interaction of many of these factors during the development of these forests.

Root growth in trees may be affected by the physical properties of soil (Taylor and Gardner 1963, Greacen, Barley and Farrell 1969) periodic anaerobic conditions due to waterlogging (Boden 1962, Greenwood 1969 and Lambert 1976), the physiological status of the tree (Burstrom 1965), allelopathy (Rice 1974), and competition from neighbouring trees (McQueen 1968). However, the effects of these factors in the causation of dieback needs to be considered in relation to the capacity of the tree to replace affected roots (Palzer 1969) or alter its root shoot relationships (Ledig, Bormann and Wenger 1970).

Many of the factors discussed above are likely to have existed during the development of these forests. As the forests are likely to have become partially adapted to them it is difficult to understand how these factors, by themselves, fully explain why dieback first develops in formerly healthy forests. Consequently the initiation of crown dieback in apparently healthy forests would appear to require some new factor such as an introduced pathogen (Newhook and Podger 1962), or a major change in previously existing factors such as long-term changes in climate (Hepting 1963, Leaphart and Stage 1971). Much research has been directed towards such factors. However it is also important to investigate the effects of changes in natural factors on the initiation of dieback. These may include changes with age which may alter the physiology of the affected tree as well as the soil environment and microbiological factors.

Changes in the physiology of trees with age may involve loss of apical dominance, increased within crown competition, decreased supplies of photosynthate to the roots, altered root/shoot ratios

and an increased sensitivity of the tree to environmental and pathological stress (Day 1960, Westing 1964, Bormann 1965, Wareing 1970, Kozlowski 1971). Bormann (1965) maintained that "for a tree to remain vigorous in a developing stand it must have a more or less constant increase in the amount of energy it fixes photosynthetically." As photosynthesis can not consistently increase due to a wide range of limiting site, environmental and host factors the physiology of the tree has to change with age. The restriction of photosynthesis may be particularly limiting for root growth which is furthest removed from the source of photosynthate. If the reduction in root growth also reduces water uptake by the tree further reductions in photosynthesis, root growth and tree vigour may be expected (Bormann 1965, Aung 1974).

While these changes may largely account for tree decline, several other factors may either alleviate or accentuate their effect. The distribution of dry matter growth from shoots to roots may alter in some trees with age to obtain a more favourable balance between the ability of the roots to supply water and the water demand of the tree (Ledig, Bormann and Wengner 1970). However, should the tree also be subjected to competition from more vigorous trees or become more susceptible to root decay as a consequence of the photosynthate or moisture stress a progressive deterioration of the root systems, and subsequently crowns can be expected. In a forest those species or genotypes which are subject to the least stress are likely to be selected for growth under limiting site conditions rather than the species or genotypes subject to high levels of stress. A natural succession of forest species may be expected at this stage of forest development from vigorous dominants with low root - shoot ratios and subject to high stress to slower growing trees with higher

root-shoot ratios which are subject to less stress (Monk 1966). In studies of pole blight this succession was most pronounced on poor soils in which fast growing species are subjected to more stress than slow growing trees. The former dominant P. monticola also exhibited the least capacity to adapt to limiting soil conditions by a reduction in shoot growth (Leaphart and Wicker 1966).

Forests and most other populations, normally undergo distinct site colonising and mature growth stages. Those tree species or genotypes naturally selected during the site colonising stage of growth need not remain the most competitive during the transition to mature forest growth (Stern and Roche 1974, Odum 1971). Consequently changes in the physiology of the tree associated with the transition of forest growth stages may be a major factor contributing to the root diseases and death of former dominant trees (Last 1971). Indeed Day (1960) observed that the limitations in growth and water uptake of the roots relative to their transpiration demands sooner or later leads to stress particularly in the former dominant trees "in which is to be found one of the basic reasons of decline in the health of trees and change in forest structure and successional development."

Changes with forest age also may affect the soil environment and thereby influence tree growth and dieback development. The inhibition of nitrification, in climax forest ecosystems has been associated with tannin accumulation (Rice and Pancholy 1973) while Ellis (1976) described the effects of accumulations of eucalypt litter extracts on the leaching of nutrients from forest soils. A substantial part of the total nutrient in a forest may also become bound within plant biomass during forest development which may

restrict subsequent nutrient supply and forest growth (Attiwill 1971). Changes in understorey development may also decrease soil temperatures and through this rates of root growth which could affect forest diebacks (Ellis 1971).

Changes may also occur in a wide range of micro-organisms including fungi, nematodes, bacteria, viruses, mycoplasmas and rickettsias during the development of the forests. These changes in the activity of micro-organisms may contribute to or cause diebacks through a variety of host disruptions including feeder root rot, structural root rot, vascular blockage, phloem dysfunction and shoot necrosis. The microbial populations, activities and interactions are also likely to be influenced by changing environmental factors, and host physiology during the development of these forests.

The role of some of these groups of micro-organisms in forest disease, including nematodes (Ruehle 1973) and viruses (Seliskar 1966, Smith 1972) has been reviewed recently. Fungi of the genera Cephalosporium and Ceratocystis have frequently been associated with trees affected by vascular wilts while members of the genera Pythium, Phytophthora, Fusarium, Cylindrocladium and Cylindrocarpon have commonly been associated with decay of the fine roots. Hendrix and Campbell (1973) reviewed the role of Pythium spp. as plant pathogens while Newhook and Podger (1972) examined the role of Phytophthora cinnamomi in forest diebacks in Australian and New Zealand. Fungi of the genera Armillaria and Fomes are often associated with structural root rots of trees, frequently during the later stages of tree decline.

Consequently the dieback of forests was often associated with moisture stress resulting from either periodic or long term changes in climatic factors or with an interacting complex of soil, physiological and microbial factors which limit the ability of the tree to absorb adequate moisture relative to its transpirational demands. Furthermore this review emphasised the dynamic nature of these interacting factors and forest growth and the need to investigate the causation of dieback in the context of the natural development of the forests.

1.2.3 Associations between Cylindrocarpon spp. and Plant Roots

Fungi of the genus Cylindrocarpon have frequently been isolated from the roots and soil of forests affected by dieback, but their role in these diebacks and their pathogenicity generally has remained illdefined. As a Cylindrocarpon species was isolated from the fine roots of E. obliqua regrowth trees the following review examined associations between Cylindrocarpon spp. and plant roots.

The genus Cylindrocarpon Wollenw. contains twenty-seven recognised species and is common and widespread throught the world (Booth 1966). Species of Cylindrocarpon have been isolated from the root zone of a wide range of plants in associations ranging from: soil inhabitant, saprophytic litter coloniser, root surface coloniser, cortical root infecting fungus, root pathogen and even as a stimulatory synergist and endomycorrhizal symbiont. Cylindrocarpon spp. have been isolated from the soils of woodland (Tresner, Backus and Curtis 1954), Matturi and Stenton (1964 a + b), Widden and Parkinson (9175) and pastures (Warcup 1957, Thornton 1965, Jackson 1965) as well as from the litter of many forest trees. These

include: ash, birch, hazel (Hering 1965), beech (Caldwell 1963), Pinus monticola (Brandsberg 1969), aspen poplar (Visser and Parkinson 1975) and Eucalyptus regnans in Victoria (Macauley and Thrower 1966), where it was referred to by its synonym Moezia (Subramanian 1971).

Cylindrocarpon spp. have been reported on the root surfaces of forest plants including: Fraxinus excelsior (Kurbis 1937, Kubikova 1963), yellow birch (Katznelson, Rouatt and Peterson 1962), Fagus sylvatica (Waid 1974) as well as on wheat and soybean (Rouatt, Peterson, Katznelson 1962), bean (Taylor 1964,1965), lupins (Papavizas and Davey 1961) and perennial ryegrass and white clover (Thornton 1965). Cortical root infection by Cylindrocarpon spp. have also been reported (Waid 1962, Taylor and Parkinson 1965, Thornton 1965, Timonin 1966). The consistency with which Cylindrocarpon spp. have been isolated from the surface and cortex of apparently healthy roots has given rise to Cylindrocarpon being classed as a root "companion" fungus (Kurbis 1937, Garrett 1956).

Cylindrocarpon spp. have also been reported as pathogens on a wide range of plants. These include alfalfa and sweet clover (Cormack 1937), white clover and ryegrass (Thornton 1965) and red clover (Ylimaki 1967). Cylindrocarpon spp. contribute to damping off of pine seedlings (Vaartaja and Bumbieris 1967, Mankea 1970, Magnani 1972) and can cause seed losses (Bloomberg 1966). C. tenue can cause root rot of coffee in India (Subramanian and Govindarajan 1968) and C. panacis has been reported to cause root rot of Ginseng (Matuo and Miyazawa 1969). C. destructans has been implicated in azalea wilt (Cox et al 1969), dieback of alder (Trutor 1947) root and flower rot of Usambara violet (Gerlach 1961) and root rot of cyclamens in Holland (Scholton 1964).

The pathogenicity of Cylindrocarpon spp. is frequently accentuated in plants stressed by frost (Hart 1965, Bojarczuk et al 1968) or wounding (Taylor 1956) and in stressed or nutrient deficient plants with lower root replacement capacities (Peterson 1958, Scholten 1964). Cylindrocarpon spp. have been found to occur frequently, and act in combination with, other micro-organisms in root rot complexes including the decline in orange (Martin 1950), avocado (Wager 1942) and peach (Weaver et al 1974). Cylindrocarpon spp. have been shown to interact with nematodes (Sutherland and Dunn 1970), bacteria (Bald and Solberg 1960) and Fusarium (Taylor and Parkinson 1965) in disease complexes.

Detailed examination of Cylindrocarpon spp. within infected and diseased roots indicates that the pathogenicity of the fungus is often related to the induction of vascular blockages. Xylem tyloses and gummosis were observed in response to Cylindrocarpon infection of Prunus spp. (Govi 1952), Quercus spp. (Urosevic 1963) and strawberries (Wilhelm 1958). Wilhelm (1958) found that C. destructans, alone or in association with Fusarium oxysporum or Phialophora spp., certain actinomycetes and bacteria, may be pathogenic to strawberries without parasitising the affected root tissue. He suggested that the fungus may saprophytically inhabit older roots in which the liberation of fungal metabolites may cause direct injury to the plant. The xylem plugging and dysfunction of strawberry roots could often be traced to affected lateral rootlets. Subsequent studies by Kluge (1966) and Evans, Cartwright and White (1967) confirmed the production of a phytotoxin by pathogenic strains, but not non-pathogenic strains, of C. destructans when grown in artificial culture. These phytotoxins caused a stunting of Eucalyptus pilularis seedling growth.

Under certain conditions, Cylindrocarpon spp. may also form root associations stimulatory to plant growth. The growth of Fraxinus excelsior seedlings in sterile soil could be greatly stimulated by reinoculation with soils containing C. destructans which is part of the rhizosphere microflora in this plant (Kurbis 1937). Recently Paget (1975) reported that C. destructans may stimulate strawberry growth in sterile soil. When inoculated onto strawberries in combination with the endomycorrhiza E_3, Glomus fasciculatus, C. destructans was able to stimulate growth and phosphorus uptake by the plant above that resulting from the E_3 inoculum. It would appear from these studies that under some conditions Cylindrocarpon may act like symbiotic endomycorrhizas.

The wide diversity of associations between Cylindrocarpon spp. with plant roots demonstrate the inadequacy of classifying members of the genus Cylindrocarpon as either soil, saprophytic or pathogenic fungi. Consequently detailed investigations need to be conducted of the Cylindrocarpon sp. isolated from the fine roots of E. obliqua to establish the nature of its associations with E. obliqua roots and regrowth dieback.

1.3 DESCRIPTION OF THE PROBLEM

1.3.1 Description of the Eucalyptus obliqua Forests

Much of the concern about the unexplained dieback of former dominant trees in E. obliqua regrowth forests in Tasmania has arisen from its variance with what has been assumed to be "normal" forest development. Consequently it is necessary to describe aspects of the forest environment, the development of these forests and the

initiation of dieback symptoms in order that the causation of dieback can be investigated in relationship to the development of these forests.

1.3.1.1 Location

These investigations of regrowth dieback were centred on 16,000 hectares of forest in South Eastern Tasmania in which Bowling and McLeod (1968) had first described regrowth dieback. The forests are typical of high quality E. obliqua and E. regnans regrowth forests throughout Tasmania (Felton 1972) and parts of Victoria which are also affected by similar diebacks. The main study area (Figure 2) was located within forests of the Lune River catchment at latitude $43^{o}54'$ south, longitude $146^{o}54'$ east. These forests comprise a series of stands of uniform age which range from one to over 250 years of age. The younger stands have been regenerated from seed trees or aerial sowing following logging and burning while the stands over 100 years of age have been naturally regenerated following wildfires.

1.3.1.2 Tree Species

The predominant tree species in these regrowth forests is E. obliqua although E. regnans, also of the subgenus Monocalyptus (Pryor and Johnson 1971) is also common in pure stands of "hybrid swarms" with E. obliqua (Ashton and Williams 1973). E. globulus of the subgenus Symphomyrtus forms an important component of some of these regrowth forests particularly on the drier sites. E. globulus is only slightly affected by regrowth dieback (Bowling and McLeod 1968), a characteristic common to other species of this subgenus in forest

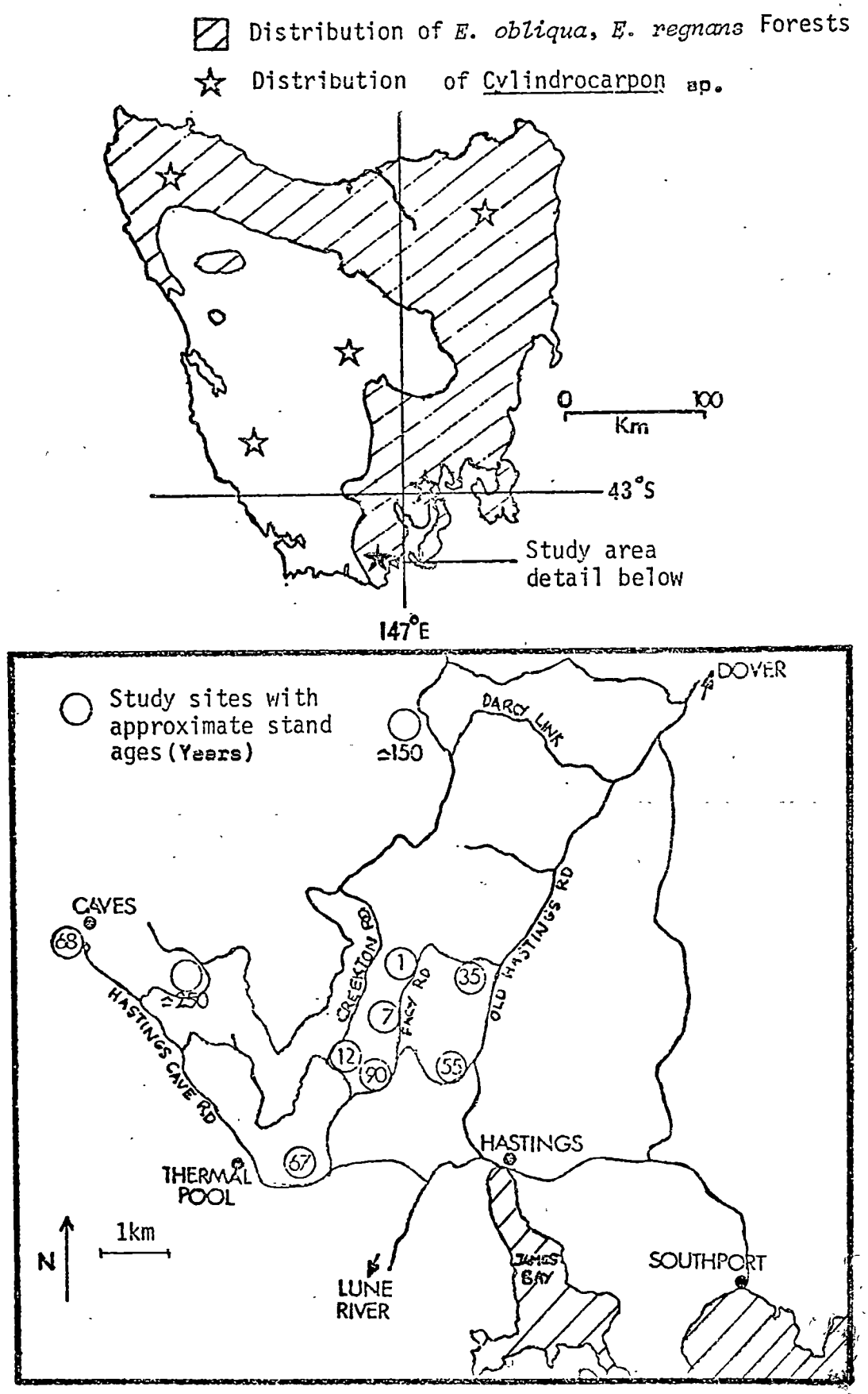


Figure 2 The location of study areas in Tasmania and the distribution of E. obliqua forests and Cylindrocarpon sp. isolations.

diebacks throughout Australia. Detailed descriptions have been published of the ecology and development of these forests in Tasmania (Gilbert 1958,1959, Jackson 1968) and in Victoria (Ashton 1958, 1975, a, b, c, 1976).

1.3.1.3 Climate

The regrowth forests of Southern Tasmania have a cool moist climate. Summer maximum temperatures generally range from 18 to 22°C while winter minimum temperatures are frequently in the range of 3 to 8°C. Diurnal fluctuations of 8-10°C are common. The average annual rainfall of 135cm occurs as persistent showers on 150-200 days per year with a slight winter maximum. However, local and seasonal variations in rainfall and temperatures may be marked depending on aspect, exposure, altitude and distance from the sea. Frosts may occur on 10 to 30 days per year, and light snow falls occasionally. Moderate to strong winds may be common on exposed slopes.

1.3.1.4. Soils

The soils of the southern regrowth forests vary widely but have broadly been mapped as podsols and yellow and grey podzolic soils (Nicolls and Dimock 1965). No detailed soil mapping has been conducted. Parent rock is variable and includes siliceous to argillaceous sandstones of Triassic age, Permian sandstones, Jurassic dolemite and limestone (Geol. survey of Tasmania). Surface soils range from sands and fine sandy to silty loams, to clay loams above gradational or duplex profiles (Northcote 1971). There are also areas of deep krasnozem soil formed on dolemite and areas of deep and friable colluvium. The relationship between soils and dieback is further examined in Chapter 3.

1.3.1.5 Forest Development and Structure

The E. obliqua forests regenerate naturally from seed falling onto ashbed soils after hot slash fires or wildfires. The even aged stands of E. obliqua regrowth grow rapidly and dominate the site colonising stage of forest growth which may last for 50 to 80 years. Depending on the site quality the regrowth forests start to undergo progressive structural changes from 40 to 150 years of age to form typical mature eucalypt forests. Mature forests are characterised by a reduction in the numbers of eucalypts and the opening of the closed eucalypt canopy. In the absence of further wildfires, forests develop to form cool closed temperate rainforests (Gilbert 1959). Regrowth dieback typically occurs in forests from 50 to 150 years of age during their transition to mature forests and the development of the rainforest understorey.

The numbers of eucalypts per hectare decrease naturally during forest development from over 2500 in five to ten year old pole thickets to less than 20 in the oldgrowth forests (Gilbert 1959, Ashton 1976). The E. obliqua forests grow vigorously in height during the initial 30-60 years with height increments often averaging one metre per annum (Jackson 1968, Gilbert 1959, Ashton 1975). The age at which height growth begins to decline appears to be closely related to site quality, with forests on the poorer sites often curtailing height growth by 30 years of age. The crowns of regrowth trees during the vigorous initial growth stage are typically conical whereas maturing trees are characterised by a decrease in apical dominance and the expansion, opening and rounding of the eucalypt crown (Jacobs 1955, Ashton 1975). The crowns of mature forests have relatively low light interception thereby

enabling the development of a closed rainforest substratum under the eucalypt canopy (Ashton 1976).

The understorey vegetation in these forests consists initially of fire weed species (Cremer and Mount 1965) and develops into thickets of Acacia dealbata, A. verticillata, Pomaderris apetala and Gahnia psittacorum. During the regrowth stage and subsequently with the opening of the eucalypt canopy the secondary stratum comprising P. apetala, A. dealbata, A. verticillata, Olearia argophylla, Phebalium squamosa and Nothofagus cunninghamii normally develops to a height of 30-40 metres. A dense stratum of ferns: Blechnum procerum, B. nudum, Dicksonia antarctica plus seedlings of rainforest species including Phyllocladus asplenifolius, Atherosperma moschatum and Drimys lanceolata develop near the forest floor. Bryophyta and lichens are abundant on exposed soils and decaying vegetation.

The growth in the diameter of regrowth trees is also rapid over the first 40 to 80 years but declines as the forests mature (Gilbert 1959). The basal area, i.e. the cross-sectional area of stems per hectare at 1.8m height, of the developing regrowth stands also increases in the developing forests. As it includes large areas of dead heartwood this parameter does not reflect the area of growing sapwood tissue in these forests.

1.3.2 Description of Crown Dieback in the E. obliqua Regrowth Forests

Dieback of E. obliqua and E. regnans regrowth forests was first officially recorded in 1945 in north western Tasmania and after a particularly wet summer in 1956 in north eastern and southern

Tasmania (Felton 1972). However these may not be the first instances of dieback as unexplained crown diebacks and tree deaths were common in 100 to 150-year-old E. regnans forests in the Florentine Valley (Gilbert 1958). Nineteenth century oil paintings, old forest photographs and discussions with older forest employees also indicate that crown dieback may have occurred in the limited areas of regrowth forests in existence last century. Crown dieback is also a characteristic feature of natural mature and oldgrowth eucalypt forests, most of which have tertiary or quaternary crowns (Jacobs 1955). Consequently it is important to determine whether the intensification of dieback of former dominant trees in regrowth forests is a relatively recent phenomena and whether it is distinct from deaths due to normal competition. Tree deaths due to competition have generally been assumed to occur in former subdominant trees due to competition for light or as a result of previous fires but few regular measurements of individual trees have been made to verify this assumption.

Symptoms of regrowth dieback first occur on trees from 35 to 150 years of age and initially involve a thinning and death of terminal shoots. The symptoms generally intensify with time, although the rate of intensification may vary with site, seasonal and stand conditions. Partial replacement of dieback affected crowns with epicormic shots, lower in the crown, frequently occurs but these may subsequently also die back. Affected trees may take from two to twenty years to die and no case of complete crown recovery has been observed. The intensification of dieback symptoms may be arrested, particularly in some of the former codominant and subdominant trees with smaller original crowns. Trees with dieback commonly exhibit a decline in diameter growth from two to ten years prior to the first

expression of crown symptoms. Consequently the causal factors are likely to be active well before the expression of crown symptoms.

Both the dieback symptoms and the pattern of dieback development in the regrowth forests indicate that the dieback may be influenced by the interactions of numerous factors associated with changes in the structure, physiology, stocking and under-storey development of these forests. The transition of regrowth forests to mature forests has been described as "one of great physiognomic change" (Ashton 1976) and is likely to provide an important context within which the aetiology of regrowth dieback needs to be understood.

Consequently this review raises several questions regarding the cause of dieback and its role in the future development of these forests.

The questions are:-

(1) Why does crown dieback occur in former dominant and codominant trees? Is this consistent with normal forest growth?

(2) Has regrowth dieback resulted in, or is it likely to result in, a reduction in tree numbers or general crown condition significantly below that expected in the natural development of these stands?

(3) What is the primary agent in the causation of regrowth dieback, how does it operate and what factors influences its action?

(4) Is regrowth dieback inconsistent with the normal development of these forests and if so what effective action may be taken to eliminate or control it?

Investigations of these four questions constitute the basis of this thesis. Investigations of the first two questions are described in the following chapter. Subsequent chapters describe investigations into the cause of regrowth dieback and the role of dieback in the natural development of these forests.

CHAPTER 2

DIEBACK AND THE NATURAL DEVELOPMENT OF E. obliqua FORESTS

The investigations described in this chapter examine the relationships between regrowth dieback and the natural development of these eucalyptus forests. This is likely to have a major bearing on the type of factors primarily responsible for the causation of dieback as well as the longterm implications of regrowth dieback in the productivity and stability of these and similar eucalypt forests. Specifically these investigations aim to establish:

(a) Whether crown dieback in former dominant and codominant trees is inconsistent with the natural development of these forests.

(b) Whether regrowth dieback has resulted in, or is likely to result in, a reduction in tree numbers per hectare or crown condition substantially below that expected in the natural development of these forests?

2.1 The Dieback of Former Dominant Trees

Studies in forests (Stern and Roche 1974) and biological systems generally (Odum 1971) have indicated that genotypes naturally selected during the site colonising or exponential growth stage may not necessarily be the genotypes which compete and survive during the mature or plateau growth stage. Such changes in dominant genotypes have been implicated in investigations into the cause of forest diebacks and succession (Leaphart and Wicker 1966, Ross 1967, McQueen 1968, Kozlowski 1971, Botkin et al 1972).

While the crown dieback of the larger, former dominant trees in the E. obliqua regrowth forests may at first appear inconsistent with our current expectations of "normal" forest growth this inconsistency is largely based on the assumption that competition between trees is predominantly for light, for which the taller dominant trees are likely to have a competitive advantage. While light may be the limiting factor in many forests at higher latitudes and during the initial stages of growth, light may not be the principle factor governing forest competition and selection in maturing eucalypt forests. Mature, 100 to 150 year old E. regnans forests with open crowns and ample light for the developing understories were noted by Gilbert (1958) to:

> "contain some eucalypts which have recently died or are about to die presumably because of competition for room in the stand."

Similarly the dieback and death of these larger surviving trees of similar age were also "fairly common" in E. regnans forests in Victoria, even though these forests had open crowns and developing understories in which competition for light is unlikely to have been a major factor (Ashton 1975). However the general acceptance that light is the principal factor governing the selection of dominants at all stages of forest development has led to the assumption that even in eucalypt forests "trees constituting the mature forests at 200 years are likely to have been present at the predominant class at 40 years" (Ashton 1976).

In contrast, if the moisture relations become limiting to the growth of maturing trees due to soil factors or root growth rather than light, markedly different competition and selection pressures may be

expected. Whereas fast growth rates, low root to shoot ratios and maximum utilization of the unlimited soil and moisture resources may contribute to the competitive selection of dominants in young regrowth forests these same features are likely to cause those dominant trees to be highly unstable in mature forests under limited moisture conditions. In contrast genotypes with lower site demands, higher root to shoot ratios and higher tolerances to moisture stress may be expected to survive better than former dominant trees under the limited site conditions associated with mature forest growth.

The validity of the latter hypothesis and its implications in the dieback of former dominant trees was investigated by examining the former growth patterns of regrowth trees and surviving oldgrowth trees.

Materials and Methods

Differences in the pattern of growth of E. obliqua regrowth dominants and surviving oldgrowth trees were examined using growth ring analyses. Discs were cut at approximately 1m from the base of five dominant regrowth trees affected by dieback and five surviving oldgrowth trees from the E. obliqua forests in southern Tasmania. Growth rings were marked on four perpendicular radii which had been planed on each disc and the mean annual increment in radial growth was measured and plotted with respect to the age of the tree.

Results and Discussion

Two distinct patterns of tree growth had occurred in the regrowth dominant and oldgrowth trees (Figure 3). The annual radial growth

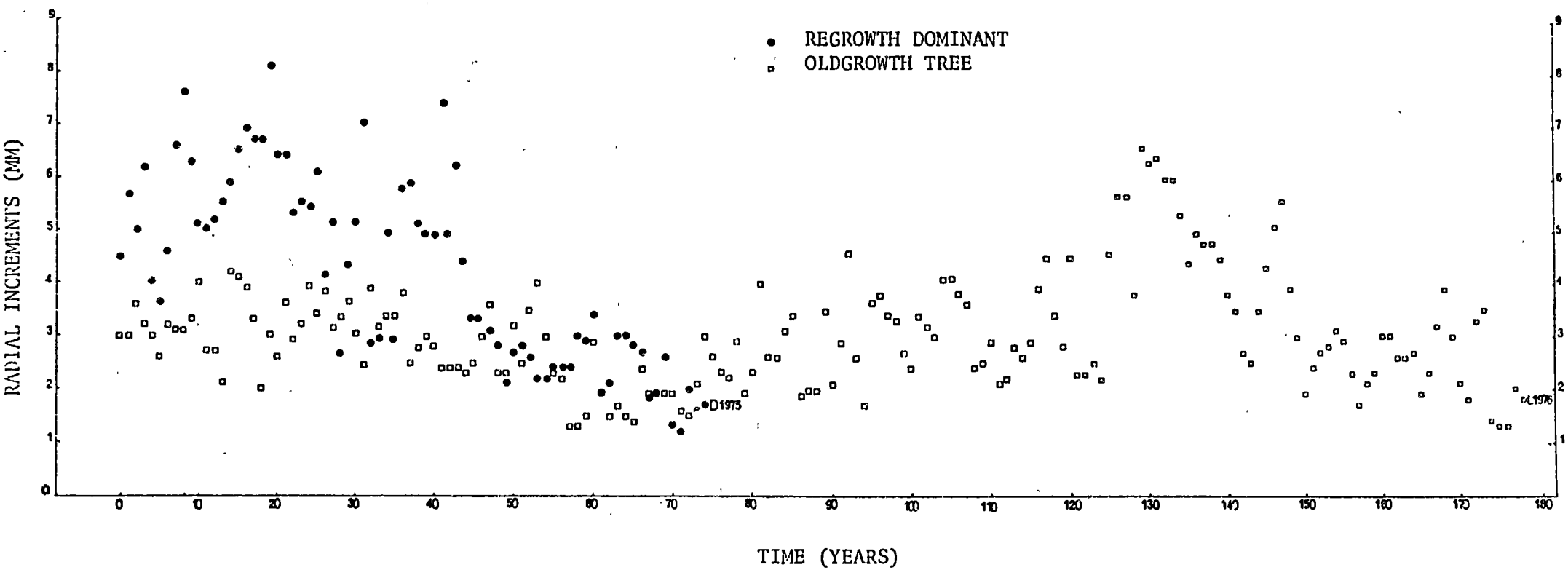


Figure 3. Patterns of previous radial growth in the bole of a regrowth tree recently killed by dieback and a surviving oldgrowth tree from the E. obliqua forests of Southern Tasmania.

rate of the former dominant regrowth trees had consistently been twice that of the surviving oldgrowth trees during their respective initial 50 years of growth. In contrast all the currently surviving oldgrowth trees had had diameters consistent with those subdominant trees in present day regrowth forests during their initial 50 years of growth.

Furthermore both types of tree underwent a marked decline in diameter increment growth when they were between 50 and 80 years of age. This period of decline was associated with the stage of forest development and was independent of the date. The period of decline in the surviving oldgrowth trees had occurred 100 years prior to the current dieback of the regrowth trees. The average radial growth per annum in the former dominant trees was reduced from 6mm to 1.5mm during the period of transition of forest growth from 50 to 80 years of age. In contrast average radial growth per annum in the oldgrowth trees over this same period of their development was only reduced from 3mm to 1.5mm. Consequently the relative level of growth reduction during this period of growth transition was greater in the former dominant trees than the former subdominant trees; growth rates having been reduced to 25 and 50% of their former levels respectively. While the actual radial growth rate of both types of trees during the period of growth transition was the same, 1.5mm/ann., the former dominant trees are likely to have been subjected to higher levels of stress than the subdominant trees during the transition from regrowth to mature forests.

Independent growth ring studies of oldgrowth trees in these same forests indicate that these trees had also grown at a rate comparable to the subdominant trees in present day regrowth forests

when they were in their regrowth growth state (CSIRO Division of Forest Research unpublished). Data from E. regnans forests in Victoria also reveals that reductions in tree numbers during the transition from regrowth to mature forests (from 50 to 80 years of age) were greater in the predominant crown class (50% reduction of tree numbers) than in the intermediate crown class (33% reduction in tree numbers) (Ashton 1976). Consequently it would appear that the slower growing subdominant trees and not the former dominants generally survive during the period of forest transition and continue growth to form the oldgrowth emergents. In contrast the former dominant trees appear to be subjected to relatively high levels of stress and growth reduction and may be less capable of surviving under the conditions existing in mature forests.

The failure to previously recognise this aspect in the development of these forests may have resulted in part from the lack of continuous measurements on individual trees during the natural development of these forests. It was only when the development of individual trees were monitored in the same stands over a period of up to 25 years that Bowling and McLeod (1968) first recorded unexplained deaths in the former dominant trees. Such deaths had previously always been assumed to have occurred in the smaller suppressed trees (Gilbert 1959, Ashton 1976).

These data also have implications regarding causation of regrowth dieback. The growth analysis indicate that both classes of trees are subject to a high level of stress during the transition of regrowth forests to mature eucalypt forests. Dominant regrowth trees are subject to a relatively higher level of stress and are more likely to die during this transition in forest growth than the

former subdominant trees. This stress does not appear to be related to competition for light but rather to the relative inability of the trees to maintain their former levels of photosynthesis and growth. However reductions in photosynthate resulting from this stress are likely to have the greatest detrimental effects on the growth of the root system which is furthest removed from the source of photosynthate . This in turn may limit the ability of the root system to supply adequate water and nutrients to the tree thereby further reducing photosynthesis, root growth and the ability of the tree to tolerate stress. Should such a reduction in photosynthesis also predispose roots to disease agents a progressive decay of the roots may be expected which could lead to dieback and death particularly in the more highly stressed trees. Consequently the dieback and death of regrowth trees could occur naturally without the involvement of any exotic factor in their aetiology.

While it is beyond the scope of this thesis these findings may have considerable relevance in forestry breeding programs in which vigorous dominant genotypes are normally selected rather than slower growing genotypes. Plantations of trees selected for rapid initial growth may be subject to more stress as site conditions become limiting for tree growth than plantations of slower growing genotypes. This may result in extensive diebacks developing. For example, Liriodendron tulipifera plantations in Georgia have developed dieback after initially growing at near maximum rates for that species (Ross 1967). These studies emphasise the need to investigate further future disease implications resulting from many of the current forest plantation programs.

2.2 Dieback and the Number of Trees per Hectare

Much of the concern over the unexplained dieback of E. obliqua regrowth forests in Tasmania has arisen from uncertainty about its future impact on what we believe to be the normal development of these eucalypt forests. It is therefore important to assess critically the current and most probable future impact of regrowth dieback in relation to natural forest development. This may enable both its degree of abnormality and the type of factors with which it may be associated to be better defined. The following investigation examined the impact of regrowth dieback on the numbers of trees per hectare and compared these data with published data on the normal forest density age relationship for similar forests. Only where dieback has resulted in a substantial reduction in tree numbers or crown condition below that normally expected in healthy forest can it be suggested that an abnormal factor is influencing forest growth.

Materials and Methods

The number of live trees per hectare in plots of regrowth forest of various ages and with various intensities of dieback, were compared with published data of normal tree densities age relationship in similar forests in Tasmania (Gilbert 1959) and Victoria (Ashton 1975). The former data had been collected from regular measurements over the preceding 20 to 30 years by Bowling and others and includes data from stands which have been worst affected by regrowth dieback. This data was kindly made available by the Commonwealth Scientific and Industrial Research Organisation, Division of Forest Research (Unpublished reports).

Results and Discussion

In none of the regrowth forest plots, including those worst affected by dieback, have the numbers of live trees per hectare fallen below levels which are regarded as normal for forests of that age (Figure 4). Furthermore there is no indication from these data that the dieback of regrowth trees will decrease the number of trees per hectare in these forests in the future below that which can be expected as normal in the development of these forests. Consequently, there is no basis, from this data, to suggest that regrowth dieback is an abnormal condition in the development of these eucalypt forests.

Much of the concern about regrowth dieback has been based on records of tree deaths on the plots used in Figure 4. These plots had been established in the 1950's for the production of a yield table, i.e. a relationship between the volume of timber in a stand and forest age, and had selected forest stands of above normal tree densities. It would appear that the severe dieback on these plots may represent their return to a more normal stocking (Figure 4). The abnormal initial density of these stands makes them unsuited for forecasting the future impact of dieback on the normal development of these forests. In fact the relative levels of dieback in these stands was substantially higher than that in the surrounding forests with 17 and 4% of the dominant trees having been severely affected by dieback in the forest plots and surrounding forests respectively (Felton and Bird 1972).

Undoubtedly the loss of even a small proprotion of the larger trees represents a considerable commercial loss as these trees represent a

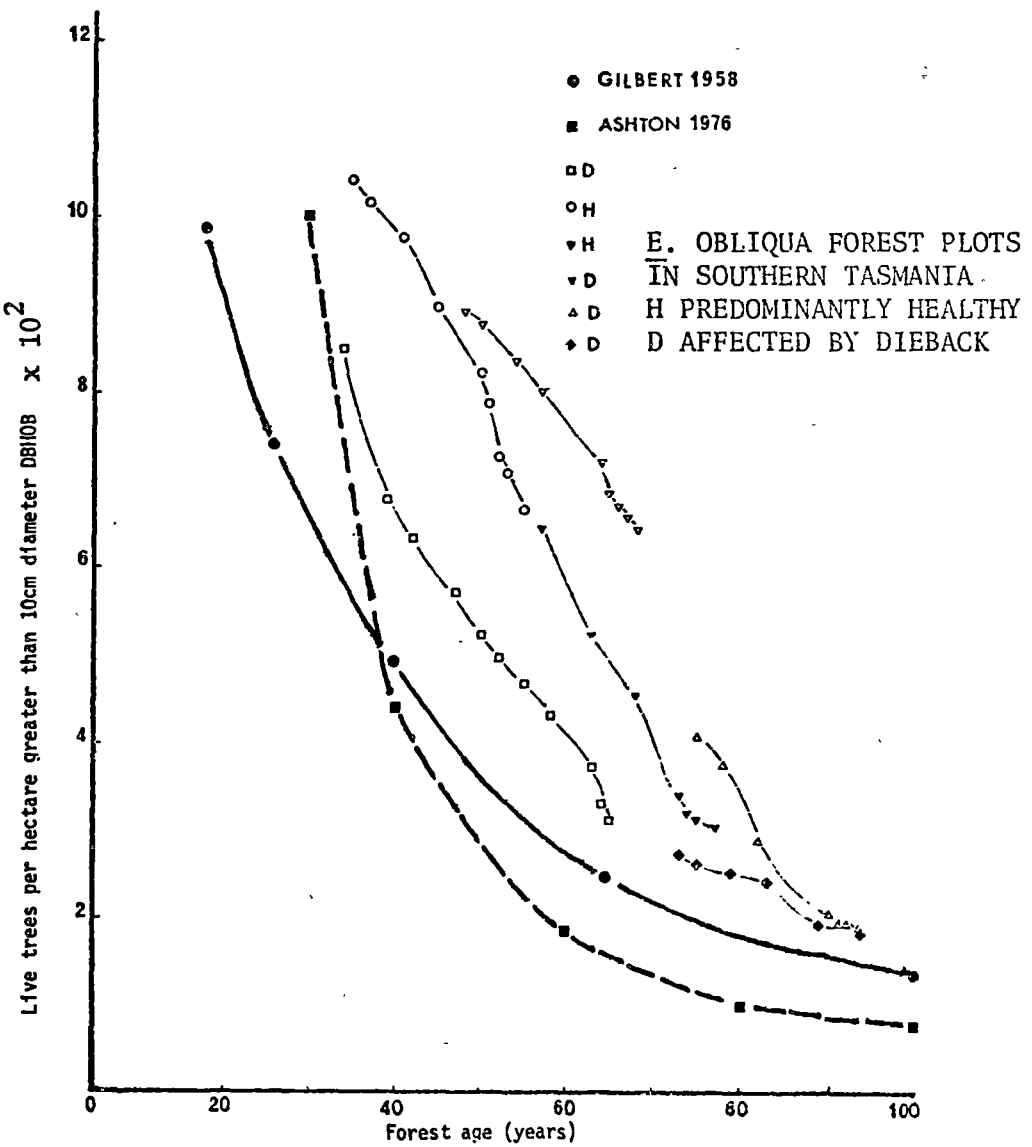


Figure 4. The number of trees per hectare on forest plots severely affected by dieback in relation to the normal density of similar aged forests in Victoria (Ashton1976) and Tasmania(Gilbert1958).

high proportion of basal area (sq. m wood/hectare). However estimates of basal area include a high proportion of dead heartwood and do not accurately reflect the impact of dieback on the current growth or stability of these forests. Extreme caution is needed in forecasting the future consequences of dieback on the productivity and stability of these forests on the basis of such basal area losses (Felton and Bird 1972).

The investigations described in this chapter examined the relationship between regrowth dieback and the normal development of these forests with the aim of providing a context within which the impact and aetiology of dieback can be studied. The studies indicate that both the level of dieback as well as the death of former dominant trees is not inconsistent with the natural development of these forests. Consequently no abnormal or introduced factor needs to be involved in the causation of regrowth dieback. While these findings provide a context for the following investigations into the cause of regrowth dieback they also raise important questions regarding the growth and stability of different tree genotypes in maturing forests which warrant further investigation.

CHAPTER 3

FACTORS ASSOCIATED WITH THE DIEBACK OF E. OBLIQUA REGROWTH FORESTS IN SOUTHERN TASMANIA

The investigations described in the following chapter were designed to examine factors and conditions which may be consistently associated with regrowth dieback and involved in its causation. Most of these investigations were conducted in the Lune River catchment (Figure 2) in regrowth forests which varied from 35 to 90 years of age and had different intensities of dieback. The methods used are outlined at the start of each investigation and, where relevant, the results of other published studies on similar diebacks are also discussed. As these investigations were of a survey nature and conducted at an early stage in the experimental program they may not all be complete beyond this particular context. Further investigations may be desirable on many of the factors independent of their association with the regrowth dieback.

3.1 Crown Factors

The crowns of a large number of standing regrowth trees were visually inspected for factors which may contribute to dieback. The crowns of over 30 regrowth trees which had been felled recently were similarly inspected. Partial defoliation by leaf-eating insects was the only crown or bole condition detected in these inspections. Defoliation occurred on trees of all ages and did not appear to be associated with tree dieback or severely dieback affected stands. The defoliation was primarily caused by the Chrysomellid beetle Chrysophtharta bimaculata (De Little pers. comm.) and similar

defoliations have been reported in E. regnans regeneration in Tasmania (Greaves 1965). Defoliation by insects is a common, often periodic, occurrence in other Australian eucalypt forests (Jacobs 1955, Campbell 1966, Carne et al 1974). Although the levels of defoliation observed in these forests (Kile et al 1974) may contribute to stress and predisposition to disease no evidence was found to indicate that defoliation is the primary or direct cause of regrowth dieback.

3.2 Aspect and Soil

Ground and air surveys were conducted throughout the southern regrowth forests but failed to detect any association between crown dieback and different topography, drainage courses or road locations. Dieback trees appeared to be randomly scattered throughout the stands although isolated cases where groups of trees were heavily affected were observed.

Dieback occurred in trees growing on a wide range of different soils ranging from krasnozems to shallow yellow podzolics with clay loam surface horizons and on deeper yellow podzolic soils or colluvium. The proportion of affected trees and the crown dieback intensity in similar aged regrowth stands was generally lower on the deeper, better drained soils. However, severe dieback also occured in older regrowth stands on deep well drained soils. While the occurrence of regrowth dieback does not appear to be directly related to either aspect or soil, particular soil characteristics may contribute to the intensity and age at which dieback develops. These are examined in the following section.

3.3 Physical Soil Properties

No attempt was made in these investigations to map and describe the various soils. Rather differences in physical properties of soils under adjacent healthy and dieback affected trees and stands were examined with the view to defining properties associated with restricted root growth and differences in tree health. These studies were concentrated on nine forest stands of variable health most of which were located in the Lune River catchment on yellow podzolic soils above poorly structured clay subsoils.

Materials and Methods

Soil pits (100cm x 40cm and up to 90cm deep) were dug under each of 35 healthy and 35 dieback affected regrowth trees and the soil profile in each pit and the depth to any apedal clay subsoils was described. Soil cores (6cm in diamter and 4cm high) were collected from the sides of each profile at 15, 30 and 45cm depth and enclosed in airtight bags for subsequent measurements of soil moisture and bulk density. The cores were weighed on return to the laboratory and then saturated by immersion in water and allowed to freely drain for 2 hours before they were reweighed. Each core was again weighed following drainage on a pressure membrane apparatus at 15 atmospheres pressure and also after drying to constant weight in an oven. The following parameters were calculated for each soil core (Table 1):-

(a) the bulk density of each soil based on the oven dry weight of the constant volume cores (g/cc).

(b) an index of the available water holding capacity which was determined by the difference between the freely drained weights and weights after drying at 15 atmospheres pressure and was expressed as a percentage of soil volume

(c) the relative soil saturation in the field at the time of collection which was the ratio obtained by dividing the field weight by the weight after saturation and free drainage.

Soil shear strength was also measured to 45cm depth at 40 locations around each tree with a cone penetrometer to detect the presence of dense subsoil horizons. Measurements are expressed as the pressure required to penetrate 45cm of soil. Each of these soil properties were examined in relation to the percentage of crown dieback in the trees (tree comparisons Table 1). The soil data was also pooled for all trees in a stand, irrespective of tree health, and the mean values related to the mean levels of crown dieback in the larger trees (plot comparisons Table 1). Crown dieback levels in individual trees were based on visual estimates of the percentage of affected crown.

Results and Discussion

The soils under trees with low intensities of crown dieback were generally friable to a greater depth and had lower bulk densities than soils from under trees in the same stands with high intensities of dieback. This was closely associated with a greater depth of fine root development and was also reflected in the measurements of shear strength (Table 1). The soil at 45cm depth under dieback affected trees also had lower capacities for storing water at

TABLE 1. Soil properties associated with the dieback of E. obliqua regrowth trees in nine forest plots[a].

SOIL PROPERTY MEAN OF 70 TREES	UNITS	PLOT COMPARISONS[b] LOW DIEBACK INTENSITY	HIGH DIEBACK INTENSITY	SIGN P0.05	TREE COMPARISONS[c] LOW DIEBACK INTENSITY	HIGH DIEBACK INTENSITY	SIGN P0.05
Depth to clay	cm	45	25	+	51	18	+
Depth of friable soil	cm	47	38		53	24	+
Depth of fine roots	cm	34	24	+	38	18	+
Depth of medium roots	cm	55	45	+	55	46	+
Boron content of fine roots	ppm	7.4	7.1		8.2	6.6	+
Bulk densities							
at 15 cm	g/cc	1.27	1.35		1.32	1.03	+
30 cm	g/cc	1.37	1.41		1.37	1.38	
% water available							
at 15 cm	%	38	28	+	34	35	
30 cm	%	37	31		36	33	
45 cm	%	47	39	+	43	34	+
Soil saturation							
at 15 cm	Ratio	1.14	1.32	+	1.17	1.25	
30 cm	Ratio	1.16	1.26		1.14	1.17	
45 cm	Ratio	1.08	1.22	+	1.10	1.18	
Shear Strength	Kg	33	43	+	37	42	+
Redox potential, 30cm	M.V.	540	547		542	542	
pH, 30 cm		5.4	5.4		5.4	5.4	

a. The plots include those examined in figure 4 while the trees examined were mostly dominants which were either visually healthy of severely affected by dieback.

b. In the plot comparisons data for all trees in those plots with low intensities of dieback were compared with data for trees in plots with high dieback intensities. Plots with low dieback intensities had less than 10% mean crown loss on dominant and codominant trees whereas plots with high intensities had greater than 20% mean crown loss.

c. In the tree comparisons all trees which currently had less than 20% of their crowns affected by dieback were compared with trees with greater than 30% crown loss irrespective of their location in the nine plots.

tensions which may be available to plants than soil from similar depths under healthy trees. While differences in physical soil properties may contribute to the intensity and age at which dieback develops in individual trees dieback was also found to occur in trees in older forests on sites with deep friable soils.

The soils under regrowth forest stands with low intensities of crown dieback similarly had deeper friable soil, deeper proliferation of fine roots and lower shear strength measurements (Table 1) than the soils under stands with high intensities of dieback. The soils from forests with low dieback intensities also had greater capacities of storing water at tensions likely to be available to plants and had lower moisture contents when collected than soil from under plots with high intensities of dieback. The physical properties of soils appear to influence the age at which regrowth forests developed dieback symptoms. Young, 35 year old forests on shallow compacted soils were often more severely affected by dieback than older forests of 150 years of age growing on a deep well drained krasnozem.

The physical properties of the soils appear to be closely associated with the amount and depth of proliferation of the fine roots of regrowth trees and influence the quantity of water available for transpiration by the tree. Consequently the physical properties of the soils may be important in influencing moisture stress in regrowth trees and their predisposition to dieback. However physical soil properties, by themselves, do not account for why some trees survive even in adverse soil microenvironments while others are affected by dieback even in deep well drained soils. Furthermore the regrowth forests have grown vigorously on these same soils and it is difficult to conceive how these soil conditions can

change to be the sole cause of dieback in scattered trees. Consequently while the physical properties of the soil are likely to be important in accentuating moisture stress and predisposing regrowth trees to dieback it is unlikely that those soils properties are the sole or primary cause of dieback in these forests.

3.4 Chemical Soil Properties

The regrowth forests of southern Tasmania had grown vigorously during their first fifty years of growth and it is likely that adequate nutrients had been available over this period particularly for the dominant trees now affected by dieback. No symptoms of nutrient deficiency were apparent in affected trees. Consequently no comprehensive studies were conducted on the nutritional status of soils from these forests. However exploratory measurements were made of soil pH and redox potentials as well as of boron concentrations in the fine roots as the latter had been associated with increased root rot and dieback (Snowden and Jehne 1975).

Materials and Methods

The pH and redox potentials were measured in each of the soil cores collected in studies of physical soil properties. Measurements were made at 20^{o}C in a 1/3 soil-water slurry with a pH meter with glass electrodes. Boron concentrations were determined using the technique of Dothie (1969) in subsamples of eucalypt fine roots which had been excavated in the soil pits dug for the previous study.

Results and Discussion

Neither the pH nor redox potentials of soil collected from trees or forest plots of different health differed significantly (Table 1). However the boron content of the fine roots from E. obliqua with low intensities of dieback were higher than those from trees with high intensities of dieback. Further studies are needed to define the role of nutrient stress in the predisposition, initiation and intensification of dieback. Particular attention should be directed at studies of the phosphorus available for tree growth at this stage of forest development as Attiwill (1971) has shown that much of the phosphorus in maturing E. obliqua forests in Victoria is fixed in organic matter and may not be available for tree growth.

3.5 Seasonal and Microclimatic Factors

A variety of microclimatic factors may influence tree growth and dieback development in these forests.The initiation and intensification of dieback in the regrowth forests has been associated with seasons of excessive high or low rainfall which may result in either waterlogging and moisture stress particularly in the forests on shallow soils which are also most affected by dieback. Crown dieback in the regrowth forest plots in southern Tasmania was first observed soon after a particularly dry season in which only 57% of the mean rainfall was recorded (Bowling and McLeod 1968). Increased dieback has also been recorded following excessively wet periods (Felton 1972).

However, periodic wet and dry seasons have occurred previously during the development of these forests and it is probable that the

forests have had to withstand and recover from previous short term deficits in rainfall or periodic waterlogging. The dieback of trees on the deeper soils with better storage of moisture as well as the failure of trees to recover from dieback on return to more favourable moisture conditions also suggests that seasonal climatic factors may not be the sole cause of regrowth dieback.

The temperatures in the surface soils of regrowth forests may also be an important factor in the growth and health of these forests. Surface soil temperatures are generally low, from 3 to 8°C in winter and 8 to 15°C in summer (Madden pers. comm.) but may vary considerably depending on canopy shading, litter depth and soil moisture. Low soil temperatures have been shown to limit rates of fine root growth (Cremer 1975), contribute to an imbalance of root and shoot metabolism (Ellis 1971) and may influence the activity of a wide range of beneficial and pathogenic soil micro-organisms. While the trees may be adapted for growth at the temperatures prevailing in regrowth forest soil, any major change in soil temperatures associated with climatic or understorey development may influence the initiation of dieback (Ellis 1971). Consequently changes in microclimatic factors in these forests may greatly influence the stress, growth and susceptibility of the fine roots of regrowth trees to infection and thereby contribute in the causation of dieback. Further studies of the influence of these factors on predisposing E. obliqua seedlings to root infections are outlined in subsequent chapters.

3.6 The Fine Root System of E. obliqua Regrowth Trees

The fine root system of E. obliqua regrowth trees appears to be the primary plant tissue affected by many of the soil, microclimatic physiological and microbial factors which may be associated with dieback. Consequently investigations were conducted of the health, depth, extent and growth of the fine root system of regrowth trees to examine possible morphological, competitive or pathological conditions which may be associated with root dysfunction, drought stress and the initiation of dieback.

