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PROGRESS REPORT FOR INTERIM FOUNDATION FOR DIEBACK RESEARCH

PROJECT : JARRAH ROOT BEHAVIOUR AND HEALTH

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Period of Research : August 1979-August 1982.

CONTENTS :

A. SUMMARY OF PROGRESS

(a) The morphology and structure of the surface root system of jarrah was examined on a number of freely drained upland sites. Components of the root pads were described.

b) The anatomy of surface feeder roots was investigated. Two types of long roots were recognized: (a) those with thick-walled epidermal cells, and (b) those with a lignified outer cortex. The hypodermis of short roots was often suberized and the inner layers of the cortex had lignified secondary walls.

(c) The timing of new surface root growth was followed for a 15-month period in the field. Roots were initiated during spring and following autumn rain. Rapid root growth occurred within 2 days of a storm in February. Much of the new root growth in jarrah coincides with the timing of Phytophthora cinnamomi activity in the soil.

(d) Inoculation of root pads during spring flush of root growth led to significant infection of surface root pads primarily by entry via fine roots. Infection of larger roots (3-4 mm) occurred via infection of fine root clusters as well as via new lateral roots initiated from the large roots. As soil moisture decreased P. cinnamomi could no longer be isolated from the soil in contrast to continued survival of the fungus in jarrah roots.

(e) A field trial is in progress to examine the susceptibility to infection of long roots and fine feeder roots of jarrah.

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B. SURFACE ROOT SYSTEM OF JARRAH

1. MORPHOLOGY

Introduction

At the time this project was undertaken there was no published information on the surface root system of jarrah. This was surprising because a number of workers had been carefully researching jarrah dieback for a number of years. In cooperation with Dr. S. Shea a very brief description of the surface root system was made. The published paper appears below.

Structure of the Surface Root System of *Eucalyptus marginata* Sm. and its Infection by *Phytophthora cinnamomi* Rands

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Abstract

The structure of the surface root system of jarrah (*Eucalyptus marginata*) trees was examined on a number of freely drained upland sites with different fire and management histories. The roots typically occurred in patches but in some stands formed extensive sheets. On excavation, this surface root system was composed of pads ranging in size from 10 cm to 1·3 m in diameter and *c.* 5 cm thick. The pads consist of short roots 1·3 mm long (which commonly form dense clusters around lateritic pebbles) which arise from small $(n-1)^{th}$ order laterals 0·5–1·5 cm long connected to $(n-2)^{th}$ order laterals 2–5 cm long and up to 0·7 mm in diameter. Mycorrhizal roots were common throughout the surface root pads. During the summer drought many of the short lateral roots die but the main framework of the roots of the surface pads is perennial. Following rains or irrigation, new, short lateral roots form rapidly from the framework of roots in the surface pads.

Phytophthora cinnamomi was consistently recovered from short lateral roots and from the perennial roots $(n-1, n-2)$ which form the framework of the root pads at a site in diseased forest where a high density of *P. cinnamomi* had been induced in the soil by irrigation.

We hypothesize that the destruction of some of the perennial components of the root pads could explain why *P. cinnamomi* can cause the decline and death of jarrah in an environment only marginally favourable for the fungus.

Introduction

Jarrah (*Eucalyptus marginata* Sm.), an important commercial timber tree of south-western Australia, is susceptible to a disease called jarrah die-back caused by the soil-borne pathogen *Phytophthora cinnamomi* Rands (Podger 1972). The disease is common in infertile lateritic podzolics (Havel 1975) in which soil temperature and moisture conditions are suitable for the growth and autonomous spread of the fungus for only a small part of the year (Shea 1975). This limited vulnerability of the host to attack and the very low rates recorded for direct recovery of the fungus from roots of diseased jarrah trees (Podger 1968; Palzer 1976; Shea, unpubl. data) may account for the slow decline of most jarrah in affected areas. This experience also raised the question of the extent to which infection by *P. cinnamomi* is responsible for the death of jarrah.

In this paper we (i) report investigations of the anatomy, morphology and distribution of the surface root systems of jarrah, which extends the preliminary work of Kimber (1974), and (ii) record high recoveries of *P. cinnamomi* from surface roots of naturally infected jarrah in a forest stand in which rainfall had been supplemented by

2. ANATOMY

Introduction

When this work commenced there was a general consensus by workers in the area that Phytophthora cinnamomi invaded the fine feeder roots of jarrah and this resulted in some unexplained process that led to the death of the tree. This process could either occur very rapidly (patch death) or very slowly (decline). It was considered that information was needed on the structure of the fine root components before detailed studies of root infection could be undertaken. Further, this information would prove useful in comparing roots of pot grown plants with field trees (see later). Details of the anatomy of the surface fine roots of jarrah are provided below.