Materials and Methods

The fine root systems of E obliqua regrowth trees which were at initial stage of dieback, but were growing in forests typically affected by dieback, were examined in a series of coring studies, as well as by the partial excavation of the surface root system of over 40 other regrowth trees. Roots were also inspected in numerous soil pits and road cuttings. Intact soil cores were collected in steel sampling tubes of various diameters. Each core segment was soaked in 1% Calgon clay dispersent and gently washed on 0.5mm sieves to expose the root systems. The roots were sorted into species and health using the criteria described below and were then ovendried and weighed. These measurements were used to provide a general assessment of the following root characteristics.

(a) Health

The health of the E. obliqua fine roots was determined in some of the root studies using criteria adopted from Leaphart and Copeland (1957). Eucalypt roots were classed as healthy if they contained a

firm, moist stele irrespective of the condition of the root cortex. Roots were classed as diseased if the stelar tissue was heavily stained and occluded, dry, brittle or obviously rotted. The health of the fine roots was also tested by incubation in 1% tetrazolium chloride, in which only healthy live roots developed a red coloration.

(b) Depth

The depth of the fine roots system of E. obliqua regrowth trees was examined using soil cores of 5cm diameter which were taken to a depth of 1m at 32 locations within a 4m radius around two regrowth trees. The roots from each 10cm segment in each core were washed free of soil and sorted into eucalypt and understorey species using the criteria described in Table 2. The health of the eucalypt roots was also determined.

(c) Extent and Root System Overlap

The extent of the fine root system of regrowth trees was determined by examining the fine root distribution along a transect from a healthy regrowth tree to a recently killed tree and on to another healthy tree. The trees were well separated from other regrowth trees. If the fine root system of E. obliqua regrowth trees occurs in a discrete zone around each tree the healthy fine roots along this transect should include a gap formerly occupied by the roots of the recently killed tree. In contrast, if the root systems normally overlap, no such gap would be expected. If the root systems are discrete then the extent and growth pattern of the fine root system may also be discernable along this transect.

TABLE 2. Characteristics used in the identification of the fine roots of plants in the E. obliqua forests.

+ positive, 0 negative, - not examined

Plant Species *	EXTERNAL CHARACTERISTICS COLOUR W	Y	R	B	FORM T	F	H	NODULES	INTERNAL CHARACTERISTICS COLOUR W	Y	G	SMELL S	N	AEREN.	STAIN
Eucalyptus obliqua		+	+			+		0		+			+	0	0
Pomaderris apetala			+		+			0	+				+	+	+
Atherosperma moschatum	+				+			0	+			+		+	-
Acacia verticillata		+				+		+	+	+		+		-	-
Acacia dealbata		+				+		+	+	+			+	-	-
Acacia melanoxylon		+				+		+	+	+		+		-	-
Melaleuca squamosa	+		+			+		+		+			+	-	-
Pittosporum bicolor	+		+			+		0	+				+	-	-
Drimys lanceolata	+				+			0	+			+		-	-
Olearia argophylla	+	+			+			0	+			+	+	-	-
Coprosma billardieri	+	+			+			0	+			+		-	-
Blechnum procerum				+			+				+		+	-	-

* SMELL S = SPICY ON CRUSHING, N = NEUTRAL
COLOUR W = WHITE, Y = YELLOW, R = REDBROWN, B = BLACKBROWN, G = GREEN
FORM T = THICK FLESHY ROOTS, R = THIN FIBROUS ROOTS, H = HAIRY ROOTS
AEREN.-Aerenchyma type tissue in root stele

STAIN- Brown stain when incubated in water

Triplicate soil cores were taken to a depth of 30cm at each 50cm interval along the transect between the three trees. The dry weight of healthy E. obliqua fine roots and understorey roots in each core was related to the location between the two healthy trees.

(d) Understorey Competition

Competition between the fine roots of understorey species and E. obliqua regrowth trees was examined in several of the coring studies by comparing the weight and length of various fine roots. Fine roots in each core were classified into species with the aid of criteria similar to those described by Gilbertson, Leaphart and Johnston (1961)(Table 2). The criteria were based on detailed inspection of fresh and preserved fine root material from each of the plant species growing in these forests and included root colour, morphology, bark structure and particularly smell.

(d) Root Growth

The rate of fine root growth in E. obliqua regrowth trees relative to that of understorey plants was examined in several soil recolonisation studies. Initial studies examined the recolonisation of fine roots of different species into soil pits which had been dug and refilled 32 months previously (Chapter 3.3). Subsequent studies examined the recolonisation of soil cores which had been cut around two healthy regrowth trees to 30cm depth with a 15cm diameter tube and left in situ. The length and weight of fine roots of different species which were recolonising the top 20cm of soil in each precut core were used as an index of the relative rate of fine root growth in the E. obliqua regrowth trees and the understorey flora.

Results and Discussion

(a) Health

Very few active roots with white unsuberised tips were found in the soil pits or cores in a range of regrowth forests (Chapter 3.3). In contrast healthy white root tips were abundant in seven year old E. obliqua regeneration on similar soils. Ectomycorrhizal rootlets were common in the surface organic horizons of regrowth forests but were uncommon in the deeper mineral soil. Non mycorrhizal fine roots of regrowth trees frequently were stained red and had vascular blockages and gummosis even though the roots remained firm.

Measurements of root health in all of these studies indicate that over 40% of the dry weight of fine roots, less than 1mm in diameter from E. obliqua regrowth trees with initial symptoms of crown dieback were moribund with consistent staining and vascular blockages. It would appear that substantial deterioration of the fine root system occurs prior to the expression of symptoms of crown dieback. Consequently, the factors responsible for the deterioration, staining and blockage of the fine roots of healthy regrowth trees may be directly associated with the dieback of these trees.

(b) Depth

Over 75% of the fine root system of regrowth trees which were growing on the yellow podzolic soils occurred in the upper 10cm of the soil profile while 99% of the fine roots occurred in the upper 20cm of the soil profile. However profiles exposed in soil pits and road cuttings indicated that some fine roots may occur to depths in

excess of 1m in deeper alluvial soils. Healthy fine roots of understorey species occurred at depths in excess of 1m even on the yellow podzolic soils. Consequently these results and those from the pit excavations (Chapter 3.3) indicate the fine roots of E. obliqua regrowth trees were frequently restricted to the surface soil horizons particularly in the yellow podzolic soils with dense subsoils.

(c) Extent and Root System Overlap

Examination of healthy fine roots along the transect between the two healthy regrowth trees indicated that over of 95% of the fine root system of each tree occurred within a 4m radius of the stem. The extent of the fine root system appeared to correspond approximately with crown spread and very few roots encroached into the soil volume of the adjacent tree (Figure 5). Healthy fine roots were common at the periphery of each root system and appeared to be recolonising the soil volume formerly occupied by the roots of the dead tree.

These observations on the depth and extent of the fine root system of E. obliqua regrowth trees in Tasmania are consistent with other studies on the roots of E. regnans in Victoria by Ashton (1975). These studies indicate that the fine root systems of regrowth trees and consequently the volume of soil exploited for water and nutrient uptake, is severely restricted, particularly in the yellow podzolic soils. This may be a major condition in predisposing these trees to periodic drought stress and crown dieback.

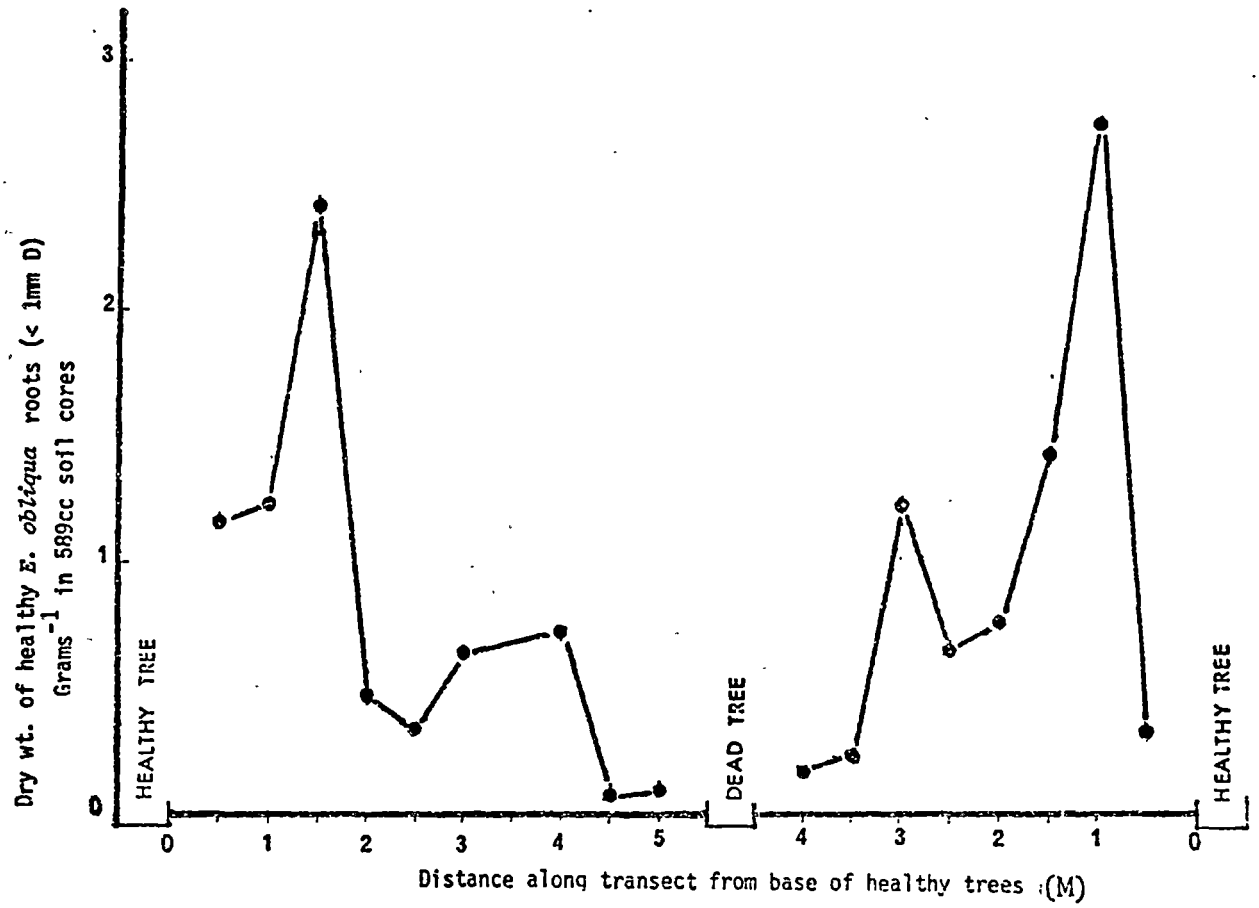


Figure 5. Extent of the fine root system between two competing E. obliqua regrowth trees.

LSD(P=0.05) =0.66

(d) Understorey Competition

Fine roots of understorey species, particularly Pomaderris apatala, consistently comprised very high proportions (50 to 80%) of the fine root biomass in the regrowth forests (Tables 3 and 4). This is in contrast to the above ground basal area of understorey vegetation in these same forests which comprises 13% of the stand (CSIRO, Division of Forest Research unpublished). Similar ratios of E. regnans and Pomaderris asperma fine root biomass have been reported in regrowth forests in Victoria (Ashton 1975).

It would appear that the fine roots of eucalypts in regrowth forests are subject to high levels of competition from the developing rainforest flora which dominate the fine root environment of these stands even though the eucalypts still maintain crown dominance. These changes in the ecology of the fine roots occur in advance of dieback symptoms or canopy change and may determine subsequent successional development above the ground. Consequently the fine root system appears to be the primary site for the initiation of the decay, and moisture stress associated with regrowth dieback and the subsequent succession of the regrowth forests to temperate rainforests.

The fine roots of understorey species appear to have a competitive advantage over that of the eucalypts in these regrowth forests which may be due to:

(a) The fine roots of understorey species being less severely affected by root decay, staining and vascular blockages than those of E. obliqua.

TABLE 3 The biomass of E. obliqua and understorey fine roots in cores from the surface soil around two "healthy" regrowth trees.

mean of 4 cores

Tree No.	Period+ of decline	%Crown dieback	Core size	Dry wt of feeder roots in cores (g) E.obliqua	Understorey	Total	% of roots E.obliqua
2	13	10	5300cm^3	1.55	15.52	17.07	9
			393cm^2	0.489	1.352	1.841	27
3	7	0	5300cm^3	6.37	8.55	14.93	43
			393cm^2	0.531	0.791	1.328	40

+ Years since the start of diameter growth decline from measurements of increment cores.

TABLE 4 Recolonization of soils under "healthy" regrowth trees by the fine roots of E. obliqua and understorey species.

A. The recolonization of soil pits excavated 32 months previously.
B. The recolonization of soil cores excavated 1 month previously.

PITS 5.4×10^4 cm^3 CORES 5.3×10^3 cm^3

A. FOREST AGE YEARS	MEAN DIEBACK IN STAND+	% CROWN DIEBACK OF TREE AND ITS DOMINANCE	DW OF NEW ROOTS <1mm diam. (g) E. obliqua	UNDERSTOREY	TOTAL	%OF NEW ROOTS BELONGING TO E. obliqua
67	LOW	20 DOMINANT	13.8	12.8	26.6	52
	SLIGHT	20 DOMINANT	8.0	17.7	25.7	31
	SEVERE	20 DOMINANT	4.5	14.1	18.6	24
	SEVERE	20 DOMINANT	9.3	37.3	46.6	20
	SEVERE	20 SUBDOMINANT	10.5	15.6	26.1	40
76	SEVERE	0 DOMINANT	7.4	13.6	21.0	35
92	SEVERE	0 DOMINANT	0.3	50.7	51.0	0.6
	INTENSE	20 DOMINANT	5.9	27.7	33.6	18

B.		LENGTH OF NEW ROOTS (MM) TOTAL IN 4 CORES			
67	SEVERE				
	10	29	63	92	32
	0	98	450	548	18

+ Based on visual assessment

(b) The fine roots of understorey species being able to grow and survive at greater depths in these soils than those of E. obliqua.

Both of these factors are likely to enable the understorey species to maintain higher water and nutrient uptake and tolerate periodic drought stress better than E. obliqua regrowth trees.

The restriction of the fine roots of E. obliqua to the surface horizons of these soils may in part result from the relative intolerance of E. obliqua roots to periodic waterlogging (Boden 1962, Podger and Batini 1971). Tolerance to partially anaerobic soil conditions has been associated with the production of various morphological (Kosceev 1953, Boden 1962, Chapter 4.2), anatomical (Burstrom 1965, Greenwood 1969, Armstrong and Read 1972, Hook et al 1972) and biochemical adaptations in plants (Grineva 1962, Crawford 1967, Lambert 1976). If some of these adapations occur in the roots of the rainforest flora but not those of E. obliqua this may account for the greater depth of root proliferation observed in the rainforest species.

(e) Root Growth

Re-excavation of roots in old soil pits and measurements of the recolonisation of in situ soil cores revealed that less than half of the fine roots were eucalypt and that the proportion of eucalypt roots relative to those of understorey species may decrease with increasing age of the regrowth forest (Table 4). These results are consistent with the studies of understorey competition and studies by Ashton (1962, 1975) on the changes in the proportion of

fine roots belonging to E. regnans and Pomaderris asperma during the transition from regrowth forests to mature forests in Victoria.

While further investigations on the fine root systems of E. obliqua regrowth forest are desirable these studies reveal several aspects which are likely to be highly relevant to investigations of the causation of regrowth dieback. These are:

1. The restriction and decay of the fine root systems of E. obliqua regrowth trees occurs in healthy regrowth trees in advance of the onset of crown dieback symptoms.

2. The root decay, root restriction plus the high level of competition from understorey flora are likely to contribute to a decreased capacity of regrowth trees to take up adequate water and nutrients particularly during periodic drier periods or from the shallow soils with lower storage capacities for available water.

3. These changes in the fine root systems are likely to predispose regrowth trees with the highest demands for water and nutrients to higher levels of drought stress.

While factors which limit the size and competitiveness of the fine root system may be important in the induction of drought stress and regrowth dieback, many of the factors examined above have existed throughout the development of these forests and may not solely account for the progressive decline of trees even after a return to favourable growing conditions or the death or removal of competing

trees. Also the restriction of the root system cannot fully explain instances of dieback on deep friable soils or the survival of individual healthy trees in severely affected stands on shallow soils.

Soil, stress and competition factors cannot fully explain the cause of the high levels of fine root decay which occurred prior to the intensification of dieback. Consequently investigations were conducted on the possible involvement of pathogenic micro-organisms in the discoloration and decay of E. obliqua root systems. While the remainder of this thesis has concentrated on these micro-organisms it is important that their pathogenicity and role in regrowth dieback must always be considered in relation to the many associated factors described above.

3.7 Micro-organisms Associated With Root Rot of E. obliqua Regrowth Trees

As the high level of staining and decay in the fine roots of E. obliqua regrowth trees appears to be a primary factor directly associated with the initiation of dieback, investigations were conducted of the factors and agents which may be responsible for this decay. These investigations were aimed at isolating possible pathogens and examining their involvement in root decay and dieback. Further detailed studies were later conducted on some of these potential pathogens.

3.7.1 Rot of Large Structural Roots

As an Armillaria sp. had been reported on the roots, collars and lower boles of E. obliqua regrowth trees which had been severely affected by dieback (Bowling and McLeod 1968) studies were conducted to examine the association of Armillaria root rot and other structural root rots with regrowth dieback. Rhizomorphs of Armillaria spp. and sapwood decay on standing dying trees and fallen timber are regarded as ubiquitous throughout wet sclerophyll forests in Tasmania (Felton 1972) and the following studies were conducted mainly to assess whether Armillaria spp may be regarded as a primary factor in the initiation of root decay and dieback in these regrowth forests.

Materials and Method

The root collar around 33 regrowth trees which were growing in two dieback affected and one predominantly healthy regrowth stands were partially excavated to expose five to ten roots per tree. These were examined to determine the occurrence and characteristics of Armillaria and other structural root rots. Root samples were collected from each tree and root tissues which had been surface sterilised for 30 seconds in 95% ethanol were plated onto agars for the isolation of fungi. The agars included: 2% malt (Oxoid); potato dextrose agar (Oxoid) and a chemically defined medium (Appendix 1).

Results and Discussion

Two structural root rots were apparent. The first, a white soft rot of the sapwood, was associated with Armillaria spp. on the basis of rhizomorphs, mycelial fans, zone lines and the isolation of a fungus consistent with descriptions of cultures of Armillaria spp. on agar plates. Localised white lesions with soft rot occurred on the roots of trees of all health classes but only increased markedly on trees already severely affected by crown dieback (Table 5). Infections were also found on moribund structural roots even in seven year old trees in younger forests. While rot of the structural root sapwood is likely to contribute to the final death of dieback affected trees its low level of development on trees in the early stages of dieback suggests it is unlikely to be the primary causal factor in regrowth dieback of E. obliqua in these forests. There was also no evidence that rot of the structural roots by Armillaria was associated with the marked discoloration and decay of fine roots prior to the initiation of dieback symptoms.

The second structural root decay involved the staining and blockage of xylem vessels in the larger roots of regrowth trees. This decay was similar to pathological heartwood (Shigo 1973) and occurred in the bole and proximal end of roots in trees of all ages. A Graphium sp was consistently isolated from the fresh margin of the affected tissue. Its ability to reproduce decay was not tested. No association between the extent of this decay and the level of crown dieback was apparent in the trees sampled (Table 5). The decay was restricted to the central tissue of large roots and did not appear to be associated with the decay and staining of fine roots.

TABLE 5 Rot of the structural root system of regrowth E. obliqua at different stages of dieback development.

DIEBACK CLASS % OF CROWNS AFFECTED	No. OF TREES EXAM.	No. OF ROOTS EXAM.	% OF ROOTS WITH ARMILLARIA SP SURFACE RHIZOMORPHS	PRIMARY INFECTION+	WHITE SAPWOOD ROT[I] %ROOTS WITH DECAY	%OF ROOT CROSS SECTION AFFECTED×	RED VASCULAR OCCULUSION[II] % ROOTS WITH DECAY	% OF ROOT CROSS SECTION AFFECTED×
0-10 (Healthy)	8	73	30	0	10	5	71	42
11-30	7	54	22	0	33	9	80	27
31-50	3	25	28	0	44	13	76	30
51-70	8	63	22	5	43	8	89	35
71-100	7	57	47	0	72	47	88	33
MEAN			30	1	40	16	81	33
TOTAL	33	272						

I - TYPICAL OF ARMILLARIA TYPE INFECTION

II - TYPICAL OF GRAPHIUM TYPE INFECTION

× - Percentage of total roots examined

\+ - Direct evidence of rhizomorphus infecting sapwood

3.7.2 Fine Root Decay Micro-organisms

The presence of potentially pathogenic nematodes and fungi which may be associated with the decay of the fine roots of regrowth trees was examined in soil and roots collected from regrowth forests. Diebacks in Australian forests have frequently been associated with isolations of Pythiaceae from soil, particularly Phytophthora cinnamomi (Podger 1965, Jehne 1971, 1972, Pratt et al 1972, Newhook and Podger 1972) and particular attention was paid to the possible involvement of these fungi in the decay of fine roots and regrowth dieback.

Materials and Methods

Soil and fine roots were collected from throughout the southern regrowth forests and examined for the presence of particular micro-organisms using the following techniques. Each soil or root sample consisted of five to ten subsamples from the surface 20cm and was bulked and stored and $2^{o}C$ for minimum periods prior to examination.

(a) Baiting for Pythiaceae

Over 250 bulked forest soil and root samples were baited for the isolation of Pythiaceous fungi using either lupin radicles (Chee and Newhook 1965), E. sieberi cotyledons (Marks and Kassaby 1974) or lupin leaves (Waterhouse and Stamps 1969) as baits. In all cases the baits were floated or suspended on the surface of soil which had been saturated with deionised distilled water and incubated for two to five days at $22^{o}C$. The baits were then removed, surface

sterilised in 1% sodium hypochlorite for thirty seconds and plated onto selective P10 VP agar (Tsao and Ocana 1969). Pythiaceae isolated from the baits were subcultured onto potato dextrose, cornmeal and lima bean agars (Oxoid) for identification.

(b) Root Plating Studies

Extensive plating studies were conducted with fine roots to isolate any potentially pathogenic fungi. E. obliqua roots which had been traced back to their tree of origin were collected from the forest, surface sterilised in the laboratory in 1% sodium hypochlorite for 30 seconds and plated on various selective agars. Over 2500 E. obliqua root tips were plated on P10 VP agar for the isolation of Pythiaceae, particularly P. cinnamomi. A similar number of root tips were also plated on a range of agars including 2% water agar (Davis), potato dextrose agar (Oxoid) and an agar selective for Fusarium (Denis et al 1966). Fungi growing from these roots following two to ten days incubation at 22°C were plated onto potato dextrose agar (Oxoid) for identification using the keys of Barnett and Hunter (1972).

(c) Root Incubation Studies

A root incubation technique was developed which enabled slower growing fungi, including Cylindrocarpon species, to be isolated from the fine roots of E. obliqua. Fine roots (5-20g wet weight), either from the field or glasshouse, were washed, surface sterilised (1% sodium hypochlorite for 30 seconds) and incubated at 10°C on sterile moist filter paper in plastic trays. Following ten days incubation the roots were examined at x 30 magnification for characteristic

conidiophores of fungi including Cylindrocarpon. The lower incubation temperatures and the sole reliance of the sporulating fungi on naturally available substrates from within the colonised root retarded the rapid proliferation of Penicillium, Mucor and Trichoderma conidiophores which had frequently obscured slower growing fungi, including Cylindrocarpon, in the agar plate studies. Cultures of sporulating fungi were obtained for identification by needle transfers of conidia.

(d) Nematode Extraction

Nematodes were extracted from E. obliqua forest soils using the Baermann funnel technique (Goodey 1963). Forty five soils representing forests of different age as well as contrasting healthy and dieback regrowth trees were examined for the numbers and types of nematodes which were identified using the keys and descriptions of Mai and Lyon (1975).

Results and Discussion

Pythiaceae: Several Pythium spp. were commonly isolated from the baits of forest soils (Tables 6 and 7) and infrequently from the surface sterilised fine root tips of E. obliqua regrowth trees (Table 7). While Pythium spp. are an important component of the soil microflora, none of the Pythium spp. were isolated with sufficient frequency from roots to be regarded as the primary pathogen causing root decay of dieback trees. However, fine root infection by Pythium spp. may contribute to root decay of regrowth eucalypts and warrant further investigation. The pathogenicity of Pythium spp. and their involvement in such root rot complex has been reviewed (Hendrix and Campbell 1973).

TABLE 6. Summary of the isolations of Pythiaceae from forest soils using the lupin baiting technique (Chee and Newhook 1965).

\- Absent + Present ++ Common

FOREST AGE AND HEALTH (YEARS)	NO. OF FOREST STANDS EXAMINED	NO. OF SOIL SAMPLES BAITED	% OF SOIL POSITIVE FOR *P. cinnamomi*	OTHER Phytophthora SPECIES	*Pythium* SPP.
1. 5	1	4	25	-	+
2. 40	1	3	33	-	+
3. 55	1	10	0	-	+
4. 60-70 Healthy	3	38	3	+	++
5. 60-70 Dieback	9	69	7	+	++
6. 80-90	3	41	12	-	+
7. Oldgrowth forests 200+	3	12	8	-	+
8. Heathlands	8	26	8	-	+
TOTAL	29	203	8		

TABLE 7 Isolation of pythiaceae from the fine roots of regrowth forest trees

Forest Type	Month of Sampling	No of trees Sampled	No of root tips plated	Sterilisation	% isolation on $P_{10}VP$ agar	
					P. cinnamomi	Pythiaceae*
E. obliqua dieback affected stands	January	6	63	0	0	2.4
	March	8	881	+	0	<1
	May	7	140	0	0	48.0
			140	+	0	16.0
	August	10	200	+	0	16.0
	October	10	200	0	0	80.0
	December	2	110	+	0	16.0
E. obliqua healthy stands	January	1	10	0	0	70.0
	March	5	363	+	0	4.0
	May	5	100	0	0	41.0
			100	+	0	2.0
	August	7	140	+	0	21.0
	October	10	200	0	0	71.0
E. globulus healthy trees	August	7	140	+	0	25.0
Banksia marginata diseased heath	January to May	21	100	+	38	frequent

*16 main cultural groups isolated

Phytophthora cinnamomi: P. cinnamomi was isolated from the lupin root baits in 8% of the forest soil and root samples (Table 6). These include soil samples collected under forests of a range of different ages and from under both dieback and healthy trees in 8 different regrowth forests. P. cinnamomi isolations were higher in the soils of young forest and heathland than regrowth forests. In no instance was P. cinnamomi isolated from the very extensive plating of surface sterilised fine roots of E. obliqua specifically conducted for this purpose (Table 7). This is in contrast to fine roots of Banksia marginata and Epacris impressa from adjoining heathland which had been plated using identical techniques and from which P. cinnamomi was isolated in 38% of the root samples.

The failure to isolate P. cinnamomi from any of the fine roots of regrowth E. obliqua indicates that P. cinnamomi is unlikely to be a consistent or major factor in fine root rot in these E. obliqua regrowth forests. It may contribute to the death of plants in adjacent heathland. The low soil temperatures occurring under regrowth forests, as distinct from those under heath, are also considered to be too low for the active development of this fungus (Roth and Kuhlman 1966) although strains of the fungus which are tolerant of low temperatures have been reported (Zentmyer et al 1976).

Nematodes

High numbers of nematodes were extraced from the forest soils, including members from 10 potentially pathogenic genera (Table 8). No relationship was apparent between the presence or increased frequency of any particular genus and the occurrence of dieback.

TABLE 8 Isolation of Nematodes from the soils of *E. obliqua* forests of different age.

\+ present ++ common +++ very abundant * H = healthy, D = dieback

NEMATODE GENUS	FOREST AGE (YEARS)											
	1	7	35		55		67		90		150	250+
*TREE HEALTH			H	D	H	D	H	D	H	D		
No. of Trees Examined	4	4	2	2	2	2	7	10	2	2	4	4
Ditylenchus	++	+	+++	+++	+	+++	+++	+++	+++	+++	+++	++
Cricenomoides							++	++				
Tylenchus	+	+	+	+		+	+	++			+	++
Rotylenchus							+	+				
Belanolaimus			+	+			+	+				
Aphelenchus	+						+	+				++
Aphelenchoides	++	+	+	++			++	++			+	
Pratylenchus				+					+		+	+
Hoptiotylus						+		+				
Paratylenchus										++		
Dorylaimus	++	+	++	++	+	++	+	++	++	+	++	++
Mononchus	+		+	+	+	+	++	++	+	+	+	+
Rhabditis	+	+	+	+	++	++	++	++	+	+	++	++
Panogrolaimus				+	+	+	++	++				+

Members of the Cricenomoides were common in soil of 67 year old regrowth forests under both healthy and dieback affected trees.

The common direct and indirect involvement of root infecting nematodes in root rot complexes of forest trees has been reviewed by Ruehle (1973) and it is possible that the numerous nematodes of root infecting genera isolated from these soils may contribute to dieback development. Nematodes have previously been described as parasitic on eucalypts in Australia (Meagher 1968) and Brazil (Lordello 1967). Species of Cricenomoides have been associated with root rot complexes in peach (Chitwood 1949, Mojtahedi and Lownsbery 1975), plum (Braun et al 1975) and Pinus echinata (Jackson 1970). Further investigations need to be conducted into the role of nematodes, including member of the Cricenomoides, in the root rot of E. obliqua.

Moniliales and Other Root Infecting Fungi

Plating of surface sterilised E. obliqua fine root tissue on a range of agars resulted in the isolation of numerous fungi including members of the genra Fusarium, Cephalosporium, Acremonium and Cylindrocarpon (Table 9). Isolation frequencies of potential pathogens were often low with this technique with only three of the plated root pieces plated yielding Cylindrocarpon isolates.

In contrast root incubation consistently resulted in the isolation of Cylindrocarpon conidiophores from the fine roots of trees of all ages but particularly those from regrowth forest (Table 10). Cylindrocarpon sporulated from the roots of both apparently healthy and dieback affected trees. Sporulation was most prolific on

TABLE 9 Summary of fungal isolations from surfaced sterilized fine roots of E. obliqua regrowth trees.

Isolations from 13 trees in dieback affected forests.

0=absent +=present ++=common +++=abundant

FUNGI ISOLATED* FROM ROOT SEGMENTS	PLATING ON AGAR			BAITING WITH HOST TISSUES		
	WATER AGAR	DENIS AGAR	$P_{10}VP$ AGAR	LUPIN ROOTS	LUPIN LEAVES	E.sieberi COTYLEDO.
NO OF ROOTS EXAM.	962	560	1244	269	246	199
Phytophthora cinnamomi	0	0	0	0	0	0
Phytophthora spp.	+	0	+	+	+	0
Pythium spp.	++	+	+++	+++	++	0
Botrydiplodia spp.	+		+		+	++
Acremonium spp.	+		+	+		
Cylindrocarpon spp.	+					
Botrytis spp.	++		+	++		
Trichoderma spp.	++					
Kellermania spp.	++				+	
Mucor spp.	+	+		+		
Gliocladium spp.	+				+	
Cephalosporium spp.	+	+	+	+	+	
Penicillium spp.	+++	++	+++	++		+
Basidiomycete sp.	++	+	+			
Fusarium spp.	++					
Trichocladium spp.	+			+		
Alternaria spp.	+					++
Rhizoctonia spp.		+				
Unidentified	++	++	++	+	+	++

TABLE 10 Isolation of Cylindrocarpon CMI196141* from the surface sterilized fine roots of E. obliqua trees from forests of different age and health.

0=absent +=present ++=common +++=abundant

AGE OF FOREST Years	NO OF TREES SAMPLED		ISOLATIONS FROM INCUBATED ROOTS				MICROSCLEROTIA IN CLEARED ROOTS			
			No. of trees +ve		Abundance		No. of trees +ve		Abundance	
	Healthy(H)	Dieback(D)	H	D	H	D	H	D	H	D
1	4	-	1	-	+	-	4	-	+	-
7	4	-	3	-	+	-	4	-	++	-
35	2	2	2	0	++	0	2	2	++	+++
55	2	2	1	1	+	+	1	2	++	++
68H	2	2	2	2	+++	+++	2	2	++	+++
68D	2	2	2	2	++	++	2	2	++	++
90	2	2	2	1	+++	+	2	2	++	++
150	-	4	-	4	-	+	-	2	-	+
250+	-	4	-	3	-	+	-	4	-	++

* isolates similar to CMI196141

Based on conidial transfer or microsclerotia morphologically consistent with Cylindrocarpon CMI 196141.

unsuberised root tips of E. obliqua (Figure 6a), but also occurred from secondary thickened roots (Figure 6b) and mycorrhizal roots (Figure 6c). In roots that had been broken prior to incubation, Cylindrocarpon sporulated from the root cortex and from numerous microsclerotia in the inner root cortex (Figure 6d).

Clearing intact fine roots with boiling KOH (Bevage 1968) revealed Cylindrocarpon microsclerotia in the inner root cortex of many roots (Table 10), (Figure 6e). The microsclerotia of Cylindrocarpon are similar in morphology and occurrence to those described by Scholten (1968), Menge (1969), Jutter (1970) and Linderman (1973) in members of the genus Cylindrocladium. The eucalypt root stele was commonly stained red and blocked but neither the Cylindrocarpon hyphae nor microsclerotia were observed within the stele of living roots.

The consistent isolations of a Cylindrocarpon sp. from surface sterilised fine roots of E. obliqua regrowth trees indicated that Cylindrocarpon spp. may be associated with the initial decay of E. obliqua roots in these forests. Consequently the following series of pathogenicity studies was conducted to examine whether Cylindrocarpon spp. or other potential pathgens could be involved in the decay of fine roots in these forests.

3.8 Testing the Pathogenicity of Fungi Isolated from E. obliqua Roots and Soil

The isolation of an organism from diseased host tissue may result from its saprophytic or parasitic colonisation and is not proof that the organism is pathogenic or the cause of any observed disease. For an organism to be regarded as a pathogen; and the cause of a

Figure 6a Isolation of Cylindrocarpon CMI196141$^{\nabla}$ from the fine roots of E. obliqua regrowth trees.

(a) Conidiophores of Cylindrocarpon CMI196141$^{\nabla}$ sporulating from surface sterilized feeder root tip of E. obliqua regrowth tree .

(b) Conidiophores of Cylindrocarpon CMI196141$^{\nabla}$ sporulating from the outer cortex as well as the surface of the inner stele of secondary thickened roots of E. obliqua regrowth tree .

(c) Conidiophores of Cylindrocarpon CMI196141$^{\nabla}$ sporulating from the surface of mycorrhizal short roots of E. obliqua regrowth tree .

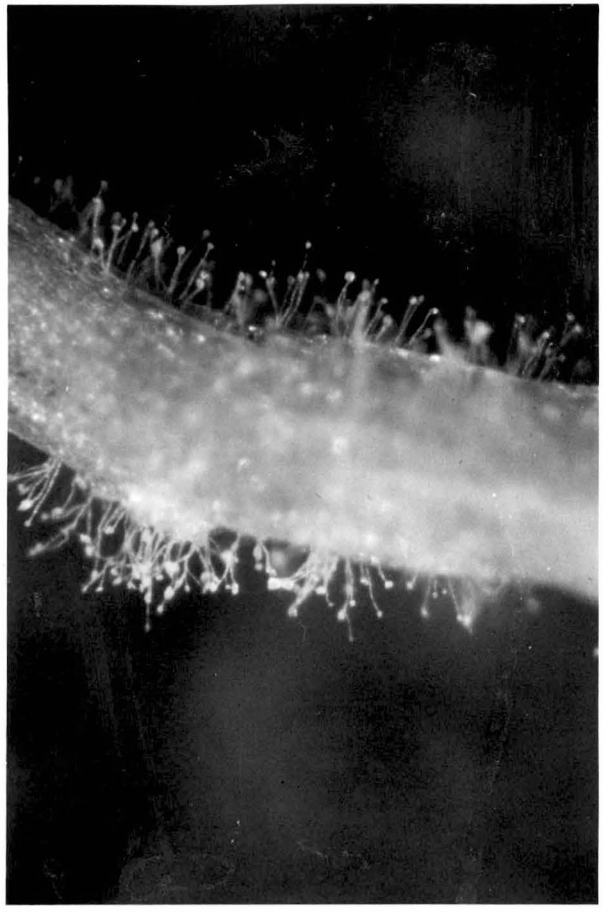
(d) Conidiophores of Cylindrocarpon CMI196141$^{\nabla}$ sporulating from microsclerotia on the surface of the inner stele of secondary thickened root of E. obliqua regrowth tree. .

(e) Microsclerotia of Cylindrocarpon CMI196141$^{\nabla}$ in the inner cortex of E. obliqua feeder root from regrowth tree following clearing of the roots in boiling KOH.

(f) Detail of microsclerotia of Cylindrocarpon CMI196141$^{\nabla}$ in the cortex of feeder root from E. obliqua regrowth tree .

$^{\nabla}$ isolates similar to type CMI196141

a



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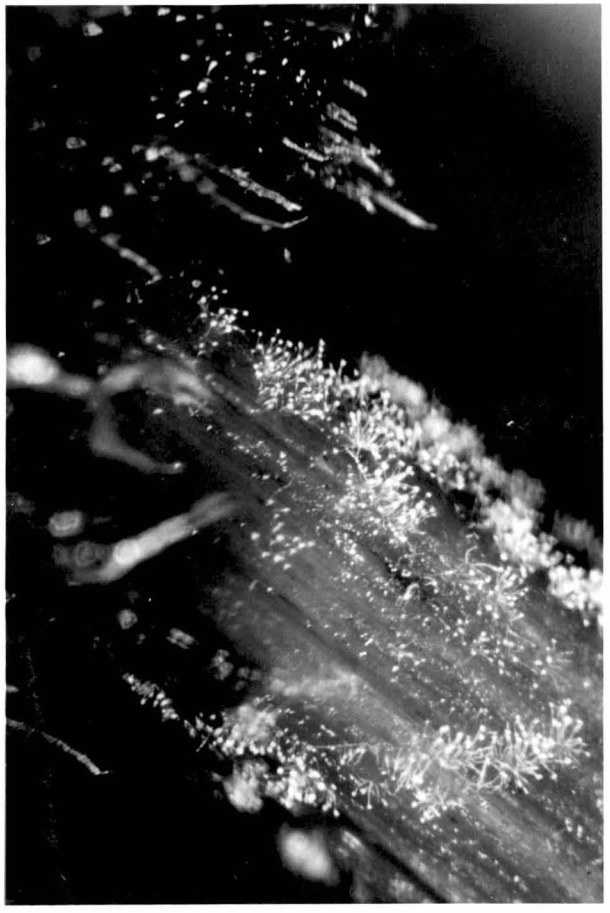
b



1 mm

c

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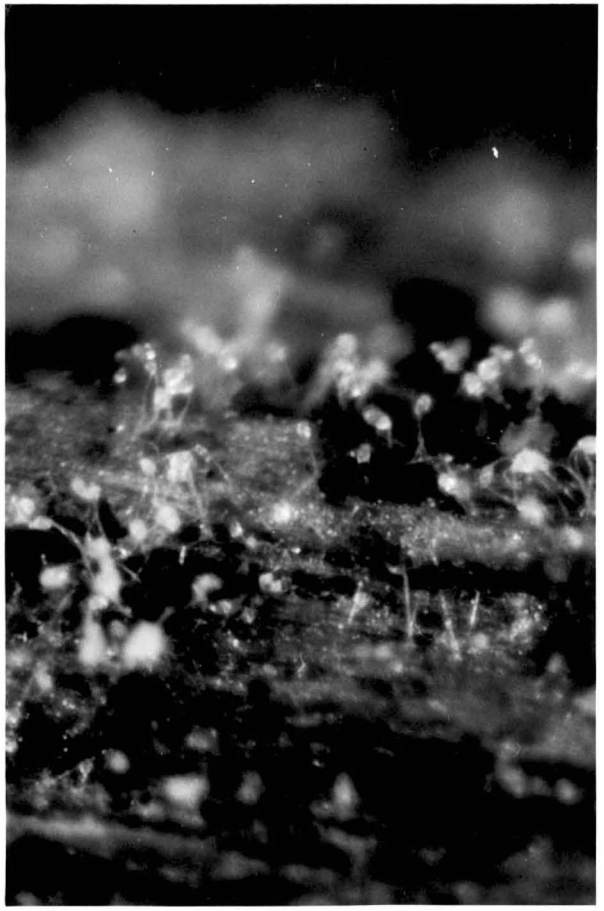
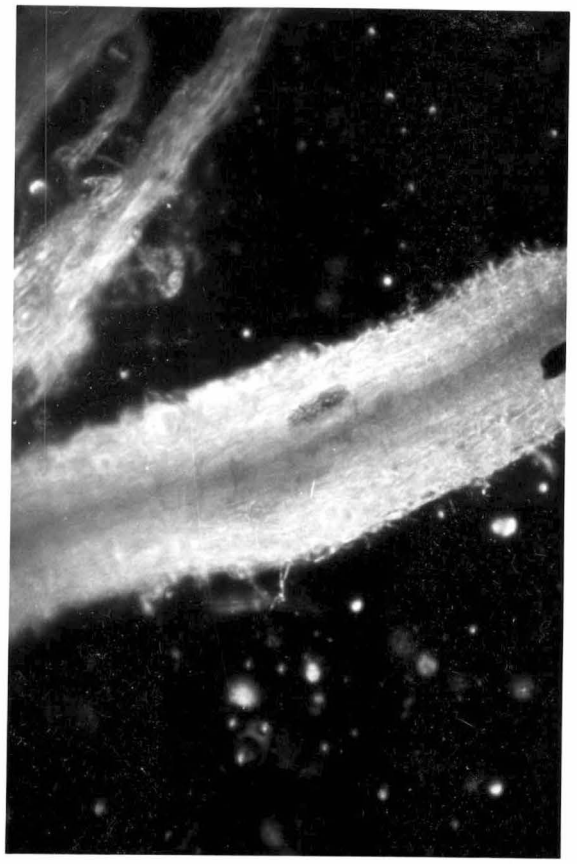
d



300 µ

e



1 mm

f

120 µ

particular disease, it needs to satisfy the following conditions which have been defined in Koch's postulates of pathogenicity (Horsfall and Dimond 1960).

1. The organism needs to be present in every case of the disease.
2. The organism must be consistently isolated from the diseased host.
3. The specific disease must be reproduced when a pure culture of the organism is inoculated into a healthy susceptible host.
4. The organism must be recoverable from the experimentally infected host.

However, disease is the expression of interactions between combinations of organisms, environmental conditions, the physiology of the host and time (Horsfall and Dimond 1960, Van der Plank 1975) and it may not always be possible to reproduce all these essential components of the disease interaction in experimental plants. This is particularly the case in diseases of trees in older forests where soil conditions, environmental factors, the physiology of the host and the population and activity of micro-organisms may all interact and change with time. In these cases it may be difficult to satisfy the third of Koch's Postulates, which requires experimental reproduction of the disease.

Attempts to reproduce a disease experimentally have included the inoculation of similar healthy hosts in the field. However difficulties may arise in that the type, application and level of experimental inoculum may differ from that occurring naturally. The time taken for expression of disease symptoms may also be so long that pathogenicity cannot be experimentally confirmed. Disease

symptoms that develop several years after inoculation of apparently healthy trees may not be caused by the applied treatment. It may also be difficult to identify disease free hosts for inoculation. Even where such trees can be found they may represent naturally resistant genotypes and pathogenicity tests on these trees may not be relevant to susceptible genotypes all of which may already be naturally affected. Furthermore many diseases may be associated with interactions and complexes between several micro-organisms and stress factors and tests of pathogenicity with one organism in the field may not be meaningful.

Alternatively the pathogenicity of a micro-organism may be tested by inoculating seedlings of the host and observing responses in the inoculated tissue or plant. While this technique may be practical and reproducible, large differences which can occur in the physiology and susceptibility of seedlings as compared with mature plants, may influence the results. However, it may be possible to partially overcome some of these differences by predisposing the seedlings to stress treatments similar to those occurring in forests. This may enable studies of the interaction between particular predisposing stress factors and the susceptibility of these plants to inoculated pathogens (Schoeneweiss 1975).

The following studies were conducted with E. obliqua seedlings, of varying ages, mindful of the limitations in relating the results to the root decay or dieback of mature regrowth trees. These studies were designed to screen fungi which had been consistently isolated from the roots of regrowth trees for their ability to infect the roots and cause disease symptoms in E. obliqua seedlings. Investigations of the pathogenicity of particular fungi under a

range of stress conditions representative of those occurring in the E. obliqua regrowth trees are described in Chapter 5.

Material and Methods

Three series of experiments were conducted in these screening studies. In the first experiment, ten one-month-old E. obliqua seedlings, and Lupinus angustifolius seedlings, which had been grown in autoclaved vermiculite, were transferred to tubes containing Petri's solution (Waterhouse and Stamp 1969). The tubes were inoculated with 1cm diameter plugs of fungal culture and were placed in natural light at 15-22°C without aeration. Seedling deaths and root infections were recorded over the following 10 days for each of the fungi (Table 11).

In the second experiment four E. obliqua seedlings which had been grown in freely drained sand peat for four months (Baker 1957) were inoculated with each of six fungi isolated from the roots of regrowth trees. The inoculum consisting of $2cm^2$ of fungal cultures on PDA or lima bean agar was placed in the surface soil next to the seedling roots. The seedlings were grown in a glasshouse for five weeks at 18 to 22°C when their roots were washed free of soil and examined for decay. Root tissue was surface sterilised in 1% sodium hypochlorite for 30 seconds and plated on PDA and P10 VP agars, for the reisolation of the inoculated fungus.

In the third experiment, intact fine root tips of two 2-year-old E. obliqua saplings were carefully excavated and placed inside glass tubes. The roots were inoculated with 9 sq. mm plugs of fungal culture on P.D.A. The inoculated root tips, and similar uninoculated control root tips which had been inoculated with water

agar plugs, were regularly inspected, and after 9 days growth at 18°C were removed, surface sterilized and plated on PDA or P10 VP for reisolation of the inoculated fungus.

Results and Discussion

Of the fungi tested in the first experiment the Cylindrocarpon sp. was the only fungus which was consistently isolated from the fine roots of E. obliqua regrowth trees which also resulted in root decay and mortality of the E. obliqua seedlings and could be reisolated from surface sterilised root tissue (Table 11). Microscopic examinations confirmed that the Cylindrocarpon sp. colonised the cortex of the roots of E. obliqua seedlings and produced microsclerotia in and from the infected tissue prior to death of the seedlings. While it had not been isolated from the roots of regrowth trees, P. cinnamomi also resulted in rapid root rot and death of the inoculated E. obliqua seedlings. Several of the Pythium spp. but not the Fusarium spp. which had been isolated from the roots of regrowth trees were also capable of causing root decay and E. obliqua mortalities. Consequently Pythium spp. should not be disregarded as factors which may contribute to root decay in regrowth forests.

No mortalities were recorded in the second experiment in 4-month-old E. obliqua seedlings which had been inoculated for five weeks in freely drained sand peat. However the Cylindrocarpon sp., P. cinnamomi, P. cactorum, and a Pythium sp. caused root rot and were reisolated from surface sterilized root tissue (Table 11). The Cylindrocarpon sp. and P. cinnamomi also caused decay in the roots 2-year-old E. obliqua saplings in the third experiment. The first

TABLE 11 Screening of fungi isolated from the roots and soils of *E. obliqua* regrowth forests for their pathogenicity to *Lupinus angustifolius* and *E. obliqua* seedlings of different ages.

Fungus	*Lupinus angustifolius* seedlings * one week old	*E. obliqua* seedlings one month old *			four month old+		24 month old+
Rating 0-3	Root infection RI	Mortality	Root rot	RI	Root rot	RI	Root infection
Cylindrocarpon CM1196141	2	3	1	2	3	2	3
Cephlasporium sp	1	0	0	0	-	-	-
Acremonium sp	1	0	0	0	-	-	-
Fusarium sp 1	2	0	0	0	0	0	-
Fusarium sp 2	2	0	0	0	-	-	-
Phytophthora cinnamomi	2	3	1	2	3	2	3
Phytophthora cactorum	1	1	1	2	2	1	-
Pythium sp U10	1	0	0	0	-	-	-
Pythium sp U5	1	3	1	1	1	0	-
Pythium sp U12	0	0	1	1	2	1	-
Pythium sp U7	1	0	1	1	-	-	-

Rating 0= No roots affected
1= 0-10% of roots affected
2= 11-40% of roots affected
3= 41%+ of roots affected

* in Petri solution (10 seedlings per treatment)

\+ in drained sand peat

Root infection (RI) based on reisolations of the inoculated fungus from surface sterilized roots.

Mortality based on seedling deaths on a similar 0-3 rating.

Root rot based on necrosis, rot or discoloration of fine roots.

and second pathogenicity studies were repeated and similar results were obtained.

Consequently, of the fungi isolated from the fine roots of E. obliqua only the Cylindrocarpon sp. was consistently isolated from affected trees, was capable of infecting and reproducing disease symptoms in seedlings of susceptible hosts and was consistently reisolated from the affected tissues. Consequently the Cylindrocarpon sp. satisfied Koch's postulates of pathogenicity. However the failure of the Cylindrocarpon sp. to cause seedlings deaths in the pot studies emphasised the need to investigate factors which contribute to its pathogenicity. Both the Pythium spp. and nematodes also warrant further study as factors which may contribute to root rot and dieback of these regrowth forests.

The investigations described in this chapter indicate that the dieback of E. obliqua regrowth forests is associated with a complex of interacting factors which result in drought stress and death of the affected trees. The restriction and decay of the fine root system of regrowth trees and its subsequent inability to absorb adequate moisture from the soil to meet the transpirational demands of the tree appear to be the direct cause of regrowth dieback. Many factors of the soil, climate, changing vegetation and microflora are likely to contribute to the soil conditions, plant stress and root health which appear to be important in predisposing particularly the former dominant trees to drought stress and crown dieback. However many of these factors have existed during the development of these forests and can not solely account for the occurrence of dieback nor its continuation under more favourable conditions. Consequently the agents responsible for the high levels of decay of the fine root

system prior to the onset of dieback symptoms appear to be of primary importance in causing dieback and preventing the recovery of the trees on return to conditions favourable for tree growth.

As the Cylindrocarpon sp. may be important in the infection and decay of the fine roots of regrowth E. obliqua the remainder of this thesis has concentrated on the microbiology and pathogenicity of the Cylindrocarpon sp. and its role in regrowth dieback and the subsequent development of these forests. However the involvement of the Cylindrocarpon sp. in E. obliqua regrowth dieback and forest development should always be considered in the context of the complex of the many associated factors described in this chapter.

CHAPTER 4

THE MICROBIOLOGY OF CYLINDROCARPON IN FOREST SOILS

4.1 Taxonomy of the Cylindrocarpon From E. obliqua Roots

The Cylindrocarpon isolate from the fine roots of Eucalyptus obliqua L'Herit CMI 196141 differs from isolates held at the Commonwealth Mycological Institute Kew (Booth pers. comm.) and has the following description:

Cylindrocarpon CMI 196141 from E. obliqua.

Cultures on PDA white turning evenly red brown with age. Aerial mycelium sparse but tall, branched conidiophores develop profusely on the surface of cultures. Conidiophores up to 500μ long with lateral primary branches. Subulate phialides 30 to 50μ x 3 to 5μ are produced at the apex and in 2 to 5 whorls along the length of the conidiophore.

Macroconidia are produced singly at tips of phialides where they may accumulate in white glistening heads. Macroconidia are straight, cylindric, hyaline one septate, 27 to 39 x 3 to 6μ and have a rounded distal and slightly conical proximal end. Microconidia are rare. Chlamydospores 10 to 30μ in diameter with smooth, brown thickened walls are produced in chains at the ends of hyphae in older culture. Conidial chlamydospores are frequently formed by macroconidial germ tubes in soil and are rounded, 8 to 13μ in diameter, thickened and may occur in chains. Microsclerotia are formed in old cultures, E. obliqua roots and forest soils and

consist of dense aggregates of brown cells with thick cell walls. Microsclerotia are irregularly rounded from 100 to 200μ in diameter and on germination produce typical conidiophores bearing macroconidia.

No perfect stage has been observed

Hosts Eucalyptus obliqua. Isolates consistent with that described above also occur on E. regnans, E. delegatensis roots as well as on leaf litter and are distributed widely in Tasmanian forest soils. Photographs and drawings of spore stages are shown in Figure 6b.

The morphology of Cylindrocarpon CMI 196141 closely resembles C. gracile which, together with C. tenue and C. reteaudii, are very close to species of the genus Cylindrocladium (Booth 1966). The main features distinguishing these members of the genus Cylindrocarpon from those of Cylindrocladium is the absence of a diagnostic lateral stipe terminating in a sterile vesicle (Booth 1966). However, isolated short appendages bearing globose heads have been infrequently observed in Cylindrocarpon CMI 196141 (Figure 6) and in C. tenue (Agnihotrudu 1959) suggesting some similarity to members of the genus Cylindrocladium. Cylindrocarpon CMI 196141 also differs from the descriptions of other members of the genus in the production of microsclerotia similar to those described in Cylindrocladium scoparium (Scholten 1968) and Cylindrocladium floridanum (Menge 1969, Jutter 1970).

Although the perfect stages of Cylindrocarpon CMI 196141, C. gracile and C. tenue are not known, C. reteaudii is so far unique within the genus Cylindrocarpon in that it has a Calonectria perfect stage as

Figure 6b The morphology of Cylindrocarpon CMI196141

a. Microsclerotia and macroconidia of Cylindrocarpon CMI196141 formed in PDA agar cultures.

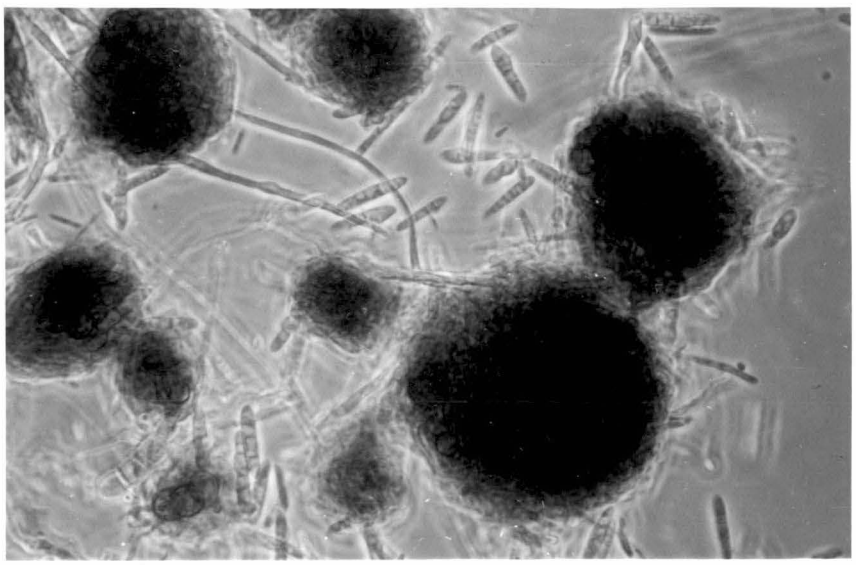
b. Macroconidia of Cylindrocarpon CMI196141. Further photographs of macroconidia and conidial chlamydospores of Cylindrocarpon CMI196141 are shown in Figure 28.

c. Tip of a compound conidiophore of Cylindrocarpon CMI196141 showing branches and the production of macroconidia in terminal heads.

d. Compound conidiophores of Cylindrocarpon CMI196141.

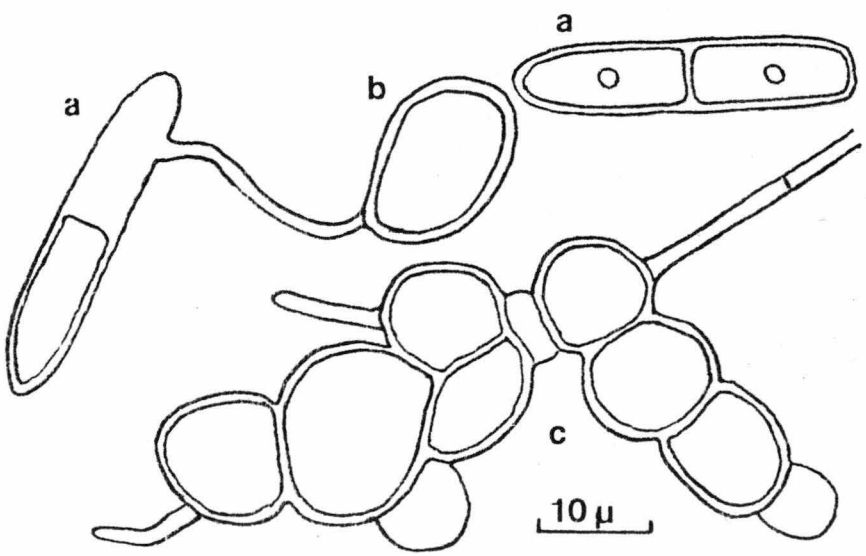
e. Rare sterile stipe with globose vesicle produced on a lateral branch of conidiophore of Cylindrocarpon CMI196141.

a



120 µ

b

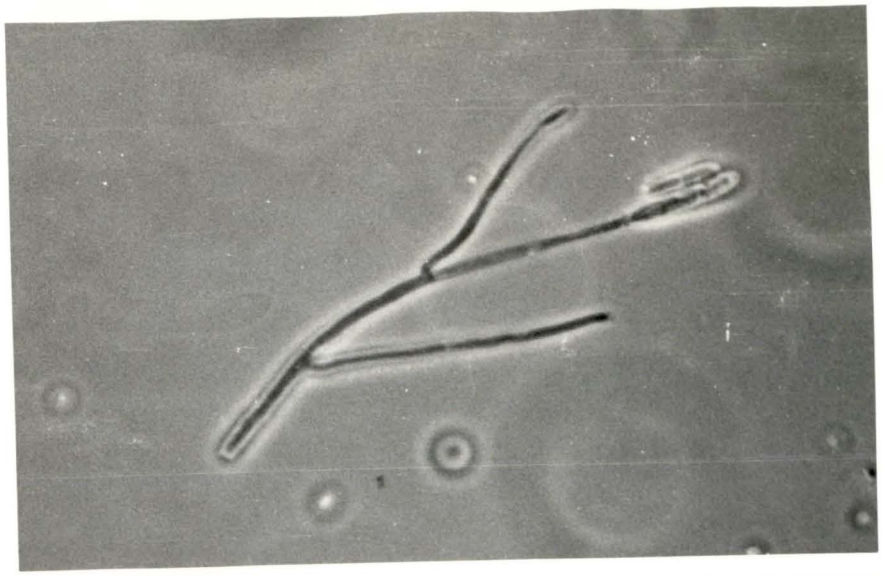


a macroconidia

b conidial chlamydospore

c microscerotial initial

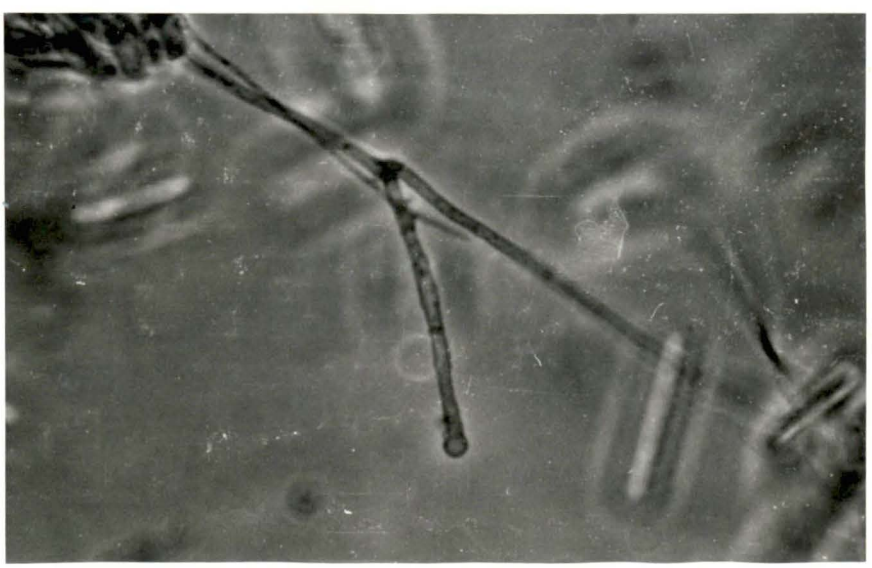
c



d

BAR = 50 µ

e



do members of the genus Cylindrocladium. All other known perfect stages in the genus Cylindrocarpon belong to the genus Nectria. The imperfect stage of some Cylindrocarpon spp. including C. destructans impinge on the mortiella group of the genus Fusarium (Booth 1966). Further descriptions of the taxonomy of Cylindrocarpon CM 196141 and its relations to other Cylindrocarpon species are being prepared by C. Booth, Commonwealth Mycological Institute, Kew, England.

Unless otherwise specified all the investigations in this thesis have been conducted using Cylindrocarpon CMI 196141 described above. Consequently this isolate will be referred to as Cylindrocarpon sp. throughout the remainder of the thesis.

4.2 Cylindrocarpon sp. in E. obliqua Forest Soils

The consistent isolation of Cylindrocarpon sp. from the fine roots of E. obliqua from forests of all ages (Table 10) indicates that the fungus is present throughout the development of these forests. Consequently investigations were conducted of the relationship between the intensification of dieback in regrowth forests and changes in the population and/or activity of Cylindrocarpon sp. on E. obliqua roots and in forest soils. The following studies examined changes in the numbers of microsclerotia and saprophytic activity of Cylindrocarpon sp. in soils from forests of different ages and dieback intensities.

Sampling Methods

E. obliqua forests of nine different ages between one and approximately 250 years were selected in the southern forests to

represent stages in forest development (Figure 2). Soil samples were collected at four sites in each of these forests. Each sample consisted of bulked subsamples of surface soil (0 to 10cm) from over five locations. Rhizosphere soil samples were also collected from each of these forests and sites. Rhizosphere soil was obtained by shaking soil off the fine roots of individual healthy or dieback E. obliqua trees. The soils were stored at 2°C for a minimum period until the population and activity of Cylindrocarpon sp. could be assessed.

Repeat series of soil samples were collected from these E. obliqua forests and assessed for the population and/or activity of Cylindrocarpon sp. on five subsequent occasions over an eighteen months period to confirm the trends obtained.

4.2.1 Numbers of Cylindrocarpon sp. Microsclerotia

Materials and Methods

The number of Cylindrocarpon sp. microsclerotia per gram of soil was determined using a wet sieving technique similar to that used by Thies and Patton (1970) for Cylindrocladium microsclerotia. Forest soil (10g) was blended at low speed in one litre of water with 5 ml of Calgon clay dispersant and the resultant soil suspension was repeatedly washed through stainless steel sieves of 180 and 99μ aperture. Preliminary studies had indicated that most Cylindrocarpon sp. microsclerotia were retained between the 180 to 99μ aperture sieves and that this technique was suitable to assess the relative differences in the numbers of Cylindrocarpon sp. microsclerotia in the different soils. The soil retained between the 180 to 99μ aperture sieves was dispersed in a known volume of

water, and aliquots (0.5ml) were pipetted onto the surface of hardened Czapeks agar containing 4% oxbile (Oxoid) and 200ppm streptomycin sulphate B (Appendix 1). The plates were incubated for 10 days at 10°C when the numbers of Cylindrocarpon sp. microsclerotia which had germinated on each plate were counted at x 40 magnification. The microsclerotia could be readily identified by the morphology of their conidiophores and macroconidia. Macroconidia were transferred onto microscopic slides or onto PDA plates in order to confirm their identity as Cylindrocarpon sp. The number of microsclerotia per gram of dry soil was determined following the measurement of the moisture content of a separate sample of soil.

Results and Discussion

The numbers of viable Cylindrocarpon sp. microsclerotia per gram of dry soil increased from very low levels in soil one year after burning and the regeneration of the forest to a maximum of above 2 x 10^4 microsclerotia per gram in the soils of 70 year old regrowth forests (Figure 7). The numbers of microsclerotia were low in forests from 0 to 30 years of age which closely corresponds with the period of rapid eucalypt growth while the period of forest growth from 30 to 90 years of age was associated with high and increasing numbers of microsclerotia and the intensification of crown dieback. Numbers of Cylindrocarpon sp. microsclerotia reached a plateau in mature forests and decreased slightly in old-growth E. obliqua forests (Figure 7). Fires appeared to markedly decrease the number of microsclerotia in soils as old-growth forests which had recently been logged and burnt contained only ~~isolated~~ occasional viable microsclerotia in distinct contrast to the soils of neighbouring unburnt oldgrowth forests.

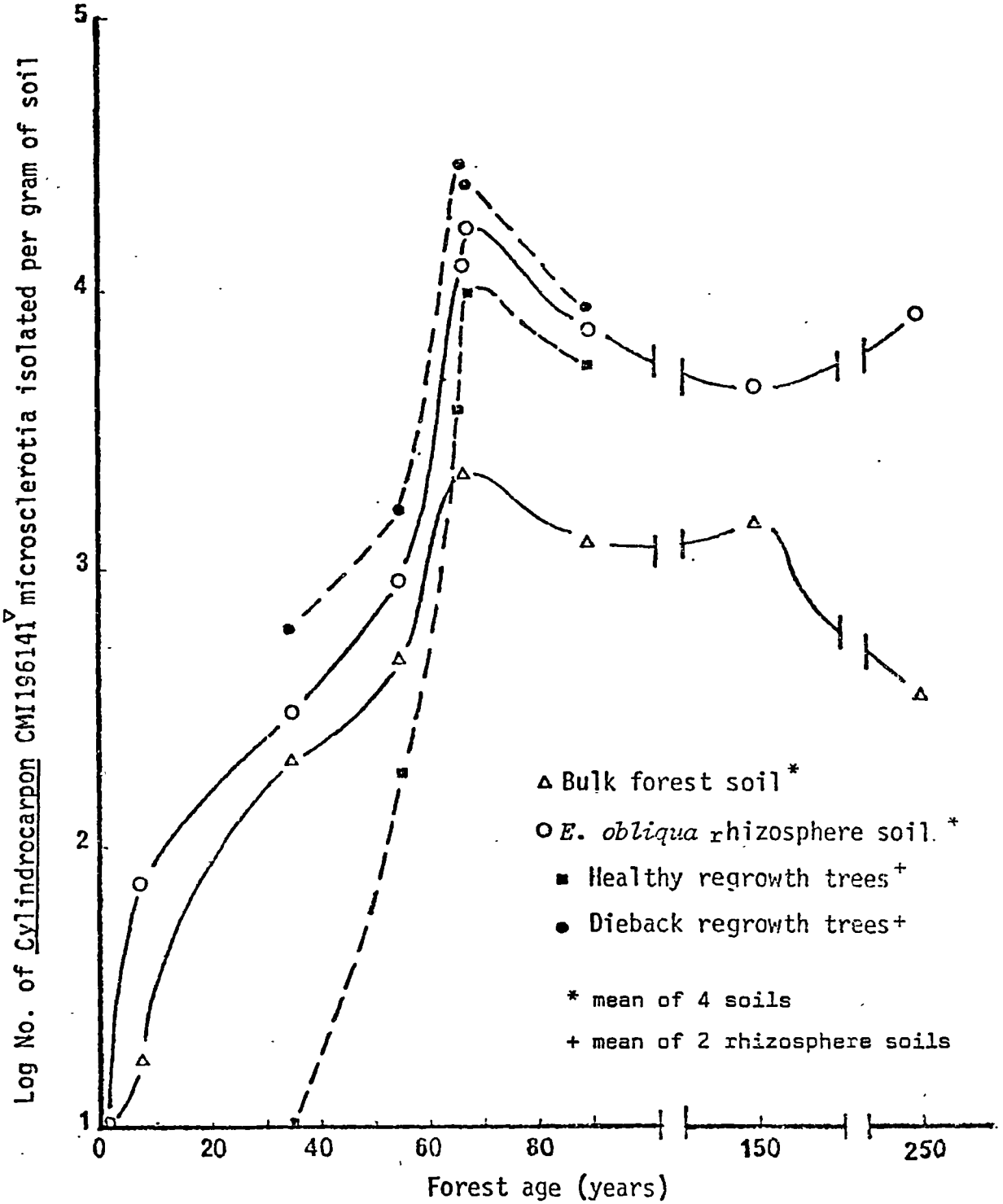


Figure 7 Changes in the numbers of Cylindrocarpon CMI196141▽ microsclerotia in the soils and rhizospheres of E. obliqua forests during their development.

▽isolates similar to CMI196141

LSD (P = 0.05)	Bulk forest soil with forest age	0.774
	Rhizosphere soil with forest age	1.429
	Difference in rhizosphere of healthy and dieback trees	0.639

The numbers of Cylindrocarpon sp. microsclerotia in the rhizosphere soils of both healthy and dieback affected E. obliqua also increased with forest age (Figure 7). However, numbers were consistently higher in the rhizosphere soils of dieback affected E. obliqua compared to the rhizosphere soil of healthy E. obliqua of the same age (Fig. 7). This was particularly evident in the younger forests.

Cylindrocarpon sp. microsclerotia commonly form in and from infected E. obliqua roots and it is likely that the higher numbers of microsclerotia in the rhizosphere of dieback affected trees may have resulted from higher levels of root infection in these trees.

4.2.2 Activity of Cylindrocarpon sp. in Forest Soils

While the soils of E. obliqua forests may contain large numbers of viable microsclerotia of Cylindrocarpon sp. much of this inoculum may be inactive and of little consequence to the growth and health of the forest. Consequently the activity of the inoculum i.e. its capacity to infect dead or live substrate also needs to be determined. The capacity of a fungus to infect or colonise host tissue may be influenced not only by the amount of inoculum, the inoculum dosage, but also be interacting effects of other micro-organisms, soil and environmental factors, the vigour and susceptibility of host tissue, the vigour of the inoculum and its ability to contact the host tissue.

The following studies, using a baiting technique, examined the capacity of Cylindrocarpon sp. in each of the rhizosphere and bulk forest soils to infect and colonise plant tissue. Changes in the activity of Cylindrocarpon sp. in these soils during forest and

dieback development were compared with the changes in the numbers of microsclerotia in the soils. In order to make this assessment as relevant as possible to the activity of Cylindrocarpon sp. in the forest soils both the host tissue and environmental conditions duplicated as far as possible those in the regrowth forests.

Materials and Methods

The relative activity of Cylindrocarpon sp. in the different forest soils was assessed using a baiting technique to measure the capacity of Cylindrocarpon sp. to saprophytically colonise dead E. obliqua leaf tissue. Between 30 to 50 autoclaved leaf baits each 5mm^2 were placed firmly in contact with moistened forest soil in a Petri dish and incubated at 10^{o}C for 10 days. The baits were then washed for 5 minutes in running water, surface sterilised in 1% sodium hypochlorite for 30 seconds, washed in sterile water and then incubated on sterile moistened filter paper in Petri dishes for a further 5 days at 22^{o}C. The number of baits from which Cylindrocarpon sp. and other fungi were sporulating were counted at x 40 magnification to determine the percentage saprophytic colonisation of baits by each fungus. The identity of the Cylindrocarpon sp. isolates was confirmed by microscopic examination and culturing of macroconidia which had been transferred from the baits.

Results and Discussion

Cylindrocarpon (CMI 196141) Cylindrocarpon tenue (CMI 196143) and Penicillium spp. were frequently found sporulating on the leaf baits. C. tenue could be distinguished from Cylindrocarpon sp. by

its shorter conidiophores with penicillate branching and its distinctly shorter macroconidia.

The percentage saprophytic colonisation of E. obliqua leaf discs, by Cylindrocarpon sp. increased during the development of regrowth forests and closely paralleled the increase in microsclerotia in both the rhizosphere and bulk forest soil series (Fig.7,8). A significant correlation ($r = 0.89$) occurred between the saprophytic colonisation of Cylindrocarpon sp. in rhizosphere soils of E. obliqua in developing forests and the number of viable microsclerotia isolated from these soils. However, the percentage saprophytic colonisation by Cylindrocarpon sp. decreased in soils from mature and oldgrowth forests even though the number of microsclerotia in these soils remained high. This decline in saprophytic colonisation was associated with increased competition by Penicillium spp. in the soils from older forests.

Comparisions of the saprophytic colonisation of Cylindrocarpon sp. in the rhizosphere and the surrounding bulk soil indicate that Cylindrocarpon sp. activities were lower in the rhizospheres of young trees compared with those in the surrounding soil. This is consistent with the numbers of Cylindrocarpon sp. microsclerotia which were also lower in the rhizospheres of healthy young trees than in their surrounding soils. However, in older trees both the saprophytic colonisation and numbers of microsclerotia of Cylindrocarpon sp. increased in the rhizospheres were greater than those in the surrounding soil. Consequently the rhizospheres of young vigorous E. obliqua appears to retard the activity and population of Cylindrocarpon sp. below that in the surrounding bulk soil. In contrast, in the rhizosphere of regrowth E. obliqua,

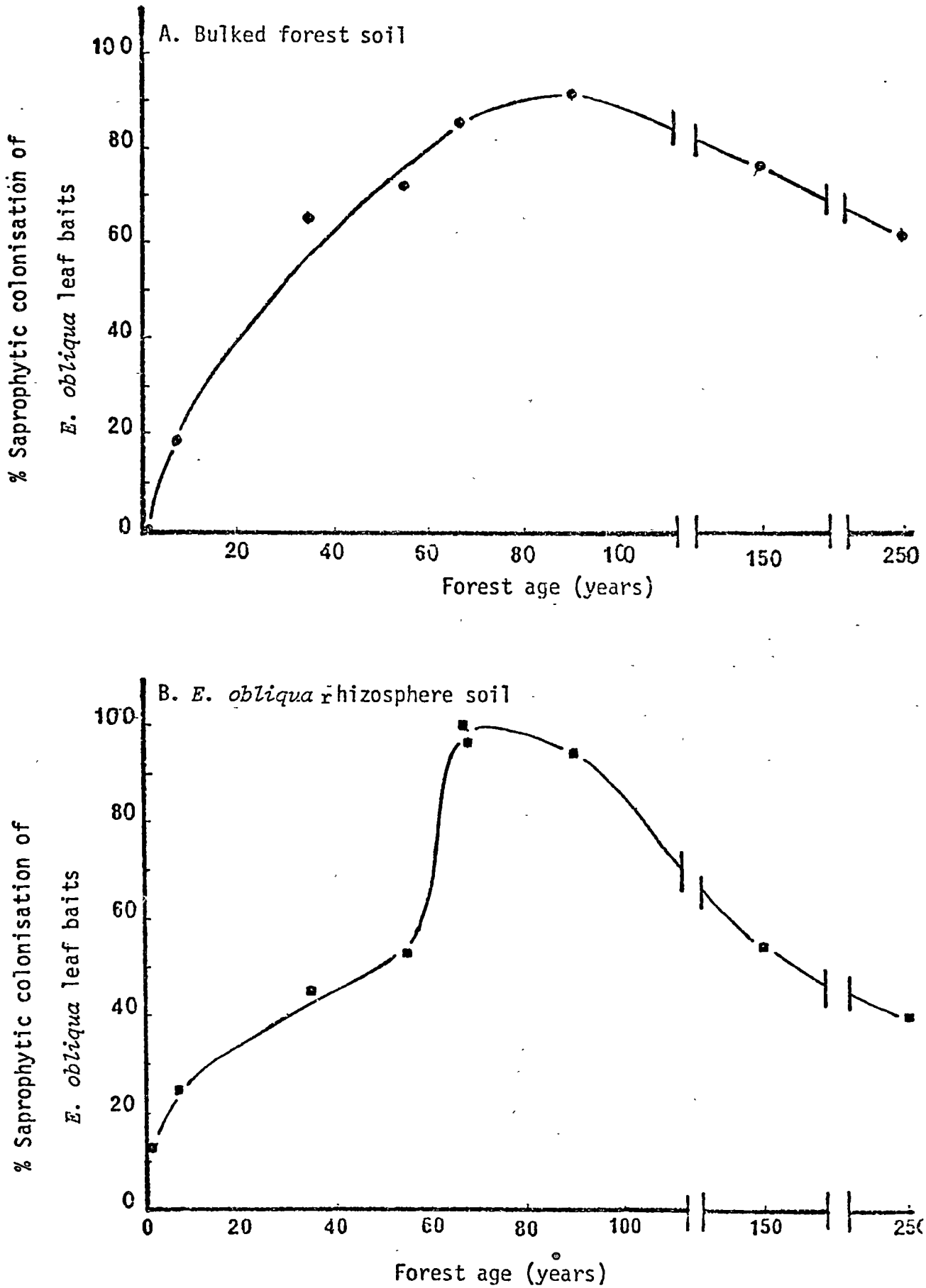


Figure 8 Changes in the competitive saprophytic colonisation activity of Cylindrocarpon CMI196141▽ in the soils and rhizospheres of E. obliqua forests during their development.

▽ isolates similar to CMI196141

particularly in trees affected by dieback, the activity and population of Cylindrocarpon sp. appears to be stimulated above that in the surrounding soils. A similar increase in the numbers of microsclerotia and saprophytic colonisation was recorded at each of the five occasions when these studies were repeated over an eighteen month period.

The two preceding experiments indicate that a close association exists between the increase in the numbers of microsclerotia and saprophytic colonisation activity of Cylindrocarpon sp. in forest soils and the intensification of crown dieback in these forests. Furthermore factors in the soils of old growth forests and the rhizosphere of young stands and healthy regrowth forests appear to be capable of retarding the activity and/or development of Cylindrocarpon sp. inoculum. Consequently studies of the pathogenicity of Cylindrocarpon sp. should pay close attention to both the quantity of inoculum present and to factors which may influence the activity of Cylindrocarpon sp. as both these factors may be important in the aetiology of regrowth dieback.

4.3 Factors Influencing Cylindrocarpon sp. in E. obliqua Forest Soils

The development and activity of Cylindrocarpon sp. inoculum in the soil of E. obliqua forests may be affected by various physical and biological factors which could influence the initiation of regrowth dieback. Consequently the effect of some of these factors on the growth and survival of Cylindrocarpon sp. was investigated. Particular attention was paid to changes in soil factors which are likely to occur during the development of these forests and which may influence the activity of Cylindrocarpon sp.

4.3.1 Physical and Environmental Factors

Physical and environmental factors which may influence the behaviour of fungi in soil include light, (Harley and Waid 1955), temperature, soil water potentials, soil aeration and hydrogen ion concentrations. Griffin (1972) has comprehensively reviewed the role of each of these factors in the ecology of soil fungi. The following studies were conducted to establish how some of these factors may influence the germination and growth of Cylindrocarpon sp. in forest soils and their implications in the aetiology of regrowth dieback.

Materials and Methods

4.3.1a Temperature

The effect of a range of incubation temperatures on the growth of Cylindrocarpon sp. was investigated in a series of agar plate studies. Potato dextrose agar (PDA) plates (Oxoid 15ml agar/9cm diam. plate) were inoculated in the centre with inverted plugs of active culture of Cylindrocarpon sp. and triplicate plates were incubated in the dark at each of 2, 6, 10, 14, 18, 22, 28 or 37°C±1°C The colony diameters were recorded daily to determine the mean daily growth in colony diameter during the phase of constant linear growth (7 to 15 days). The effect of each of the above incubation temperatures on the germination of Cylindrocarpon sp. macroconidia was also examined. Macroconidia from an active culture were inoculated onto PDA agar plugs which were incubated at the above temperatures above sterile moist filter paper. Germination percentages were determined after 48 hours of incubation.

The effect of incubation temperature on the competitive saprophytic colonisation of E. obliqua leaf discs by Cylindrocarpon sp. was also examined using the baiting technique previously described. Eight soils from under E. obliqua regrowth trees were baited with leaf discs and replicate dishes of each soil were incubated at either 6, 10, and 18°C for a total of 108 degree days. The percentage colonisation of the baits by Cylindrocarpon sp. was assessed at the end of each incubation period as previously described. Incubation of the baits for a similar total of degree days enabled the effect of temperature on the competitive saprophytic colonisation activity of Cylindrocarpon sp. and other fungi to be assessed independent of the rate of colonisation.

4.3.1b Water Activities

The effect of moisture stress on the growth of Cylindrocarpon sp. was examined using agar plates which had been adjusted to a range of water activities by the addition of mixtures of salts (Dubé, Dodman and Flentje 1971) (Appendix 1). Cylindrocarpon sp. was also grown on glass slides using Kouyeas' (1964) technique to overcome possible effects of solute toxicity in the previous technique. In this technique plugs of Cylindrocarpon sp. culture on 1% water agar were grown on glass slides in the sealed headspace above the same agars of varying water activity but not in contact with them. The plates in both experiments were incubated at 18°C±1°C and colony diameters on the plates and glass slides were periodically recorded either directly or at x 200 magnification with a stage micrometer. Mean colony growth rates during the phase of constant linear growth (7 to 15 days) were calculated for all treatments.

The effect of water potentials on the germination of Cylindrocarpon sp. macroconidia and microsclerotia was also examined by incubating propagules from active PDA cultures on water agars above agars of a range of water activities. The percentage germination of macroconidia and microsclerotia were measured after 25 and 144 hours incubation at $18^{o}C \pm 1^{o}C$ respectively.

4.3.1c Hydrogen Ion Concentration

The influence of hydrogen ion concentrations on the growth of Cylindrocarpon sp. cultures and the germination of microsclerotia was examined on Czapek's agar which had been amended to different pH values by $K\ H_2PO_4$/ $Na_2H\ PO_4$ and Citric Acid/KH_2PO_4 buffers (Appendix 1). The buffers had resulted in a pH range in the Czapek's agar after autoclaving of pH 5.4 to 8 and 2.6 to 8.0 respectively. Triplicate plates of each of the agars (15ml agar/9cm diam. plate) were centrally inoculated with an inverted plug of Cylindrocarpon sp. culture obtained from a growing colony on Czapek's agar and incubated at $18^{o}C$. The mean daily diameter growth rates during the phase of constant linear growth (7 to 15 days) was calculated for each of the agars. The percentage germination of Cylindrocarpon sp. microsclerotia was also determined on agars of different pH after 13 days incubation at $10^{o}C$.

The hydrogen ion concentration of rhizosphere soils from forest of different ages was also determined in a 1/4 soil/water mixture at $22^{o}C$ using a radiometer 22 pH meter with glass electrodes. Changes in soil pH during the development of these forests were examined in relation to the pH optima for the germination and growth of Cylindrocarpon sp. The organic matter content of these soils was

also determined from the loss of their dry weights following ignition.

Results and Discussion

4.3.1a Temperature

Cylindrocarpon sp. grew on PDA agar at temperatures ranging from 2 to 28°C, with maximum growth rates occurring between 14 and 22°C (Figure 9). Macroconidia germinated over a similar range of temperatures with close to 100% germination being recorded after 48 hours incubation at temperatures as low as 6°C. The percentage of germinated macroconidia which formed conidial chlamydospores increased at incubation temperatures above 18°C.

The competitive saprophytic colonisation activity of Cylindrocarpon sp. was higher at the lower temperatures (Figure 9) in contrast to colonisation by Penicillum spp. which increased with increasing temperature.

The prevailing temperatures in the surface soils of E. obliqua forests in southern Tasmania are low, ranging from 3 to 15°C (Madden pers. comm.). Consequently these temperatures are compatible with the germination, growth and competitive saprophytic colonisation by Cylindrocarpon sp. in these forests. These results are also consistent with previous studies by Matturi and Stenton (1964) and Taylor (1964) which indicate that Cylindrocarpon destructans has a similarly low temperature optimum.

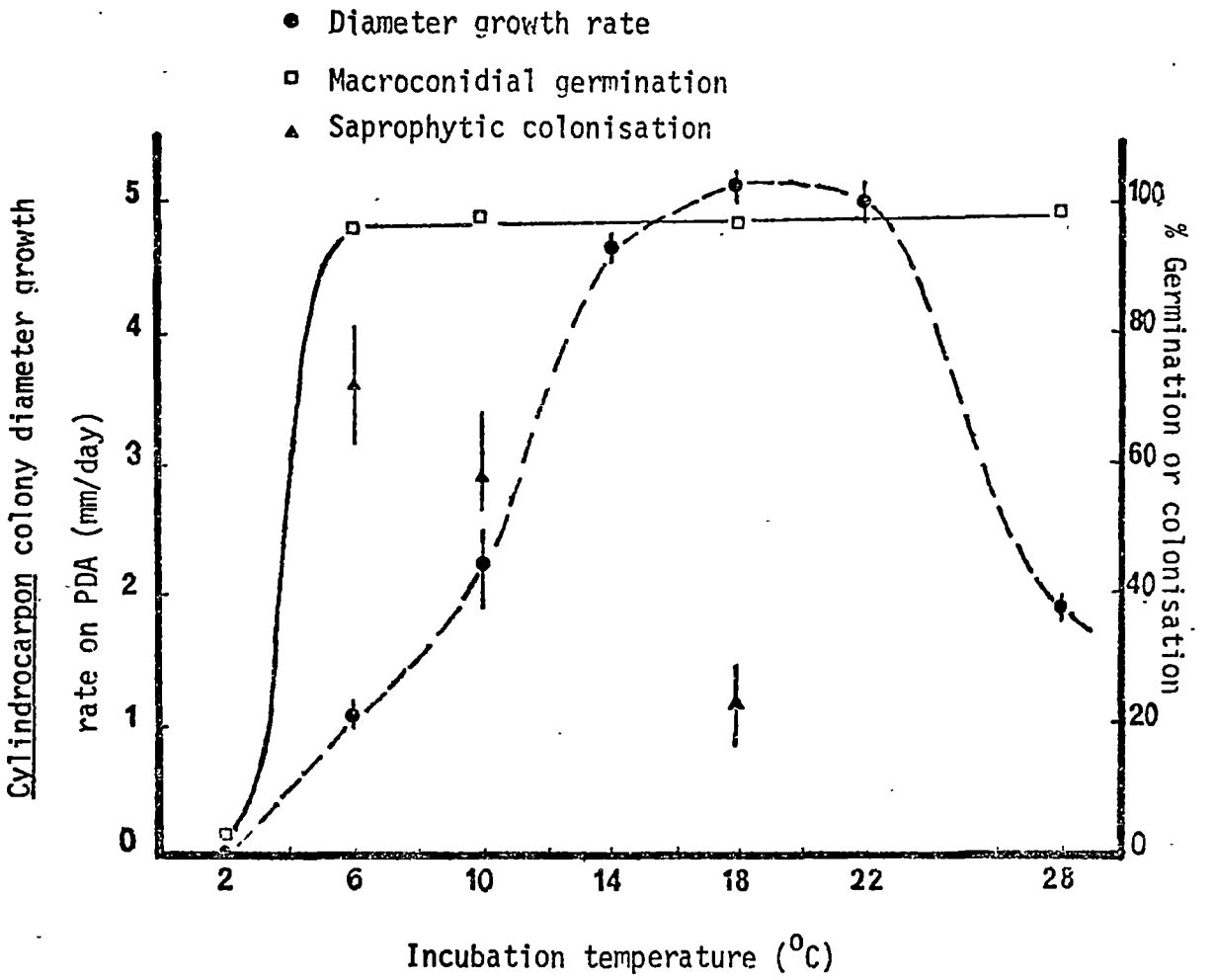


Figure 9 The effects of temperature on the growth, macroconidial germination and competitive saprophytic colonisation activity of Cylindrocarpon sp.

Vertical lines indicate twice the standard error of each treatment mean.

The cooler soil temperatures may be an important factor contributing to the high activity of Cylindrocarpon sp. in regrowth forest soil and on the roots of regrowth trees. Apart from the suitability of low temperatures for the germination and growth of Cylindrocarpon sp. low soil temperature appear to decrease the activity of competing fungi such as Penicillium and also decrease the activity of fungistatic and lytic microorganisms in the eucalypt rhizosphere (Chapter 6.4.2). Root growth of E. regnans is also reduced at lower temperatures (Cremer 1975) which may influence the capacity of trees to replace fine roots infected by Cylindrocarpon sp. and maintain adequate uptake of water.

4.3.1b Water Activities

Cylindrocarpon sp. was able to grow on agars with water activities ranging from a_w 0.999 to a_w 0.960 (1.3 to 56 bars tension) (Figure 10) while maximum growth occurred at a_w 0.990 (16 bars tension). Growth was depressed at the very high water activites a_w 0.999 (1.3 bars tension) which is consistent with other soil fungi (Griffin 1972). The relationships between water activity and growth of Cylindrocarpon sp. were similar in both the glass slide and agar plate techniques, indicating that solute toxicities were unlikely to have influenced fungal growth in the plate studies. Germination of Cylindrocarpon sp. macroconidia and microsclerotia occurred over a similar range of water activities to that in the colony growth studies.

Cylindrocarpon sp. can grow over a range of water activities which are often higher than tensions at which many plants wilt but are consistent with the growth of other soil fungi including

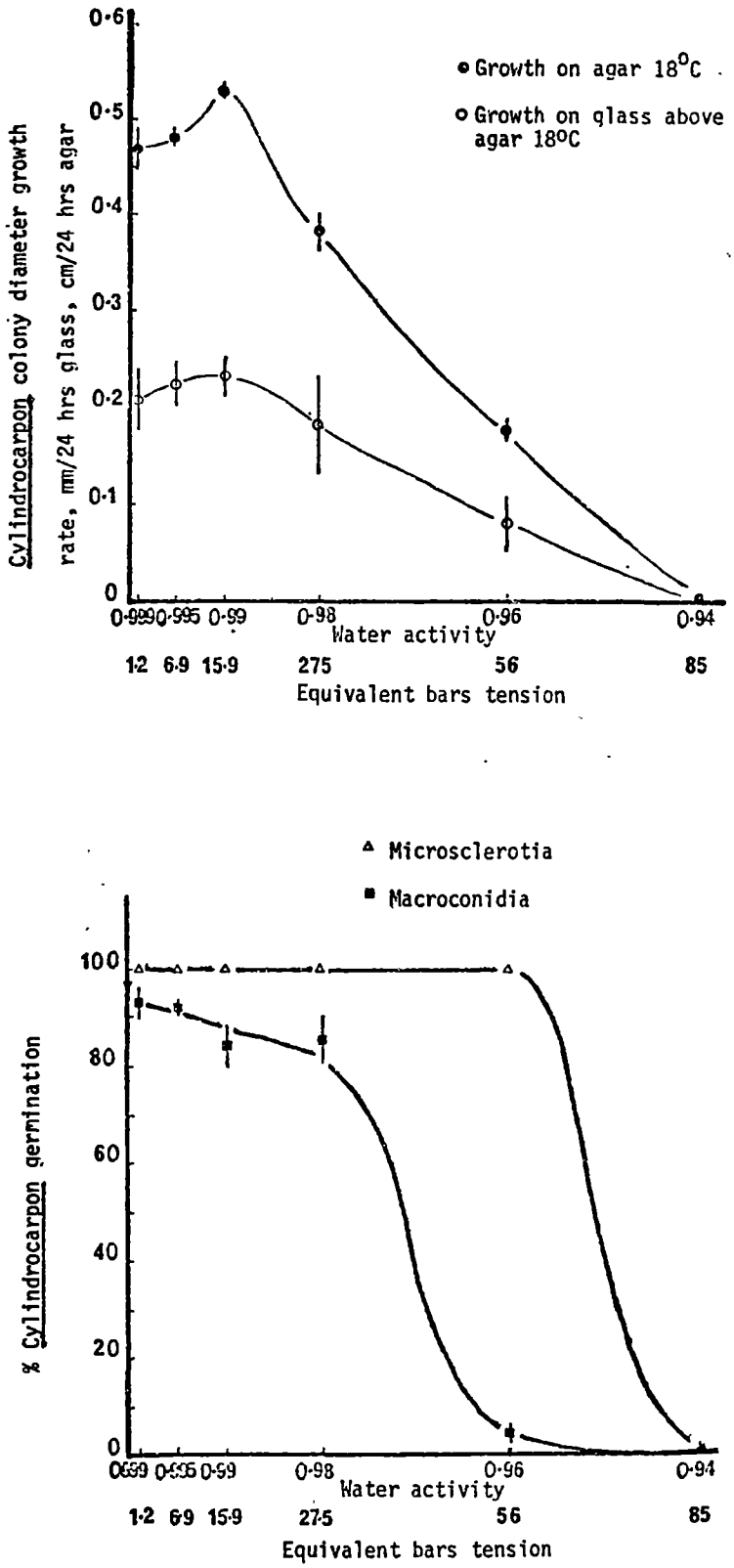


Figure 10 The effects of water activity on the growth and germination of macroconidia and microsclerotia of Cylindrocarpon sp.

Vertical bars indicate twice the standard error of each treatment mean.

Rhizoctonia solani (Dube~ et al 1971) and Gaeumannomyces graminis (Griffin 1972). As in the case of Phytophthora cinnamomi (Sommers et al 1970) and Fusarium culmorum (Griffin 1972) maximum growth rates occurred at water activities comparable to those at which many mesic plants wilt. Consequently Cylindrocarpon sp. germination and growth appeared to be optimal at the range of water potentials likely to occur in the soils, and in the root zone of E. obliqua forests affected by dieback.

4.3.1c Hydrogen Ion Concentration

Cylindrocarpon sp. grew on Czapek's agar over the range of pH from 4.0 to 6.0 in both of the buffer systems tested (Figure 11). Similar pH optima occurred for the germination of Cylindrocarpon sp. microsclerotia on Czapek's agar (Figure 11). Both the colony growth rates and percentage germination of Cylindrocarpon sp. microsclerotia declined sharply below pH 4.0 while colony growth but not germination of microsclerotia occurred up to pH 8.0.

These results are consistent with those of Cylindrocarpon destructans which also had a pH optimum from pH 4.0 to 6.0 with decreasing growth rates to pH 9.0 (Taylor 1964).

The pH of rhizosphere soils of E. obliqua forests declined during the development of the forest from pH 5.6±0.1 one year following burning to a pH of about 4.0 in regrowth forests (Table 12). The decrease may in part be associated with the initial increase in soil organic matter contents and is consistent with other studies of pH changes in forest soils following recent burning (Renbuss et al 1971). The rhizosphere soils of developing E. obliqua regrowth

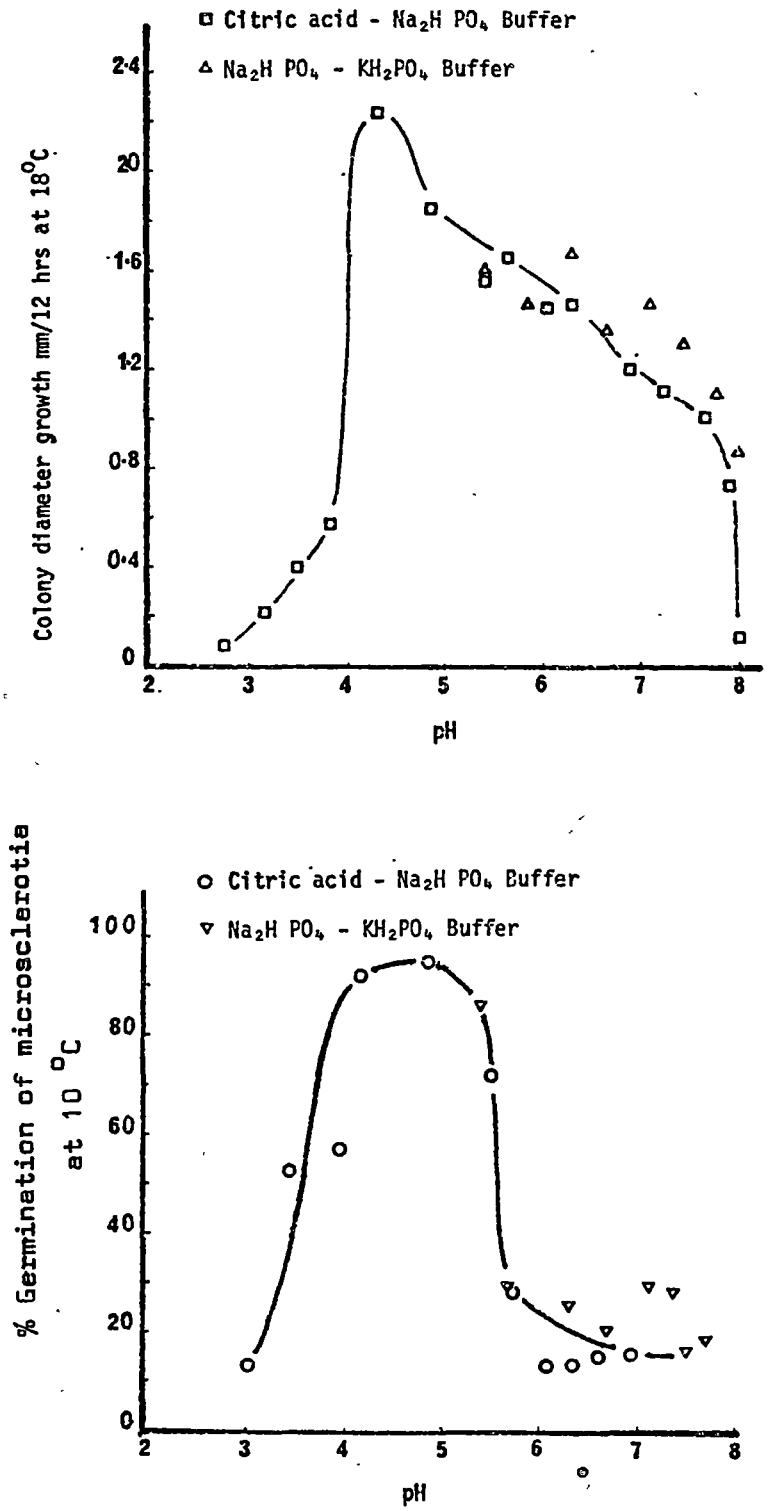


Figure 11 The effect of hydrogen ion concentration on the growth and microsclerotial germination of Cylindrocarpon sp.

TABLE 12 Changes in the hydrogen ion concentration and organic matter in E. obliqua rhizosphere soils during forest development.

Forest Age	Soil pH	Organic matter % loss of dry weight on ignition
1	5.7	14 a
7	4.3	16 a
35	3.9	48 b
55	4.0	47 b
67 Dieback	4.4	24 a
67 Healthy	4.0	-
90	3.7	50 b
150	3.6	42 b
250 +	3.6	58 b
	LSD(P=0.05)=0.3	a differs from b at (P=0.05)

forests from 1 to 70 years of age are at a pH range optimal for the germination and increase in Cylindrocarpon sp. microsclerotia and saprophytic colonisation activities (Figure 7 and 8). This period is also consistent with the period during which the intensification of root decay and crown dieback normally occurs. In constrast pH levels in mature and oldgrowth forests are less suited for the germination and growth of Cylindrocarpon sp. which is consistent with the decline in the activity and population of Cylindrocarpon sp. in these forest soils. Consequently the changes in the hydrogen ion concentration of the soil surrounding E. obliqua roots may have an important influence on the activity of the Cylindrocarpon sp. inoculum.

4.3.2 Biological Factors

The ecology of soil fungi may also be influenced by a range of biological factors in the soil environment. These include: the availability of substrate, the availability of specific nitrogen and vitamin requirements, the fungistatic and competitive effects of other soil micro-organisms and the nature of excretions from host and non-host plants. The following experiments examined the effect of some of these properties of the E. obliqua regrowth forests on the activity of Cylindrocarpon sp. and their possible implications in the development of dieback.

Materials and Methods

4.3.2a Substrate

One of the major factors which may influence the activity of Cylindrocarpon sp. in E. obliqua forest soils is the availability of a substrate. Cylindrocarpon spp. commonly colonise leaf litter and the high levels of colonisation of E. obliqua leaf baits by Cylindrocarpon sp. suggest that further investigation should be conducted of the role of leaf litter as a substrate for Cylindrocarpon sp. in these forests.

The dominant fungi which colonised E. obliqua litter in the regrowth forests were investigated in two experiments. In the first experiment E. obliqua leaf litter at various stages of decomposition was collected from regrowth forests, surface sterilised in 1% sodium hypochlorite for 30 seconds and incubated on moist, sterile filter paper for 12 days at 10°C. Fungi sporulating from the litter were recorded. In the second experiment, intact blocks ($20cm^3$) of surface litter and soil were removed from four sites in the regrowth forests and carefully separated into six litter and soil horizons which represented different stages of litter incorporation into the soil. The saprophytic colonising activity of Cylindrocarpon sp. was determined in each of these horizons using the leaf baiting technique previously described.

4.3.2b Nitrogen and Vitamin Sources

The forms of available nitrogen frequently change during the development of forests and grasslands (Florence and Crocker 1962,

Rice 1969) and may influence the ecology of soil fungi (Huber and Watson 1973). Similar changes may occur in the availability of essential vitamins. Consequently the nitrogen and vitamin requirements of Cylindrocarpon sp. were investigated to determine whether specific requirements existed for the growth of Cylindrocarpon sp.

Cylindrocarpon sp. was grown in Czapek's liquid culture in a factorial experiment with five nitrogen and four vitamin treatments. Treatments were replicated five times and comprised no nitrogen, nitrate nitrogen, ammonium nitrogen, l-asparagine and cas-amino acids. Vitamin treatments comprised thiamine HCl, biotin, thiamine HCl plus biotin and no vitamins. The experiment was conducted in 100ml flat bottles which were filled with 15ml of nutrient solution, sterilised and inoculated with washed plugs of Cylindrocarpon sp. culture which had been grown on water agar. The bottles were incubated horizontally for 15 days at 22°C with loosely fixed caps to allow adequate aeration. The dry weights of the washed mycelial mats were determined for the different treatments.

4.3.2c Soil Fungistasis

The factors in a soil which govern the germination of fungal spores are likely to be of major importance in the activity of soil fungi. The factors responsible for this fungistatis are illdefined (Watson and Ford 1972), although Balis (1976) indicated that volatiles, including allyl alcohol and acrylic acid which are produced by micro-organisms in soils may be a major contributing factor in soil fungistasis. Consequently the level of fungistasis in different E. obliqua forest soils was investigated to determine whether

fungistasis can influence the activity of Cylindrocarpon sp. and the development of regrowth dieback.

Soil from eight dieback and five healthy regrowth trees were collected from the forest and placed in Petri dishes. Sterile water agar plugs were enclosed in folded sterile cellophane and were incubated on the surface of each moistened soil for 48 hours at 10°C. The plugs were then inoculated by brush with Cylindrocarpon sp. macroconidia and replaced on their respective soils for a further 60 hours incubation at 10°C. The percentage germination of macroconidia was measured on each of the forest soils and on controls which had been incubated on sterile moistened filter paper for a similar period.

The levels of fungistasis was also determined in rhizosphere soils from E. obliqua forests of eight different ages. Cylindrocarpon sp. microsclerotia from old PDA cultures were placed onto sterile cellophane films which were placed in contact with the moistened surface of each of the soils in four replicate Petri dishes. The soils were incubated for 10 days at 10°C when the percentage germination of microsclerotia was measured. The viability of the ungerminated microsclerotia was tested by removing the cellophane films bearing the microsclerotia from the soil and incubating them for a further 48 hours at 10°C on glass slides above moistened filter paper when their germination was remeasured.

The influence of soil volatiles on the fungistasis of Cylindrocarpon sp. was also examined in each of the rhizosphere soils in the initial study. Water agar plugs were preincubated for 48 hours at 22°C in the confined headspace above each soil by placing the plugs

Results and Discussion

4.3.2a Substrate

Cylindrocarpon sp., Cylindrocarpon tenue, Penicillium, Mucor and Sympodiella spp. were the dominant fungi sporulating on the surface sterilized E. obliqua leaf litter from regrowth forests. The frequency of Cylindrocarpon sp. isolations decreased with increasing decompositon of the litter whereas Penicillium spp. increased on the more decomposed litter. Similar results were obtained when the different litter and soil horizons from regrowth forests were baited for Cylindrocarpon sp. The saprophytic colonisation activities of Cylindrocarpon sp. decreased with increasing litter decomposition and soil depth (Table 13) while Penicillium spp. increased with increasing depth of the litter horizons.

Cylindrocarpon sp. appears to be a primary coloniser of dead E. obliqua leaf litter in these forests. This is consistent with observations overseas on other Cylindrocarpon spp. and studies by Macauley and Thrower (1962) in E. regnans forests in Victoria who also recorded a Moezia sp., synonymous with Cylindrocarpon (Subramaniam 1971) as one of the dominant litter colonisers. Isolations of Penicillium and Mucor spp. were relatively higher in the Victorian studies possibly due to the higher incubation temperatures, differences in isolation technique or lower activities of Cylindrocarpon spp. in the warmer Victorian soils.

The litterfall in Victorian E. regnans forests is at a maximum of 2.9 tons per hectare in 70 to 90 year old forests (Ashton 1975a). If similar litterfalls occur in Tasmanian forests this litter could

TABLE 13 Activity of Cylindrocarpon CMI196141$^{\nabla}$ in litter horizons at different stages of decomposition and in the underlying E. obliqua regrowth forest soil.
∇ isolates similar to CMI196141

Litter Horizon	Depth (cm)	% Saprophytic Colonisation of Leaf Baits		
		Healthy forest	Dieback forest	Mean
A. UNDECOMPOSED LEAF LITTER	0-0.5	35	52	43[a]
B. DECOMPOSED ORGANIC LITTER	0.6-1	50	86[+]	68[b]
C. SURFACE OF ORGANIC SOIL	1-2	90	100	95[b]
D. FEEDER ROOT ZONE	2-4	58	84[+]	71[ab]
E. SURFACE SOIL	4-10	52	79[+]	65[ab]
F. DEEPER SOIL	10+	37	12	25[a]

+ colonisation in soil from dieback forest differs from that in healthy forest at (P=0.05)

a differs from b at (P=0.05)

account for the large and increasing supply of substrate that would be required for the development of the Cylindrocarpon sp. inoculum observed in the regrowth forests. Cylindrocarpon sp. microsclerotia are produced both in and from colonised plant substrates including leaf litter.

4.3.2b Nitrogen and Vitamin Sources

An exogenous source of nitrogen was essential for the growth of Cylindrocarpon sp. in contrast to vitamins for which no essential requirement was detected (Table 14). Growth of Cylindrocarpon sp. was greatest an l-asparagine nitrogen while growth on nitrate nitrogen exceeded that on ammonium nitrogen. There is no evidence from this experiment, that the form of nitrogen is a critical factor which may limit the growth of Cylindrocarpon sp.

As both Cylindrocarpon sp. and E. obliqua (Carrodus 1969) are able to grow on both nitrate and ammonium nitrogen it is unlikely that the form of nitrogen has a major effect on the growth of either the plant or the fungus in the regrowth forests.

4.3.2c Soil Fungistasis

Rhizosphere soil from E. obliqua regrowth forests inhibited germination of the Cylindrocarpon sp. macroconidia to various degrees with germination pecentages ranging from 6 to 82% of the sterile water control. The level of fungistasis measured in these soils did not appear to be related to the health of the tree from which the soil had been collected. Variable levels of fungistasis were also recorded in Cylindrocarpon sp. microsclerotia incubated on

TABLE 14 The effects of nitrogen and vitamin sources on the growth of Cylindrocarpon sp. in liquid culture.

Dry Wt of washed mycelial mats in mg.

	No vitamins added	Thiamine HCl	d biotin	Thiamine HCl plus d biotin
No nitrogen added	0	0	0	3.5
$NaNO_3$	46.0	67.2	63.1	54.6
$(NH_4)_2SO_4$	42.5	37.4	19.4	39.1
L asparagine	62.0	68.1	66.3	61.7
Cas - amino acids	46.0	49.8	49.3	24.0

LSD (P0.05) = 6.0

rhizosphere soils from E. obliqua forests of different ages (Table 15). Soils from 67 year old forests severely affected by dieback had the lowest levels of fungistasis in contrast to soils from mature forest which had high levels of fungistasis. The high level of variation in fungistasis obscures any statistical differences in these studies. All of the microsclerotia germinated when reincubated above moist filter paper which indicated that the inhibition had been due to fungistasis and not differences in the viability of the microsclerotia.

Variable levels of fungistasis were also recorded when Cylindrocarpon sp. macroconidia were incubated in volatiles in the confined headspace above rhizosphere soils from E. obliqua forests. Germination percentages ranged from 3 to 100% of the moist filter paper controls but were not related to tree health in these studies.

While these three experiments demonstrate that a general fungistasis does occur in regrowth forest soils no direct relationship was apparent in these initial studies between the level of fungistasis of Cylindrocarpon sp. and regrowth dieback. However, the high levels of fungistasis observed may be an important factor influencing the behaviour of Cylindrocarpon sp. in these forest soils and should receive further study.

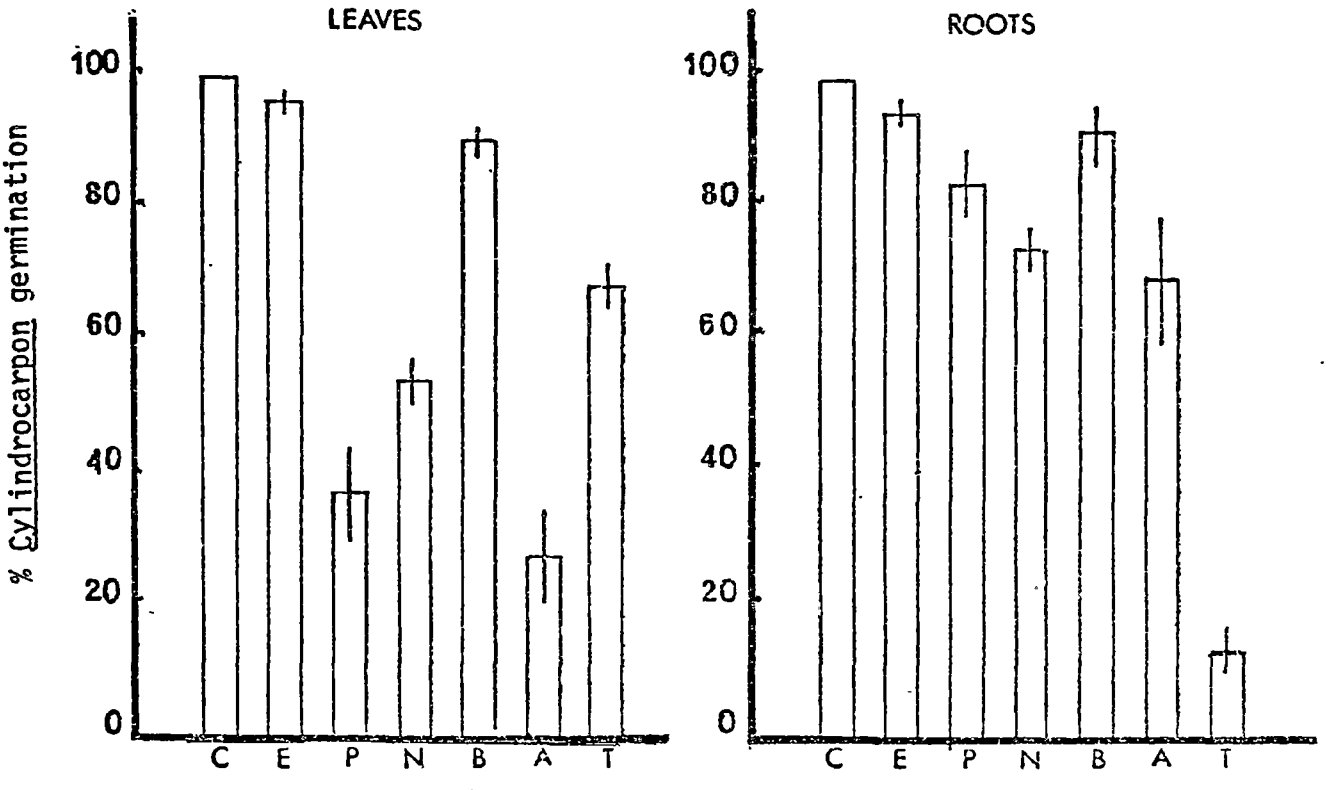
4.3.2d Vegetation

The headspace volatiles above the leaves and roots of a range of forest plants resulted in variable levels of fungistasis in Cylindrocarpon sp. macroconidia (Figure 12). The leaves and roots of dominant rainforest flora resulted in consistent reductions in

TABLE 15 Fungistasis of *Cylindrocarpon* sp. microsclerotia in soils of *E. obliqua* forests of different age.

Forest Age Years	% Germination of Microsclerotia Incubated on Forest soil	When transferred to moist filter paper
1	31	100
7	38	100
35	35	100
55	17	100
67	45	100
90	20	100
150	12	100
250+	34	100

LSD (P = 0.05) = 22



C = Filter paper controls

Regrowth forest flora

E = Eucalyptus obliqua

B = Blechnum procerum

Rainforest flora

P = Pomaderris apetala

N = Nothofagus cunninghamii

A = Atherosperma moschatum

T = Drimys lanceolata

Figure 12 The effect of fungistatic volatiles from understorey vegetation on germination of macroconidia of Cylindrocarpon sp.

Vertical bars indicate twice the standard error of each treatment mean.

germination whereas volatiles from E. obliqua and ferns, which dominate the flora of regrowth forests did not appear to have any fungistatic effect. The chemical nature of the fungistatic volatiles was not studied but the fungistatic effect did diminish with storage of the plant material.

Consequently fungistatic volatiles liberated by the roots and leaves of the rainforest flora or from their associated micro-organisms may influence the microbiology of Cylindrocarpon sp. in these soils. This may contribute to the decline in the activity of Cylindrocarpon sp. observed in mature and oldgrowth forest soil.

These investigations demonstrate the important influence that the environmental, physical and biological properties of soil can have on the microbiology of Cylindrocarpon sp. Cylindrocarpon sp. appears to be well adapted for germination and growth in the conditions existing in the soils of regrowth forests, in which it may have a major competitive advantage relative to other soil fungi. In contrast the physical, substrate and competitive environment of oldgrowth forests may be less conducive to Cylindrocarpon sp. and this may contribute to the decline of saprophytic potentials recorded therein.

The compatibility of Cylindrocarpon sp. with the conditions existing in the soils of E. obliqua forests, its consistent isolations from forest soils and its close assocation with forest development (Chapter 4.2), indicate that Cylindrocarpon sp. is a major natural component of the fungal microflora in these forests.

4.4 The Survival of Cylindrocarpon sp.

The activity and development of a fungus in soil is governed not only by factors influencing its germination and growth, but also by factors which influence its survival. Fungi may survive in soils in several ways (Garrett 1970) which include:

(a) Dormant survival of resting propagules.
(b) Competitive colonisation of dead organic substrates.
(c) Saprophytic survival within substrates infected during a previous parasitic stage.
(d) Parasitic survival on susceptible hosts.
(e) Parasitic survival on non susceptible hosts.

The role of each of the mechanisms was investigated in the survival of Cylindrocarpon sp. in the soils of E. obliqua regrowth forests.

4.4.1 Dormant Survival

Both the microsclerotia and macroconidial chlamydospores of Cylindrocarpon sp. may contribute to dormant survival. Microsclerotia from forest soils remained viable after 24 months storage in aqueous suspensions at 2°C but were rapidly killed when similar suspensions were heated for 1 hour at temperatures above 30°C. Cylindrocarpon destructans had also been shown to be sensitive to heating (Bollen 1969).

The survival of conidial chlamydospores of Cylindrocarpon sp. was not tested although these spores were effective in dormant survival in Cylindrocarpon destructans (Matturi and Stenton 1964) and

Fusarium oxysporum f sp. cubense (Garrett 1970). Macroconidia of Cylindrocarpon sp. were readily lysed by bacteria in forest soils (Chapter 6) and rapidly lost viability when incubated in air at 10°C and do not appear to be important in dormant survival.

4.4.2 Survival by Competitive Colonisation of Dead Organic Substrate

A fungus may survive in soil by the continued colonisation of dead organic substrates (Garrett 1970) provided that it:

(1) has access to a sustained supply of suitable substrate;
(2) has the ability to colonise that substrate in competition with other micro-organisms;
(3) has adequate inoculum potential at the surface of the substrate to be colonised.

Cylindrocarpon sp. has been shown to colonise E. obliqua leaf litter in these forests and it is likely that the large quantities of litter falling in such forests (Ashton 1975a) may consitute a major source of substrate for Cylindrocarpon sp. in forest soils. Cylindrocarpon sp. has a high inoculum potential in regrowth forest soils capable of infecting leaf litter (Chapter 4.2.1, 4.2.2). The high level of colonisation of E. obliqua leaf baits by Cylindrocarpon sp. also suggests that Cylindrocarpon sp. has a high competitive saprophytic colonising ability for E. obliqua leaf substrate but this ability was further examined under controlled conditions.

Materials and Methods

Soil from E. obliqua regrowth forest and sterile washed sand were each mixed with Cylindrocarpon sp. inoculum to produce mixtures consisting of 0, 2, 10, 50, 80 and 100% inoculum. The inoculum consisted of a 3% cornmeal, 97% sand mixture in which Cylindrocarpon sp. had been grown for 6 weeks at 22°C. The soil and sand inoculum mixtures were placed in Petri dishes, moistened and the E. obliqua leaf disc technique was used to determine the saprophytic colonisation activity of Cylindrocarpon sp. in each of the soil or sand inoculum mixtures.

Results and Discussion

The percentage of leaf baits which were colonised by Cylindrocarpon sp. increased rapidly in the sands mixed with increasing amounts of Cylindrocarpon sp. inoculum but more slowly in the forest soils (Figure 13). This may have been due to the higher levels of antagonistic and competing micro-organisms existing in forest soil than in sterile sand. Cylindrocarpon sp. appeared to have a high saprophytic colonisation ability under these conditions relative to that reported for Cylindrocarpon destructans (Matturi and Stenton 1964) and other soil fungi (Balis 1975).

Consequently the high substrate supply, inoculum potential and competitve saprophytic colonisation ability indicate that Cylindrocarpon sp. should have a high capacity to survive in these forest soils via the saprophytic colonisation of substrate.

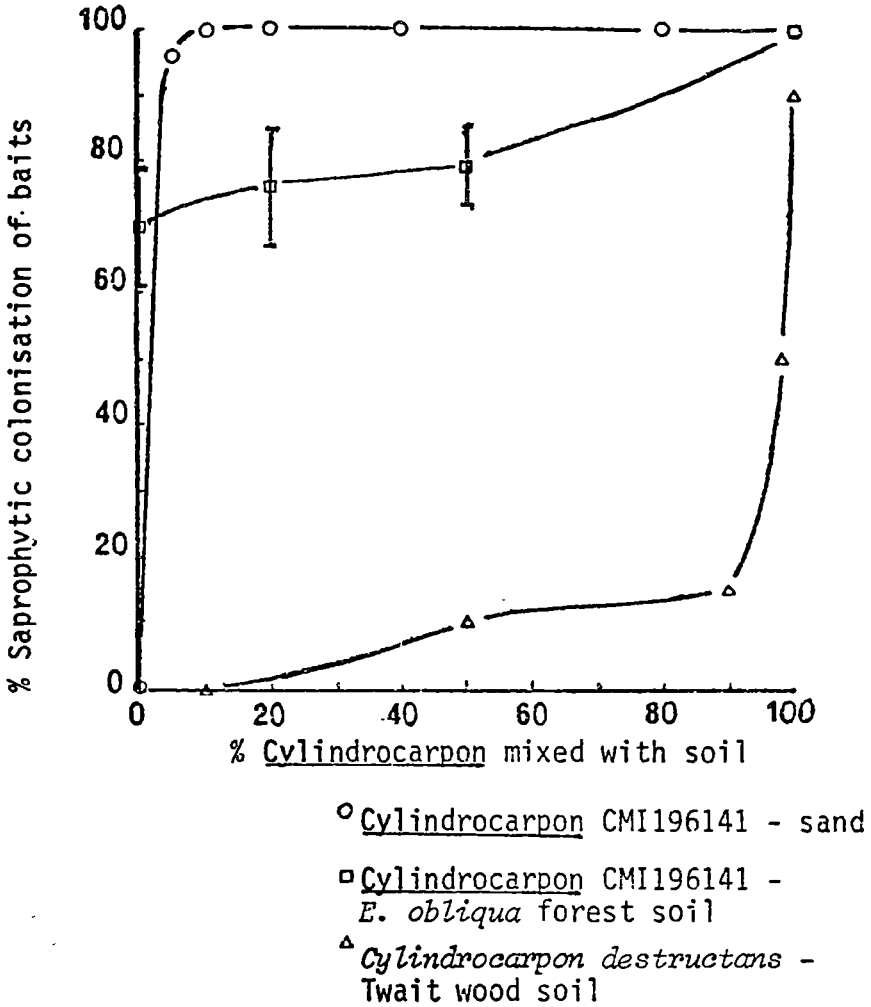


Figure 13

The competitive saprophytic colonisation activity of Cylindrocarpon sp. in relation to inoculum concentrations in sand and E. obliqua forest soils.

Percentage saprophytic colonisation by Cylindrocarpon destructans (C. radicicola) in Twait wood soil after 72 hours incubation (Matturi and Stenton 1964).

Vertical bars indicate twice the standard error of each mean.

4.4.3 Saprophytic Survival in Infected Substrate

A fungus may survive within colonised substrate for varying lengths of time depending on the rate at which the substrate is utilised, the cellulolysis rate, and the relationship between the cellulolysis rate and the growth requirements of that fungus: the cellulolysis adequacy index (Garrett 1970, 1975a,b). To maximise saprophytic survival the fungus should utilise substrate at the slowest rate which is still sufficient to maintain its growth and competitiveness relative to other fungi. While these principals may govern the saprophytic survival of a fungus other factors such as the production of fungal toxins, the requirement of external nutrients and the composition of the substrate also influence this form of survival. Cylindrocarpon sp. does have a slow growth rate, a low cellulolysis rate and is capable of producing fungal toxins (Chapter 7.4) all of which are consistent with Cylindrocarpon sp. having a high capacity for saprophytic survival in infected substrate.

4.4.4 Parasitic Survival

The parasitic growth of a fungus, either as non pathogenic infections or as latent infections may also constitute a means of survival. Cylindrocarpon sp. colonises the cortex of the fine roots of E. obliqua throughout the development of the forests (Table 10) which may represent a form of parasitic survival. This is further examined in Chapter 7.

The experiments in this chapter have examined factors which influence the germination, growth and survival of Cylindrocarpon sp.

in forest soils and may account for the observed increase in population and activity of Cylindrocarpon sp. throughout the growth and dieback of E. obliqua forests. Cylindrocarpon sp. is very well adapted for growth and survival under the conditions existing in these E. obliqua regrowth forests and appears to be a natural microbiological component closely associated with the development, dieback and succession of E. obliqua in these forests. However further studies, which are described in the following chapters, are needed to determine how Cylindrocarpon sp. may be involved in the aetiology of dieback in the E. obliqua regrowth forests of southern Tasmania.

CHAPTER 5

THE PATHOGENICITY OF CYLINDROCARPON SP. ON E. OBLIQUA SEEDLINGS

The involvement of Cylindrocarpon spp. in plant disease has not been clearly defined. Whereas some studies have demonstrated primary pathogenicity (Scholten 1964, Kluge 1966), many pathogenicity studies have remained inconclusive (Hann and Eno 1966) or have given inconsistent results (Paget 1975). Similarly some of the pathogenicity studies with E. obliqua seedlings and Cylindrocarpon sp. also failed to result in seedling mortalities even though root infection was consistently confirmed (Chapter 3.8).

Most of the pathogenicity studies have been conducted on seedlings in the glasshouse and with low levels of pure culture inocula. Results from these studies may have little relevance to the causation of diseases of older perennial plants in the field which are often associated with a complex of contributing stress factors (Chapter 3). Consequently it may be important to study the pathogenicity of potential disease agents on plants which have been preconditioned to the stresses and conditions existing in the field. The experiments in this chapter attempt to examine the pathogenicity of Cylindrocarpon sp. on E. obliqua seedlings under the inoculum, environmental and plant conditions likely to occur in E. obliqua regrowth forests. In this way an attempt was made to relate the pathogenicity of Cylindrocarpon sp. on seedling plants to an understanding of the involvement of this fungus in root decay and dieback of maturing regrowth forests.

5.1 The effect of increasing inoculum levels on the pathogenicity of Cylindrocarpon sp.

The numbers of microsclerotia and activity of Cylindrocarpon sp. increase greatly in the soils of developing regrowth forests and this increase closely parallels the intensification of crown dieback in these forests (Chapter 4.2). As the level of inoculum may influence the initiation and expression of disease (Van der Plank 1975), investigations were conducted of the effects of increasing levels of inoculum of Cylindrocarpon sp. on its pathogenicity to E. obliqua seedlings.

Materials and Methods

Soil from healthy E. obliqua regrowth forests and washed sterile sand, were mixed with different proportions by weight of Cylindrocarpon sp. inoculum. The inoculum consisted of a 3% cornmeal sand mixture on which Cylindrocarpon sp. had been grown for six weeks at 22°C. Each of the soil or sand inoculum mixtures was placed in six replicated pots, at 25g per pot, and five E. obliqua seedlings which had been grown from surface sterilised seed on sterile vermiculite for two months were transplanted into each pot. The seedlings were grown at 12°C in natural light and were regularly watered to field capacity. Seedling mortalities were recorded over the following 50 days, when the roots of the remaining live seedlings were removed from the soil, surface sterilised in 1% sodium hypochlorite for 30 seconds and incubated on sterile moist filter paper for 5 days at 22°C. The percentage of roots from which Cylindrocarpon sp. was reisolated was determined at x 50 magnification.

The same soils were then replanted with a further four, 2 month old E. obliqua seedlings which were grown under similar conditions for a further 37 days. Mortality and root infection levels were similarly recorded in these seedlings.

Results and Discussion

Mortalities in the E. obliqua seedlings increased in both the sand and forest soils with increasing levels of added Cylindrocarpon sp. inoculum (Figure 14). Forest soils had higher mortality levels than the sand at low levels of added inoculum which may be due to the high natural populations of Cylindrocarpon sp. which were present in the unsterilized forest soil. However the rate of increase in mortalities with increasing additions of inoculum was similar for both the sand and forest soil treatments and it is highly likely that seedling mortalities in the forest soil were due to the Cylindrocarpon sp. inoculum.

The level of Cylindrocarpon sp. root infection was distinctly higher than the mortality levels in both the forest soil and sand series. The rates with which root infection increased with added inoculum also differed between the sand and forest soil. Root infections at the lower additions of inoculum were lower in the forest soil, which contained other micro-organisms, than in the sand which had been sterilised to remove competing micro-organisms.

Mortalities in the replanted E. obliqua seedlings were markedly lower than in the initial planting in both the sand and forest soil mixtures. However, root infection levels by Cylindrocarpon sp. were similar to those in the first planting. The decrease in seedling

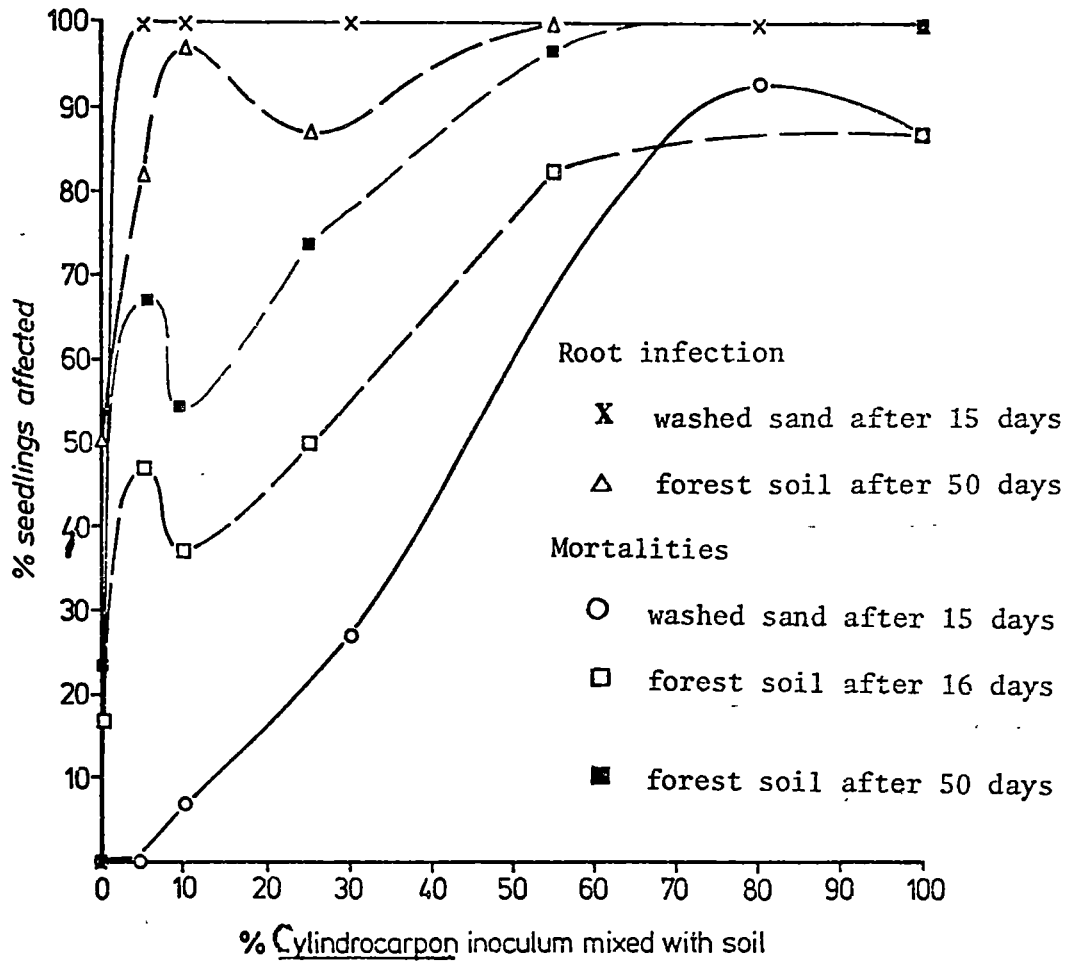


Figure 14 Root infection and mortalities of E. obliqua seedlings in sand and regrowth forest soil mixed with Cylindrocarpon sp. inoculum.

mortalities appeared to be related to a decrease in the pathogenicity but not the infectivity of the Cylindrocarpon sp. inoculum with age.

Infection levels in the roots of E. obliqua seedlings were similar to the levels of saprophytic colonisation of E. obliqua leaf substrate in similar sand or forest soil inoculum mixtures (Chapter 4.4.2). It would appear that the infection of the roots of living E. obliqua seedlings by Cylindrocarpon sp. may be influenced by similar factors to those which affect the saprophytic colonisation of dead leaf baits. However, root infections alone did not account for root decay and mortalities in these E. obliqua seedlings as moderate to high infection levels occurred even in apparently healthy seedlings at the low levels of added inoculum. This is consistent with the isolation of Cylindrocarpon sp. from the fine roots of young and healthy forest trees (Chapter 3.7) and the non-pathogenic infections in previous studies (Chapter 3.8).

The distinct patterns of root infection and seedling mortalities with increasing levels of Cylindrocarpon sp. inoculum suggest that a root infection and a seedling mortality stage may exist in the pathogenicity of Cylindrocarpon sp. on E. obliqua seedlings. Root infection levels appeared to vary with soil factors but were not greatly affected by the level or age of added inoculum. In contrast seedling moratlities were less affected by soil factors but were closely related to the level and age of added Cylindrocarpon sp. inoculum. The differences between two apparent stages in the pathogenicity of Cylindrocarpon sp. on E. obliqua seedlings is further examined in Chapter 7.

This experiment has demonstrated that the amount of Cylindrocarpon sp. inoculum may have a major influence on the mortality of E. obliqua seedlings and that at high concentrations, Cylindrocarpon sp. may be pathogenic on E. obliqua seedlings. Consequently it is highly likely that the marked increase in the population and activity of Cylindrocarpon sp. in the soils and rhizosphere of E. obliqua regrowth forests may also be a major factor which could contribute to the increased root decay and dieback recorded in the regrowth forests.

5.2 Effects of Soil and Environmental Factors on the Pathogenicity of Cylindrocarpon sp.

The development of dieback in the E. obliqua regrowth forests is associated with a range of soil and environmental stress factors which may influence tree growth directly and indirectly by influencing factors such as the pathogenicity of Cylindrocarpon sp. and other soil fungi. The following experiments examined the effects of waterlogging, drought and defoliation stresses and low temperatures on the pathogenicity of Cylindrocarpon sp. on E. obliqua seedlings. The stress treatments were applied to the E. obliqua seedlings at the time of inoculation thereby affecting both the host plant and the fungal inoculum.

5.2.1 Waterlogging, Drought and Defoliation Stress

Materials and Methods

Waterlogging, drought or defoliation stress treatment were imposed in potted E. obliqua seedlings which had been grown for 5 months in

freely drained sand peat (1:1 mixture)(Baker 1957). The waterlogging was imposed by standing 16 seedlings in individual containers of water so that the water level was 2cm below the soil surface and then allowing the water to be transpired to field capacity (after 10-14 days) before reimposing the same treatment. The drought treatment involved repeatedly withholding water from sixteen seedlings until wilting was apparent and then rewatering them to field capacity. Defoliation consisted of removing all but the upper expanded leaf pair and growing tip from each of the sixteen seedlings. The defoliated seedlings and the 16 unstressed control seedlings were regularly watered with deionised distilled water to free drainage. All seedlings were grown in a glasshouse at 18-22^{o}C and received regular additions of Hoagland's nutrient solution as part of their watering treatment.

Four seedlings in each stress treatment were inoculated just prior to the imposition of the stress treatments with either Cylindrocarpon sp., Phytophthora cinnamomi, the two fungi combined or left uninoculated. P. cinnamomi was included in this experiment to enable comparisons of its pathogenicity with that of Cylindrocarpon sp. The inoculum consisted of half a fully grown culture (9cm diameter) of the respective fungus which was mixed into the surface 1cm of soil around each seedling. Mixed quarter cultures were used in the dual inoculation to maintain similar total amounts of fungal inoculum in all the treatments. The fungal cultures had been grown on PDA and CMA for Cylindrocarpon sp. and P. cinnamomi respectively. Disease symptoms in the plants were recorded for 80 days when all plants were harvested and the washed root systems assessed for root decay by a visual rating method (Table 16). Subsamples of root tissue were surface sterilised in 1%

sodium hypochlorite for 30 seconds and plated on PDA and P10 VP (Tsao and Ocana 1969) agars for the reisolation of Cylindrocarpon sp. and P. cinnamomi respectively. The dry weights of leaves and fine roots were measured for all seedlings.

Results and Discussion

Cylindrocarpon inoculation resulted in mortalities in single seedlings in both the defoliation and waterlogging treatments. These occurred after 72 and 11 days respectively. No mortalities were recorded in the uninoculated control seedlings with these treatments (Table 16). Mortalities were highest in the waterlogged seedlings inoculated by P. cinnamomi. The imposition of a drought stress at the time of inoculation did not result in seedling mortalities in any of the inoculation treatments above that in the uninoculated droughted seedlings.

Root rot increased significantly as a consequence of Cylindrocarpon sp. inoculation in the defoliated seedlings whereas the smaller increase in the waterlogged or unstressed seedlings was not significant. Cylindrocarpon sp. appeared to be less damaging in this respect than P. cinnamomi, which resulted in increased root decay in stressed and unstressed seedlings. Waterlogging alone also resulted in increased root decay but at lower levels than in the inoculated and waterlogged seedlings. The survival of seedlings in all waterlogged treatments was associated with the production of adventitous surface roots. Both Cylindrocarpon sp. and P. cinnamomi were consistently reisolated from the surface sterilised roots taken from the root systems of inoculated living seedlings.

TABLE 16 Mortality and root rot of E. obliqua seedlings following inoculation with Cylindrocarpon sp. and P. cinnamomi under various conditions of stress

Inoculum	STRESS TREATMENT			
	Normal	Waterlogged	Defoliated	Droughted
A. Seedling Mortalities at 80 days Days after inoculation when seedlings died				
a. Uninoculated	0	0	0	45,45,49,6
b.+ Cylindrocarpon	0	11	72	49,64,64,78
c.+ P. cinnamomi	0	28,28,31	0	45,78
d.+ Cylind. + P. cinn	0	28,35,64	0	45,45,45,64
B. Root Rot at 80 days Mean of Rating 0-5* LSD (P0.05) 0.9				
a. Uninoculated	1.5	2.5A	1.3	1.0
b.+ Cylindrocarpon	2	3.0 A	2.8	1.8
c.+ P. cinnamomi	2.5 A	5.0	2.5	1.8
d.+ Cylind. + P. cinn.	2.5	3.8 A	1.0	1.5
C. Reisolations of Cylindrocarpon from surfaced sterilized roots				
a. Uninoculated	0	0	0	0
b.+ Cylindrocarpon	+	+	+	+
c.+ P. cinnamomi	0	0	0	0
d.+ Cylin. + P. cinn.	+	+	+	+
D. Reisolations of P. cinnamomi from surface sterilized roots				
a. Uninoculated	0	0	0	0
b.+ Cylindrocarpon	0	0	0	0
c.+ P. cinnamomi	+	+	+	+
d.+ Cylin. + P. cinn.	+	+	+	+

* Rating 1 =no rot, 2 < 10%, 3 = 11-40%, 4 = 41-90%, 5 = > 90%

A Adventitous Roots formed in Surviving Seedlings Only

Inoculation with Cylindrocarpon sp. decreased the root and leaf dry weight in the defoliated and waterlogged seedlings, but had no effect in the unstressed controls (Figure 15). These results are consistent with those of previous experiments (Chapter 3.8, 5.1) which indicate that under conditions suitable for healthy seedling growth, root infections but no mortalities or growth reductions occur as a result of Cylindrocarpon sp. inoculation. Inoculation with P. cinnamomi caused reductions in leaf and root growth in both the stressed and unstressed seedlings.

There was no evidence of any synergism between Cylindrocarpon sp. and P. cinnamomi in the dual inoculation treatments. Rather root and shoot weights some of the plants with dual inoculation were higher than in singly inoculated plants. Further studies on the interaction of these two fungi are described in Chapter 7.

The results of this experiment indicate that Cylindrocarpon sp. was capable of infecting the roots of stressed and unstressed E. obliqua seedlings but only caused reductions in seedling growth, root decay or mortalities in E. obliqua seedlings which had been stressed by waterlogging or defoliation. In contrast, P. cinnamomi was capable of causing root decay, growth reductions or death in the unstressed as well as the stressed E. obliqua seedlings.

5.2.2 Low Temperature Stress

As the low temperatures in the soils of E. obliqua forests may reduce root growth (Cremer 1975) and affect the activity of Cylindrocarpon sp. (Chapter 4.3.1a) investigations were conducted on the pathogenicity of Cylindrocarpon sp. on E. obliqua seedlings at

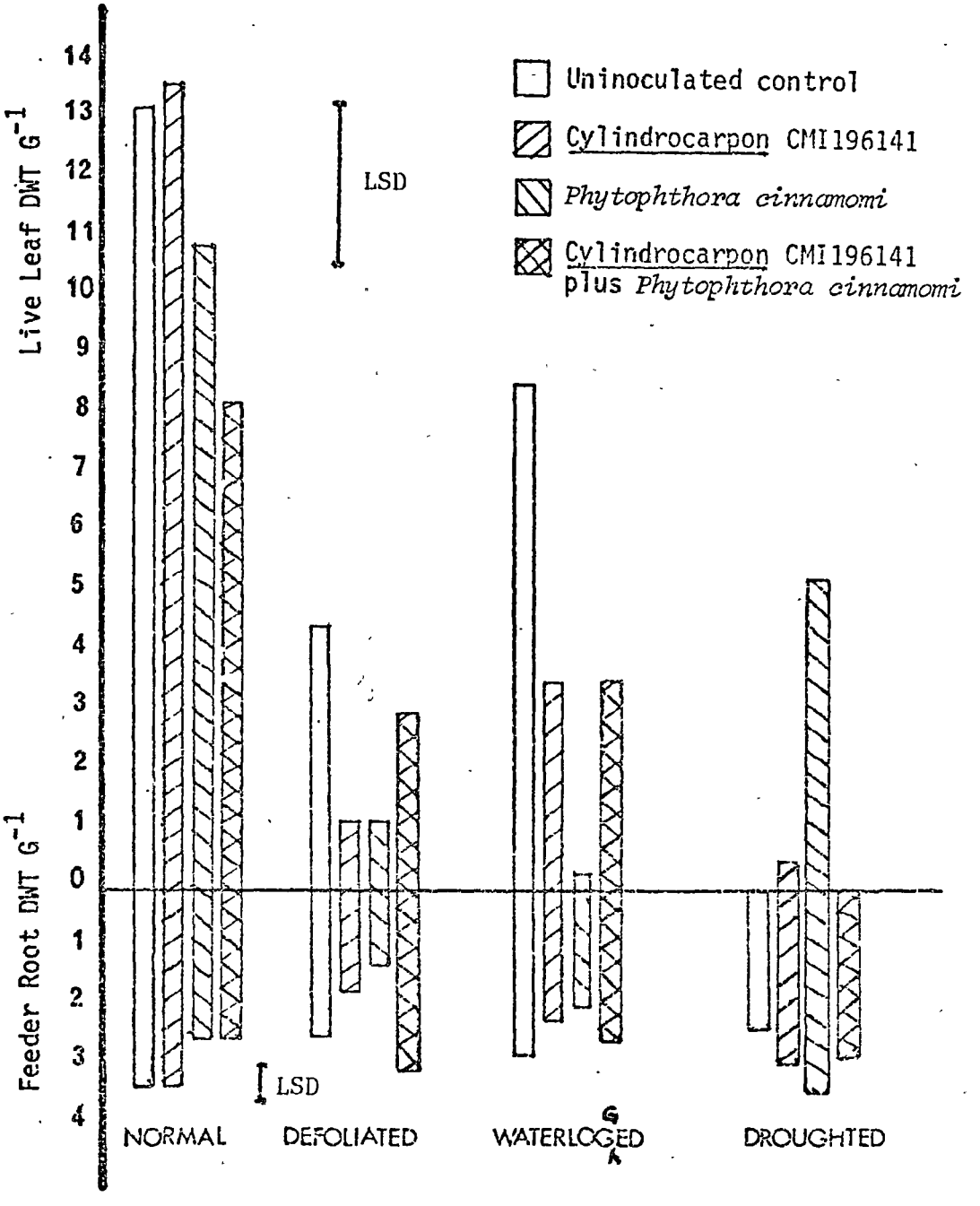


Figure 15 Root and leaf growth in waterlogged, defoliated and drought stressed E. obliqua seedlings inoculated with Cylindrocarpon sp. and or Phytophthora cinnamomi.

LSD Shoots (P0.05) 0.264
Roots (P0.05) 0.058

low soil temperatures similar to those occurring in the regrowth forests.

Materials and Methods

E. obliqua seedlings, which had been grown in autoclaved vermiculite for one month were transplanted singly into the surface soil of twenty pots containing 500g of steamed sand peat mix (Baker 1957). Ten of these pots had been inoculated with a 3mm thick layer of Cylindrocarpon sp. inoculum at 75% of the pot height while the other ten pots had been inoculated with a similar layer of sterile sand. The inoculum consisted of mixtures of sand and 3% cornmeal on which Cylindrocarpon sp. had been grown for 6 weeks at 22°C. The seedlings were grown in a naturally lit growth cabinet for 18 weeks at 10°C when they were transferred to a glasshouse at 20-22°C for a further 13 weeks. The seedlings were watered daily with free drainage and received weekly additions of Hoagland's nutrient solution. The mortality and growth of seedlings was recorded periodically and the reisolation of Cylindrocarpon sp. from surface sterilised roots as well as the leaf area and leaf and root dry weights were determined after 31 weeks growth.

Results and Discussion

Two mortalities occured in the E. obliqua seedlings which had been inoculated with Cylindrocarpon sp. whereas no mortalities occurred in the uninoculated seedlings during the 18 weeks growth at 10°C. The rate of height growth over 18 weeks at 10°C was also substantially lower in the inoculated seedlings, 4.2mm/week relative to 7.6mm/week in the control seedlings (Figure 16). When these

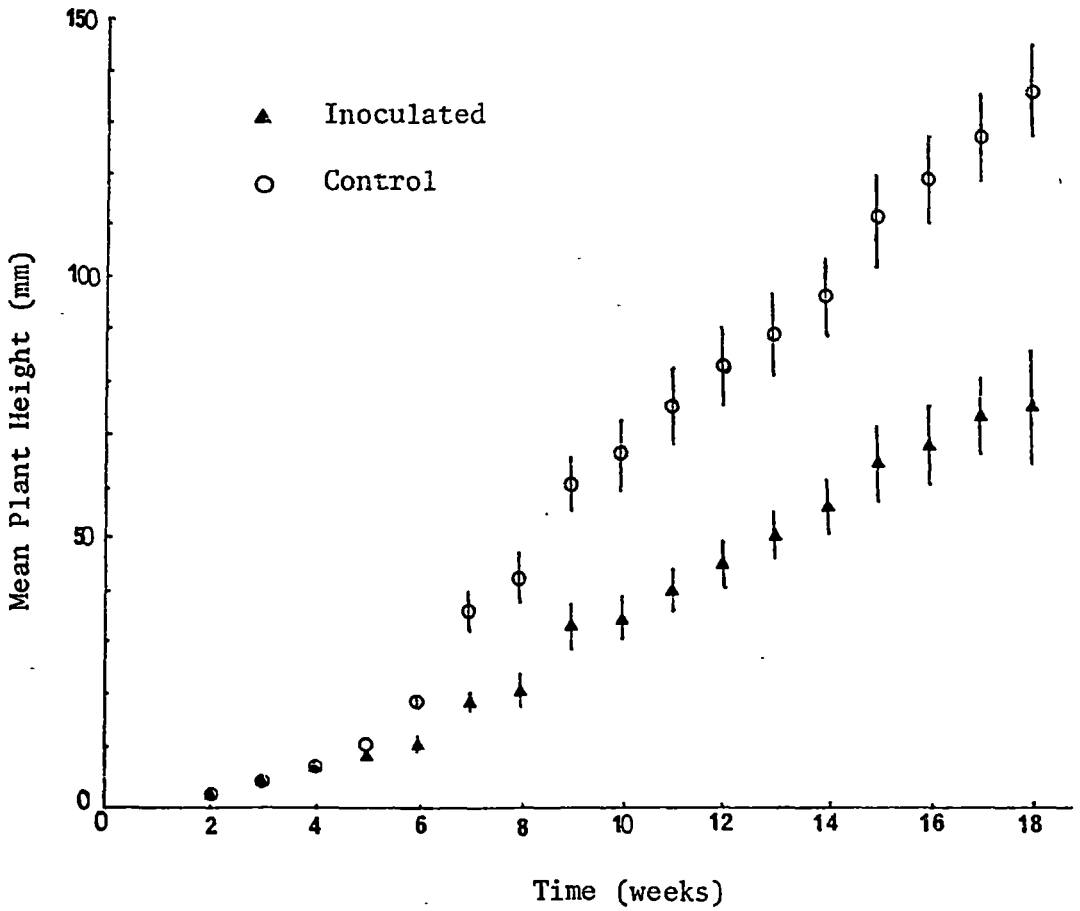


Figure 16 The effects of Cylindrocarpon sp. inoculation on the growth of E. obliqua seedlings at 10°C.

Vertical bars indicate twice the standard error of each mean.

seedlings were transferred to 22°C for a further 13 weeks the mean height increment of all seedlings increased but remained proportionally lower in the inoculated seedlings (Table 17). Leaf areas, leaf dry weights and root dry weights at the end of the experiment were all lower in the inoculated seedlings (Table 17). Cylindrocarpon sp. was consistently reisolated from the surface sterilised roots of the live inoculated seedlings.

The mortality of E. obliqua seedlings and the reduction in seedling growth due to Cylindrocarpon sp. inoculation at 10°C in this experiment contrasts with the results of previous experiments conducted at 18-22°C (Chapter 5.2.1). This may be associated with higher rates of root replacement at the higher temperatures. However once debilitating infections have developed it would appear that the infections and their effects on plant growth may not be reduced simply be a return to temperatures favourable for plant growth.

These results show that Cylindrocarpon sp. is capable of infecting and rotting the fine roots of E. obliqua seedlings under soil and environmental conditions similar to those existing in regrowth forests affected by dieback. However the pathogenicity of Cylindrocarpon sp. on E. obliqua seedlings appears to be greatly influenced by the effects of these soil and environmental factors and they are likely to play a major role in the aetiology of dieback in the E. obliqua forests in southern Tasmania.

TABLE 17 The effect of Cylindrocarpon sp. inoculation on the dry weight, leaf area and growth rates of E. oblique seedlings grown for 18 weeks at 10°C and then transferred to 18-22°C for a further 13 weeks.

Growth Parameter	Seedlings Inoculated with Cylindrocarpon sp.	Uninoculated Contro Seedlings
1. Height Increment mm/week		
0-18 weeks at 10°C	4.2 [a]	7.6 [b]
19-31 weeks at 18-22°C	18.7 [c]	33.5 [d]
2. Leaf Area Week 31 $mm^2 \times 10^4$	2.81 ± 0.57	5.03 ± 0.43
3. Leaf Dry Weight (g) week 31	2.00 ± 0.27	2.93 ± 0.32
4. Root Dry Weight (g) week 31	0.57 ± 0.11	0.96 ± 0.15
5. Root Shoot Ratio week 31	0.27	0.32

a differs from b at (P=0.05)

c differs from d at (P=0.05)

5.3 The Effect of Host Physiology on the Pathogenicity of *Cylindrocarpon* sp.

Some insight into the aetiology of diseases of mature trees may be gained by imposing physiological conditions similar to those existing in maturing trees in seedlings and studying the effects of these induced stresses on the pathogenicity of fungi. The following three experiments investigated the effects of a variety of preinoculation stress treatments on the pathogenicity of *Cylindrocarpon* sp. in *E. obliqua* seedlings. The stress treatments were all imposed prior to inoculation in order to test the effects of the treatments on the physiology of the seedlings rather than the effects of the treatments themselves on the activity of *Cylindrocarpon* sp. In this way an attempt was made to increase the relevance of these pathogenicity tests to the aetiology of dieback in the *E. obliqua* regrowth forests. The stress treatments include root pruning to decrease root shoot ratios, preinoculation drought stress and stress associated with different growth stages in *E. obliqua* seedlings of similar age.

5.3.1 Root Shoot Ratios

The transition of regrowth forests to mature forests may result in a decline in the photosynthate available to fine roots which may influence; the root shoot ratio, the ability of roots to take up water; the replacement of fine roots and patterns of root exudation (Bormann 1965, Aung 1974). The stresses resulting from these changes may influence the pathogenicity of fungi. The following experiment examined the effects of stress, associated with differences in the root shoot ratio of *E. obliqua* seedlings on the pathogenicity of *Cylindrocarpon* sp.

Materials and Methods

E. obliqua seedlings which had been grown in 500g of sand/peat for 9 months were given one of four root pruning treatments. Either 0, 25, 50 or 75% of the root systems and soil of ten seedlings per treatment were cut off vertically in sectors and replaced into their respective pots inside a plastic liner. The forty seedlings were then grown in a naturally lit growth cabinet at 10°C for 5 days to allow them to recover from the immediate effects of the root pruning treatments and develop physiological stress associated with their altered root shoot ratios.

Ten fine root tips in five seedlings from each pruning treatment were then inoculated with $2mm^3$ plugs of Cylindrocarpon sp. culture on PDA. The ten unsuberised root tips which had not been severed from the seedling were inoculated by placing the inoculum through flaps cut in the plastic liner around the roots and soil. Five control seedlings were similarly inoculated with plugs of water agar.

Two seedlings, which had their tops cut off 5 days previously, were also inoculated with either Cylindrocarpon sp. or water agar using the same technique to establish the relative level of root infection in decapitated plants.

The plants were grown in a growth cabinet at 10°C with natural light and were watered daily to field capacity. The plants were inspected regularly for disease symptoms and after 10 days all the inoculated and control roots from each pruning treatment were removed, surface sterilised (1% sodium hypochlorite for 30 seconds), and incubated on

moist filter paper for 5 days at 22°C. The roots were examined for the level of root infection by Cylindrocarpon sp.

The water tension within each of the seedlings was measured with a pressure bomb (Boyer 1967) following the pathogenicity study to establish the effects of the different root pruning and inoculation treatments on plant moisture stress.

Results and Discussion

Neither the root pruning nor inoculation treatments resulted in the death of E. obliqua seedlings. However, the percentage of inoculated roots from which Cylindrocarpon sp. was reisolated increased with increasing intensities of preinoculation root pruning (Figure 17). All the roots of the decapitated seedlings were infected by Cylindrocarpon sp. Seedling water tensions increased in the 50% root pruning treatment.

Consequently the partial pruning of the roots of E. obliqua seedlings appears to have resulted in the induction of stresses within the seedlings which increased the susceptibility of the remaining intact roots to infection by Cylindrocarpon sp. Further investigations of the factors involved in the changes in the level of root infection on these seedlings are described in Chapter 6.1

5.3.2 Preinoculation Drought

While drought stress may be an important factor contributing to regrowth dieback (Chapter 3), previous pathogenicity studies failed to demonstrate any increase in root rot or seedling mortalities in

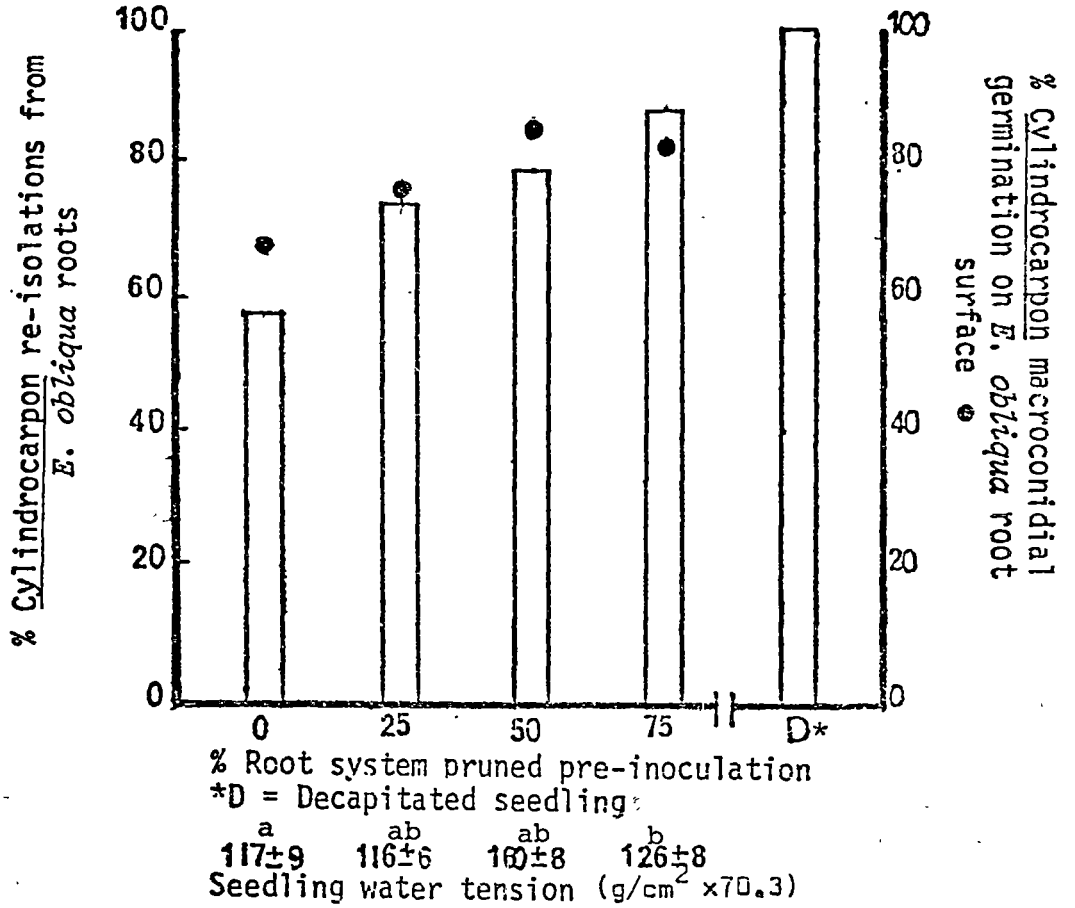


Figure 17a Histogram illustration Cylindrocarpon sp. infection of the roots of E. obliqua seedlings which had been stressed by different pre-inoculation root pruning.

17b Percentage germination of Cylindrocarpon sp. macroconidia on the surface of roots of seedlings in the preinoculation root pruning treatments (Chapter 6.1).

a differs from b at(P=0.05)

seedlings subjected to drought stress at inoculation (Chapter 5.2.1). The following experiment examined the effects of preinoculation drought stress on the pathogenicity of Cylindrocarpon sp. to E. obliqua seedlings on their return to field capacity.

Materials and Methods

E. obliqua seedlings which had been grown for 9 months in 500g of sand peat in pots with plastic liners were given one of seven different drought stress treatments. The treatments involved withholding water from each of ten seedlings for 12, 10, 8, 6, 4, 2 and zero days prior to rewatering all seedlings to field capacity. Five seedlings in each preinoculation drought stress treatment were then inoculated with Cylindrocarpon sp. on each of ten roots per seedlings as described in the last experiment. Water agar blocks were placed on ten roots of the five control seedlings per stress treatment. The seedlings were grown in a growth cabinet a $10^{o}C$ for 10 days when the level of root infection by Cylindrocarpon sp. was determined as described in the last experiment.

The water tensions in the seedlings were measured using the pressure bomb following the pathogenicity studies and the reimposition of the original drought treatments in individual seedlings. The reimposition of a second drought stress may result in different water tensions in the seedlings than in the first treatment and should only be used as an indicator of relative stress levels.

Results and Discussion

Neither the drought treatments nor the low level of root inoculation with Cylindrocarpon sp. resulted in the death of E. obliqua seedlings. However, root infection by Cylindrocarpon sp. was lower in the unstressed seedlings and increased in seedlings receiving increasing periods of preinoculation drought stress (Figure 18). Seedlings which had been daily rewatered to field capacity also had higher levels of infection than seedlings which were not stressed but were growing in better drained soil which had been withheld from water for two days (Figure 18). Water tensions only increased in seedlings which had been withheld from watering for more than four days.

The results suggest that physiological changes within the E. obliqua seedlings as a consequence of previous drought stress, increase the susceptibility of the roots to infection by Cylindrocarpon sp. Further investigations of the changes induced by previous drought stress and how they may influence Cylindrocarpon sp. root infection are described in Chapter 6.1.

5.3.3 Seedling Growth Stage

Changes in the physiology of trees during the transition of forests from exponential to mature growth stages (Kozlowski 1971, Woolhouse 1967) may influence the natural selection of the surviving genotypes (Stern and Roche 1974) and the susceptibility of individual trees to disease (Day 1960). Consequently an experiment was designed to examine the effect of stress associated with changes in the stage of growth of E. obliqua seedlings on the pathogenicity of

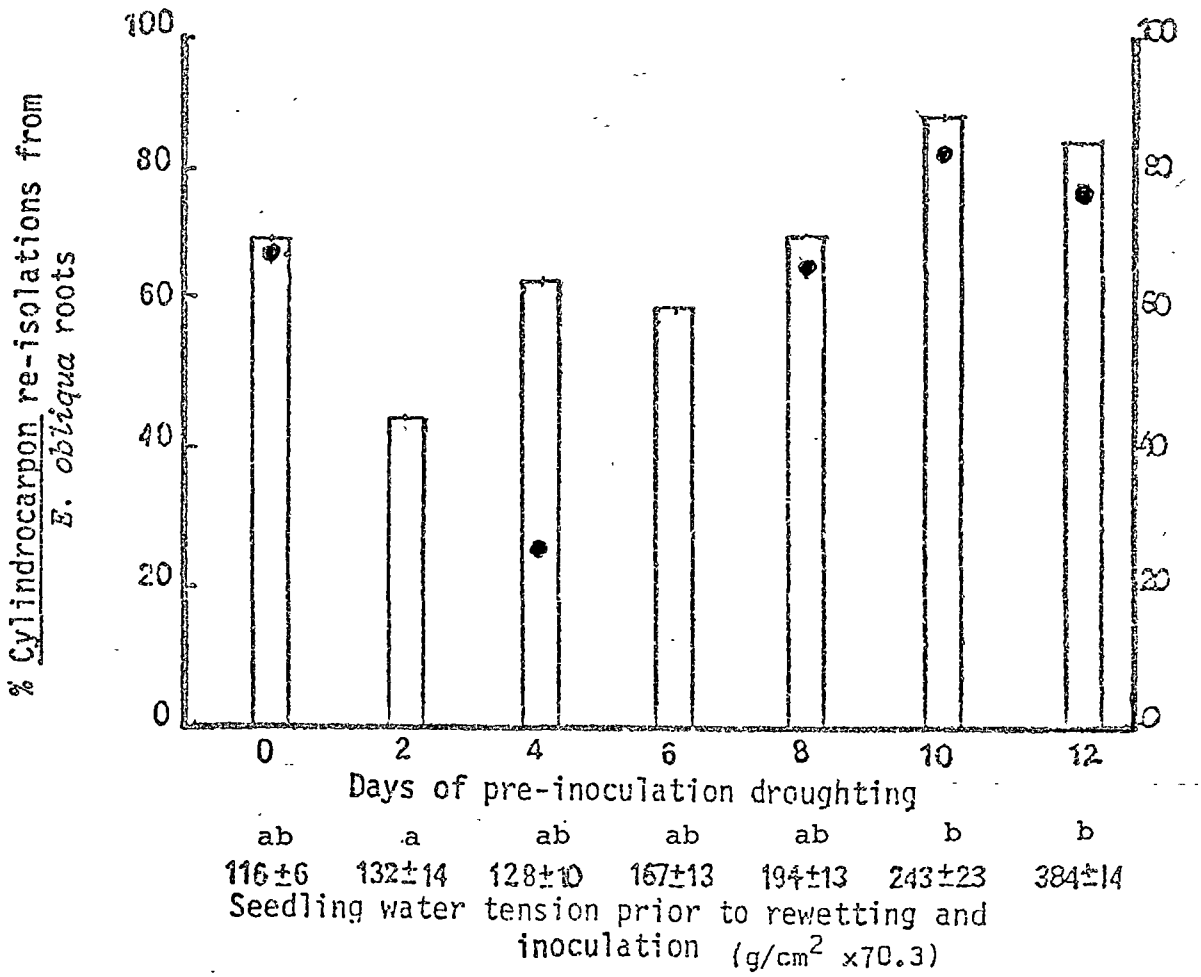


Figure 18a Histogram illustrating Cylindrocarpon sp. infection of the roots of E. obliqua seedlings which had been stressed by different pre-inoculation drought stress treatments.

18b Percentage germination of Cylindrocarpon sp. macroconidia on the surface of roots of seedlings in the preinoculation drought stress treatments (Chapter 6.1).

a differs from b at (P=0.05)

Cylindrocarpon sp. Different growth stages were induced in E. obliqua seedlings of the same age by growing them in different volumes of soil so that conditions for seedling growth may become limiting at different times. The relative susceptibility of the seedlings at the different stages of growth was then tested by simultaneously inoculating them with Cylindrocarpon sp.

Materials and Methods

Plastic tubes of various sizes were filled with either 10, 15, 25, 50 or 100g of autoclaved sand peat and a month old E. obliqua seedling was planted in each of the twenty drained tubes per treatment. The seedlings were grown at 18-22°C in natural light and were regularly watered by weight to field capacity with distilled water or periodically with Hoagland's nutrient solution. The height of individual seedlings was measured every week and height increment for each treatment plotted to obtain a series of growth curves (Figure 19).

After 74 days growth, when the seedlings in the smaller soil volumes had ceased exponential growth but the seedlings in the larger soil volumes were still growing actively 16 seedlings in each treatment were inoculated with Cylindrocarpon sp. The inoculum comprised an aqueous suspension of Cylindrocarpon sp. macroconidia (96,000/ml) which was applied to the surface soil of each inoculated seedling to bring that seedling back to its field capacity weight. This inoculation was designed to give all seedlings a comparable inoculum dose relative to their current growth or water use. The smaller seedlings received approximately 30% of the inoculum, of the larger, vigorous seedlings. The seedlings were grown for a further 21 days

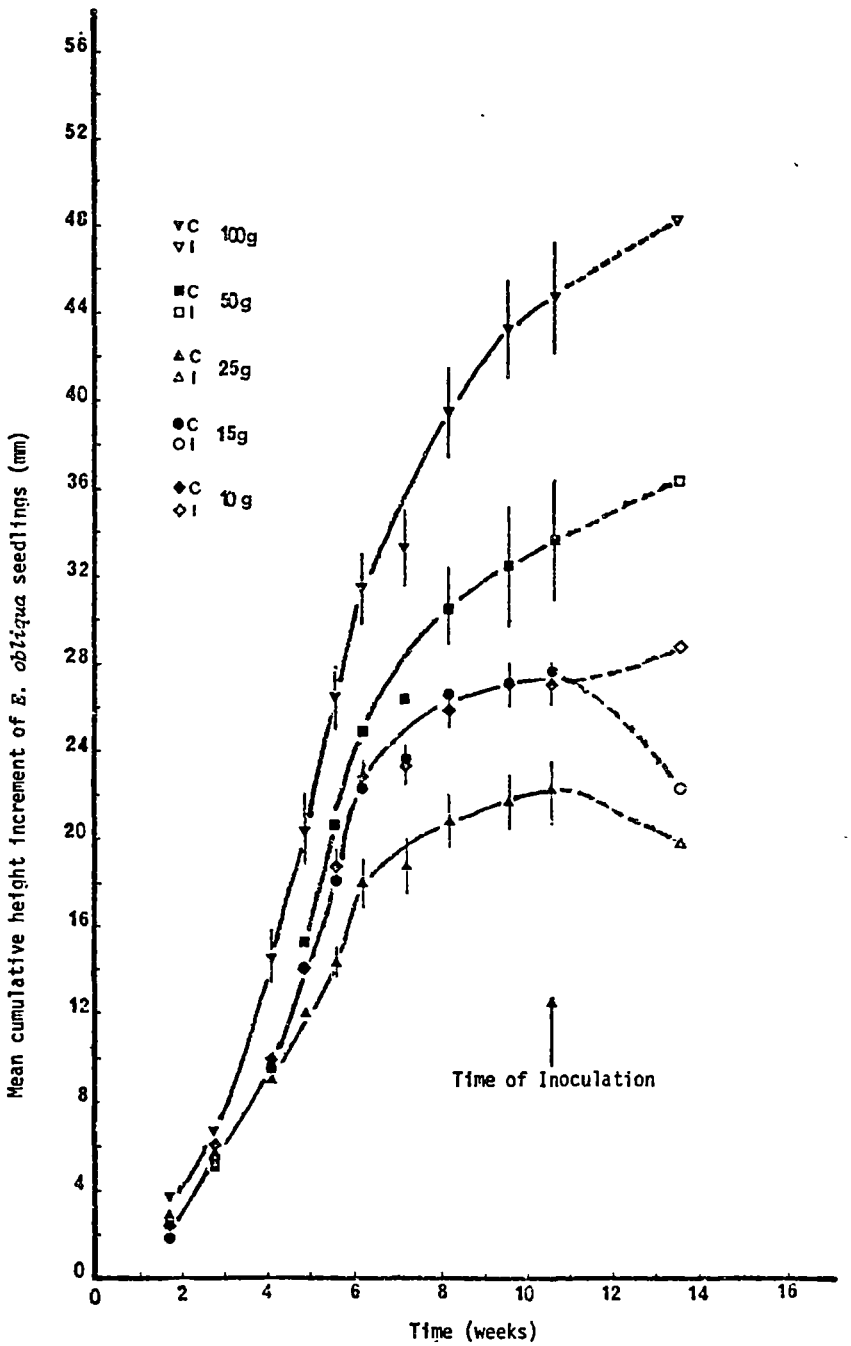


Figure 19 Cumulative height increment of E. obliqua seedlings growing in different weights of soil to induce different growth stages prior to inoculation with Cylindrocarpon sp.

C = prior to inoculation, I = after inoculation.

Vertical bars indicate twice the standard error of each treatment mean.

at 18-22°C when their roots were washed free of soil, surface sterilised in 1% sodium hypochlorite for 30 seconds and incubated on filter paper for 5 days at 22°C. Root infections by Cylindrocarpon sp. were assessed at x 40 magnification. The root and shoot dry weight of each seedling was also determined.

Results and Discussion

Changes in the growth rate of the different treatments were calculated from Figure 19 and were expressed as the height increment over the 17 days prior to inoculation as a percentage of height increment over the preceding exponential growth phase. The reduction in height growth following inoculation was greatest in seedlings in the 15g soil treatments and least in seedlings in the 100g treatments (Table 18). Seedlings in the 25g treatments were smaller than those in the 10g or 15g treatments possibly due to the shallower soil depth in their larger diameter tubes.

Cylindrocarpon sp. inoculation resulted in low levels of mortalities in seedlings approaching the plateau growth stage (21% mortality in the 25g treatment) as well as in some of the seedlings at the transition from exponential to plateau growth (25% mortality in the 15g treatment). No mortalities were recorded in seedlings which were still in the exponential growth stage (50 and 100g treatments) in spite of these seedlings having received higher doses of Cylindrocarpon sp. inoculum. The absence of mortalities in seedlings in the 10g treatment may be related to the comparative low dosage of Cylindrocarpon sp. inoculum relative to the size of these seedlings.

TABLE 18 The pathogenicity of Cylindrocarpon sp. on E. obliqua seedlings at different stages of growth. Mean response in 16 seedlings per treatment.

Plant Response	Soil Quantity to Induce Various Stages of Growth (g)				
	10	15	25	50	100
A. Growth Stage at Inoculation					
Height increment mm/week					
(a) Current growth rate	0.5	0.4	0.5	1.2	2.1
(b) Former growth rate	3.5	3.8	2.8	4.3	5.5
Current growth (a) as a % of former growth rate (b)	14	10.5	18	28	38
B. Plant Characteristics at Harvest					
Height (mm)	44 ± 1	45 ± 2	39 ± 1	51 ± 3	62 ± 3
Shoot Dwt (mg)	25 ± 2	20 ± 3	14 ± 2	24 ± 3	52 ± 6
Root Dwt (mg)	6.1 ± 0.7	5.9 ± 0.6	4.3 ± 0.5	6.9 ± 1.0	14.7 ± 1.6
Root Shoot Ratio	0.23	0.31	0.31	0.28	0.29
C. Inoculum Added (ml)					
	2.2	2.8	3.3	4.4	9.3
D. Response to Inoculation					
Root Infection					
No. examined	158	185	198	217	233
% Infected	73 ± 7	77 ± 7	73 ± 7	49 ± 9	35 ± 5
% Infected/ml inoculum	33	28	22	11	4
Mortalities					
% seedlings killed	0	25	21	0	0

The reisolations of Cylindrocarpon sp. from the roots of the E. obliqua seedlings was highest in seedlings during the transition from exponential to plateau growth (10, 15 and 25g treatments) and decreased in seedlings which were still growing actively (50 and 100g treatments). The level of root infection per unit of Cylindrocarpon sp. inoculum was also highest in the seedlings at the transition from exponential to plateau growth and decreased in seedlings which were still growing actively. Intermediate levels of root infection per unit of Cylindrocarpon sp. inoculum occurred in the seedlings approaching their mature growth stage (25g treatment).

Consequently stress associated with the stage of growth may influence the susceptibility of E. obliqua seedlings to infection by Cylindrocarpon sp. Seedlings which were still growing actively were less susceptible to infection by Cylindrocarpon sp. than seedlings at the transition from exponential to plateau growth in spite of the substantially higher levels of inoculum which were added to the larger seedlings.

Each of the last three experiments indicate that the physiology of E. obliqua seedlings may change as a result of stress and that this change may affect their susceptiblity to infection by Cylindrocarpon sp. The physiological changes and increased susceptibility to Cylindrocarpon sp. occurred within the prestressed plants which had been inoculated at field capacity and are likely to be independent of the effects of these stress treatments on the activity of the fungus. While caution is needed in extending the results of seedling pathogenicity studies to the regrowth forest previous studies indicate that similar stress factors occur in regrowth trees where they may also influence infection of fine roots by Cylindrocarpon

sp. inoculum. The increased root decay and dieback in trees on shallow soils with restricted root growth, the continuation of dieback even on return to soil moisture conditions favourable for tree growth and the high level of dieback in former dominant trees during the transition of regrowth forests to mature forests may not be inconsistent with the increased susceptibility of E. obliqua seedlings subjected to similar stresses in these experiments.

The experiments in this chapter indicate that Cylindrocarpon sp. can infect the fine roots of E. obliqua seedlings, cause growth reductions, and under some conditions, the death of E. obliqua seedlings. Furthermore these studies demonstrate the important role that particular site, inoculum and stress factors may play in the pathogenicity of Cylindrocarpon sp. on E. obliqua seedlings. The stress conditions investigated in these experiments have attemped to predispose the E. obliqua seedlings to conditions similar to those in the E. obliqua regrowth forests and thereby increase the relevance of these pathogenicity studies to the understanding of the aetiology of dieback in maturing E. obliqua forest in southern Tasmania.

When considered in the context of the various environmental, host and inoculum conditions previously associated with regrowth dieback the results of these and previous pathogenicity studies suggest that Cylindrocarpon sp. may be a major contributing factor in the root decay and dieback of the E. obliqua regrowth forests in southern Tasmania. However Cylindrocarpon sp. should be regarded as one factor in the dieback complex and not as a single, highly destructive or exotic causal agent.

CHAPTER 6

CYLINDROCARPON SP. IN THE E. OBLIQUA RHIZOSPHERE

The previous pathogenicity studies indicate that Cylindrocarpon sp. can be pathogenic on E. obliqua seedlings. However these studies do not show how this occurs nor what factors influence the process of root infection and decay. Consequently the following experiments examined factors affecting Cylindrocarpon sp. inoculum in rhizosphere soil from E. obliqua regrowth trees to determine how they may influence root infection, root decay and dieback under the conditions likely to occur in regrowth forests.

6.1 The Association Between Fungistasis in the Rhizosphere of E. obliqua and Root Infection by Cylindrocarpon sp.

The infection of E. obliqua roots by Cylindrocarpon sp. may be influenced by factors which affect either or both the susceptibility of root tissue or the activity of Cylindrocarpon sp. on the root surface. However the activity of the large quantity of Cylindrocarpon sp. inoculum in the root zone of regrowth trees is affected by numerous fungistatic or competitive factors (Chapter 4) which may influence the infection of E. obliqua roots by Cylindrocarpon sp. (Chapter 5.1). Consequently the relationship between physiological stress, fungistasis and root infection of E. obliqua by Cylindrocarpon sp. needs to be investigated. The following experiment examined this relationship in conjunction with the previous preinoculation root pruning and drought stress pathogenicity studies.

Materials and Methods

The fungistasis of Cylindrocarpon sp. macroconidia was examined on the surfaces of roots in two uninoculated E. obliqua seedlings from each of the root pruning treatments and all but two of the preinoculation drought stress treatments (Chapters 5.3.1, 5.3.2). Strips of cellophane which had been washed, autoclaved and brush inoculated with Cylindrocarpon sp. macroconidia were carefully folded and placed across five of the roots which had not been inoculated with water agar plugs. The centre of the strip was placed in direct contact with the root surface while the two ends were in contact with the surrounding soil. The strips were held in place by the plastic liner for 25 hours and were then carefully removed and stained in lactophenol trypan blue. The percentage germination of Cylindrocarpon sp. macroconidia was assessed at both the root contact zone and 8mm to either side of this zone to record fungistasis at the rhizoplane and in the surrounding soil respectively. The seedlings were then maintained for a further 9 days as part of the pathogenicity study.

Results and Discussion

The percentage germination of Cylindrocarpon sp. macroconidia was higher in the root zone of unstressed seedlings when compared with the adjacent soil (Table 19). With increasing stress prior to inoculation the relative differences between germination on the roots and in the surrounding soil declined to the extent that no differences were apparent at the highest stress levels.

TABLE 19 Fungistasis of macroconidia of Cylindrocarpon sp. on the surface and in the soil surrounding roots of E. obliqua seedlings which had received different pre-inoculation stress treatments.

Pre inoculation Stress Treatment	% Germination of Cylindrocarpon macroconidia On Root Surface	In Adjacent Soil
A. Root pruning stress		
0% of roots pruned	68	56
25% of roots pruned	76	69
50% of roots pruned	85	87
75% of roots pruned	81	82
Decapitated	71	66
B. Pre inoculation drought		
Water withheld		
0 days	68	56
4 days	37	50
8 days	61	51
10 days	75	74
12 days	77	82

LSD =10 (P=0.05)

The percentage of macroconidia which germinated on the root surface increased with increasing levels of preinoculation stress in both experiments (Table 19) (Figure 17, Figure 18). This increase was associated (r^2 = 0.79) with the increase in the level of root infection by Cylindrocarpon sp. in these experiments. Consequently root infection by Cylindrocarpon sp. was inversely associated with the fungistasis of Cylindrocarpon sp. on the root surface.

The E. obliqua seedlings in both of these experiments were of similar age, had been grown in similar soils and environmental conditions and are likely to have had similar rhizosphere microfloras prior to the stress treatments. The differences in Cylindrocarpon sp. fungistasis on the root surfaces of these seedlings are therefore most likely to have resulted from physiological differences in these seedlings due to the preinoculation stress treatments. Root exudates increase following stress (Katznelson 1955, Martin 1957, Beute and Lockwood 1968, Bowen 1969, Vancura and Garcia 1969) and it is possible that differences in root exudation by the E. obliqua seedlings may have influenced the fungistasis of Cylindrocarpon sp. macroconidia and the level of root infection in these experiments.

The higher germination of Cylindrocarpon sp. macroconidia on the root surface of the unstressed seedlings relative to those in the surrounding soil, the increase in germination on the root surface with increasing stress and the progressive extension of this stimulation into the surrounding soil with increasing stress are all consistent with an increase in the release and spread of root exudates as a result of the previous stress treatments. However further studies need to be conducted on the relationship between

stress, root exudates and the fungistasis of Cylindrocarpon sp. in order to confirm this possible explanation.

6.2 Changes in Root Exudates Following Stress in E. obliqua Seedlings

The intensification of dieback in the E. obliqua regrowth forests has been associated with factors which may contribute to periodic moisture stress (Chapter 3). If such stress factors alter root exudation in E. obliqua it is conceivable that this may influence the fungistasis of Cylindrocarpon sp. and thereby affect root infection. Consequently the relationship between previous drought stress, increased root exudation in E. obliqua seedlings and the fungistasis of Cylindrocarpon sp. inoculum was investigated in the following experiments.

Materials and Methods

E. obliqua seedlings were grown for one month from surface sterilised seed on sterile filter paper moistened with Hoagland's nutrient solution. Drought stresses were imposed in the seedlings by transferring them onto agars of varying water activities, a_w 0.999, 0.990, 0.980 for 72 hours at 22°C (Appendix 1). The seedlings were then transferred onto 1% water agar for a further 48 hours to allow root exudates to diffuse from the stressed seedlings into the water agar. The root exudates released during the 48 hour post stress period were tested for their capacity to stimulate germination of Cylindrocarpon sp. macroconidia. The root exudates were also extracted for identification by thin layer chromatography.

The effect of the root exudates on the germination of Cylindrocarpon sp. macroconidia was examined by applying known quantities of the crude exudate or chromatographically separated exudate fractions to pieces of washed sterile filter paper ($1cm^2$) on glass slides. The five replicate filter paper squares of each of the extracts were moistened with sterile distilled water and overlain with sterile cellophane which had been brush inoculated with Cylindrocarpon sp. macroconidia from a 7 day old PDA agar culture. The glass slides were incubated for 20 hours at $22^{o}C$ in Petri dishes above filter paper which had been saturated with allyl alcohol solutions of different concentrations (see Figure 20). The allyl alcohol suppressed the germination of macroconidia on the control slides and allowed any stimulation of germination as a result of the root exudates to be accurately measured (Balis 1976).

The root exudates were extracted from the water agars by freezing, thawing, centrifugation and decanting the aqueous suspension or by methanol extraction. The root exudates were separated and identified by thin layer chromatography using cellulose and silica gel G plates and the methods, reagents and identification criteria described by Stahl (1965). Thesea are outlined in Table 20. The detection and characterisation of an unknown fluorescent compound in the exudate was made using a Unicam SP800 scanning U.V. spectrophotometer.

Results and Discussion

The germination of Cylindrocarpon sp. macroconidia was consistently stimulated by water or methanol extracts from drought stressed E. obliqua seedlings when compared with similar extracts from

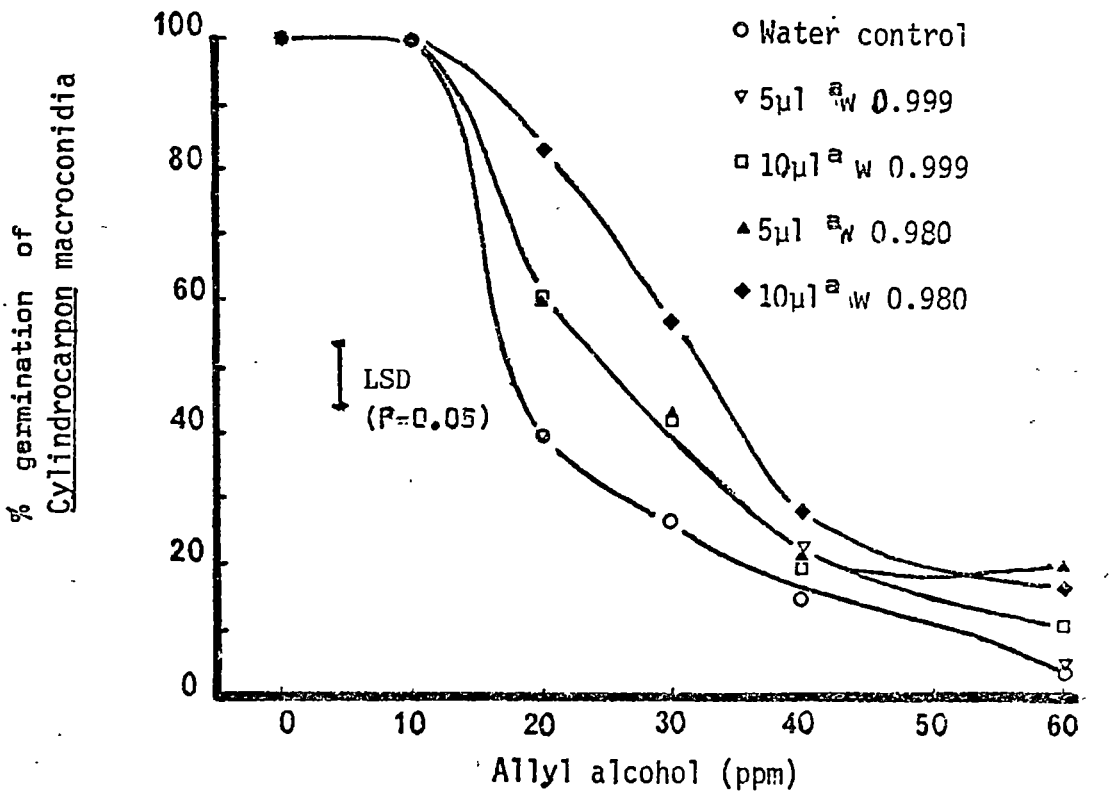


Figure 20 The effects of exudates from the roots of drought stressed E. obliqua seedlings on the germination of Cylindrocarpon sp. macroconidia in the headspace above allyl alcohol solutions.

unstressed seedlings or water controls (Figure 20). Germination percentages in the headspace above 20ppm allyl alcohol were 83, 60 and 39% for 10µl of unconcentrated extracts from the drought stressed, unstressed and water controls respectively.

Thin layer chromatography of the root exudates consistently resulted in the detection of increased quantities of five substances from the drought stressed seedlings relative to those exuded in the unstressed controls. The properties of these substances are summarised in Table 20.

Each of the purified exudates stimulated germination of Cylindrocarpon sp. macroconidia when tested above allyl alcohol. Positive identification of the unknown fluorescent compound by nuclear magnetic resonance and infra-red spectroscopy was not successful due to the small quantity of material available. Increased coumarin exudation has been reported from the roots of drought stressed plants (Martin 1957) and the properties and U.V. spectra of these coumarins appear to be not unlike those of the unknown compound isolated in these studies.

The consistent exudation of increased quantities of these compounds from the roots of drought stressed E. obliqua seedlings is consistent with results of Katznelson et al (1955) and Martin (1957) They support the hypothesis that the increased germination of Cylindrocarpon sp. macroconidia on the surface of E. obliqua roots in the previous experiment (Chapter 6.1) may have resulted from increased levels of root exudation by the E. obliqua seedlings as a consequence of stress treatments. The level and nature of exudates from the roots of E. obliqua seedlings may be an important factor

TABLE 20 The effects of prior drought stress on the levels and types of root exudates from E. obliqua seedlings.

Seedlings had been placed on agars of various water activities prior to transfer to water agar and extraction of the exudates as described in the text. The table summarizes details of exudate fractions which increased in response to previous drought stress.

Exudate No.	Isolation on cellulose thin layer plates	rf	Tentative Identification	Levels of exudation after transfer from agars of water activity +				
				0.999H	0.999W	0.990H	0.990W	0.980W
1	Blue flourescence with U.V.	0.58	Postive to $FeCl_3$ $KFe(CN)_6$ - possible phenolic	1	1	3	6	10
2	Purple spot with ninhydrin spray	0.43	Cochromatographed with aspartic acid	4	6	8	10	10
3	Purple spot with ninhydrin spray	0.52	Cochromatographed with glutamic acid	0	0	0	3	5
4	Brown spot with $AgNO_3$-NH_4OH	0.18	Cochromatographed with glucose	1	2	2	4	6
5	Unstained spots with $AgNO_3$-NH_4OH	0.15	Positive to $FeCl_3$ H_2O_2 possible coumarin	1	0	5	2	10

+ Relative levels of exudation (0-10) based on spot size and intensity in healthy (H) and wilting (W) E. obliqua seedlings.

which can influence fungistasis of Cylindrocarpon sp. on the root surface and thereby the level of root infection. If similar responses occur in the roots of regrowth trees it may explain part of the relationship between moisture stress, root infection by Cylindrocarpon sp. and the aetiology of dieback in E. obliqua regrowth trees.

6.3 Changes in the Population of Rhizosphere Micro-organisms in Forest Trees

Changes in root exudates in forest trees are likely to influence not only Cylindrocarpon sp. but also the other micro-organisms in the E. obliqua rhizosphere. As these micro-organisms may greatly affect the activity of Cylindrocarpon sp. in the E. obliqua rhizosphere studies were conducted of how the populations of rhizosphere micro-organisms may change in relation to increasing age of the E. obliqua forests, changes in the growth rate of dominant regrowth trees, and in eucalypt species which are susceptible or resistant to dieback.

Materials and Methods

The following procedure was used in each of three following experiments. Eucalypt root samples, less than 1mm in diameter, were collected from 8 to 10 locations around each tree and stored overnight at 2°C. Loosely adhering soil was shaken free from the roots and 0.5g of fresh roots were agitated in a tube containing 10ml of sterile distilled water with a vortex mixer to disperse micro-organisms from the roots. Aliquots of this suspension were plated on a range of media (Appendix 1) using the dilution plating technique. The numbers of bacteria, actinomycetes and fungi growing

on the respective agars were counted after incubating the plates for 120 hours at 22°C.

While the limitations of the dilution plating technique are recognised, these studies examined the relative differences in population of rhizosphere micro-organisms between similar root materials and are likely to be some guide to the changes occurring on the surface of E. obliqua roots.

The first experiment examined the effects of increasing forest age on the numbers of rhizosphere micro-organisms. E. obliqua roots (0.5g) were collected from four trees in each of nine forests ranging in age from 1 to 250 years. The relative numbers of bacteria, actinomycetes and fungi on these roots were determined on dilution plates of glycerol asparagine agar.

The second experiment examined changes in the relative numbers of actinomycetes in the rhizosphere of E. obliqua regrowth trees which were at various stages of incipient dieback. Ten E. obliqua regrowth trees for which diameter growth data was available (CSIRO Division of Forest Research pers. comm.) were selected in each of the three regrowth forests. The forests were growing on similar yellow podzolic soils but were either 52, 64, or 90 years of age. The number of actinomycetes on the surface of 0.5g of five roots from each of the trees was determined by dilution plating on glycerol-asparagine agar and related to an index of incipient dieback. This index was based on the current diameter increment of the tree as a percentage of the diameter increment over the preceding five years and had been found to indicate the current vigour of the tree and its likely predisposition to regrowth dieback.

The third experiment compared the population of micro-organisms on the surface of roots of healthy E. obliqua, E. regnans and E. globulus regrowth trees of similar age which were growing adjacent to each other on the same soil. E. globulus, a member of the subgenus Symphomyrtus, is only slightly affected by dieback (Bowling and McLeod 1968) whereas E. obliqua and E. regnans of the subgenus Monocalyptus are severely affected by dieback. This is consistent with the susceptibility of other members of these eucalypt subgenera to dieback throughout Australia (Tippett et al 1976). Soil and root samples (0.5g fresh weight) were collected from under the respective trees and the numbers of fungi, bacteria and actinomycetes were determined by dilution plate studies or soil plate studies using a range of agar media (Appendix 1, Table 23).

Results and Discussion

6.3.1 Changes in Rhizosphere Micro-organisms during the Development of E. obliqua forests

The numbers of bacteria and actinomycetes in the rhizospheres of E. obliqua decreased from high levels in vigorous young seedlings on recently burnt soils to low levels in 55 to 70 year old regrowth forests (Figure 21). The numbers of actinomycetes, but not bacteria, increased in the rhizospheres of mature and oldgrowth forests. The numbers of fungi also decreased in the rhizosphere of regrowth trees relative to the numbers in very young or mature forests. The decrease in the numbers of bacteria and actinomycetes in the rhizospheres of E. obliqua trees from 0 to 70 years of age was inversely associated (r^2 = 0.79) with the development of Cylindrocarpon sp. microsclerotia in rhizosphere soils from the same trees (Chapter 4.1).

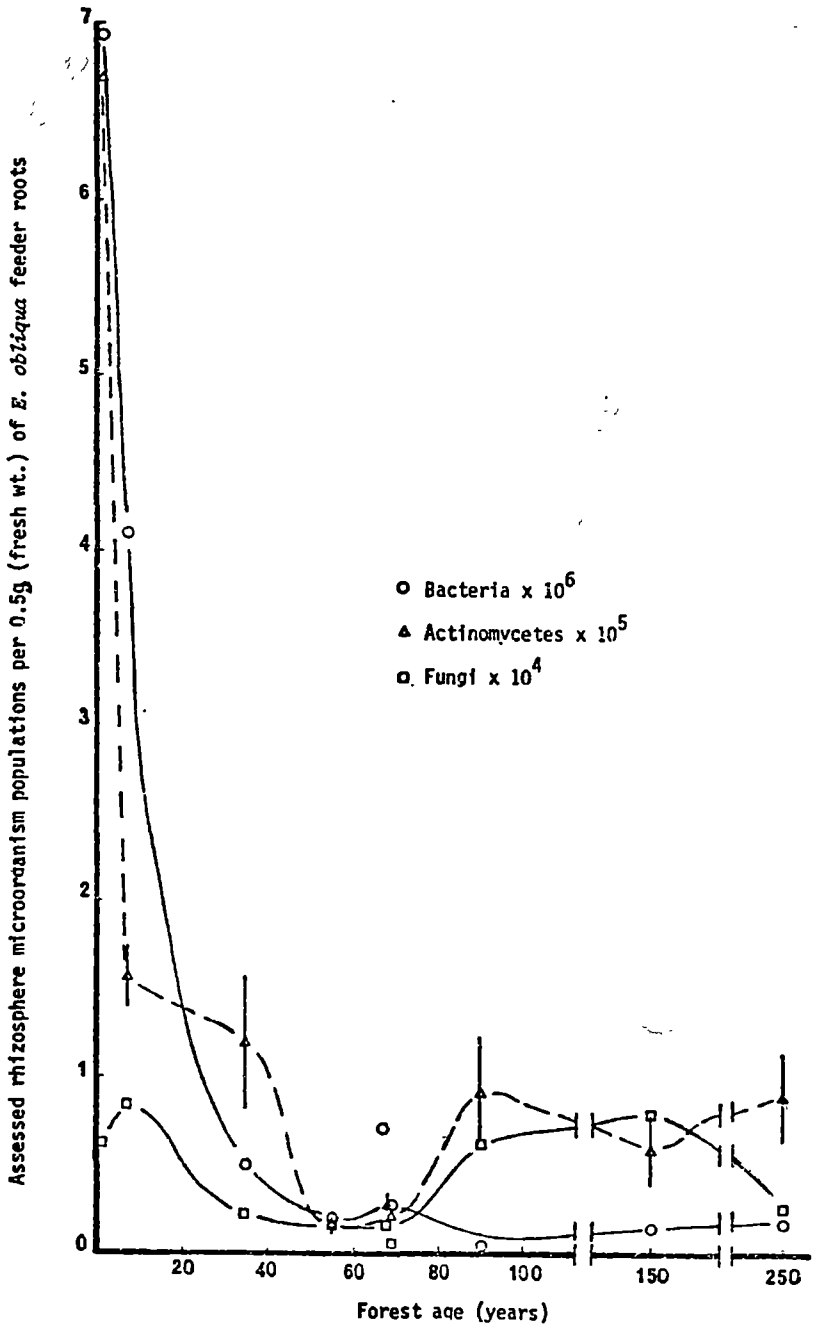


Figure 21 Changes in the populations of rhizosphere microorganisms during the development of E. obliqua forests in southern Tasmania.

Vertical bars indicate twice the standard error of each treatment mean.

Changes in rhizosphere micro-organisms during the development of these forests may influence the activity of Cylindrocarpon sp. on the E. obliqua root surface. Many of the rhizosphere bacteria and actinomycetes when grown on agar produced volatiles which were fungistatic to Cylindrocarpon sp. when tested in agar plate studies (Chapter 4). If similar fungistatic responses also occur in these soils in the E. obliqua forests maximum fungistasis of Cylindrocarpon sp. inoculum may be expected in young forests on recently burnt ground while minimum fungistasis may be expected in regrowth forests from 55 to 70 years of age. The Cylindrocarpon sp. inoculum may therefore be most active during the transition of regrowth forests to mature forests which is consistent with the period of intensification of dieback in some of the former dominant trees.

6.3.2 Changes in the Numbers of Actinomycetes in the Rhizosphere of Regrowth Trees at Various Stages of Incipient Dieback

The current diameter growth in individual regrowth trees varied from 40 to 230% of their previous average growth rate. Current growth rates were generally high due to above average rainfall. This index of current growth was higher in the younger, 52 year old forest, and lowest in the 64 year old regrowth forest which was severely affected by dieback. The index of current growth in individual trees in the 90 year old forests varied widely and ranged from 70 to 230% of their former growth rates. This index of current diameter growth was not related to the size or former dominance of the tree. Many of the smaller subdominant and codominant trees were growing faster, relative to their former growth rates, than some of the larger dominant trees. This is consistent with the growth ring studies on E. obliqua in these forests (Chapter 2).

The numbers of actinomycetes in the rhizosphere of E. obliqua regrowth trees were directly associated with the current diameter growth index in each of the three forests (Figure 22). Trees which were growing vigorously had high numbers of actinomycetes in their rhizospheres whereas trees with poor growth had lower numbers of actinomycetes in their rhizospheres.

These changes in the population of rhizosphere actinomycetes during the decline in diameter growth may reflect changes in root exudation during the transition of regrowth forests to mature forests. Former dominant trees are likely to be subject to relatively higher levels of growth decline than former subdominant trees (Chapter 2) and may have greater reductions in the level of sustainable root exudate than former subdominant trees. These changes in root exudation are likely to affect the numbers and activity of micro-organisms in the E. obliqua rhizosphere. Cultures of actinomycetes from the rhizosphere of E. obliqua regrowth trees did produce volatiles fungistatic to Cylindrocarpon sp. If similar fungistatic volatiles are produced in regrowth forests they may greatly affect the activity of Cylindrocarpon sp. on the roots of E. obliqua regrowth trees.

6.3.3 Micro-organisms in the Rhizosphere of Euclaypts Species which are Susceptible or Resistant to Dieback

The numbers of bacteria and actinomycetes isolated from the rhizosphere of regrowth trees were consistently higher on E. globulus than on either E. obliqua or E. regnans (Table 21). No clear differences were apparent in the numbers of fungi isolated from the roots of these three species on the range of media and methods used. Differences in the micromorphology of E. globulus, E.

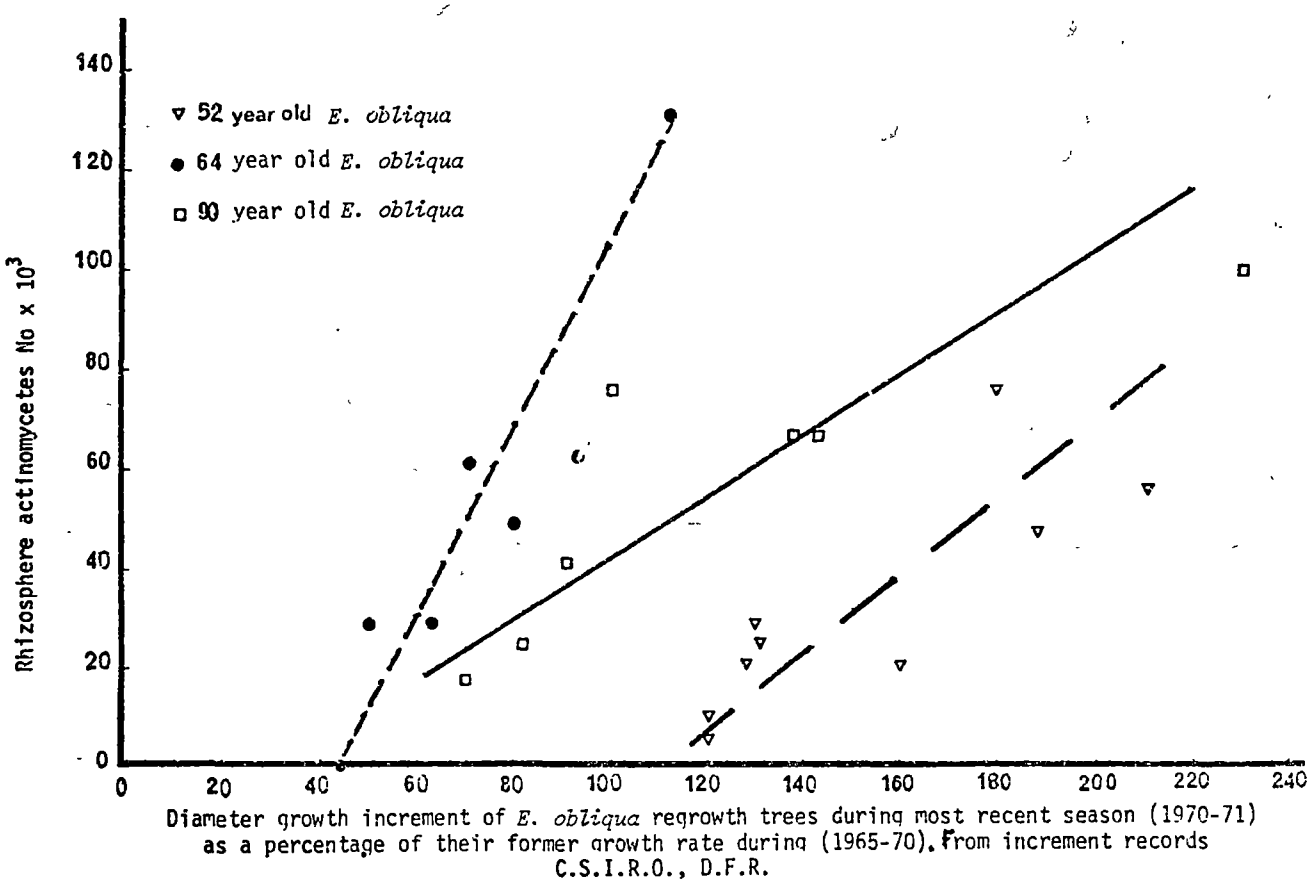


Figure 22 Changes in the numbers of actinomycetes in the rhizosphere soil of healthy E. obliqua regrowth trees in relation to their initial decline in diameter growth.

Correlations significant at P = 0.01 52 year old
P = 0.001 64 year old
P = 0.05 90 year old

TABLE 21 Numbers of microorganisms in the rhizosphere and soil surrounding regrowth trees of different eucalypt species and health. Mean of seven samples per treatment.

Organisms & Media for Isolation	Dilution	E. obliqua healthy forest		E. regnans dieback forest		E. globulus healthy forest	
		Soil	Rhizosphere	Soil	Rhizosphere	Soil	Rhizosphere
1 a FUNGI - TOTAL							
.1 Nutrient agar*	10^3	4.5	32	5.4	85	5.2	61
.2 Soil extract agar	10^3	6.4	7.6	11.0	38	25.0	53
.3 Basal agar	10^3	-	20	-	43	-	2
b Pythiaceae							
$P_{10}VP$ - dilution pl.	10^3	-	4.0	-	6.4	-	1.1
$P_{10}VP$ - Warcup soil pl.	-	87%		90%		65%	
c Phytophthora cinnamomi							
$P_{10}VP$ - dilution pl.	10^3	0	0	0	0	0	0
$P_{10}VP$ - Warcup soil pl.	-	0	-	0	-	0	-
2 BACTERIA							
.1 Nutrient agar	10^5	12	18	12	11	12	36
.2 Soil extract agar	10^5	62	33	68	38	57	42
.3 Basal agar	10^5	-	6.7	-	2.0	-	94
.4 $P_{10}VP$ agar	10^5	-	13	-	14	-	48
.5 Warcup soil pl.	-	50%	-	47%	-	70%	-
3 ACTINOMYCETES							
.1 Glycerol asparagine agar	10^4	-	4.1	-	6.6	-	290
.2 Basal agar	10^4	-	0.9	-	0.8	-	300

* Details of media in Appendix I

regnans and E. obliqua roots were not detected and are unlikely to have greatly affected these results.

The substantially higher numbers of actinomycetes and bacteria in the rhizosphere of E. globulus regrowth trees relative to those in the rhizosphere of E. obliqua and E. regnans may be an important factor influencing the susceptibility of fine roots of these species to infection by fungi including Cylindrocarpon sp. The rhizosphere micro-organisms are capable of producing volatiles fungistatic to Cylindrocarpon sp. in plate studies and if these also occur under forest conditions they may contribute to the lower levels of root decay and dieback observed in E. globulus in regrowth forests. Similar studies have also revealed higher populations of rhizosphere micro-organisms, which are antagonistic towards P. cinnamomi in culture studies, in E. calophylla of the subgenus Symphomyrtus which is more resistant to dieback than in E. marginata of the subgenus Monocalyptus which is sensitive to dieback (Malajczuk and McComb 1979).

Consequently the numbers of micro-organisms in the rhizosphere of E. obliqua may change markedly during the development of the forests and in individual trees during their transition from regrowth to mature growth stages. Actinomycetes in particular were most numerous in the rhizospheres of young vigorous regeneration, in healthy regrowth trees not affected by incipient declines in diameter growth and in species of eucalypts which are resistant to dieback. These microorganisms can affect the activity of Cylindrocarpon sp. in the rhizosphere. Therefore the changes in the numbers and types of micro-organisms in the rhizosphere of E. obliqua, and the factors which govern their population and activity,

are likely to be important in influencing the behaviour of Cylindrocarpon sp. inoculum on the surface of E. obliqua roots and on the basis of earlier studies (Chapter 6.1) the level of root infection.

6.4 A Hypothetical Model of Cylindrocarpon sp. in the Rhizosphere of E. obliqua Regrowth Trees

Factors in the rhizosphere of E. obliqua regrowth trees appear to greatly affect the numbers (Chapter 4.2.1), activity (Chapter 4.2.2) and infectivity (Chapter 5.1) of Cylindrocarpon sp. The infection of E. obliqua roots by Cylindrocarpon sp. was found to be closely related to the fungistasis of inoculum on the root surface (Chapter 6.1) and may be influenced by changes in root exudates and the micro-organisms in the rhizosphere. It would appear that three components are involved in this rhizosphere effect; the inoculum dosage of Cylindrocarpon sp., the level and composition of root exudates and the qualitative and quantitative characteristics of the rhizosphere microflora. Each of these may interact with the other and change as a consequence of age, growth and stress factors. As the resultant effect of their interaction may be important in determining the activity of Cylindrocarpon sp. in the rhizosphere of E. obliqua regrowth trees a hypothetical model was formulated to try to explain some of the possible consequences of these interactions. The following section describes this hypothetical model and the experiments designed to test its validity using rhizosphere soils from regrowth forests.

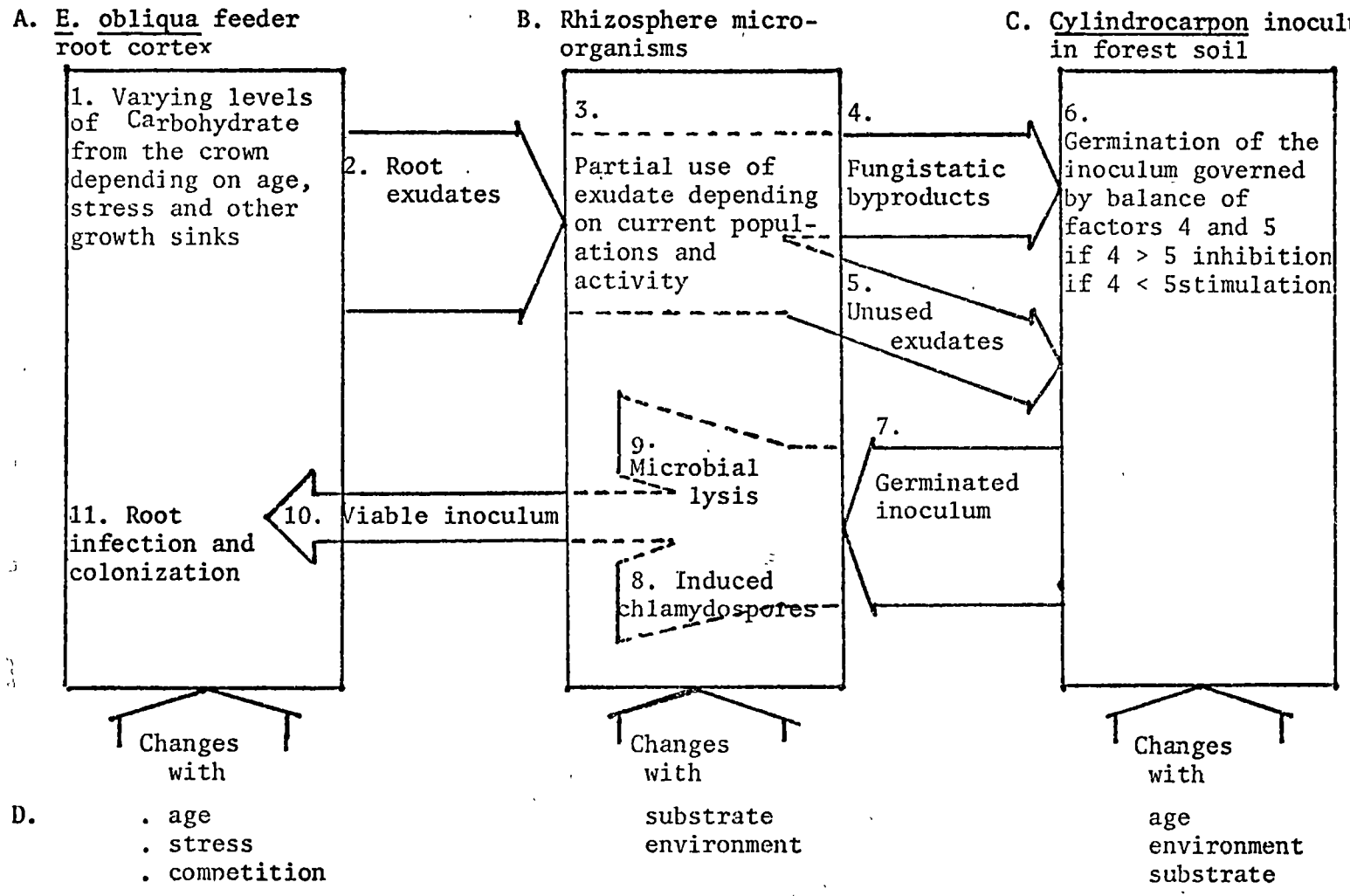


Fig. 23 Hypothetical model of Cylindrocarpon sp. interactions in the rhizosphere of E. obliqua trees.

6.4.1 Description of the Model

The hypothetical model (Figure 23) is based on the following observations and assumptions.

(a) Compounds are exuded from the roots of plants at various compositions and levels depending on plant and environmental factors.

(b) These exudates enter the rhizosphere where they may be utilised and thereby influence the germination and activity of a wide range of rhizosphere micro-organisms.

(c) The degree of which the germination and activity of the fungal inoculum in the rhizosphere is stimulated or retarded is likely to depend on the nett effects of stimulatory root exudate and fungistasis by rhizosphere micro-organisms utilising some of the exudates. The sustained exudation of high levels of exudate may be expected to sustain a high population of active rhizosphere micro-organisms. This would fully utilise the exudate and result in high levels of fungistasis. As a result most of the fungal inoculum may remain inactive as indicated in the rhizosphere of healthy young E. obliqua regeneration (Chapter 4.2.2).

In contrast, where there is a decline in root exudates, such as due to sustained decreases in photosynthate, the population and activity of rhizosphere micro-organisms may also decline and result in a decreased production of fungistatic metabolites. This could allow an increase in fungal activity. Short term increases in root exudates due to stress or wounding of roots, may result in exudate levels above those which can not be fully

utilised by the depleted rhizosphere microflora. The excess substrate may then stimulate the germination and growth of the fungal inoculum on the root surface. This is consistent with the effects of moisture stress on root exudation by E. obliqua and the observed effect on Cylindrocarpon sp. fungistasis and root infection (Chapter 6.2, 6.1). Consequently the germination of fungal inoculum in the rhizosphere would depend on the balance between levels of stimulatory exudates from the roots and fungistasis resulting from the use of these exudates by the rhizosphere micro-organisms.

(d) Once the fungal inoculum has germinated it may either infect the host root, be lysed, or reform survival propagules. The level of lysis and induction of survival propogules may similarly be governed by the activity of micro-organisms in the rhizosphere and the levels of root exudates needed to sustain their activity. In rhizospheres with low levels of exudate and low microbial activity, lysis by micro-organisms may be low. In contrast, in rhizospheres with high levels of exudation and active lytic micro-organisms, much of the fungal inoculum may be killed. The latter is consistent with the low numbers of Cylindrocarpon sp. microsclerotia in the rhizosphere of healthy E. obliqua regeneration relative to those in the surrounding soil (Chapter 4.2.1). Consequently the survival of the germinated fungal inoculum and its capacity to infect the root would also depend on the balance between exudates stimulating the growth of the fungus and the activity of lytic micro-organisms.

While this hypothetical model of rhizosphere interactions may be an oversimplification it is consistent the observed behaviour of Cylindrocarpon sp. in soils from the rhizospheres of regrowth forests. The validity of the model and its application to understanding the behaviour of Cylindrocarpon sp. on E. obliqua roots was tested in the following studies.

6.4.2 Testing of the Hypothetical Model

The validity of the model was tested by examining the fungistasis and lysis of Cylindrocarpon sp. inoculum on rhizosphere soils to which different levels of simulated root exudates had been added. The first experiment examined fungistasis and lysis in rhizosphere soils from E. obliqua regrowth trees and seedlings which had different populations of rhizosphere micro-organisms. The second experiment examined soil from a single dieback tree in which the activities of the micro-organisms had been varied by incubation at different temperatures.

Material and Methods

6.4.2a Fungistasis and Lysis in the Rhizosphere Soil from Different E. obliqua Trees

Rhizosphere soil was collected from two E. obliqua regrowth trees, one of which was from a dieback and another from a predominantly healthy forest, as well as from two, one-year-old E. obliqua seedlings, one of which had been growing on a previously burnt soil (ashbed soil) and another from a mildly disturbed soil (nonashbed soil). The numbers of actinomycetes and bacteria in the

rhizospheres of these trees was determined using the dilution plating technique (Chapter 6.3). Each soil was placed in twelve Petri dishes (15g per dish) and was moistened and preincubated at 15°C for 24 hours. Soil in three of the twelve plates was then thoroughly mixed with 3ml of either 2,000ppm glucose, 2,000ppm cas-aminoacids, 2000ppm glucose plus 2,000ppm cas-aminoacids or sterile distilled water. These amendments brought moisture contents close to field capacity. Cellophane films which had been washed, sterilised and inoculated with Cylindrocarpon sp. macroconidia were placed firmly in contact with the soil at five locations on each plate and incubated for a further 19 hours at 22°C. The cellophane films with the macroconidia were lifted from the soils and replaced by a new film which had been freshly inoculated with Cylindrocarpon sp. macroconidia. The new films were incubated for a further 19 hours at 22°C. This procedure was repeated four times for each soil treatment to enable the germination and lysis of Cylindrocarpon sp. macroconidia to be measured at mean incubation times of 9.5, 33, 80 and 145 hours after the initial nutrient addition. Lysis and germination was examined in 15 fields of approximately 100 macroconidia for each soil-nutrient-incubation time combination and compared with the lysis and germination of macroconidia which had been similarly incubated on control plates containing sterile moist filter paper.

6.4.2b Fungistasis and Lysis at Different Incubation Temperatures

The second experiment examined fungistasis and lysis of Cylindrocarpon sp. macroconidia in rhizosphere soil from the regrowth tree affected by dieback (Chapter 6.4.2a) which was incubated at different temperatures in an attempt to alter the

activity of the rhizosphere micro-organism. Thirty-six Petri dishes of moistened soil were preincubated at 15°C for 25 hours and nine dishes were amended with 3ml of either 0, 500, 1000 or 2000ppm glucose. Films inoculated with Cylindrocarpon sp. macroconidia were placed on each of the soils and nine filter paper control dishes and three of the nine plates of each treatment were then incubated at 6, 14 or 22°C. The incubation periods were extended to 40 hours to enable germination at lower temperatures and the effects of lysis to become more apparent. The germination and lysis of Cylindrocarpon sp. macroconidia were measured at mean incubation times of 20, 60 and 130 hours after the addition of the nutrients as in the previous experiment.

Results and Discussions

6.4.2a Fungistasis and Lysis in the Rhizosphere Soil from Different E. obliqua Trees

Maximum fungistasis occurred for each of the nonamended soils following 80 hours incubation (Table 22). The maximum level of fungistasis, the fungistasis potential, was highest in the ashbed soils from young regeneration which also contained high populations of soil micro-organisms. Fungistasis was lowest on the soil from dieback affected regrowth forests which had low populations of actinomycetes and bacteria but relatively high populations of soil fungi. Disturbed soil with eucalypt regeneration had intermediate levels of fungistasis and rhizosphere actinomycetes. Cylindrocarpon sp. macroconidia consistently germinated on the sterile filter paper controls indicating that the inhibition of germination was due to factors in the various forest soils.

TABLE 22 Fungistasis of *Cylindrocarpon* sp. in soils from *E. obliqua* forests of different age and health and with different populations of rhizosphere microorganisms.

	E. obliqua Rhizosphere Soil				Filter Paper
	1	2	3	4	Control
Soil Origin					
Forest Age	68	68	1	1	-
Forest Health	Dieback	Healthy	Ashbed	Non Ashbed	
Populations of rhizosphere					
* bacteria $x_5 10^5$	2	1	50	72	0
fungi x 10^3	18	3	38	109	0
actinomycetes x 10^4	2	1	135	51	0
Germination of macroconida on unamended soil which was incubated for:					
9.5 hours	42 ± 3	46 ± 5	92 ± 2	66 ± 3	100
33 hours	36 ± 6	39 ± 8	32 ± 4	38 ± 8	100
80 hours	34 ± 4	25 ± 4	3 ± 0.4	21 ± 4	100
145 hours	44 ± 6	41 ± 7	58 ± 8	22 ± 5	100
Maximum level fungistatis	66	75	97	79	0

* From dilution plates on Glycerol-Asparagine agar

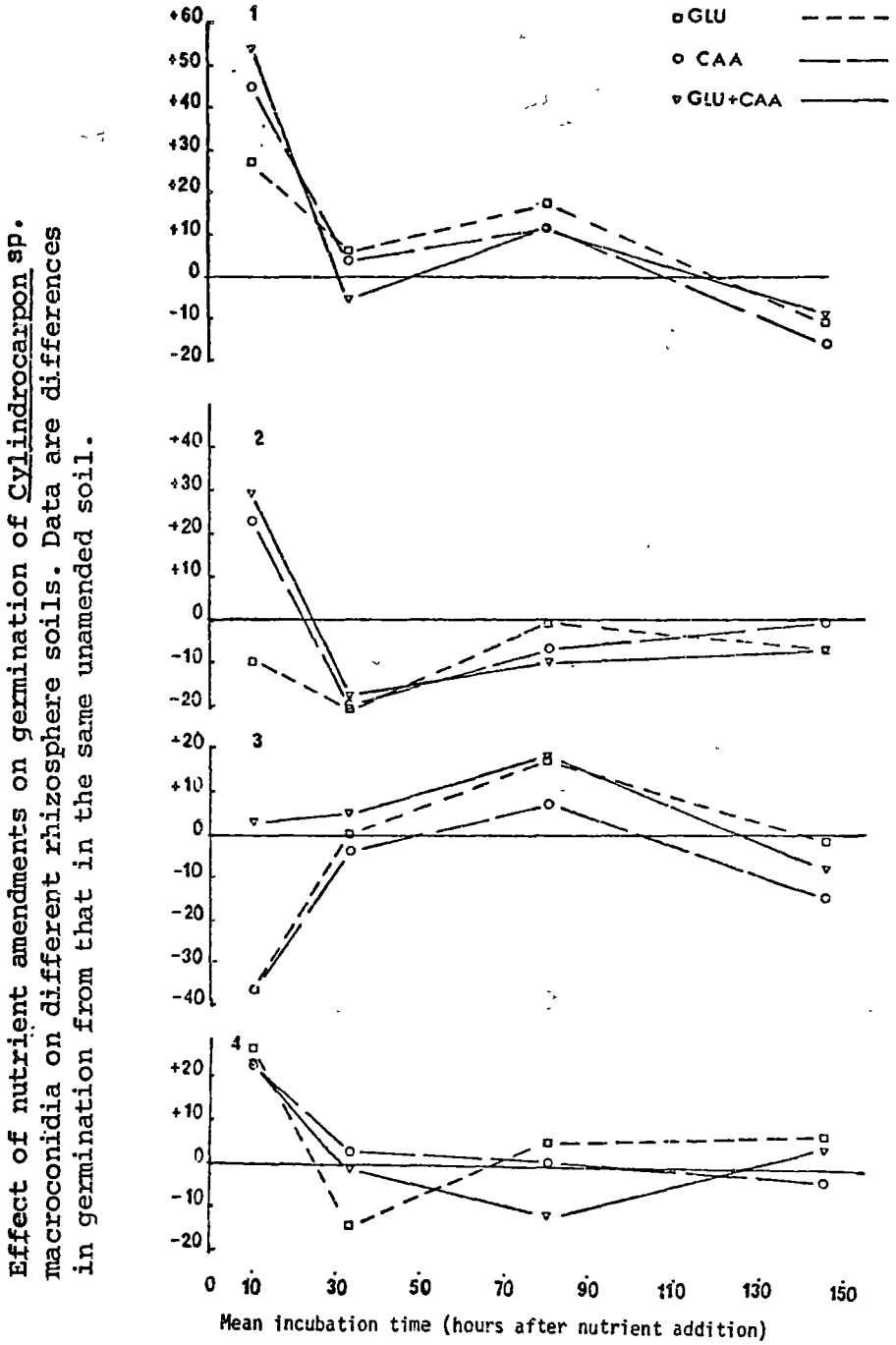


Figure 24 Fungistasis of Cylindrocarpon sp. macroconidia on rhizosphere soil from different E. obliqua forests which have been amended with various simulated exudates.

The soils were collected from the rhizospheres of E. obliqua in:
1. Dieback affected regrowth forests
2. Healthy regrowth forests
3. One year old regeneration on ashbed soil
4. One year old regeneration on non ashbed soil

The simulated exudate amendments consist of:
GLU - 3 ml of Glucose solution at 2000 ppm
CAA- 3 ml of Cas amino acid solution at 2000 ppm
GLU + CCA - 3 ml of Glucose at 2000 ppm plus 3 ml of Cas amino acids

The stimulation or inhibition of Cylindrocarpon sp. germination in response to the nutrient amendments was determined relative to germination in the same soil for each incubation period (Figure 24). The addition of either glucose or cas-aminoacids resulted in a marked initial depression in Cylindrocarpon sp. germination in the ashbed soil from young regeneration with high fungistatic potential (Soil 3) relative to that in the same unamended soil (Figure 24). This response is consistent with high fungistatis by the active microflora in response to the nutrient additions. Where both glucose and cas-aminoacids had been added there was an initial nett stimulation of germination relative to the unamended soils. This is consistent with some of the nutrients not being utilised by the microflora and consequently stimulating the germination of the macroconidia. The effects of nutrient amendments did not persist long in this microbiologically active soil.

In marked contrast nutrient amendments to rhizosphere soil from regrowth trees affected by dieback (Soil 1) resulted in the stimulation of Cylindrocarpon sp. germination relative to that in the same unamended soils. This is consistent with the low populations of micro-organisms in these soils not having the capacity to utilise all the added nutrients which consequently were available for stimulating the germination of the Cylindrocarpon sp. Very high levels of germination occurred in these soils with low fungistatic potentials particularly when they were amended with both glucose and cas-aminoacids. The addition of nutrient amendments to soils from E. obliqua regeneration on disturbed soil and healthy regrowth trees, which had intermediate levels of micro-organisms and fungistatic potentials, also resulted in the initial stimulation of Cylindrocarpon sp. germination but at a lower level than in the

soils from dieback trees. However with continued incubation an inhibitory effect developed, particularly in the soil from healthy regrowth forest. This response is consistent with excess nutrients initially stimulating both Cylindrocarpon sp. and the microbial activity of the soil, which subsequently resulted in an increase in fungistasis and/or nutrient depletion thereby causing a nett inhibitory response. Glucose amendments which appeared to be utilised the fastest, also resulted in the earliest inhibitory effect in these soils whereas glucose plus cas-aminoacids amendments resulted in the longest sustained inhibition of germination.

The lysis of Cylindrocarpon sp. germ tubes by micro-organisms in the rhizosphere soils incubated for 19 hours was least in the unamended soil from regrowth forests affected by dieback and highest in the soils from young regeneration on ashbed soils (Figure 25). The addition of glucose stimulated lytic activity in all soils, but to a relatively lesser extent in soils from regrowth forests (Figure 25). Whereas the soil from eucalypt regeneration on ashbeds had the highest fungistatic potential and lysis in the unamended state the soil from eucalypt regeneration on disturbed soil with a mild ashbed developed higher lytic activities in the presence of glucose.

These interactions between rhizosphere micro-organism, simulated root exudates and the fungistasis and lysis of Cylindrocarpon sp. inoculum are in agreement with the responses predicted by the hypothetical model of Cylindrocarpon sp. interactions in the E. obliqua rhizosphere (Figure 23). Although these responses were not quantified they strongly support the validity of the model of Cylindrocarpon sp. behaviour in the rhizosphere of E. obliqua.

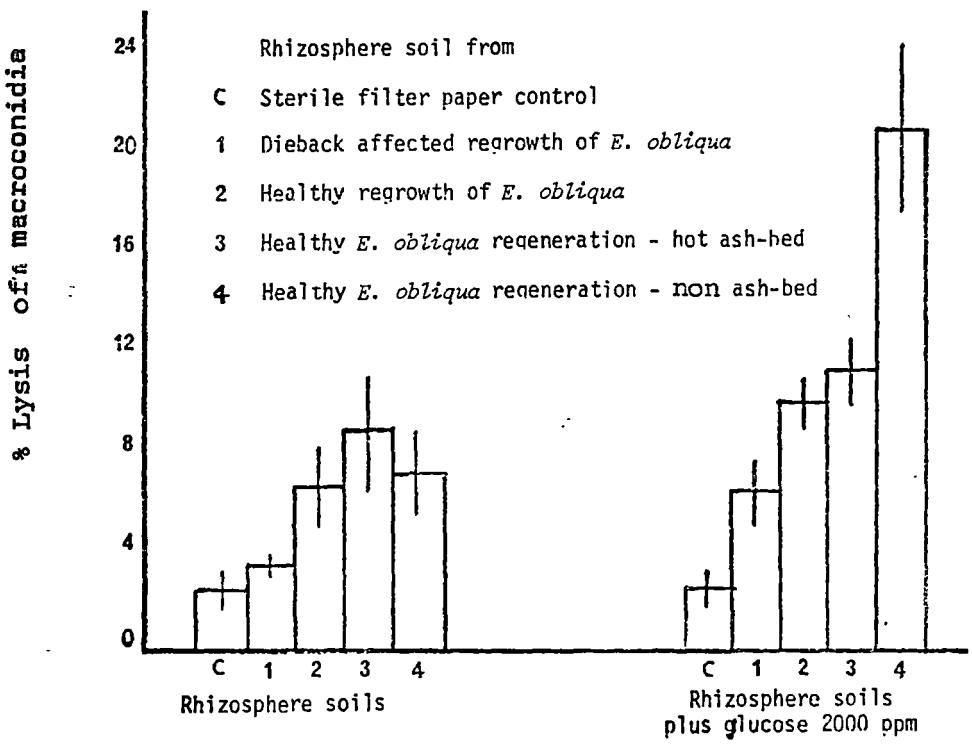


Figure 25 Lysis of Cylindrocarpon sp. macroconidia after 19 hours on rhizosphere soils from different E. obliqua forests with and without glucose amendments.

Vertical bars indicate twice the standard error for each treatment mean.

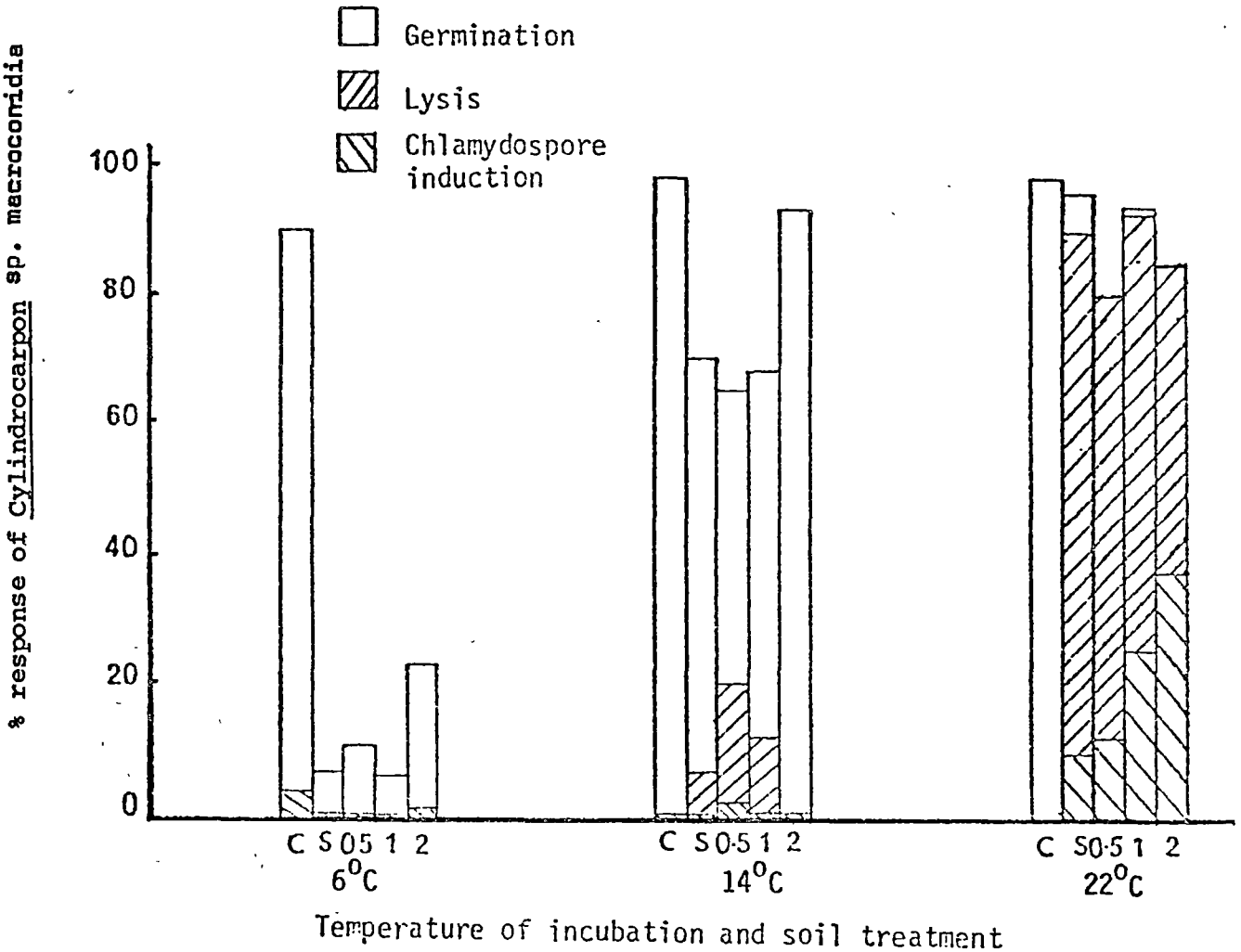


Figure 26 Germination. lysis and chlamydospore induction in Cylindrocarpon sp. macroconidia on rhizosphere soil which was incubated for 20 hours at different temperatures and had been amended with different levels of glucose.

C = response on moist filter paper

S = response on unamended forest soil

0.5 = response on soil amended with 500 ppm glucose

1 = response on soil amended with 1000 ppm glucose

2 = response on soil amended with 2000 ppm glucose

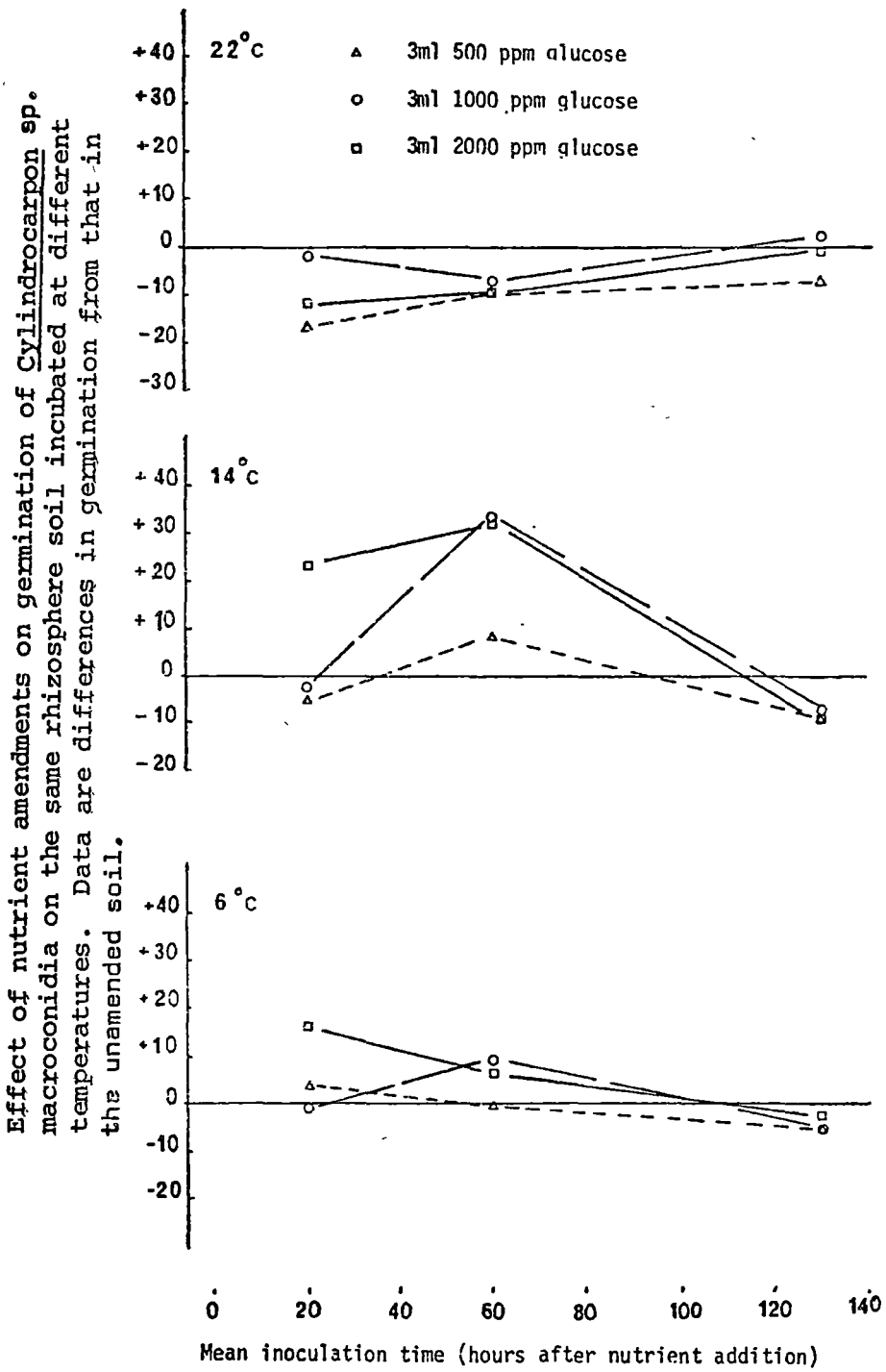


Figure 27 Fungistasis of Cylindrocarpon sp. macroconidia on the same rhizosphere soil which was incubated at different temperatures and amended with different levels of simulated exudates.

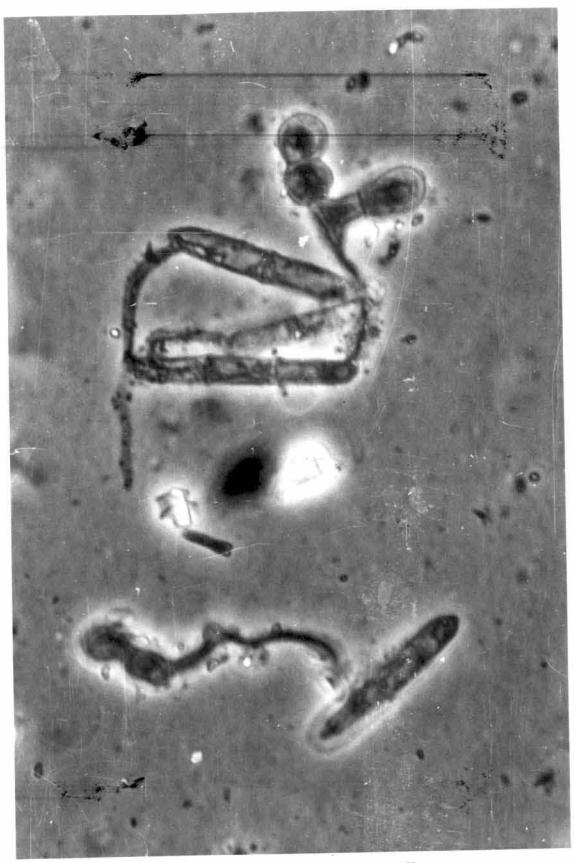


Figure 28 Macroconidia of Cylindrocarpon CMI196141 illustrating germination, germ tubes, lysis and chlamydospore induction following incubation on E. obliqua rhizosphere soil.

6.4.1b Fungistasis and Lysis at Different Incubation Temperatures

The germination of Cylindrocarpon sp. macroconidia increased on the soil from the rhizosphere of regrowth trees affected by dieback with increasing temperatures of incubation (Figure 26). The stimulation or inhibition of Cylindrocarpon sp. germination in response to nutrient amendments at the various incubation temperatures were again examined relative to the germination on the unamended soil at that incubation (Figure 27). The addition of nutrients to rhizosphere soil, which had been incubated at 22°C to promote maximum microbial activity, resulted in an initial decrease in germination relative to their unamended controls. In contrast the addition of the same nutrients to the same soil which had been incubated at 6°C resulted in a stimulation of Cylindrocarpon sp. germination relative to the unamended control. Soil incubated at 14°C stimulated germination only at the highest level of nutrients addition relative to that in the unamended soil.

The stimulation of Cylindrocarpon sp. germination in the amended soil incubated at 6°C to induce with low microbial activities and fungistasis and on the inhibition of germination in the same soil treatments which had been incubated at 22°C to induce high microbial activities and fungistasis are entirely consistent with the responses predicted in the hypothetical model.

The lysis of Cylindrocarpon sp. macroconidia and the induction of conidial chlamydospores in this experiment (Figure 26) were also consistent with those predicted in the model. Very few of the germinated macroconidia remained viable and active on soil incubated at 22°C due to the very high lysis and induction of conidial

chlamydospores (Figure 28). In contrast most Cylindrocarpon sp. macroconidia remained viable in soils incubated at 6°C. The higher levels of microbial activities in soils incubated at 22°C are likely to have resulted in a high level of lysis and induction of conidial chlamydospores whereas the lower incubation temperature are likely to have resulted in decreased microbial activities and therefore lower levels of lysis.

Maximum germination and survival of Cylindrocarpon sp. inoculum occurred in the regrowth forest soils incubated at 14°C. This had previously been found to be the optimum temperatures for the growth and saprophytic colonisation of Cylindrocarpon sp. (Chapter 4.3). The addition of nutrients to soils incubated at 22°C increased the proportion of macroconidia which were able to produce conidial chlamydospores in response to the very high levels of lysis.

The results of both of these experiments strongly support the validity of the hypothetical model of the interactions of Cylindrocarpon sp. in the rhizosphere of E. obliqua. The germination, lysis and survival of Cylindrocarpon sp. inoculum in the rhizosphere of E. obliqua would appear to be largely governed by the resultant of interactions between stimulatory root exudates and fungistasis resulting from the utilisation of those exudates by the rhizosphere micro-organisms. The changes in either the level or nature of the root exudates or the population or activity of rhizosphere micro-organisms in relation to stress, age, and species are likely to greatly affect the stimulation and lysis of the Cylindrocarpon sp. inoculum in the rhizosphere of E. obliqua and thereby influence its capacity for root infection.

Consequently the capacity of Cylindrocarpon sp. to infect E. obliqua roots appears to be largely governed by the interactions in the rhizosphere which affect the fungistasis and lysis of the inoculum on the root surface. The experiments in this chapter have defined some of the factors which may contribute to the interactions involved in the rhizosphere and their influence on the behaviour of Cylindrocarpon sp. on the surface of E. obliqua roots. A hypothetical model of the interaction of these factors and their effect on Cylindrocarpon sp. in the rhizosphere of E. obliqua was tested and may have application in understanding the microbial ecology of dieback in the E. obliqua regrowth forests as well as in other root diseases.

CHAPTER 7

CYLINDROCARPON SP. INFECTION AND DECAY OF THE FINE ROOTS OF E. OBLIQUA

The previous pathogenicity studies indicate that Cylindrocarpon sp. can infect and decay the fine roots of E. obliqua seedlings and may, under certain conditions, be pathogenic to E. obliqua seedlings (Chapter 5). Previous studies have also examined the interactions between Cylindrocarpon sp., other micro-organisms and root exudates in the rhizosphere of E. obliqua regrowth trees and studied how these may govern the behaviour of Cylindrocarpon sp. inoculum on the surface of E. obliqua roots (Chapter 6). However the mechanisms involved in the infection and decay of E. obliqua roots by Cylindrocarpon sp. and their relationship to the pathogenicity of this fungus have not been adequately described. The experiments in this chapter examine these mechanisms and their role in the pathogenicity of Cylindrocarpon sp. and the dieback of E. obliqua regrowth forests in southern Tasmania.

7.1 Cylindrocarpon sp. Infection and Colonisation of the Cortex of Fine Roots of E. obliqua

The similarity in the relationships between Cylindrocarpon sp. infection of the roots of E. obliqua seedlings and infection of dead leaf bait with increasing Cylindrocarpon sp. inoculum (Chapter 4.4, Chapter 5.1) and the close association between root infection and the fungistasis of Cylindrocarpon sp. on the surface of E. obliqua roots (Chapter 6.1) indicates that the cortex of E. obliqua roots may have little resistance to infection by Cylindrocarpon sp.

Rather it would appear that resistance to infection is governed by the fungistasis and lysis of Cylindrocarpon sp. inoculum in the rhizosphere (Chapter 6) and that once the fungus has overcome these factors it can readily infect the fine roots of E. obliqua. However, as possible defence reactions in the cortex of E. obliqua roots may also influence the infection, by Cylindrocarpon sp. investigations were conducted to determine the existence and nature of any such host reactions.

Materials and Methods

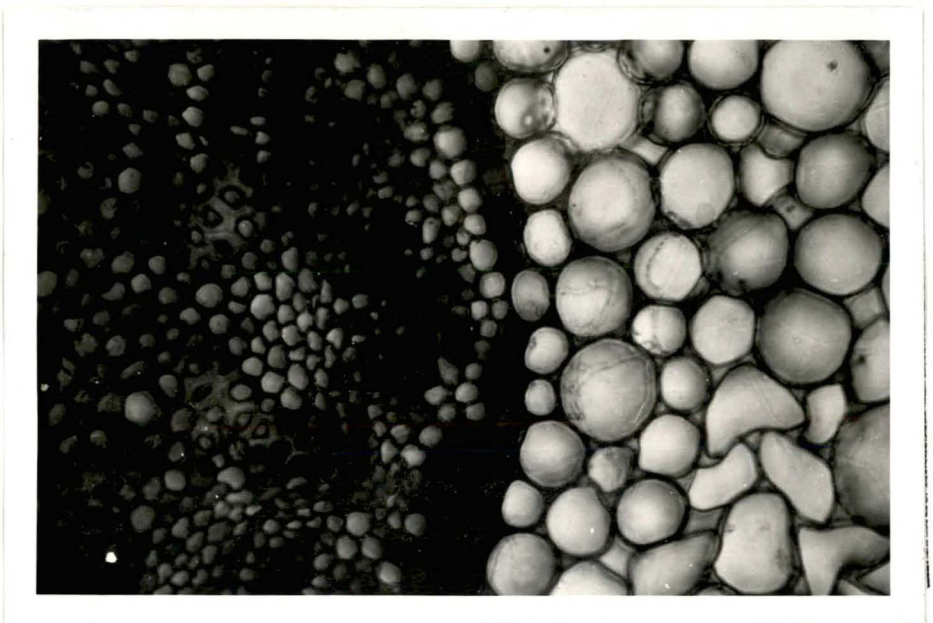
The roots of of E. obliqua seedlings which had been infected by Cylindrocarpon sp. in the various pathogenicity studies were examined microscopically for the presence of host defence reactions. Large numbers of transverse and longitudinal root sections, 12-30μ thick, were cut from fresh root tissue with a Hooker microtome and stained in lactophenol trypan blue. Particular attention was paid to roots from the pathogenicity studies in Chapter 3.8 and Chapter 5.3.

Results and Discussion

Cylindrocarpon sp. hyphae readily penetrated the epidermis and cortex of E. obliqua roots without any evidence of infection cushions. Hyphal growth within the root cortex was both inter and intracellular and although generally extensive, resulted in only minimal structural damage to the root cortex (Figure 29). Some breakdown of cortical walls and separation of parenchyma cells was observed. Cylindrocarpon sp. was capable of decreasing the viscosity of media containing pectin which suggests that it can produce pectolytic enzymes as does C. destructans (Milko and Melvik

Hyphae in inner cortical cells, transmitted light x 150.

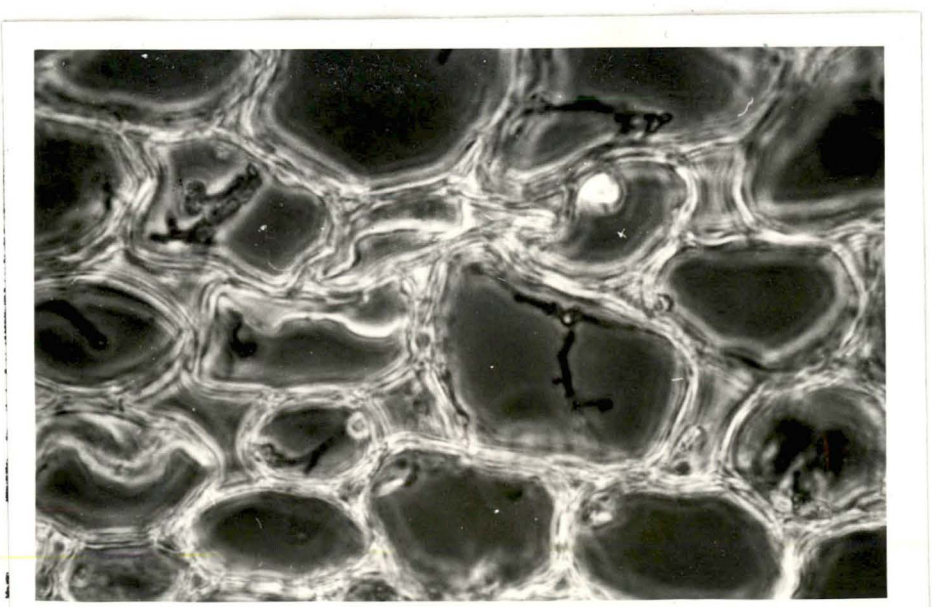
a



50µ

Detail of hyphae in cortical cells, phase contrast x 400.

b



10µ

Figure 29 Hyphal infection of the cortex of fine roots of E. obliqua by Cylindrocarpon sp.

1960). No anatomical features or accumulation of materials which may indicate physical resistance in the host to cortical root infection were observed in these sections.

The failure to detect physical defence barriers within the cortex of E. obliqua roots suggests that infection by Cylindrocarpon sp. is largely governed by chemical inhibitions or by factors in the rhizosphere (Chapter 6.4). The latter is consistent with observations in previous investigations on E. obliqua roots infected by Phythophthora cinnamomi (Tippett et al 1976).

Low levels of infection of the E. obliqua root cortex by Cylindrocarpon sp. did not appear to be detrimental to the growth and function of the roots. Indeed Kurbis (1937) and Paget (1975) have suggested that similar root infections by C. destructans may at times benefit plant growth and nutrient uptake in a manner not unlike that in endomycorrhizas. However, no studies were conducted to test whether beneficial responses may occur in E. obliqua infected by Cylindrocarpon sp.

7.2 Cylindrocarpon sp. in the Stele of E. obliqua Roots

Cylindrocarpon sp. hyphae were never found infecting the stele of living E. obliqua roots in these examinations. Colonisation of the stele was prevented by either physical and/or chemical barriers which included suberised endodermal tissue, pericycle tissues and zones of phenolic materials below the pericycle (Figure 29). While the cortex of E. obliqua roots which had been inoculated with plugs of Cylindrocarpon sp. culture was often macerated, transverse sections through these infections revealed that colonisation of the stele was prevented in all cases by the development of multi

pericycle layers and/or by accumulations of phenolic-like materials below the point of inoculation (Figure 30). However, inspection of the infected roots revealed that many of the xylem vessels were blocked with tyloses and gum (Figure 31). The dark red staining, tyloses and gummosis extended above and below the point of inoculation and into the xylem vessels of branch roots. No such blockages or staining were observed in comparable uninfected E. obliqua roots.

It would appear that Cylindrocarpon sp. may be capable of producing a substance in the root cortex of E. obliqua which may be transported across the endodermis and into the xylem vessels where it may induce vascular staining, tyloses and gum blockages of the xylem vessles and consequently restrict the capacity of the xylem to conduct water. Investigations of Cylindrocarpon spp. on apricots (Rieuf 1952, Prunus, Govi 1956), strawberries (Wilhelm 1958) and Quercus (Uroseric 1963) had previously demonstrated instances of similar vascular staining, tyloses and gummosis of xylem tissue without the Cylindrocarpon spp. infecting the xylem tissue. Subsequent studies by Kluge (1966) and Evans, Cartwright and White (1967), on Pinus and Eucalyptus respectively, confirmed that pathogenic strains of C. destructans could produce phytotoxins in culture which were capable of stunting and killing test plants at low concentrations. Non pathogenic isolates of C. destructans failed to produce similarly high levels of this phytotoxin. The phytotoxin was isolated and identified as a macrolide antibiotic similar to Brefeldin A (Sigg 1964). However both the production of the phytotoxin within plants (Whilhelm 1959, Evans 1966) and the relationship between phytotoxin production and vascular dysfunction requires further investigation.

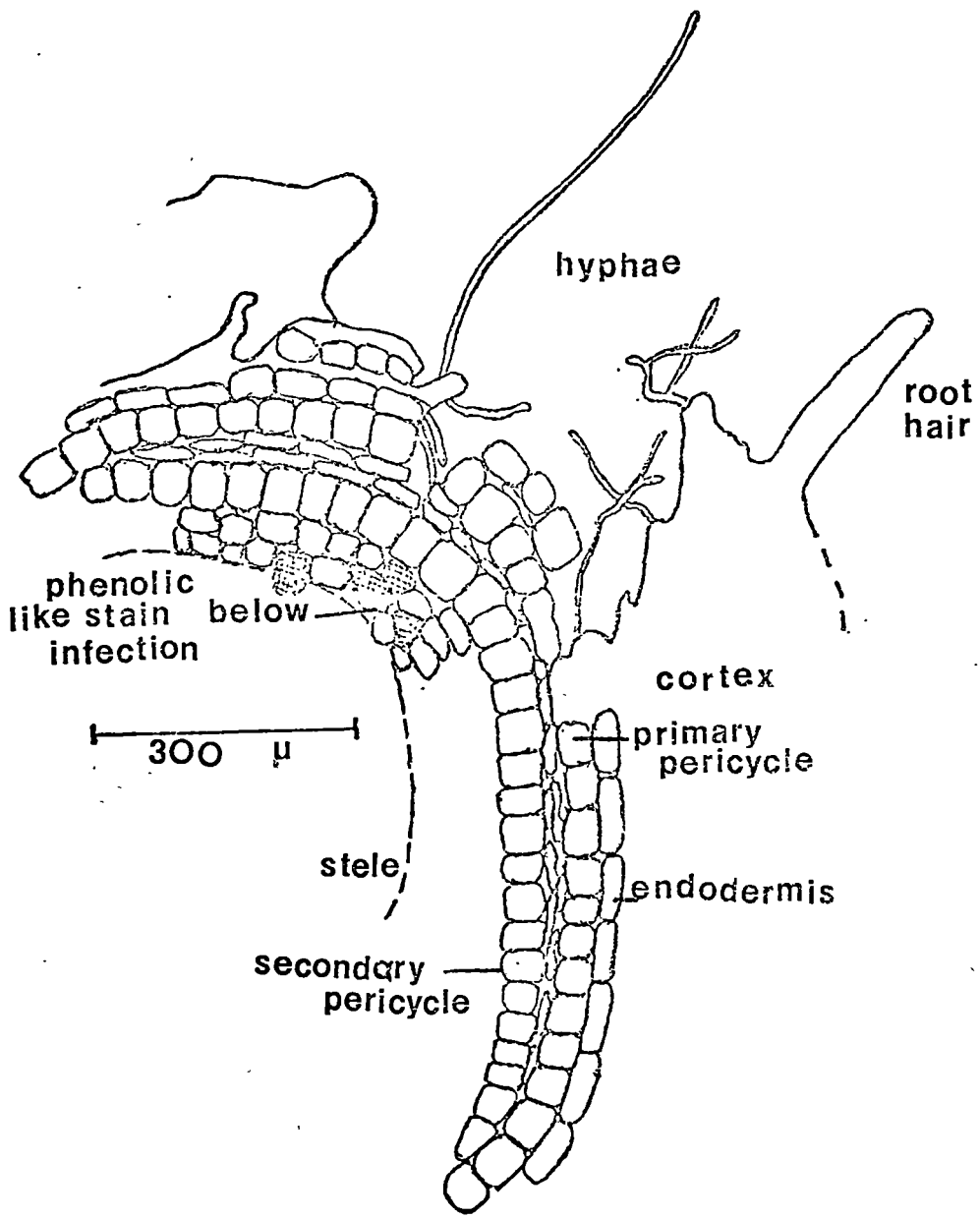


Figure 30 Secondary pericycle formation in the stele of E. obliqua roots in response to inoculation with Cylindrocarpon sp.

Figure 31.

Tyloses and blockages of the xylem of E. obliqua roots without fungal colonisation of the affected stelar tissue.

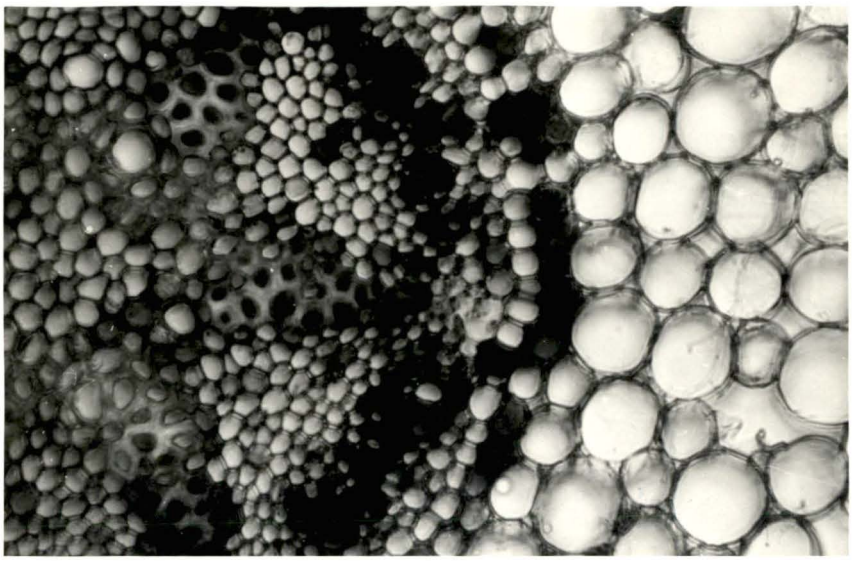
a. Extensive blockages of xylem initials by gum and tyloses with Cylindrocarpon CMI 196141 hyphae in the cortex (transmitted light x 150 magnification.

b. As above phase contrast x 150 magnification.

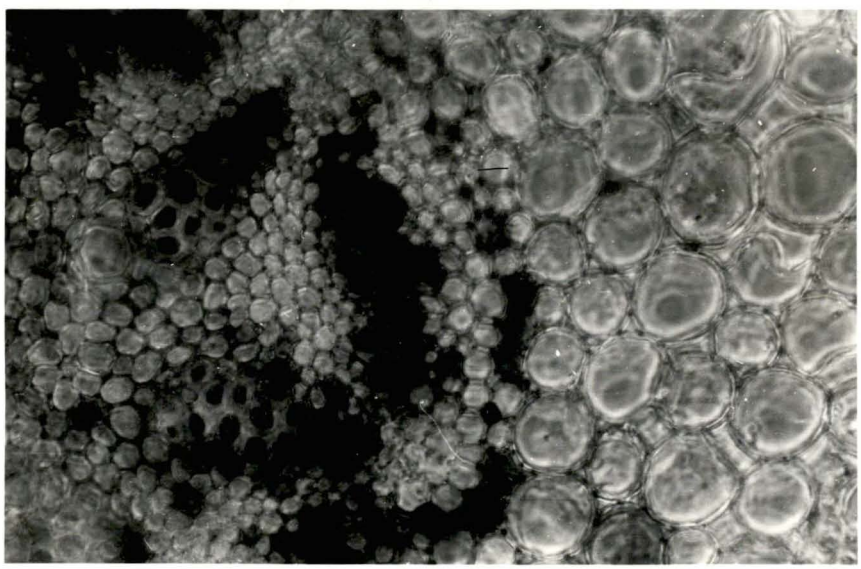
c. Vascular connection of a lateral root showing xylem blockages in the main root and their extension along the vessels of the latter root x 150 magnification.

120 µ

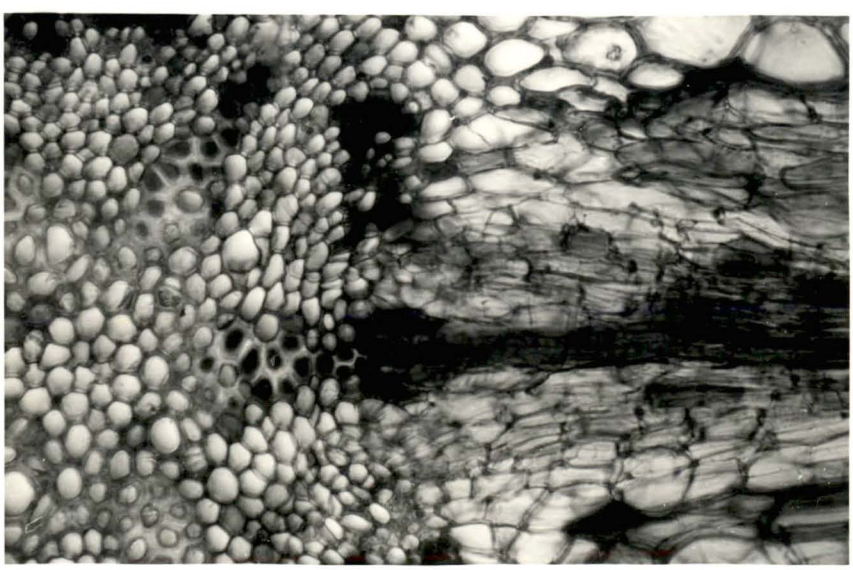
a



b



c



Consequently the ability of Cylindrocarpon sp. to produce similar phytotoxins in culture and in E. obliqua roots was investigated as were the factors which influence its production and effects within the plant.

7.3 Phytotoxin Production by Cylindrocarpon sp.

A series of experiments were conducted to investigate the presence of phytotoxins in sterilised culture filtrates of Cylindrocarpon sp. and study their effects on E. obliqua seedling growth and root dysfunction.

Materials and Methods

In the first experiment three E. obliqua seedlings which had been grown for 2 weeks in autoclaved vermiculite were placed in each of four replicate tubes containing one of the following treatments:-

1. Sterile Hoagland's nutrient solution
2. Viable liquid culture of Cylindrocarpon sp.
3. Membrane filter sterilised (0.45μ) liquid culture of Cylindrocarpon sp.
4. Autoclaved sterilised liquid culture of Cylindrocarpon sp.
5. Hoagland's nutrient solution containing viable mycelium of Cylindrocarpon sp. which had been washed free of any culture filtrate.

The seedlings were grown at 10°C in a naturally lit growth cabinet and were inspected daily for mortalities. The three most affected seedlings per treatment were progressively removed after 3, 7 and 14

days incubation and placed in F.A.A. (Formalin, Acetic acid, Alcohol, 10;5;85) for microscopic examination of root infection and vascular dysfunction. The experiments were repeated using Czapek's nutrient solution and Czapek's culture solutions.

The root tips of 12-month-old E. obliqua seedlings were also inserted into glass tubes containing culture filtrates of either Cylindrocarpon sp. or sterile nutrient solutions. The roots which were still attached to the seedling had been carefully excavated at the soil-pot interface and were cut from the plant after 12 days growth at 18°C and examined for vascular staining and tyloses.

Results and Discussion

Mortalities occurred in the E. obliqua seedlings which had been placed in the Cylindrocarpon sp. liquid cultures and in the heat or filter sterilised culture filtrates. No mortalities were recorded during the experimental period in the seedlings growing in Hoagland's solution or in the seedlings inoculated with washed Cylindrocarpon sp. mycelium (Table 23). A heat stable, filtrable phytotoxin appeared to be present in the Cylindrocarpon sp. culture filtrates which was capable of killing E. obliqua seedlings at normal culture concentrations.

Tyloses and gum blockages occurred in the xylem vessles of E. obliqua roots grown in Cylindrocarpon sp. culture and in the heat and filter sterilised treatments. No such blockages were observed in the control seedlings. Seedlings in Hoagland's solution with added washed Cylindrocarpon sp. mycelium had extensive hyphae in the cortex but no evidence of vascular tyloses or gummosis in their root

TABLE 23 The effect of *Cylindrocarpon* sp. culture treatments on the mortality and infection of *E. obliqua* seedlings.

Growth Media for 10 *E. obliqua* seedlings per Treatment	Number of Seedlings with Symptoms Day 3 Wilted	Day 3 Dead	Day 7 Wilted	Day 7 Dead	Day 10 Wilted	Day 10 Dead	Cortical Infection Day 14	Vascular Staining & Tylose
1 Hoaglands nutrient solution	0	0	0	0	0	0	0	0
2 Viable culture of *Cylindrocarpon* sp.	1	2	2	8	0	10	+	+
3 Membrane filtered *Cylindrocarpon* sp. culture	1	0	4	0	6	0	0	+
4 Autoclaved culture of *Cylindrocarpon* sp.	0	0	3	2	6	4	0	+
5 Viable washed mycelium of *Cylindrocarpon* sp. in hoaglands solution	0	0	0	0	0	0	+	0

steles. Similar results were obtained in the repeat experiment using Czapek's nutrient and culture solutions.

Microscopic examination of the roots of 12 month old E. obliqua seedlings also confirmed that those grown in sterilised culture filtrates consistently developed vascular tyloses and gummosis. Consequently vascular dysfunction which is associated with mortalities of E. obliqua roots and seedlings appears to result from a phytotoxin produced by cultures of Cylindrocarpon sp. While mycelium of Cylindrocarpon sp. can result in the infection of the cortex of E. obliqua roots this did not result in root dysfunction or the mortality of E. obliqua seedlings.

7.3.1 Isolation of the Phytotoxin from Cylindrocarpon sp.

Both Kluge (1966) and Evans, Cartwright and White (1967) extracted and identified the phytotoxin from C. destructans which in the latter case was called Nectrolide. The phytotoxin from Cylindrocarpon sp. culture filtrates was extracted using an identical technique to that described by Evans et al (1967) and its properties compared with those of Nectrolide.

One hundred mls of Cylindrocarpon sp. culture filtrate, which had been grown for 20 days at 22^{o} in Czapek's liquid media (Appendix 1) while stationary in the dark, was extracted twice with 50ml of anhydrous ethyl ether. The combined ether fractions were washed with 50ml 1% sodium bicarbonate and dried under vacuum. A white amorphous residue, frequently contaminated with a red brown gum, was consistently recovered. Weighed amount of this residue were redissolved in water for subsequent bioassay studies. As these

bioassay studies were based on solutions made from known dry weights of residues which contained variable traces of gum the phytotoxin concentrations in different extractions may vary and should not be compared between experiments.

7.3.2 Bioassay of Cylindrocarpon sp. Phytotoxin

The biological activity of the extract from the Cylindrocarpon culture filtrates was examined in a series of bioassay experiments.

Materials and Methods

7.3.2a E. obliqua Seedling Growth

E. obliqua seedlings which had been grown for 2 weeks in autoclaved vermiculite were transferred to tubes containing either 0, 10 or 25ppm phytotoxin or tubes with heat and filter sterilised Cylindrocarpon sp. culture filtrates. The seedlings were grown at 10°C in natural light and mortalities were recorded daily. The lengths and numbers of lateral roots formed from the initial single taproot after 18 days growth were measured on each seedling with a stage micrometer at x 100 magnification. The experiment was repeated using similar seedlings which were grown at 18-22°C in natural light in replicated tubes containing 0, 6, 12, 25 and 50ppm phytotoxin. Mortalities, shoot growth, root growth and seedling height were recorded after 28 days growth.

7.3.2b Tomato Shoot Symptoms

Lycopersicum esculentum shoots from mature plants were placed in replicated tubes of freshly prepared phytotoxin of either 0, 100 or 1000ppm and in phytotoxin of similar concentrations which had been stored in the light for 6 weeks at 22°C (Kluge 1966). The wilting and mortality of shoots and the length of shoot necrosis was recorded after 4 days growth at 18-22°C.

7.3.2c Root Growth of Medicago sativa Seedlings

Approximately 30 M. sativa seeds were seeped in 0.5ml of aqueous phytotoxin for 16 hours in the dark and then transferred onto sterile moistened filter paper and incubated in the dark for 4 days at 22° when the mean length of the seedling radicles was measured.

Results and Discussion

4.3.2a E. obliqua Seedling Growth

In the first E. obliqua bioassay both the length and total number of lateral roots which had formed over the 18 days decreased with increasing phytotoxin concentration (Table 24a). Root growth in the sterilised culture filtrates was more severely reduced. In the second of the bioassays with E. obliqua, seedling mortalities and significant reductions in height, leaf area and root growth were recorded after 28 days at phytotoxin concentrations at and above 12ppm (Figure 32, Table 26b). Tyloses and gummosis of xylem tissue occurred in the wilted seedlings.

TABLE 24 Seedling responses to phytotoxins extracted from *Cylindrocarpon* sp. cultures.

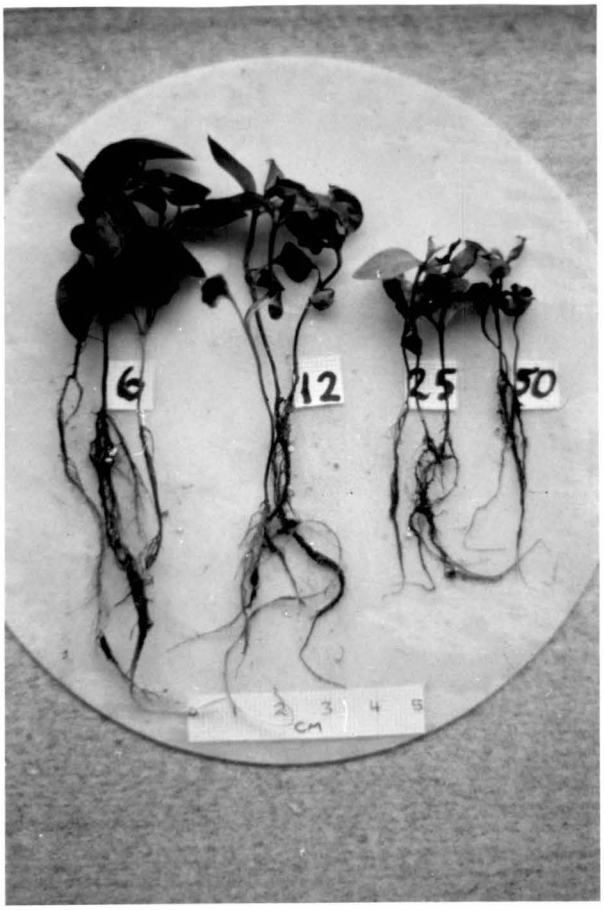
a. Root growth in *E. obliqua* seedlings. Mean of 12 seedlings per treatment.

Treatment for growth of *E. obliqua* seedlings	No. of lateral roots produced	Mean length of lateral roots	Cumulative length of lateral roots
		MM	MM
1. Czapeks nutrient solution as control	34 ± 4	2.21	75 ± 17
2. *Cylindrocarpon* sp. culture in Czapeks solution	12 ± 1	1.00	12 ± 1
3. Filter sterilized culture solution of *Cylindrocarpon* sp.	2.4 ± 1	1.08	3 ± 2
4. Autoclaved culture solution of *Cylindrocarpon* sp.	11 ± 3	1.00	11 ± 3
5. Phytotoxin extracted from *Cylindrocarpon* sp. culture, 10ppm	30 ± 5	2.03	60 ± 16
6. Phytotoxin from *Cylindrocarpon* sp. culture, 25ppm.	23 ± 1	1.15	26 ± 4

b. Growth of *E. obliqua* seedlings. Mean of 8 seedlings per treatment.

Phytotoxin concentration in Hoaglands solution (ppm)	Height MM	Leaf Area MM^2	% Seedlings with Symptoms Chlorosis	Wilting	Death	Total
0	51 ± 6	402 ± 69	0	0	0	0
6	53 ± 3	277 ± 80	0	0	0	0
12	43 ± 3	214 ± 51	24	0	12	36
25	34 ± 3	199 ± 44	63	12	12	87
50	27 ± 3	122 ± 34	0	12	88	100

a The growth of E. obliqua seedlings



b Stem necrosis and death of Lycopersicum esculentum shoots

Figure 32 Effect of phytotoxins from Cylindrocarpon sp.

Both of these bioassays confirmed that extracts from Cylindrocarpon sp. culture filtrates were phytotoxic to E. obliqua seedlings. The phytotoxin appears to be similar in its effect as nectrolide isolated from C. destructans which reduced growth of E. pilularis seedlings in similar bioassays at concentrations as low as 6ppm. However the presence of gum residues in the extract does not allow accurate quantitative comparisons of dosage responses.

7.3.2b Tomato Shoot Symptoms

Tomato shoots placed in fresh phytotoxin at 1000ppm were killed within 4 days while wilting occurred in shoots placed in phytotoxin at 100ppm (Figure 32, Table 24c). In contrast tomato shoots placed in phytotoxin which had been stored in the light for 6 weeks at 22°C were unaffected at 100ppm and only wilting at 1000ppm. The length of shoot necrosis after 4 days was highest in the fresh extract at 1000ppm and intermediate in the fresh extract at 100ppm and the stored extract at 1000ppm (Table 24c). Petiole necrosis extended well above the level of immersion and sections through the necrotic petioles consistently revealed tyloses and gum blockages of the vascular tissues. Tomato shoots in the control treatments remained turgid and healthy. The results of this bioassay are consistent with those in pathogenic isolates of C. destructans (Kluge 1966).

7.3.2c Root Growth of Medicago sativa Seedlings

Root growth in Medicago sativa seedlings decreased as the concentration of phytotoxins increased above 20ppm (Table 24d). This bioassay was highly reproducible, simple and quick and was used in some of the following studies.

Table 24 c Stem necrosis and wilting of Lycopersicum esculentum shoots

Original Concentration of phytotoxin (ppm)	Freshly extracted phytotoxin		Phytotoxin stored at 20°C in light	
	Length of stem necrosis (mm) *	Symptoms	Length of stem necrosis (mm) *	Symptoms
0	0	0	0	0
100	16	wilting	10	0
1000	47	death	23	slight wilting

* stems were imersed 10mm in the phytotoxin

TABLE 26 d Root Growth in Medicago sativa seedlings. Mean length of 20 seedlings per treatment (mm).

Phytotoxin concentration in imbibing solution (ppm)	Root length of M. sativa seedlings $LSD_{(p0.05)} = 2$
0	17
10	16
20	14
100	12
200	11
1000	8
2000	7

7.3.3 Chemical Properties of the Phytotoxin from Cylindrocarpon sp.

The chemical properties of the phytotoxin from Cylindrocarpon sp. were examined using the methods described by Evans et al (1967) and were compared with some of the chemical properties of Nectrolide and Brefeldin A which have been described by Evans et al (1967) and Sigg (1964).

Silica gel G thin layer plates were spotted with Cylindrocarpon sp. phytotoxin and run to 10cm in 10% ether in methanol and then dried and sprayed with 36N sulphuric acid. Black spots appeared at rf 0.60 to 0.66 which are consistent with those described for nectrolide, rf 0.66. A larger black diffuse spot associated with gum coloration prior to spraying was also observed.

The ultra violet absorption spectra of freshly prepared Cylindrocarpon sp. phytotoxin in ethanol was examined using a Unicam SP 800 scanning spectrophotometer. Distinct absorption maxima occurred at 237.5, 243, 248.5, 254 and 260.5mμ while mimima occurred at 240, 246, 252, 258 and 266mμ (Figure 33). The absorbance at each of the maxima was closely related to the concentration of phytotoxin in the ethanol suspension (Figure 34). Consequently the U.V. absorption at each of these wavelengths could be used to measure the relative concentrations of phytotoxin but only in fresh ethanol extracts.

The U.V. absorption spectra of the phytotoxin from Cylindrocarpon sp. was unstable. Rescanning of phytotoxin solutions which had been stored for 30 days at 22°C in natural light revealed that the stored phytotoxin had a large absorption peak between 214 and 220mμ with smaller rounded maxima at 241, 246, 250, 256.5 and minima at 243,

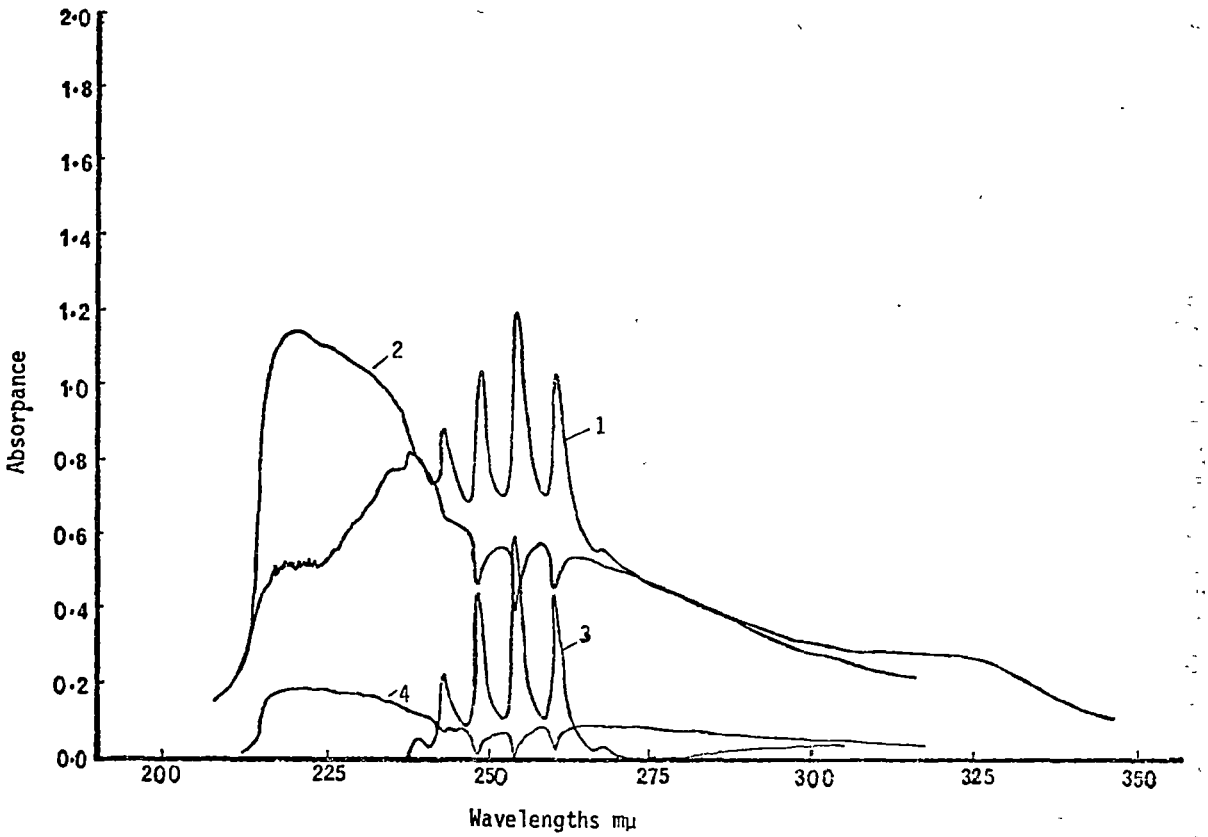


Figure 33 - Ultraviolet absorption spectra of phytotoxin from Cylindrocarpon CMI 196141 and changes following storage.

U.V. absorption spectrum of Cylindrocarpon CMI 196141 phytotoxin extracted from Czapeks culture solution with 2% sucrose as carbon source.

1. Absorption spectrum of phytotoxins from still incubated cultures measured just after extraction.

2. Absorption spectrum of phytotoxin from still incubated cultures measured after 7 days storage in ethanol in light.

3. Absorption spectrum of phytotoxin from shake incubated cultures measured just after extraction (see Table 27).

4. Absorption spectrum of phytotoxin from shake incubated cultures measured after 7 days storage in ethanol in light.

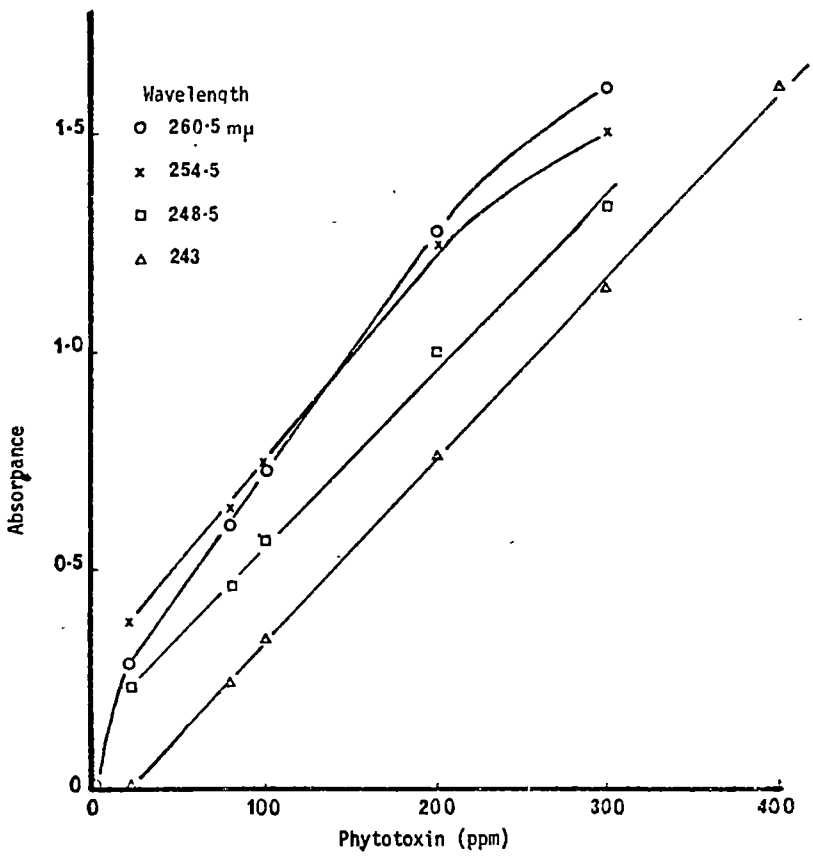


Figure 34 Relationship between the concentration of fresh phytotoxin and its U.V. absorption at diagnostic wavelengths.

248, 254 and 260.5mμ (Figure 33). These new minima occurred at the same wavelengths as the maxima in the freshly extracted phytotoxin. Consequently only freshly extracted phytotoxins were used in comparative studies. The U.V. absorption characteristics reported for Brefeldin A (Sigg 1964) and Necrolide (Eavns 1966) are consistent with those of partially degraded phytotoxin from Clyindrocarpon sp. Numerous structural and chemical changes occur during the oxidation of Brefeldin A (Harri and Sigg 1963, Kluge 1966) and may in part account for these changes in the U.V. absorption.

As the activity of the phytotoxin from Cylindrocarpon sp. may alter with environmental factors investigations were conducted of the stability of the phytotoxin in natural light or darkness both with or without oxygen. Tubes containing freshly extracted phytotoxin in ethanol were either enclosed in aluminium foil to exclude light or left unsealed. Replicate tubes were incubated either in air or in a sealed anaerobic jar for 14 days at 22°C in the light. The U.V. absorbtion spectra of each treatment was then compared with that of the freshly extracted phytotoxin to examine changes during storage. Phytotoxin stored under dark, anaerobic conditions had the least change in its absorbtion spectra while the greatest change occurred following aerobic storage in natural light.

Consequently environmental conditions may influence the rate of phytotoxin breakdown. It is therefore possible that the level of root dysfunction may also be influenced by the environmental conditions in the inner cortex of the E. obliqua roots. Anaerobic conditions in particular may be important in limiting the rate of breakdown of phytotoxins within E. obliqua roots. The higher level

of root decay in E. obliqua seedlings (Chapter 5.2) or forest trees (Chapter 3.6) under partially anaerobic soil conditions may be associated with the effect of these environmental factors on the production and stability of the Cylindrocarpon sp. phytotoxin.

7.3.4 Factors Influencing the Production of Phytotoxins by Cylindrocarpon sp.

While Cylindrocarpon sp. can produce phytotoxins in artificial culture investigations need to determine whether phytotoxins are also produced in substrates which may be present in regrowth trees.

Materials and Methods

7.3.4a Natural Substrates and Carbon Sources

The capacity of Cylindrocarpon sp. to produce phytotoxins from a range of natural substrates including fresh leaves, excised roots or live roots of E. obliqua as well as from sucrose, starch and pectin was examined in the following experiment. Fresh substrates equivalent to 2.5g of dry leaves and 1.6g of dry roots or 1g of either sucrose, starch or citrus pectin were added to four conical flasks (150ml) containing 50ml of Czapek's nutrient solution and were sealed and autoclaved. The roots of four living E. obliqua seedlings were surface sterilised in 0.1% $HgCl_2$ for 60 seconds, repeatedly washed in sterile water and placed into similar flasks. Three flasks of each of E. obliqua substrate and all of the flasks in the other carbon substrates were inoculated with Cylindrocarpon sp. macroconidia and incubated while stationary in the dark at 22°C. Flasks with live seedlings were incubated with the shoots in natural

light. An additional four flasks with sucrose were also incubated on an orbital shaker to enhance the aeration of the liquid culture.

After 10, 20 and 27 days incubation, 10, 20 and 30ml respectively of liquid culture were withdrawn from each flask and the concentrations of phytotoxins in these aliquots was determined using the M. sativa bioassay and U.V. absorption at 260.5mμ. Phytotoxin production in the E. obliqua substrates was assessed by comparing differences in absorption by the inoculated substrates relative to the corresponding uninoculated controls.

7.3.4b Nitrogen Sources

The effects of different nitrogen sources on phytotoxin production by Cylindrocarpon sp. was also investigated. Czapek's nutrient solution (50ml) with 2% sucrose and 1mg per ml nitrogen equivalents of either $NaNO_3$, $(NH_4)_2SO_4$ or L-asparagine was placed in four 150ml conical flasks, autoclaved and inoculated with Cylindrocarpon sp. macroconidia. After 14 days still incubation at 22°C in the dark the Cylindrocarpon sp. mycelium was filtered off and the remaining culture filtrates individually extracted and tested for phytotoxins. The dry weight of Cylindrocarpon sp. mycelium was also determined.

Results and Discussion

7.3.4a Natural Substrates and Carbon Sources

Cylindrocarpon sp. produced phytotoxins in the E. obliqua substrates as well as on artificial carbon sources (Table 25). Phytotoxin production was higher on the roots of dead E. obliqua seedlings than

TABLE 25 The production and activity of phytotoxins from culture filtrate of Cylindrocarpon sp. in relation to carbon and nitrogen sources.

Substrate Treatment in still culture	DWt(mg) of mycelium	Phytotoxin Production DWt(mg) of Residue	ppm of phytotoxin in extract UV[a] at 260.5 mμ	Activity M. sativa bioassay of contr
A. NATURAL SUBSTRATES [b]				
1. Dead E. obliqua leaves	-	5	2	114
2. Dead E. obliqua roots	-	190	13	57
3. Live E. obliqua roots	-	5	10	-
B. CARBON SUBSTRATES				
1. Sucrose (shake)[c]	67 ± 4	5	59	98
2. Sucrose	219 ± 19	18	102	81
3. Pectin	139 ± 50	15	107	52
4. Starch	300 ± 10	19	209	76
C. NITROGEN SOURCES				
1. NO_3-N_2	172 ± 29	3.5	3.6 ± 1	44 [d]
2. NH_4-N_2	270 ± 18	3.0	15 ± 6	43
3. L-asparagine	340 ± 79	2.5	37 ± 11	34

a. based on U.V. absorpance of culture filtrate in ethanol.

b. based on differences in absorbance between inoculated and uninoculated substrate

c. incubated on a shaker for increased aeration. (see Fig 33).

d. bioassay of phytotoxin of 50 x concentration of culture filtrate.

on the live root or leaf substrates. This is consistent with results from the M. sativa root growth bioassay. Phytotoxin production by Cylindrocarpon sp. was high in the starch and pectin carbon sources (Table 25). Both starch and pectin are present in the cortex of E. obliqua roots and may contribute to phytotoxin production within roots.

The growth of Cylindrocarpon sp. mycelium and phytotoxin production was greater in the sucrose cultures which had been still incubated when compared with the shake cultures which had been better aerated. Partial anaerobic conditions appear to aid growth and phytotoxin production by Cylindrocarpon sp. This is consistent with responses in the growth of C. destructans (Waid 1962). These results are supported by the inhibition of M. sativa root growth in bioassay studies.

7.3.4b Nitrogen Sources

Phytotoxin production by Cylindrocarpon sp. was higher in the organic nitrogen source than in either of the inorganic sources, both in actual amounts and per mg dry weight of fungal culture (Table 25). Phytotoxin production was higher in NH_4 nitrogen treatment than in the NO_3 nitrogen treatment. The lower dry weights of Cylindrocarpon sp. mycelium in the NO_3 nitrogen treatments in this experiment when compared with those in a previous study (Chapter 4.3) may be associated with the relatively poor aeration in the culture solutions used in this experiment.

7.3.5 Mode of Action of the Cylindrocarpon sp. Phytotoxin

Fungal phytotoxins may influence the physiology of the host plant in a variety of ways (Wood et al 1972) including the induction of xylem dysfuntion via tyloses and gummosis. Tylosis consist of invaginations of the damaged pit membranes into the lumens of adjacent xylem vessels and may be formed as a response to damage of the plant membranes by the phytotoxin. Tyloses may block the root xylem and lead to reduced water flow and drought stress in the affected plant even under favourable soil moisture conditions.

The degree of membrane damage as a consequence of a phytotoxin may be assessed from the degree of protoplasm leakage through that membrane (Page 1972). Membrane leakage may be determined from changes in the electrical conductivity of a solution of low electrolyte concentration in which the tissue is bathed and may be measured with a wheatstone bridge. The effect of phytotoxin from Cylindrocarpon sp. on membrane damage in the fine roots of E. obliqua seedlings was measured using this technique in a series of repeat experiments.

Materials and Methods

E. obliqua seedlings which had been grown for one month in sterile vermiculite were carefully washed and left to desorb free ions for 25 hours in sterile, deionised, distilled water. The roots of five intact seedlings were then placed in aqueous solutions of 0, 5, 10, 50, 100 and 500ppm phytotoxin from Cylindrocarpon sp. culture filtrates for 2 hours at 20^{o}. The seedling roots were then carefully washed to remove the phytotoxin and transferred to new tubes of sterile, distilled, deionised water. Changes in the

conductivity of these bathing solutions were measured over the following 6 hours with a wheatstone bridge conductivity meter. Differences in the rates with which the conductivity of the bathing solutions changed in the period from one to six hours after transfer of the seedlings were compared for the different phytotoxin treatments.

Results and Discussion

The rate of electrolyte leakage from the roots of E. obliqua seedlings was highest in the 500ppm phytotoxin treatment and decreased with decreasing phytotoxin concentration (Figure 35a). Substantial membrane damage would appear to have occurred in solutions of phytotoxin at concentrations in excess of 50ppm. Microscopic examination confirmed the induction of tyloses in xylem vessels of E. obliqua seedlings which had been placed in aqueous phytotoxin from Cylindrocarpon sp. (Figure 35b). Subsequent repetition of this experiment with E. obliqua and M. sativa seedlings consistently confirmed the above results.

Consequently the blockages and tyloses of xylem vessels observed in E. obliqua roots inoculated with Cylindrocarpon sp. and in regrowth forests affected by dieback could have resulted from damage of the root membranes by the Cylindrocarpon sp. phytotoxin. The action of a phytotoxin could readily account for the extensive dysfunction of xylem tissue observed in E. obliqua roots without Cylindrocarpon sp. hyphae colonising the affected stelar tissues (Chapter 3.8). The increased membrane damage and nutrient leakage from E. obliqua roots affected by Cylindrocarpon sp. phytotoxins may also result in increased leakage of nutrients into the rhizosphere with subsequent effects on further infection by Cylindrocarpon sp. (Chapter 6.4).

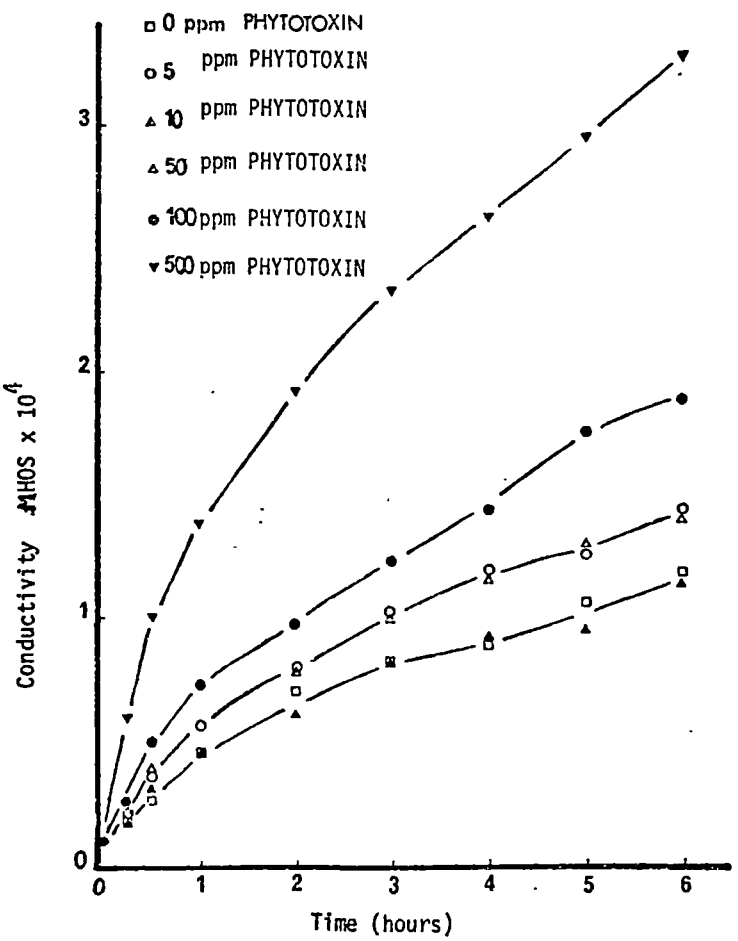
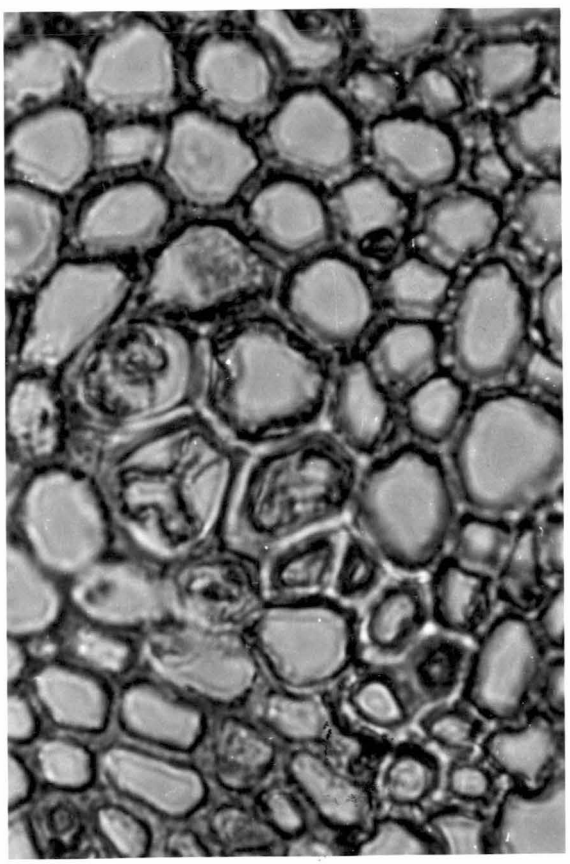


Figure 35 Effects of phytotoxin from Cylindrocarpon CMI 196141 on the leakage of electrolytes from the roots of intact E. obliqua seedlings.

a. Changes in the conductivity of bathing solutions measured for 6 hours following the transfer of washed E. obliqua seedlings which had been given pretreatments in phytotoxin solutions of varying concentrations.



10μ

Figure 35b Effects of phytotoxin from Cylindrocarpon CMI 196141 on the formation of tyloses in the xylem initials of E. obliqua seedlings.

This is consistent with the increase in the population and activity of Cylindrocarpon sp. in the rhizosphere of regrowth trees affected by dieback relative to those in the surrounding soil (Chapter 4.1).

7.4 Fungitoxin Production by Cylindrocarpon sp.

The interactions of Cylindrocarpon sp. with the roots of E. obliqua may also be influenced by competition from other fungi which colonise the roots of regrowth E. obliqua (Chapter 3.8). However C. destructans has been shown to produce fungitoxins, Radicicol and Radidicolin, in cultures which may influence these interactions with other soil micro-organisms (Evans et al 1966). Investigations were conducted to establish whether Cylindrocarpon sp. also produced similar fungitoxins and how these may affect competitive micro-organisms and the aetiology of dieback.

Materials and Methods

A series of antagonism studies on Czapek agar were conducted to study the production and effects of Cylindrocarpon sp. fungitoxins on a range of competing fungi. Filter paper discs (6mm in diameter) were sterilised and dipped in Czapek's culture solution of Cylindrocarpon sp. which had been filter sterilised. The discs were placed at a corner of a square with sides of 5cm which had been marked on the underneath of each agar plate. Plates of Czapek's agar were also inoculated with 6mm diameter plugs of live Cylindrocarpon sp. cultures in a similar manner. Three plates were then inoculated at the centre with 6cm diameter plugs of each of eight soil or root infecting fungi which had been taken from the growing margin of cultures on Czapek's agar. The fungi included

Botrytis cinerea, Penicillium italicum, Aspergillus nigra, Trichoderma sp., Fusarium oxysporum, Pythium ultimum, Phytophthora cinnamomi and Rhizoctonia solani from the University of Tasmania culture collection. The plates were incubated at 22°C and the perimeters of the colonies of Cylindrocarpon sp. and the test fungi were marked each day both towards and away from the Cylindrocarpon sp. inoculum or culture filtrate discs. All the fungi were also grown singly on Czapek's agar to measure colony growth in the absence of Cylindrocarpon sp.

The daily growth of each soil fungus towards the Cylindrocarpon sp. culture or disc was calculated as a percentage of the growth of that fungus away from the Cylindrocarpon sp. culture or disc and was plotted in relation to the distance between the colony fronts or filter paper disc at that measurement period. Colony growth of the soil fungi away from the Cylindrocarpon sp. culture was similar to that in plates which had only been inoculated with the soil fungus.

The above experiment was repeated to investigate whether Cylindrocarpon sp. was able to produce similar fungitoxic responses when grown on malt agar, leaf extract agar or soil extract agar (Appendix 1). The capacity of sterilised Cylindrocarpon sp. culture filtrates or live cultures to inhibit growth of Bacillus subtilis and Pseudomonas fluorescens was also investigated on nutrient agar plates which had been seeded with the respective bacteria and inoculated with Cylindrocarpon sp.

Results and Discussion

Three types of interactions occurred between the fungi and Cylindrocarpon sp. cultures or sterile culture filtrates (Figure 36). The growth of pythiaceous fungi, Pythium ultimum and Phytophthora cinnamomi towards the sterile Cylindrocarpon sp. culture filtrates and live cultures was strongly inhibited. This suggests that neither of these fungi are likely to be important synergists or competitors with Cylindrocarpon sp. in infected roots. This may in part account for the decrease in the pathogenicity of P. cinnamomi on E. obliqua seedlings inoculated together with Cylindrocarpon sp. (Chapter 5.2).

The growth of Botrytis cinerea and Trichoderma were also reduced by Cylindrocarpon sp. but to a lesser degree than in the Pythiaceae. Growth of the third group of fungi which include Fusarium oxysporum, Rhizoctonia solani as well as the Penicillium and Aspergillus was not greatly affected by Cylindrocarpon sp. cultures or sterile culture filtrates. Only in the Penicillium and Rhizoctonia plates did the Cylindrocarpon sp. culture fail to overgrow those of the test fungi.

Similar fungitoxic responses between Cylindrocarpon sp. and the various soil and root fungi were obtained in studies on malt agar, leaf extract agar and soil extract agar. Neither Bacillus subtilis nor Pseudomonas fluorescens growth on nutrient agar was inhibited by either Cylindrocarpon sp. cultures or culture filtrates.

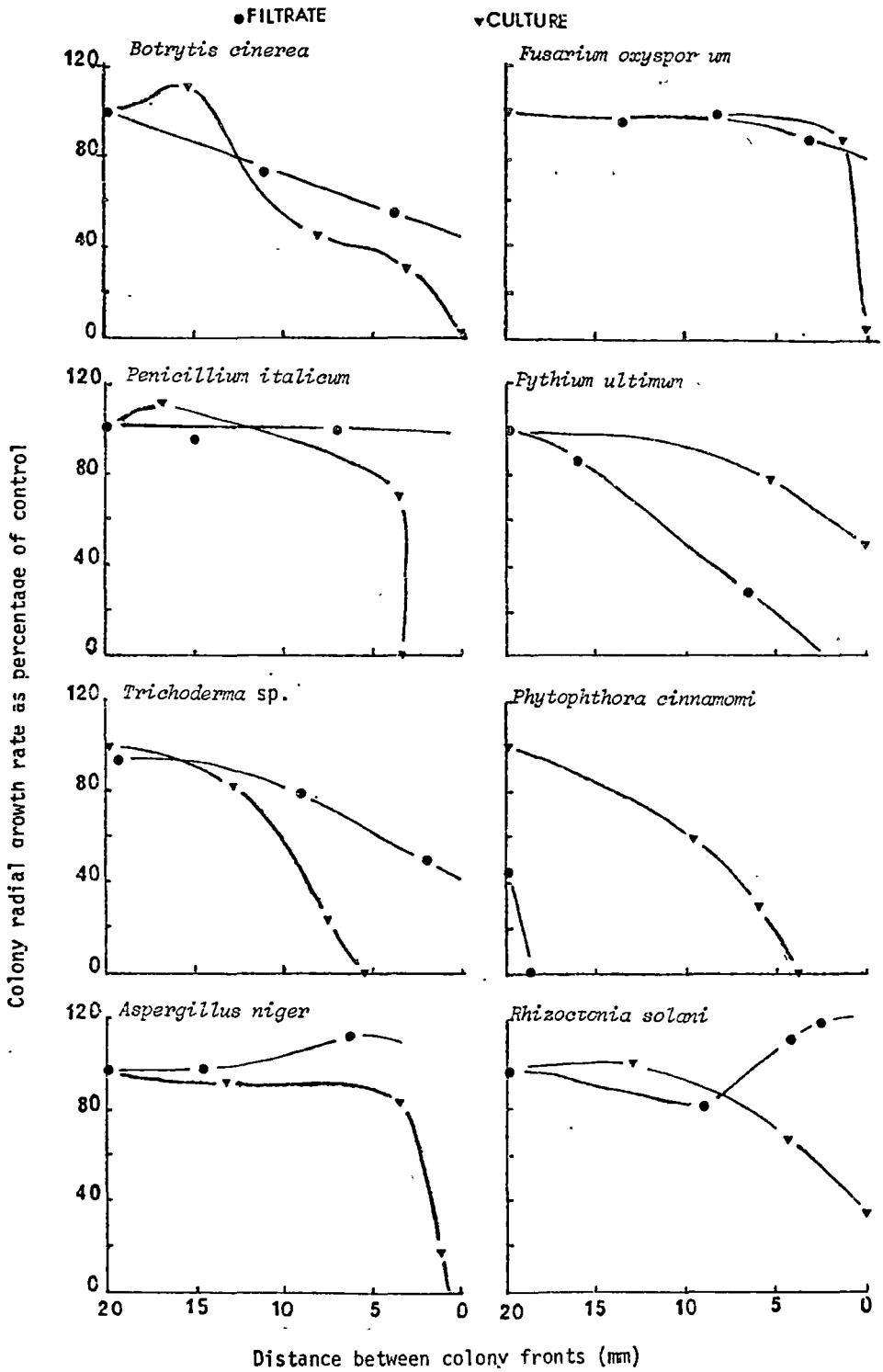


Figure 36 Fungitoxic responses by cultures and culture filtrates of Cylindrocarpon CMI 196141 against a range of soil and root infecting fungi.

Consequently Cylindrocarpon sp. appears to be capable of producing fungitoxins in both artificial and more natural substrates which can markedly influence the growth of different groups of soil and root colonising fungi. The nature of the fungitoxins extracted and their action appear to be very similar to those described in C. destructans by Evans et al (1966). The capacity of Cylindrocarpon sp. to produce fungitoxins, may provide it with a competitive advantage in saprophytic and parasitic colonisation and parasitic survival relative to other soil fungi. This appears to be particularly the case with respect to the faster growing Pythiaceae which may otherwise be potential competitors in the colonisation of E. obliqua roots in these forests (Chapter 2.7). The possiblity that root colonisation by Cylindrocarpon sp. may cross protect E. obliqua roots from subsequent infections by fungi such as P. cinnamomi warrants further investigation. The results of these antagonism studies are consistent with the coexistence of Cylindrocarpon sp. and Fusarium in the roots of plants (Matturi and Stenton 1964, Parkinson 1965).

The experiments in this chapter have examined the relationships between Cylindrocarpon sp. and E. obliqua roots. It appears that there may be two stages in this relationship. The first stage involves colonisation of the root cortex by hyphae of Cylindrocarpon sp. and is nonpathogenic and causes little root damage or malfunction. The colonisation of E. obliqua roots by Cylindrocarpon sp. may occur throughout much of the life of the tree (Chapter 3.7) and appears to be controlled by the interaction of factors in the rhizosphere (Chapter 6.4).

The second stage involves the production of a phytotoxin by Cylindrocarpon sp. in the root cortex which results in tyloses, gummosis and xylem blockage in the E. obliqua roots. The dysfunction of the fine roots contributes to increased drought stress and dieback of the affected tree even in soils and seasons favourable for the growth of E. obliqua. This stage in the decay of E. obliqua roots occurs without Cylindrocarpon sp. colonising, and consequently being recoverable from the affected tissue.

These two stages in the relationship between Cylindrocarpon sp. and the roots of E. obliqua may account for several unexplained aspects of previous experiments and regrowth dieback. The reisolation of Cylindrocarpon sp. from surface sterilised fine roots of E. obliqua trees of widely variable health and ages is likely to reflect the nonpathogenic infection of the root cortex by Cylindrocarpon sp. throughout the development of these forests (Chapter 3.7). Similarly the distinct root infection and seedling mortality responses in pathogenicity studies with increasing Cylindrocarpon sp. inoculum (Chapter 5.1) may also reflect these two stages in the association of Cylindrocarpon sp. with E. obliqua. The lower mortality in the second planting of E. obliqua seedlings may have resulted from the partial deactivation of phytotoxins in the Cylindrocarpon sp. inoculum with time.

The experiments in this chapter indicate that a close relationship may exist between Cylindrocarpon sp. and the roots of E. obliqua throughout the development of these forests. Cylindrocarpon sp. is able to colonise the surface and cortex of E. obliqua roots throughout much of the healthy life of the tree without causing major damage to the host. Fungitoxins produced by Cylindrocarpon

sp. may help it to maintain its competitive advantage within the E. obliqua roots relative to other potentially pathogenic fungi in the root zone. However during the transition of regrowth forests to mature forests highly stressed trees may become heavily infected by Cylindrocarpon sp. This high level of infection under environmental and host conditions suitable for the production and stability of its phytotoxin, may cause extensive root dysfunction, accentuated drought stress and dieback even in seasons and sites favourable for the growth of E. obliqua.

This may result in the natural removal of stressed, former dominant trees which are no longer adapted for growth under the limiting conditions existing in mature eucalypt forests.

GENERAL DISCUSSION

The investigations described in this thesis are aimed at understanding the cause of a crown dieback which had developed throughout large areas of natural E. obliqua and E. regnans forests in southern Tasmania. The unexplained dieback occurred in scattered trees, many of which were former dominant trees, and was of concern with respect to the future productivity and stability of these high quality forests. The initial investigations examined aspects of the previous growth of these and older E. obliqua forests in an attempt to provide an overall perspective of forest development within which the occurrence, cause and consequences of regrowth dieback could be studied. The initial investigations also examined a wide range of factors to determine their possible association with regrowth dieback. While further detailed investigation of some of these associated factors is desirable the studies did enable the aetiology of regrowth dieback to be examined in relationship to the natural development of these eucalypt forests rather than simply as a disorder of individual trees.

The examination of the growth rings of former dominant regrowth trees, which had been killed by dieback, and surviving oldgrowth trees, indicated that the death of dominant trees may not be abnormal during the transition of regrowth forests to mature forests. Growth rings were reduced to a relatively greater degree in the former dominant trees than in subdominant trees during this period of transition and it is likely that the resultant stress within these trees largely contributed to the reduced root growth and higher moisture stress apparent in trees affected by dieback. Comparisons of the number of trees per hectare in forests affected

by dieback with published data of the natural decline in tree numbers during the development of similar forests also indicated that not even in the most severely affected forests had regrowth dieback resulted in a reduction in tree numbers below those regarded as normal for forests of that age. Consequently the dieback of scattered trees throughout the E. obliqua regrowth forests appeared to be part of the natural decline in tree numbers during the transition of regrowth forests to mature forests and it is unlikely that these deaths threaten either the future productivity or stability of these forests.

These investigations of the relationship of dieback to the natural development of the E. obliqua forests greatly influenced subsequent studies on the aetiology of dieback. Rather than requiring the presence of new or abnormal factors the dieback of regrowth trees may be associated with changes in natural, pre-existing factors and equal emphasis was placed on examining a wide range of both new and natural factors for their association with regrowth dieback. Periodic drought stress, the reduced storage of moisture in the soils of some regrowth forests, periodic waterlogging, competition from the fine roots of understorey plants and the general decline of the fine roots of E. obliqua trees with age may all contribute to moisture stress and dieback in some of the regrowth trees. However the failure of trees to recover from dieback on return to more favourable soil conditions, the dieback of trees on deep, well drained soils and the high level of root decay observed in the fine roots of E. obliqua regrowth trees indicate that drought stress is unlikely to be the sole factor in the causation of regrowth dieback. Rather it appeared that factors responsible for the deterioration in the size, health and capacity of fine roots of regrowth trees to

absorb adequate water from the soil were directly involved in the causation of the dieback. Extensive investigations for potential pathogens of the fine roots of E. obliqua regrowth trees revealed that the fungus, Cylindrocarpon CMI 196141, was the only potential pathogen which was both consistently isolated from the fine roots of E. obliqua regrowth trees and was capable of causing root infections and death of E. obliqua seedlings. Although Phytophthora cinnamomi was isolated from the soil of some forests it was not isolated from any of the roots of E. obliqua regrowth trees and there is no evidence to suggest that it is a major factor in the causation of root decay and dieback. As Cylindrocarpon CMI 196141, a previously undescribed fungus, appeared to be a potential major factor in root decay and the dieback of regrowth trees detailed studies were conducted of its microbiology within forest soils and its pathogenicity on E. obliqua seedlings. While these investigations have concentrated on the Cylindrocarpon sp., its role in the dieback of these forests must at all times be considered in the context of the wide range of factors which the initial studies had shown to be associated with the root decay complex and the development of these forests.

In order to increase the relevance of pathogenicity studies with E. obliqua seedlings to the understanding of the dieback complex in maturing E. obliqua forests particular emphasis was placed on studying the pathogenicity of Cylindrocarpon sp. under the inoculum, soil, environmental and physiological stress conditions which are known or likely to occur in the regrowth forests. Of particular relevance to these studies was the production of microsclerotia by Cylindrocarpon sp. in forest soils and the increase in their numbers and activity during the development of the forests to reach maximum

levels in regrowth forests affected by dieback. Microsclerotia have not previously been reported in the genus Cylindrocarpon but do occur in the closely related genus Cylindrocladium (Jutte 1970, Menge 1969, Scholten 1967, Linderman 1973). The mortality of E. obliqua seedlings increased in regrowth forest soil to which increasing levels of Cylindrocarpon sp. inoculum had been added although high levels of root infection occurred even at low levels of added inoculum. Consequently the quantity and activity of Cylindrocarpon sp. inoculum in forest soils are likely to be important factors which can influence the level of root decay, moisture stress and dieback in regrowth trees.

Cylindrocarpon sp. was able to infect and kill E. obliqua seedlings in pathogenicity studies in which the inoculum, soil, environmental and physiological stress conditions had been modified to represent those occurring in the regrowth forests. Sublethal root infection by Cylindrocarpon sp. but no mortalities were observed in seedlings tested under conditions ideal for healthy seedling growth. Consequently the pathogenicity of Cylindrocarpon sp. depends upon the interaction of suitable inoculum, environment and host conditions. The failure of other workers to confirm the pathogenicity of other Cylindrocarpon species in forest diebacks, even though the fungus had consistently been isolated from decayed roots, may have been due to the use of environmental conditions and seedlings which are not relevant to the conditions in the dieback affected forests (Hann and Eno 1956).

The importance of physiological stress in predisposing roots of E. obliqua seedlings to high levels of infection by Cylindrocarpon sp. was repeatedly demonstrated. Of particular interest were studies in

which infections by Cylindrocarpon sp. were higher on the roots of E. obliqua seedlings which were approaching their plateau of growth than in seedlings of the same age which were still growing actively. If the roots of E. obliqua regrowth trees are also more susceptible to infection by Cylindrocarpon sp. during stress periods associated with the transition of regrowth forests to mature forests this could readily account for the higher levels of Cylindrocarpon sp. infection, root decay and dieback observed in regrowth forests. This may also account for the high levels of root decay and dieback in former dominant trees which are likely to be subject to greatest stress during this transition of forest growth. Forests on shallow, periodically waterlogged soils frequently are affected by dieback at an earlier age and at greater intensities than forests on deeper soils and it is possible that these differences in soils also result in different levels of stress which can affect the relative susceptibility of roots to infection and decay by Cylindrocarpon sp.

Whereas the early studies demonstrated that Cylindrocarpon sp. could infect and kill E. obliqua seedlings subsequent studies examined the processes and factors affecting the infection and decay of E. obliqua roots. The interactions between soil and rhizosphere micro-organisms, environmental conditions, root exudates from stressed seedlings and Cylindrocarpon sp. inoculum were examined to determine how they may influence the behaviour of Cylindrocarpon sp. on the roots of E. obliqua. A hypothetical model of these interactions in the rhizosphere of E. obliqua roots was tested and found to account for much of the resultant effect on Cylindrocarpon sp. inoculum. The germination, survival and activity of Cylindrocarpon sp. on the surface of E. obliqua roots appears to be influenced by a balance between stimulatory root exudates and

fungistatic and lytic products resulting from the utilisation of these exudates by the rhizosphere microflora. The sustained decrease in exudate which may occur during a decline in tree growth could result in decreased fungistasis and lysis by micro-organisms in the rhizosphere leading to the increased germination of Cylindrocarpon sp. Irregular higher levels of exudation, such as those following stress or wounding may not be fully utilised on the surface of roots with low microbial activities and may further stimulate Cylindrocarpon sp. The model of the interactions between root exudates, rhizosphere micro-organisms and the fungistasis and lysis of fungal inoculum on the surface of plant roots may be relevant to a wider understanding of rhizosphere microbiology and root diseases.

Cylindrocarpon sp. readily colonised the cortex of roots of E. obliqua seedlings without any apparent resistance by the plant apart from that afforded by the fungistasis and lysis of Cylindrocarpon sp. in the rhizosphere. This is consistent with studies on eucalypts in Western Australia (Malajczuk and McComb 1979) and Victoria (Marks et al 1973) in which resistance of roots to infection by P. cinnamomi were associated with differences in rhizosphere micro-organisms and not root defence reaction (Tippett 1976). While the infection of the cortex of E. obliqua roots by Cylindrocarpon sp. did not result in major damage many of the infected roots had extensive discolorations and blockages of their xylem vessels. It appeared that two stages may occur in the association of Cylindrocarpon sp. with the fine roots of E. obliqua seedlings which together would account for the various associations between Cylindrocarpon sp. and the infection and decay of E. obliqua roots.

The first stage involves infection of the cortex of roots by Cylindrocarpon sp. and is nonpathogenic. This stage is consistent with the common role of Cylindrocarpon spp. as root companion fungi (Kurbis 1937, Garrett 1970) and occurs throughout the healthy development of the E. obliqua forests. The second stage involves the production of phytotoxins by Cylindrocarpon sp. within the cortex of infected roots which induce tyloses and blockage of the xylem vessels and thereby greatly increase moisture stress in the affected plants even under optimal soil moisture conditions. The dysfunction of the xylem occurs without Cylindrocarpon sp. colonising the affected tissue. Substrate and environmental factors may influence the production and stability of the phytotoxin. Anaerobic conditions appear to increase the stability of the phytotoxin. This may account for the higher incidence of root decay and dieback in forests on shallow periodically waterlogged soils relative to forests of similar age which have similar levels of Cylindrocarpon sp. infection on their roots but occur on well aerated soils.

Consequently Cylindrocarpon sp. appeared to be a natural fungus in these forest soils which was highly adapted to the environmental, soil and biotic factors existing in E. obliqua regrowth forests. Cylindrocarpon sp. was capable of infecting and killing E. obliqua seedlings under the conditions existing in regrowth forests. The dieback of former dominant trees during the transition from regrowth to mature forests also appear to be a natural phenomena in the development of these forests. The dieback appears to be caused by an interaction between physiological stress, the level and activity of Cylindrocarpon sp. inoculum, the environmental conditions and their resultant effects on the level of root decay and moisture

stress in the affected trees. While root decay by Cylindrocarpon sp. appears to be a primary factor in both the initiation of regrowth dieback and the failure of stressed trees to recover on return to more favourable conditions the role of Cylindrocarpon sp. in dieback should always be considered as one of several factors which contributes to the dieback complex.

The investigations of the aetiology of regrowth dieback raise several questions regarding the natural development of these and other eucalypt forests. It would appear that the dieback of former dominant trees is natural and has occurred previously in the transition of regrowth forests to mature forests. The close involvement of the different stages of Cylindrocarpon sp. root infection and decay during the growth, and selective dieback of E. obliqua trees suggests that Cylindrocarpon sp. may be an important mechanism in the natural succession of these forests. While it has been suggested that fungal and/or insect parasites are involved in species differentiation during the natural development of eucalypt forests (Burden and Chilvers 1974) the involvement of Cylindrocarpon sp. in the dieback of these E. obliqua forests represents some of the first evidence in support of this hypothesis.

The regeneration and development of these and many other eucalypt forests have been associated with previous fires (Jackson 1978, Gill 1975) and it is therefore feasible that interrelationships may exist between fire and Cylindrocarpon sp. which may influence the development of these forests. Indeed the increase in the numbers of microsclerotia and activity of Cylindrocarpon sp. in the soils of E. obliqua forests with time since regeneration fires suggests such an interrelationship exists. So does the decrease in the numbers and

activity of Cylindrocarpon sp. microsclerotia in recently burnt forest soil or soil heated above 45°C (Jehne unpublished). Further studies (Jehne unpublished) support that a close relationship exists between fire and Cylindrocarpon sp. in the soils of these forests. This is consistent with studies in other forests (Visser and Parkinson 1975) and with studies of some of the responses in eucalypt seedlings attributed to previous fires (Florence and Crocker 1962).

The close involvement of Cylindrocarpon sp. in the dieback and development of natural E. obliqua forests in Tasmania also raises questions about the involvement of Cylindrocarpon spp. in the ecology of eucalypt forests throughout Australia which have similar fire histories. Cylindrocarpon sp. was consistently isolated from the soils of tall open eucalypt forests from throughout Tasmania (Figure 2) and is associated with the dieback and development of similar E. delegatensis forests in northeastern Tasmania (Jehne unpublished). A Moezia sp., synonymous with Cylindrocarpon (Subramanian 1971), was found to be a common coloniser of the leaf litter in E. regnans forests in Victoria (Macauley and Thrower 1962). C. destructans was found to be the dominant fungus colonising the rhizospheres of E. fastigata and E. viminalis seedlings growing in forest soil in the Australian Capital Territory (Johnston 1976 pers. comm.). C. destructans was also found to be common on the roots of E. pilularis seedlings growing in soils from forests in northern New South Wales (Evans, Cartwright and White 1967). Consequently Cylindrocarpon spp. appear to be common in the root zones of many eucalypt forests throughout eastern Australia. Further studies need to be conducted to establish whether these Cylindrocarpon spp. similarly influence the root systems, stress and development of eucalypt forests throughout Australia.

The investigations of the aetiology of regrowth dieback in southern Tasmania may also contribute to a wider understanding of dieback complexes in other forests throughout the world. In particular the involvement of Cylindrocarpon spp. in the unexplained dieback complexes in birch and Pinus monticola forests in North America may warrant reinvestigation. These investigations on the death of former dominant trees also raise questions about the future health and stability of the large areas of forest monoculture which have been established with tree genotypes which have been selected for rapid initial growth. This may be particularly relevant to the large areas of eucalypt plantations which have recently been established throughout the world and whose development may not be unlike that of natural regrowth forests of uniform age.

These investigations may also help to clarify the involvement of Cylindrocarpon spp. in soils, on the surface of plant roots and in plant disease. While Cylindrocarpon spp. have frequently been isolated from a wide range of soils, litter, root surfaces and host plants the involvement of Cylindrocarpon spp. in disease had remained illdefined. The demonstration that Cylindrocarpon sp. can be pathogenic on E. obliqua seedlings under suitable conditions and the identification of the two stages in its association with E. obliqua roots may aid in the interpretation of the role of Cylindrocarpon spp. in other root diseases. The demonstration of the importance of inoculum concentration, environmental factors and physiological stress in the pathogenicity of Cylindrocarpon sp. may also aid in relating the results of pathogenicity studies with seedlings to an understanding of diseases in older and more complex natural forests. Finally these studies on the aetiology of regrowth dieback emphasise the value of investigating this "unexplained"

dieback of natural forests not simply as a new phenomena in isolated trees, but as part of a wider perspective which also considered the natural development of these forests and the many factors which may contribute to the aetiology of this and many other disease complexes.

REFERENCES

AFIFI, A.F. (1975) Effect of volatile substances from species of Labiatae on rhizospheric and phyllospheric fungi of Phaseolis vulgaris. Phytopath. Z. 83: 296-302.

AGNIHOTRUDU, V. (1959) Notes on fungi from North east India III Cylindrocarpon tenue Bugnicourt (syn Gliocladiopsis sagariensis Sak Sena). Trans. Br. mycol. Soc. 42: 458-62.

ARMSTRONG, W., READ, D.J. (1972) Some observations on oxygen transport in conifer seedlings. New Phytol. 71: 55-62.

ASHTON, D.H. (1962) Some aspects of root competition in Eucalyptus regnans. Paper to 3rd Gen. Conf. Inst. of Foresters of Aust. Melbourne 1962.

ASHTON, D.H., WILLIAMS, G. (1973) The occurrence of gum topped stringy bark in the Trentham forests. Victorian Nat. 90: 90-2.

ASHTON, D.H. (1975) The seasonal growth of Eucalyptus regnans F. Muell. Aust. J. Bot. 23: 239-52.

ASHTON, D.H. (1975) Studies of Flowering Behaviour in Eucalyptus regnans F. Muell. Aust. J. Bot. 23: 399-411.

ASHTON, D.H. (1975) Studies of litter in Eucalyptus regnans F. Muell forests. Aust. J. Bot. 23: 413-433.

ASHTON, D.H. (1976) The development of evengaged stands of Eucalyptus regnans F. Muell in central Victoria. Aust. J. Bot. 24: 397-414.

ASHTON, D.H. (1976) Phosphorus in forest ecosystems at Beenak Victoria. J. Ecol. 64: 171-186.

ATTIWILL, P.M. (1971) Phosphorus adsorption isotherms and growth responses for a highly weathered eucalypt forest soils. Proceedings of Australian Forest Tree Nutrition Conference Canberra 1971 P 125-34.

ATTIWILL, P.M. (1971) On the cycling of the elements in mature Eucalyptus obliqua forest. Proceedings of Australian Forest Tree Nutrition Conference Canberra 1971. P 39-46.

AUNG, R.H. (1974) Root Shoot relations in CARSON, E.W. ed. The plant root and its environment Univ. press of Virginia: P29-63.

BAKER, K.F. (1957) The U.C. system of producing healthy container grown plants. Calif. Agr. Expt. Sta. Manual 23: 1-332.

BAKER, R., MAURER, C.L., MAURER, R.A. (1967) Ecology of plant pathogens in soil VII Mathematical models and inoculum density Phytopathology 57: 662-66.

BALD, J.G., SOLBERG, R.A. (1960) Antagonism and synergism among organisms associated with the scale tip rot of lilies. Phytopathology 50: 615-20.

BALIS, C. (1975) A Theoretical approach to competitive saprophytic colonization of substrates as a function of inoculum density. Annls. Inst. Phytopath. Benaki N.S. 11: 74-93.

BALIS, C. (1976) Ethylene induced volatile inhibitors causing soil fungistasis. Nature 259: 112-114.

BARNETT, H.L., HUNTER, B.B. (1972) Illustrated genera of imperfect fungi. 3rd Edition Burgess Publ. Co.

BATINI, F.E., HOPKINS, E.R. (1972) Phytophthora cinnamomi Rands - a root pathogen of the Jarrah forests. Aust. For. 36: 57-68.

BATISTA, A.C. (1951) Cylindrocladium scoparium Morgan var brasiliensis a new fungus on ecualypts. BOL. SEC. AGRIC. PERN. AMBUCO. 18: 188-91.

BEGA, R.V. (1974) Phytophthora cinnamomi, Its distribution and possible role in Ohio decline on the Island of Hawaii. Pl. Dis. Reptr. 58: 1069-73.

BEUTE, M.K., LOCKWOOD, J.L. (1968) Mechanisms of increased root rot in virus infected peas. Phytopathology 58: 1643-1651.

BEVAGE, D.J. (1968) A rapid technique for clearing tannins and staining intact roots for detection of mycorrhizas caused by Endogone spp and some records of infection in Australasian plants. Trans. Br. mycol. Soc. 51: 808-10.

BLOOMBERG, W.J. (1966) The occurrence of endophytic fungi in Douglass Fir seedlings and seed. Can. J. Bot. 44: 413-20.

BODEN, R.W. (1962) Adaption of eucalypts to the waterlogged environment. MSc Thesis Botany Univ. of Sydney.

BOJARCZUK, M., BOJARCZUK, J., KROLIKOWSKI, Z. (1968) Investigations of resistance in maize to the action of soil fungi under cold test conditions. Hodowla Rosl. Aklin. Nasienn. 12: 15-36. (in Rev. Appl. Mycol. 48: 792.

BOLLEN, G.J. (1969) The selective effect of heat treatment on the microflora of a greenhouse soil. Neth. J. Pl. Path. 75: 157-63.

BOOTH, C. (1966) The genus Cylindrocarpon Mycol. pap. 104, 56pp.

BORMANN, F.H. (1965) Changes in the growth pattern of white pine trees undergoing suppression. Ecology 46: 269-77.

BOTKIN, D.B., JANAK, J.F. WALLIS, J.R. (1972) Some ecological consequences of a computer model of forest growth. J. Ecol. 60: 849-72.

BOWEN, G.D. (1969) Nutrient status effects on loss of amides and amino acids from pine roots. Pl. Soil 30: 139-142.

BOWLING, P.J., McLEOD, D.E. (1968) A note on the presence of Armillaria in second growth eucalypt stands in Southern Tasmania. Aust. For. Res. 3: 38-40.

BOYER, J.S. (1967) Leaf water potentials measured with a pressure chamber Pl. Physiol. 42: 133-7.

BRANDSBERG, J.W. (1969) Fungi isolated from decomposing conifer litter. Mycologia 61: 373-81.

BRAUN, A.L., MOJTAHED, I.H., LOWNSBERRY, B.F. (1975) Seperate and combined effects of Pratylenchus neoamblycephalus and Cricenemoides xenoplex on Myrobon plum. Phytopathology 65: 328-30.

BRAUN, E.J., SINCLAIR, W.A. (1976) Histopathology of phloem necrosis in Ulmus americana. Phytopathology 66: 598-607.

BROADBENT, P., BAKER, K.F. (1974) Behaviour of Phytophthora cinnamomi in soils suppressive and conductive to root rot. Aust. J. Agric. Res. 25: 121-39.

BURDON, J.I., CHILVERS, G.A. (1974) Fungal and insect parasites contributing to niche differentiation in mixed stands of eucalypt saplings. Aust. J. Bot. 22: 103-15.

BURSTROM, H.G. (1965) The physiology of plant roots. in SNYDER, W.C., BAKER, K.F. ed. Ecology of Soil Borne Pathogens: Prelude to biological control. U of Calif. Press p 154-166.

BUXTON, E.W. (1960) Effect of pea root exudate on the antagonism of some rhizosphere microorganisms towards Fusarium oxysporum. f. pisi. J. Gen. Microbiol. 22: 678-689.

CALDWELL, R. (1963) Observations in the fungal flora of decomposing beech litter in soil. Trans. Br. mycol. Soc. 46:249-261.

CAMPBELL, K.G. (1966) Aspects of insect v tree relationships in forests of Eastern Australia. in GERHOLD, H.D. et. al. ed. Breeding Pest Resistant Trees, Pergaman Press Lond p 239-250.

CAMPBELL, W.A. (1951) Fungi associated with the roots of littleleaf-diseased and healthy shortleaf pine. Phytopathology 41: 439-446.

CAMPBELL, W.A., COPELAND, O.L. Jr. (1954) Little leaf disease of shortleaf and loblolly pines. U.S.D.A. Cir. 940, 41pp.

CARNE, P.B., GREAVES, R.J.G., McINNES, R.S. (1974) Insect damage to plantation grown eucalyptus in North Coastal N.S.W. with particular reference to christmas beetles Coleoptera, Scarabaeidae. J. Aust. Ent. Soc. 13: 189-206.

CARRODUS, B.B. (1969) Studies on the metabolism of Eucalyptus obliqua I Inorganic sources of nitrogen and the factors affecting the uptake of ammonia. New Phytol. 68: 1031-9.

CARTLEDGE, G., SHAW, D.E., STAMPS, D.J. (1975) Studies in relation to dead patches of Nothofagus in Papua New Guinea. Dept. Agr. Stk. Fish. P. Moresby Res. Bull. 13: 1-26.

CHEE, K.H., NEWHOOK, F.J. (1965) Improved methods for use in studies of Phytophthora cinnamomi Rands and other Phytophthora species. N.Z. J. Aric. Res. 8: 88-95.

CHITWOOD, B.G. (1949) Ring nematode (Criconematinae) a possible factor in decline and replanting problems of peach orchards. Proc. Helminth, Soc. Wash. 16: 6-7.

COLEY-SMITH, J.R., KING, J.E. (1969) The production by species of Allium of alkyl sulphides and their effect on germination of scleratia of Sclerotium cepivorum. Ann. Appl. Biol. 64: 289-301.

COOPER, J.I. (1976) Viruses of Hardwoods. Annual Rep. 1974. Inst. of Terrestrial Ecology. Nat. Env. Res. Council H.M.S.O.

COPELAND, O.L., Jr (1952) Root mortality of shortleaf and loblolly pine in relation of soils and littleleaf disease. J. For. 50: 21-25.

COPELAND, O.L., Jr, McALPINE, R.G. (1962) Soil characteristics associated with spot die out in loblolly pine plantations. For. Sci. 8: 12-15.

CORMACK, M.W. (1937) Cylindrocarpon ehrenbergi Wr and other species as root parasites of alfalfa and sweet clover in Alberta. Can. J. Res. 9: 403-24.

COX, E.A., MANNING, W.J., CAMPBELL, F.J. (1969) Cylindrocarpon radicicola found associated with wilt of Azalea. Pl. Dis. Reptr. 53:620.

CRAWFORD, R.M.M. (1967) Alcohol dehydrogenase activity in relation to flooding tolerance of plants. J. Exp. Bot. 18: 458-64.

CREMER, K.W., MOUNT, A.B. (1965) Early stages of plant succession following the complete felling and burning of Eucalyptus regnans forest in the Florentine valley Tasmania. Aust. J. Bot. 13: 303-32.

CREMER, K.W. (1975) Temperature and other climatic influences on shoot development and growth of Eucalyptus regnans. Aust. J. Bot. 23: 27-45.

CUNNINGHAM, T.M. (1960) The natural regeneration of Eucalyptus regnans. Bull. No. 1, School of Forestry, University of Melbourne.

DAY, W.R. (1959) Observations of eucalypts in Cyprus. 2 Root development in relation to soil conditions. Emp. For. Rev. 38: 186-197.

DAY, W.R. (1960) The influence of pathogenic factors in the rooting space on the development of evenaged plantations. Emp. For. Rev. 39: 38-53.

DELATOUR, C. (1969) Root rot of Eucalyptus spp in Northern Tunisia. Annls. Inst. Natn. Rech. For. Tunis. 2: 23pp.

DENIS, S.J., SNODGRASS, C.J., KOBURGER, J.A., ELLIOT, E.S. (1966) An azide-rose bengal medium for the isolation of Fusarium species from red clover roots. Phytopathology 56: 584 abstr.

DISANCO. C.P., ROHDE, R.A. (1969) Xiphinema americanum associated with maple decline in Massachusetts. Phytopathology 59: 279-284.

DOTHIE, H.J. (1969) Foliar Analysis. Trop. Sci. 9: 49-58.

DUBÉ, A.J., DODMAN, B.L., FLENTJE, N.T. (1971) The influence of water activity on the growth of Rhizoctonia solani. Aust. J. Biol. Sci. 24: 57-65.

ELLIS, R.C. (1964) Dieback of Alpine Ash in North Eastern Tasmania. Aust. For. 28: 75-90.

ELLIS, R.C. (1971) Dieback of Alpine Ash as related to changes in soil temperature. Aust. For. 35: 152-163.

ELLIS, R.C. (1971) The mobilization of iron by extracts of eucalypt leaf litter. J. Soil Sci. 22: 8-22.

EVANS, G. (1964) The antibiotic activity of Cylindrocarpon radicicola. PhD Thesis University of Sydney.

EVANS, G., WHITE, N.H. (1966) Radicicolin and radicicol, two new antibiotics produced by Cylindrocarpon radicicola. Trans. Br. mycol. Soc. 49: 563-76.

EVANS, G., CARTWRIGHT, J.B., WHITE, N.H. (1967) The production of a phytotoxin nectrolide by some root surface isolates of Cylindrocarpon radicicola. Pl. Soil 26: 253-260.

FELTON, K.C. (1972) Eucalypt diebacks in Tasmania. Appita 26: 207-8.

FELTON, K.C., BIRD, T. (1972) Economic effects of eucalypt diebacks in Tasmania. Paper to 44th ANZAAS Congress SYDNEY.

FLORENCE, R.G., CROCKER, R.L. (1962) Analysis of blackbutt seedling growth in a blackbutt forest soil. Ecology 43: 620-9.

FRIES, N. (1973) Effects of volatile organic compound on the growth and development of fungi. Trans. Br. mycol. Soc. 60: 1-21.

GARRETT, S.D. (1956) Biology of root infecting fungi. Cambridge University Press.

GARRETT, S.D. (1970) Pathogenic root infecting fungi. Cambridge University Press.

GARRETT, S.D. (1975) Cellulolysis rate and competitive saprophytic colonization of wheat straw by root rot fungi. Soil Biol. Biochem. 7: 323-9.

GARRETT, S.D. (1976) Influence of nitrogen on cellulolysis rate and saprophytic survival of some cereal root rot fungi. Soil Biol. Biochem. 8: 229-35.

GERLACH, W. (1961) Contributions to the knowledge of the genus Cylindrocarpon. IV C. radicicola its phytopathological importance and its occurrence as the pathogen of a rot of the Usumbra violet. Phytopath. Z. 41: 361-9.

GERRETTSON-CORNELL, L. (1973) A preliminary study on the morphology of Phytophthora cinnamomi Rands from Ourimbah State Forest Wyong N.S.W. Info. Batan Italiano 5: 78-80.

GIESE, R.L. et. al. (1964) Studies of maple blight IV Defoliation and the the genesis of maple blight. Univ. of Wisconsin Res. Bull. 250: 80-113.

GILBERT, J.M. (1958) Eucalypt-rainforest relationships and the regeneration of the eucalypts. PhD thesis University of Tasmania.

GILBERT, J.M. (1959) Forest succession in the Florentine valley Tasmania. Proc. R. Soc. Tasm. 93: 129-151.

GILBERTSON, R.L., LEAPHART, C.D., JOHNSON, F.D. (1961) Field identification of roots of conifers in the inland empire. Forest Sci. 7: 352-6.

GILL, A.M. (1975) Fire and the Australian flora. A Review. Aust. For. 38: 4-26.

GOODEY, J.B. (1963) Laboratory methods for work with plant and soil nematodes. Minist. Agric. Fish. Food Tech. Bull. No. 2 H.M.S.O.

GOVI, G. (1952) Two species of Cylindrocarpon isolated from fruit trees. Ann. Sper Agr. N.S. 6: 793-804.

GREACEN, E.L., BARLEY, K.P., FARRELL, D.A. (1969) The mechanics of root growth in soils with particular reference to the implications for root distribution. p 256-269 in Whittington, W.J. ed. Root Growth Butterworths London.

GREAVES, R.J.G. (1966) Insect defoliation of eucalypt regrowth in the Florentine valley Tasmania. Appita 19: 19-26.

GREENIDGE, K.N.H. (1953) Further studies of birch dieback in Nova Scotia. Can. J. Bot. 31: 548-559.

GREENWOOD, D.J. (1969) Effects of oxygen distribution in the soil on plant growth. p. 202-223 in WHITTINGTON, W.J. ed. ROOT GROWTH Butterworths London.

GRIFFIN, D.M. (1972) Ecology of soil fungi. Chapman and Hall London.

GRINEVA, G.M. (1962) Excretion by plant roots during brief periods of anaerobiosis. Plant Physiol. U.S.S.R. 8: 549-52.

HAMILTON, C.D. (1951) The dying of jarrah Eucalyptus marginata in Western Australian forests. Progress on work to 1948. Unpublished Report, Forestry and Timber Bureau, Canberra.

HANN, G.G., ENO. H.G. (1956) Fungus association with birch dieback and its significance. Pl. Dis. Reptr. 40: 71-9.

HARLEY, J.L., WAID, J.S. (1955) The effect of light upon the roots of beech and its surface population. Pl. Soil 7: 96-112.

HARRI, E. et. al. (1963) Uber die isolierung neuer Stoffwechselprodukte aus Penicillium brefeldianum Dodge Helv. chim. Acta. 46: 1235-43.

HART, J.H. (1965) Root rot of oak associated with Cylindrocarpon radicicola seedlings killed following a severe winter. Phytopathology 55: 1154-5.

HAWBOLDT, L.S., SKOLKO, A.J. (1948) Investigations of yellow birch dieback in Nova Scotia. J. For. 46: 659-71.

HENDRIX, F.F., CAMPBELL, W.A. (1973) Pythiums as plant pathogens. A. Rev. Phytopathol. 11: 77-98.

HEPTING, G.H. (1963) Climate and forest disease. A. Rev. Phytopathol. 1: 31-50.

HERING, T.F. (1965). Succession of fungi in the litter of a lake district oakwood. Trans. Br. mycol. Soc. 48: 391-408.

HIBBEN, C.R. (1964) Identity and significance of certain organisms associated with sugar maple decline in New York woodlands. Phytopathology 54: 1389-92.

HIBBEN, C.R., BOZARTH, R.F. (1972) Identification of the ash strain of tobacco ringspot virus associated with declining ash trees Fraxinus americana. Phytopathology 62: 1023-9.

HODGES, C.S., MAY, L.C. (1972) A root disease of pine, Araucaria and eucalypts in Brazil caused by a new species of Cylindrocladium. Phytopathology 62: 898-901.

HOOK, D.D., BROWN, C.L., WETMORE, R.H. (1972) Aeration in Trees. Bot. Gaz. 133: 443-54.

HOPKINS, E.F. (1973) Crown dieback of eucalypt forests p 1-16 in MARKS, G.C., IDZAK, R.M. eds. Eucalypt diebacks in Australia. Proc. of the Lakes Entrance Seminar 1973 Vict. For. Comm. Publ.

HORSFALL, J.G., DIMOND, A.E. (1960) Plant pathology. An advanced Treatise. Academic Press New York.

HUBER, D.M., WATSON, R.D. (1974) Nitrogen form and plant disease. A. Rev. Phytopathol. 12: 139-65.

JACKSON, L.W.R. (1945) Root defects and fungi associated with the litleleaf disease of Southern Pine. Phytopathology 35: 91-105.

JACKSON, L.W.R. (1970) Nematode parasitic on shortleaf pine roots. Pl. Dis. Reptr. 54: 465-6.

JACKSON, R.M. (1965) Studies of fungi in pasture soils II. Fungi associated with plant debris and fungal hyphae in soil. N.Z. J. Aric. Res. 8: 865-77.

JACKSON, R.M. (1965) Studies of fungi in pasture soils III Physiological studies on some fungal isolates from the root surface and from organic debris. N.Z. J. Aric. Res. 8: 878-888.

JACKSON, W.D. (1968) Fire, air, water and earth an elemental ecology of Tasmania. Proc. Ecol. Soc. Aust. 3: 9-16.

JACOBS, M.R. (1955) Growth habits of the eucalypts. Comm. of Aust. Government Printer.

JEHNE, W. (1971) The occurrence of Phytophthora cinnamomi and tree dieback in the A.C.T. Aust. For. Res. 5 (1): 47-52.

JEHNE, W. (1971) Soil conditions and the occurrence of Phytophthora cinnamomi in relation to the deaths in young plantations of Radiata Pine near Jervis Bay. Aust. For. Res. 5 (3): 39-46.

JEHNE, W. (1972) An instance of association between Exocarpus cupressiformis and dieback of Eucalyptus dives. Aust. For. Res. 5 (4): 51-56.

JUTTER, A.S. (1970) Biology of the sclerotia of Cylindrocladium floridanum and its relation to root rot of yellow poplar. M.Sc. Thesis Duke University North Carolina 116pp.

KATZNELSON, H., ROUATT, J.W., PAYNE, T.M.B., (1955) The liberation of amino acids and reducing compounds by plant roots. Pl. Soil 7: 35-48.

KATZNELSON, H., ROUATT, J.W., PETERSON, E.A. (1962) The rhizosphere effect of mycorrhizal and non mycorrhizal roots of yellow birch seedlings. Can. J. Bot. 40: 377-82.

KILE, G.A. (1974) Insect defoliation in the eucalypt regrowth forests of southern Tasmania. Aust. For. Res. 6 (3): 9-18.

KLIEJUNAS, J.T., KO, W.H. (1974) Deficiency of inorganic nutrients as a contributing factor to Ohia decline. Phytopathology 64: 891-896.

KLUGE, E. (1966) Pathogenicity of Cylindrocarpon radicicola to pine seedlings and it production of toxins. Phytopath. Z. 55: 368-88.

KOSCEEV, A.L. (1952) The silvicultural significance of adventitous roots on tree species in waterlogged clear felled areas. Tr. Inst. Ces. 13: 116-129, For. Abstr. 16: 3886 (1955).

KOUYEAS, V. (1964) An approach to the study of moisture relations of soil fungi. Pl. Soil 20: 351-363.

KOZLOWSKI, T.T. (1971) Ageing. p 117-163 in Growth and Development of Trees I Academic Press New York.

KUBIKOVA, J. (1968) Fusarium oxysporum Schlect - a dominant fungus species on the root surface of woody plant seedlings. Pl. Soil 28: 306-12.

KUBIKOVA, J. (1963) The surface mycoflora of ash roots. Trans. Br. mycol. Soc. 46: 107-114.

KURBIS, P. (1937) Mycological investigations on the rhizosphere of ash Fraxinus excelsior L. Flora Jena 31: 129-175.

LAMBERS, H. (1976) Respiration and NADH oxidation of the roots of flood tolerant and flood intolerant Senecio species as affected by anaerobiosis. Physiologia Pl. 37: 117-123.

LAST, F.T. (1971) Epidemiology of some nonfoliar pathogens. A. Rev. Phytopathol. 9: 341-62.

LEAPHART, C.D., GILL, L.S. (1955) Lesions associated with pole blight of western white pine. Forest Sci. 1: 232-9.

LEAPHART, C.D., COPELAND, O.L. Jr (1957) Root and soil relationships associated with the pole blight disease of western white pine. Proc. Soil Sci. Soc. Am. 21: 551-4.

LEAPHART, C.D., WICKER, E.F. (1966) Explanation of pole blight from responses of seedlings grown in modified environments. Can. J. Bot. 44: 121-137.

LEAPHART, C.D., STAGE, A.R. (1971) Climate a factor in the origin of the pole blight disease of Pinus monticola. Ecology 52: 229-39.

LEAPHART, C.D., FOILES, M.W. (1972) Effect of removing pole blighted western pine trees on growth and development of a mixed conifer stand. U.S.D.A. For. Ser. Res. Note INT. 161 6pp.

LEAPHART, C.D., JOHNSON, H.E. (1973) Pole blight: also a disease of western white pine plantations. Pl. Dis. Reptr. 57: 948-51.

LEDIG, F.T., BORMANN, F.H., WENGER, K.F. (1970) The distribution of dry matter growth between shoot and roots in loblolly pine. Bot. Gaz. 131: 349-359.

LINDERMAN, R.G. (1973) Formation of microsclerotia of Cylindrocladium spp. in infected azalea leaves, flowers and roots. Phytopathology 63: 187-191.

LORDELLO, L.G.E. (1967) A root lesion nematode found infesting eucalypt trees in Brazil. Pl. Dis. Reptr. 51: 791.

MACAULEY, B.J., THROWER, L.B. (1966) Succession of fungi in leaf litter of Eucalyptus regnans. Trans. Br. mycol. Soc. 49: 509-20.

MADER, D.L., THOMPSON, B.W. (1969) Foliar and soil nutrients in relation to sugar maple decline. Proc. Soil Sci. Soc. Am. 33: 794-800.

MAGNANI, G. (1972) Damping off of Pinus radiata seedlings caused by fungal parasites. Publ. del Centrodi Sper. Agric. Far. 11: 307-13, Rev. Pl. Path. 52: 3089.

MAI, W.F., LYON, H.H. (1975) Pictorial key to genera of plant parasitic nematodes. Cornell Univ. Press London N.Y. 4th ed.

MALAJCZUK, N., McCOMB, A.J. (1979) The unsuberized roots of Eucalyptus calophylla and Eucalyptus marginata seedligns grown in soil suppresive and conducive to Phytophthora cinnamomi Rands I Rhizosphere bacteria, Actinomycetes and Fungi. Aust. J. Bot. 27: 235-54.

MANKA, K. (1970) Parasitic seedling diseases of forest trees and soil fungi. For. Abstr. 33: 4654.

MARCU, G. (1966) Causes prevention and control of the dieback of Oak Centru de Doc. Tech. Bucherest, For. Abstr. 28: 6085.

MARKS, G.C., KASSABY, F.Y. (1974) Detection of Phytophthora cinnamomi in soils. Aust. For. 36: 198-204.

MARKS, G.C., KASSABY, F.Y., REYNOLDS, S.T. (1972) Dieback in the mixed hardwood forests of eastern Victoria: A preliminary report. Aust. J. Bot. 20: 141-154.

MARKS, G.C., KASSABY, F.Y., FAGG, P.C. (1973) Dieback tolerance of eucalypt species in relation to fertilization and soil population of Phytophthora cinnamomi. Aust. J. Bot. 21: 53-65.

MARKS, G.C., ALMOND, G.A., EDGAR, J.G., KILE, G.A. (1976) Spread of Armillaria spp in the bark of Eucalyptus obliqua and Eucalyptus bicostata. Aust. For. Res. 7: 115-9.

MARTIN, J.P. (1950) Effects of soil fungi on germination of sweet orange seeds and development of the young seedlings. Proc. Soil Sci. Soc. Am. 14: 184-8.

MARTIN, P. (1957) Die abgabe von organischen verbindugnen ins besondere von scopoletin aus dem keimwurzeln des Hafers. Z. Bot. 45: 475-506.

MATTURI, S.T., STENTON, H. (1964) Distribution and status in the soil of Cylindrocarpon species. Trans. Br. mycol. Soc. 47: 577-87.

MATTURI, S.T., STENTON, H. (1964) The behaviour in the soil of four species of Cylindrocarpon. Trans. Br. mycol. Soc. 47: 589-99.

MATUO, T., MIYAZAWA, Y. (1969) Cylindrocarpon panacis sp. nov causing root rot of Ginseng. Trans. mycol. Soc. Jap. 9: 109-112.

MAZANEC, Z. (1968) Influence of defoliation by the phasmatid Didymuria violescens on the seasonal growth of diameter and on the pattern of growth rings in alpine ash. Aust. For. 32: 3-14.

McMINN, R.G. (1955) Studies on the root systems of healthy and pole blight affected white pine (Pinus monticola). Bimonthly Res. Notes For. Biol. Div. Canada Dept. Agric. 12(6): 3.

McQUEEN, D.R. (1968) The quantitative distribution of absorbing roots of Pinus sylvestris and Fagus sylvatica in a forest succession. Oecologia Plantarum 3: 83-99.

MEAGHER, J.W. (1968) Acontylus vipriensis NGNSP (Nematoda Haplolaimidae) parasitic on Eucalypt spp in Australia. Nematologia 14: 94-100.

MENGE, J.A. (1969) The ecology and survival of Cylindrocladium floridanum in soil. M.Sc. Thesis University of Mimmesota, St. Paul 117pp.

MILKO, A.A., MELNIK, A.V. (1960) A comparative study of pectolytic enzymes of some fungus species. Rev. Appl. Myc. 41: 439.

MOJTAHEDI, H., LOWNSBERY, B.F. (1975) Pathogenicity of Cricenomoides xenoplax to prune and plum root stock. J. Nematology 72: 114-119.

MONK, C. (1966) Ecological importance of root shoot ratios. Bull. Torrey Bot. Club 93: 402-6.

MOUNT, A.B. (1969) Eucalypt ecology as related to fire. Proc. Tall Timbers Fire Ecology Conf. 13: 975-1008.

NEWHOOK, F.J., PODGER, F.D. (1972) The role of Phytophthora cinnamomi in Australian and New Zealand forests. A. Rev. Phytopathol. 10: 299-326.

NICHOLLS, K.D., DIMOCK, G.M. (1965) Soils. In Atlas of Tasmania Mercury Press, Hobart.

NICHOLS, J.O. (1968) Oak mortality in Pennsylvania. A ten year study. J. For. 66: 681-694.

NORTHCOTE, K.H. (1971) A factual key for the recognition of Australian soils. 3rd Ed. Rellim Press Adelaide 123p.

ODUM, E.P. (1971) Fundamentals of Ecology 3rd Ed. W.B. Saunders Philadelphia.

OLD, K.M., KILE, G.A., OHMART, O.P. ed. (1981) Eucalypt dieback in Forests and woodlands. C.S.I.R.O. Australia.

PAGE, O.T. (1972) Effects of phytotoxins on the permeability of cell membranes. In WOOD, R.K.S. et. al. Phytotoxins and Plant Disease Academic Press N.Y., London p211-224.

PAGET, D.K. (1975) The effect of Cylindrocarpon on the plant growth response to vesicular arbuscular mycorrhiza. In Sanders, F.E., Mosse, B., Tinker, P.B., ed. Endomycorrhizas. Academic Press London, N.Y. p. 593-606.

PALZER, C.R. (1969) Is Phytophthora cinnamomi a threat to Karri forests. Aust. For. 33: 38.

PAPAVIZAS, G.C., DAVEY, C.B. (1961) Extent and nature of the rhizosphere of lupinus. Pl. Soil 14: 215-236.

PETERSON, E.A. (1958) Observations of fungi associated with plant roots. Can. J. Microbiol. 4: 257-265.

PETRESCU, M. (1974) Dieback of oak in Rumania. Eur. J. For. Path. 4: 222-7.

PODGER, F.D., DOEPEL, R.F., ZENTMYER, G.A. (1965) Association of Phytophthora cinnamomi with a disease of Eucalyptus marginata forest in Western Australia. Pl. Dis. Reptr. 49: 943-47.

PODGER, F.D., BATINI, F. (1971) Susceptibility to Phytophthora cinnamomi root rot of thirty six species of eucalypts. Aust. For. Res. 5 (3): 9-20.

PODGER, F.D., ASHTON, D.H. (1970) Phytophthora cinnamomi in dying vegetation on the Brisbane ranges Victoria. Aust. For. Res. 4 (3): 33-36.

PODGER, F.D. (1972) Phytophthora cinnamomi. A cause of lethal dieback in indigenous plant communities in Western Australia. Phytopathology 62: 972-81.

POMERLEAU, R., LORTIE, M. (1962) Relation of dieback to the rooting depth of white birch. Forest Sci. 8: 219-224.

PRATT, B.H., HEATHER, W.A. (1973) The origin and distribution of Phytophthora cinnamomi Rands in Australian native plant communities and the significance of its association with particular plant species. Aust. J. Biol. Sci. 26: 559-574.

PRYOR, L.D., JOHNSON, L.A.S. (1971) A classification of the Eucalypts. 102 pp A.N.U. Press.

READSHAW, J.L., MAZANEC, Z. (1969) Use of growth rings to determine past phasmatid defoliations of alpine ash forests. Aust. For. 33: 29-36.

REDMOND, D.R. (1957) Observations on rootlet development in yellow birch. For. Chron. 33: 208-212.

REDMOND, D.R. (1955) Studies in forest pathology XV Rootlets, mycorrhiza and soil temperature in relation to birch dieback. Can. J. Bot. 33: 595-627.

RENBUSS, M.A., CHILVERS, G.A., PRYOR, L.D. (1972) Microbiology of an ashbed. Proc. Linn. Soc. N.S.W. 94: 302-310.

RICE, E.L. (1974) Allelopathy. Academic Press N.Y. London 353pp.

RICE, E.L., PANCHOLY, S.K. (1973) Inhibition of nitrification by climax ecosystèms II Additional evidence of possible role of tannins. Am. J. Bot. 60: 691-703.

RIEUF, P. (1950) Apricot wilt I State of researches in France and abroad. Fruits and Primeurs 20: 354-357.

RIEUF, P. (1950) Apricot wilt II The situation and researches in Morocco. Fruits and Primeurs 20: 393-395.

RISHBETH, J. (1955) Fusarium wilt of banana in Jamacia I Some observations on the epidemiology of the disease. Ann. Bot. 19: 293-328.

ROSS, E.W. (1967) Association of Cylindrocladium scoparium with mortality in a 27 year old yellow poplar plantation. Pl. Dis. Reptr. 51: 38-39.

ROSS, E.W. (1966) Etiological developmental studies on a dieback disease of Fraxinus americana. Dissert. Abstr. 26 (8): 4163-4164.

ROTH, L.F., KUHLMAN, E.G. (1966) Phytophthora cinnamomi, an unlikely threat to Douglas Fir forestry. For. Sci. 12: 147-160.

ROUATT, J.W., PETERSAN, E.A., KATZNELSON, H. (1963) Microorganisms in the root zone in relation to temperature. Can. J. Microbiol. 9: 227-236.

ROVIRA, A.D. (1969) Plant root exudates. Bot. Rev. 35: 35-57.

RUEHLE, J.L. (1973) Nematodes and forest trees. A. Rev. Phytopathol. 11: 99-118.

SCHOENEWEISS, D.F. (1975) Predisposition, stress and plant disease. A. Rev. Phytopathol. 13: 193-211.

SCHOLTEN, G. (1964) Nectria radicicola on Theilaviopsis basicola als parasieten von Cyclamen persicum. Neth. J. Pl. Path. Suppl 2. 68pp.

SCHOLTEN, H. (1968) Factors affecting the infection of black spruce (Picea mariana) transplants by Cylindrocladium scoparium. Disert. Abstr. 28(8) B 3131. PhD thesis U of Minn.

SELISKAR, C.E. (1966) Virus and virus like disorders of forest trees. F.A.O./ I.U.F.R.O. Symp. OXFORD 1964.

SHEA, S.R. (1975) Environmental factors of the northern Jarrah forests in relation to pathogenicity and survival of Phytophthora cinnamomi. For. Dept. W.A. Bulletin 85, 83pp.

SHEA, S.R., MALAJCZUK, N., KITT, R.J. (1976) Promotion of understorey native legumes - a possible method of control of Phytophthora cinnamomi in the northern Jarrah forests of Western Australia. Aust. Plant Pathol. Newsl. 5 (Suppl). 5.

SHIGO, A.L., HILLIS, W.E. (1973) Heartwood, discoloured wood and microorganisms living in trees. A. Rev. Phytopathol. 11: 197-222.

SIGG, H.P. (1964) Die konstitution von Brefeldin A. Helv. Chim. Acta. 47: 1401-1415.

SMITH, A.M. (1973) Ethylene as a cause of soil fungistasis. Nature 246: 311-313.

SMITH, K.M. (1972) Plant virus diseases. Longman 3rd Ed.

SNOWDEN, P., JEHNE, W. (1975) The effects of boron and other nutrients on the susceptibility of lupin roots to infection by Phytophthora cinnamomi. Proc. 3rd Aust. Spec. Conf. in Soil Biol. Adelaide 1975.

SOMMERS, L.E., HARRIS, R.F., DALTON, F.N., GARDNER, W.R.(1970) Water potential of three root infecting Phytophthora species. Phytopathology 60: 932-934.

SPECHT, R.L. (1970) Vegetation in LEEPER, G.W. Ed. The Australian Environment 4th Ed., CSIRO Melb. Univ. Press.

STAHL, E. (1965) Thin layer chromatography. A laboratory handbook. Springer-Verlag Academic Press London N.Y. 553 pp.

STALEY, J.M. (1965) Decline and mortality of red and scarlet oaks. Forest Sci. 11: 2-17.

STERN, N.K., ROCHE, L. Ed. (1974) Genetics of Forest Ecosystems. Ecological Studies 6 Springer-Verlag New York Berlin.

SUBRAMANIAN, C.V. (1971) Hyphomycetes. Indian Council Agricultural Research.

SUBRAMANIAN, S., GOVINDARAJAN, T.S. (1968) A new root rot of coffee in India. Pl. Dis. Reptr. 52: 773-774.

SUTHERLAND, J.R., DUNN, T.G. (1970) Nematodes in coastal British Colombia forest nurseries and associations of Xiphinema bakeri with a root disease of Douglas Fir seedlings. Pl. Dis. Reptr. 54: 165-168.

TAYLOR, G.S. (1964) Fusarium oxysporum and Cylindrocarpon radicicola in relation to their association with plant roots. Trans. Br. mycol. Soc. 47: 381-391.

TAYLOR, G.S., PARKINSON, D. (1965) Studies on fungi in the root region IV Fungi associated with the roots of Phaseolus vulgaris. Pl. Soil 22: 1-20.

TAYLOR, H.M., GARDNER, H.R. (1963) Penetration of cotton seedling taproots as influenced by bulk density, moisture content and strength of soil. Soil Sci. 96: 153-156.

TAYLOR, R.H. (1956) Cylindrocarpon radicicola Wr the recording of a new fungus on grapes in Victoria. J. Aust. Inst. Agric. Sci. 22: 291-292.

THIES, W.G., PATTON, R.F. (1970) An evaluation of propagules of Cylindrocladium scoparium in soil by direct isolation. Phytopathology 60: 599-601.

THORNTON, R.H. (1960) Fungi of some forest and pasture soils. N.Z. J. Agric. Res. 3: 699-711.

THORNTON, R.H. (1965) Studies of fungi in pasture soils I Fungi associated with live roots. N.Z. Jl. Agric. Res. 8: 417-449.

TIMONIN, M.I. (1966) Rhizosphere effect of healthy and diseased lodgepole pine seedlings. Can. J. Microbiol. 12: 531-537.

TIPPETT, J.T, HOLLAND, A.A., MARKS, G.C., OBRIEN, T.P. (1976) Penetration of Phytophthora cinnamomi into disease tolerant and susceptible eucalypts. Arch. Microbiol. 108: 231-242.

TRESNER, H.D., BACKUS, M.P., CURTIS, J.T. (1954) Soil microfungi in relation to the hardwood forest continuum in Southern Wisconsin. Mycologia 46: 314-333.

TRUTER, S.J. (1947) A preliminary investigation of the dieback of Alnus glutinosa (L) Gaertner. Rev. Appl. Mycol. 26: 516.

TSAO, P.H., OCANA, G. (1969) Selective isolation of Phytophthora from natural soil on an improved antibiotic medium. Nature 223: 636-638.

UROSEVIC, B. (1963) Some new and little known diseases of oak plants in plantations and nurseries. Commun. Inst. For. Cechosl. 3: 122-134.

VAN ARSDEL, E.P., HALLIWELL, R.S. (1970) Progress in research on live oak decline. Pl. Dis. Reptr. 54: 669-672.

VANCURA, V., GARCIA, J.L. (1969) Root exudates of reversibly wilted millet plants Panicum milliaceum. Oecol. Plant. 4: 93-98.

VAN DER PLANK, J.E. (1975) Principles of plant infection. Academic Press N.Y. London

VAARTAJA, O., BUMBIERIS, M. (1967) Organisms associated with root rot of conifers in South Australian nurseries. Pl. Dis. Reptr. 57: 473-476.

VINCENT, G., KANTOR, J. (1971) Das frühzeitige Tannensterben, seine Ursachen und Vorbeugung. Cbl. ges. Forstwesen. 88: 101-115.

VISSER, S., PARKINSON, D. (1975) Fungal succession on aspen poplar leaf litter. Can. J. Bot. 53: 1640-1651.

WAGNER, V.A. (1942) Phytophthora cinnamomi and wet soil in relation to the dying back of avocado trees. Hilgardia 9: 519-532.

WAID, J.S. (1962) Influence of oxygen upon growth and respiratory behaviour of fungi from decomposing rye grass roots. Trans. Br. mycol. Soc. 45: 479-487.

WAID, J.S. (1974) Biology of plant litter decomposition in DICKINSON, C.H., PUGH, G.J.F. ed Decomposition of Roots Academic Press N.Y., London.

WARCUP, J.H. (1950) The soil plate method for isolation of fungi from soil. Nature 166: 117.

WARCUP, J.H. (1957) Studies on the occurrence and activity of fungi in a wheat field soil. Trans. Br. mycol. Soc. 40: 237-262.

WAREING, P.F. (1970) Ageing of the tree. In LUCKWILL, L.C., CUTTING, C.V. ed. Physiology of Tree Crops Academic Press N.Y., London.

WATERHOUSE, G.M., STAMPS, D.J. (1969) Isolation of Phytophthora and Pythium. in SHAPTON, D.A., GOULDS, G.W. ed Isolation methods for microbiologists. Soc. Appl. Bacteriol. Tech. Serv. No. 3, Academic Press N.Y., London 178pp.

WATSON, A.G., FORD, E.G. (1972) Soil fungistasis a reappraisal. A. Rev. Phytopathol. 10: 327-349.

WEAVER, D.J.W.E., HUNT, E.J., DOWLER, W.M. (1974) Association of tree site, Pseudomonas syringe, Cricenomoides xenoplax and pruning date with short life of peach trees in Georgia. Pl. Dis. Reptr. 58: 76-79.

WESTE, G.M., LAW, C. (1973) The invasion of native forest by Phytophthora cinnamomi III Threat to the national park Wilsons Promontary Victoria. Aust. J. Bot. 21: 31-51.

WESTING, A.H. (1964) The longevity and ageing of trees. Gerantologist 4: 10-15.

WESTING, A.H. (1966) Sugar maple decline: An evaluation. Econ. Bot. 20: 196-212.

WIDDEN, P., PARKINSON, D. (1975) The effects of forest fire on soil microfungi. Soil Biol. Biochem. 7: 125-139.

WILHELM, S. (1959) Parasitism and pathogenesis of root disease fungi p356-366 in HOLTON, C.S. et. al. Ed Plant Pathology Problems and Progress 1908-1958 A.P.S. Univ. of Wisc. Press.

WNEKOWSKI, S. (1963) Studies on the actinomycete strain A52 from the Globispora group and its fungal antagonisms. Biul. Inst. Ochr. Rosl. Pozanan 19: 193-217. Rev. Appl. mycol. 43: 33.

WOOD, R.K.S., BALLIO, A., GRANITI, A. (1972) Phytotoxins in plant disease. Academic Press N.Y., London.

WOOLHOUSE, H.W. (1967) The nature of senescence in plants. p171-215 in WOOLHOUSE, H.W. ed. Aspects of the biology of ageing. Camb. Univ. Press.

YLIMAKI, A. (1967) Root rot as a cause of red clover decline in leys in Finland. Ann. Aric. Fenn. 6 SUPPL. 1 59pp.

ZAK, B. (1961) Aeration and other soil factors affecting southern pines as related to little leaf disease. U.S.D.A. Tech. Bull. 1248 30pp.

ZENTMYER, G.A., LEARY, J.V., KLORE, L.J., GRANTHAM, G.L. (1976) Variability in growth of Phytophthora cinnamomi in relation to temperature. Phytopathology 66: 982-986.

APPENDIX 1.

COMPOSITION OF MEDIA

1. Standard Mycological Media.

a. Malt extract agar *

b. Cornmeal Agar *

c. Potato dextrose agar *

d. Lima Bean agar *

* Commercial preparations by Oxoid or Difco used throughout.

e. Czapeks agar or liquid media in the absence of agar.

$NaNO_3$	2g
K Cl	0.5g
Mg $SO_4.7H_2O$	0.5g
K_2 H PO_4	0.35g
Sucrose	30g
Agar	25g
Distilled water	1000 ml

2. Media for the isolation of structural wood decay fungi.

L Asparagine	2g
K H_2 PO_4	1.75g
Mg SO_4 $7H_2O$	0.75
Glucose	10g
Agar	10g
Thiamine HCl	1mg
Benomyl	10µg
Streptomycin Sulphate	200µg
ethanol	0.5ml
distilled water	1000ml

3. Media for the isolation of pythiaceae P_{10}VP Tsao + Ocana (1969)

Cornmeal Agar (Difco)	17g
Pimaricin "Pimafucin", 2.5% sterile	10ppm
Vancomycin (Vancocin HCl)	200ppm
P.C.N.B. (Pentachlor nitrobenzene)	100ppm

4. Media for the isolation of Fusarium spp. Denis et al. 1966.

tryptone	15g
Soya peptone	5g
Na Cl	5g
Yeast extract	5g
$K H_2 PO_4$	1.5g
Agar	15g
Rose Bengal	0.035g
Sodium Azide	0.025g
distilled water	1000ml

5. Media for the isolation of Cylindrocarpon microsclerotia.

Czapeks agar	as above
Oxgall "oxbile" (Oxoid)	40g
Streptomycin sulphate	200ppm

6. Media for water activity studies Dubé et al. 1971.

Basic media	$Na NO_3$	0.68g
	$KH_2 PO_4$	0.68g
	$K_2 H PO_4$	1.4g
	$Mg SO_4 .7H_2O$	0.12g
	Sucrose	4.0g
	Agar	10.0g
	Distilled water	1000ml

Additions of salt mixtures (g/l) to obtain water activity of:

a_w	Na Cl	K Cl	$Na_2 SO_4$
0.995	3.36	2.57	3.27
0.990	7.56	5.78	7.35
0.980	16.60	12.50	15.90
0.960	33.90	25.90	33.00
0.940	50.80	38.80	49.50

7. Buffers used in pH response studies.
Mixtures of these buffer solutions at the various compositions were added to moulten Czapeks agar minus $K_2 HPO_4$ to make agars of half normal solute concentrations.

0.2M $Na_2 HPO_4.H_2O$:0.1M Citric Acid.

pH			
2.2	2	:	98
2.6	10.9	:	89.1
3.0	20.5	:	79.5
3.4	28.5	:	71.5
3.8	35.5	:	64.5
4.2	41.4	:	58.6
4.6	48.2	:	51.8
5.0	51.5	:	48.5
5.4	55.7	:	44.3
5.8	60.4	:	39.6
6.2	66.1	:	33.9
6.6	72.7	:	27.3
7.0	82.3	:	17.7
7.4	90.8	:	9.2
7.8	95.7	:	4.2
8.0	97.2	:	2.8

0.067M $Na_2 HPO_4 .2H_2O$: 0.067M $KH_2 PO_4$

pH			
5.4	3.1	:	96.9
5.8	8.0	:	92
6.2	18.5	:	81.5
6.6	36.0	:	64
7.0	61.0	:	39
7.4	80.8	:	19.2
7.8	91.5	:	8.5
8.0	94.5	:	5.5

8. Liquid media used in cellulolysis studies Garrett(1970).

$Na No_3$	5g
$KH_2 PO_4$	1g
$Mg SO_4.7H_2O$	0.5g
Iron Citrate	1mg
Thiamine HCl	0.1mg
Biotin	0.01mg
Distilled water	1000ml

9. Media used for studies on rhizosphere micro-oganisms.

a. Nutrient agar

Yeast extract	3g
Peptone	5g
Agar	12g
Distilled water	1000ml

b. Soil extract agar

Filtered suspension of 5 g of soil in 1000ml distilled water.

KH_2PO_4	2g
Agar	12g

c. Basal media

KNO_3	0.5g
K_2HPO_4	1.0g
$MgSO_4.7H_2O$	0.2g
$CaCl_2$	0.1g
NaCl	0.1g
$FeSO_4.7H_2O$	0.01g
Glucose	1.0g
Agar	15g
distilled water	1000ml.

10. Media for fungitoxicity studies.

a. Soil extract agar

Filtered suspension of 30g soil in 1000ml distilled water.

KH_2PO_4	2g	
Agar	20g	pH 5.7

b. Leaf extract agar.

Filtered macerate of 30g eucalypt leaves in 1000ml distilled water.

KH_2PO_4	2g	
Agar	20g	pH 5.7

11. Petri solution.

$Ca(NO_3)_2.4H_2O$	0.4g
$MgSO_4.7H_2O$	0.15g
KH_2PO_4	0.15g
KCl	0.06g
distilled water	1000ml

12. Gylcerol-Asparagine agar for the isolation of Actinomycetes in the E. obliqua rhizosphere.

Glycerol	20g
L Asparagine	2.5g
NaCl	1.0g
$CaCO_3$	0.1g
$FeSO_4.7H_2O$	0.1g
$MgSO_4.7H_2O$	0.1g
Agar	20g
distilled water	1000ml

APPENDIX 2.

STATISTICAL ANALYSIS

The replicated measurements made in experiments with factorial designs were analysed using standard analysis of variance tests. Least significant differences for these data were calculated at the probabilities indicated in each figure.

Standard regression analyses were used to test the association between data and obtain co-relation coefficients.

The variability of measurements from replicated samples for individual data points in series of samples of different ages on types of materials is represented by bars in some of the figures which indicate twice the standard error of that mean.