Surface Root System of *Eucalyptus marginata* Sm.: Anatomy of Non-mycorrhizal Roots

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Abstract

The anatomy of surface feeder roots of *Eucalyptus marginata* was investigated. Two types of long roots were recognized: (*a*) those with thick-walled epidermal cells, and (*b*) those with a lignified outer cortex. The hypodermis of short roots was often suberized and the inner layers of the cortex had lignified secondary walls. The occurrence of lignified and suberized layers is discussed in relation to possible infection by *Phytophthora cinnamomi*.

Introduction

Jarrah (*Eucalyptus marginata* Sm.) is an important timber tree in south-western Australia. On lateritic podzols the trees have an extensive surface root system from which sinker roots pass into the pallid zone (Kimber 1974). Many of the fine surface roots occur in dense pads (Shea and Dell 1981). Since prolonged feeder root destruction by the pathogen *Phytophthora cinnamomi* Rands may result in the decline and eventual death of jarrah, detailed investigations of the surface feeder roots were initiated. This paper reports on the anatomy of the non-mycorrhizal feeder roots. The ectomycorrhizae of jarrah have recently been described (Malajczuk and Hingston 1981).

Materials and Methods

Jarrah roots were collected in September from an upland site in the jarrah forest near Dwellingup, W.A. Pieces of root were fixed at 21 °C in 3% glutaraldehyde in 0·025 M phosphate buffer (pH 7·0), dehydrated and embedded in glycol methacrylate as previously described (Dell *et al.* 1980). Sections 1–1·5 μm thick were stained with toluidine blue O (TB 0·05% in benzoate buffer, pH 4·4) for 1 or 2 min; Sudan black B (sat. soln in 70% ethanol) for 30 min, and by the periodic acid–Schiff's (PAS) reaction.

For scanning electron microscopy (SEM), roots fixed in glutaraldehyde and dehydrated in ethanol were dried to the critical point in amyl acetate, coated with gold and examined in a Philips electron microscope at 25 kV.

3. PERIODICITY OF GROWTH

ABSTRACT

The timing of new surface root growth in jarrah (Eucalyptus marginata) was followed for a 15-month period in the field. The periodicity of new root growth was similar for long roots, non-mycorrhizal and mycorrhizal root clusters. Roots were initiated during two peak periods of growth in spring (September-October) and following autumn rain (May-June). Little new root activity was recorded in late winter (August) or during summer drought. Rapid root growth occurred within 2 days of a storm (47 mm rain) in February. In addition short roots formed after very light showers of rain (< 5 mm) in late summer. Much of the framework for fine feeder roots was built up after autumn rain. In contrast to new long root growth which was equally spread between spring and late autumn, the majority of new mycorrhizal roots were produced from May to July. The periodicity of new root growth appears to be influenced by both soil moisture and soil temperature. Root growth ceased when warm surface soils dried out and commenced when rain fell. Lack of root growth in August may be due to low soil temperatures. Much of the new root growth in jarrah coincides with the timing of Phytophthora cinnamomi activity in the soil.

INTRODUCTION

Jarrah (Eucalyptus marginata Donn ex Sm.) grows extensively on the deep upland lateritic soils of the dissected Darling Plateau. In recent years this important timber tree has received attention from a number of workers as a result of widespread destruction of the jarrah forest from disease, especially Phytophthora cinnamomi, and clearing

for mining and other land uses. The ability for a high forest to develop on highly leached and infertile soils which experience long periods of summer drought must be due in part to the specialized root system of jarrah, the dominant tree species. Jarrah roots have two major components, namely deep penetrating descending or sinker roots which pass through the caprock into the pallid zone, and an extensive array of fine feeder roots above the caprock close to the soil surface. Aspects of the root system have been described by Dell and Wallace (1981), Kimber (1974), Malajczuk and Hingston (1981) and Shea and Dell (1981). The surface root system often occurs in dense mats where the litter layer is repeatedly removed by burning. Where the litter layer is allowed to accumulate over many years a larger proportion of the fine roots are mycorrhizal and the mats are more diffuse. Within these layers occasional observations indicated that the timing of new root growth was seasonal. This study was undertaken to give a qualitative assessment of new long and short root production in the surface mats. It forms part of a long term study on nutrition of jarrah and infection of jarrah roots by P. cinnamomi.

MATERIALS AND METHODS

Five plots each 5 x 5 m were established in an upland jarrah pole stand on a well-drained laterite near Dwellingup, W.A. (32° 34'S, 116° 14'E). The site was last burnt in 1977, two years prior to the commencement of this study. Over a 15 month period, beginning in July, three samples (approx. 30 x 30 cm and 5 cm deep) were removed from each plot every 14 days, or monthly during summer. The samples, which included all roots in the A_0 and sandy gravel A_1 horizons and part of the A_2 horizon, were placed in plastic bags and stored at 4°C before processing.

Soil was removed from roots by hand washing with water. Roots were classified into the following seven categories for the purpose of this study (Fig. 1).

A. Long roots - (i) unbranched linear extensions from dormant long root t
(ii) unbranched laterals from parent roots with periderms.
(iii) branched long roots.

B. Non-mycorrhizal root clusters (classification after Dell & Wallace, 198
(i) nth order short roots
(ii) n-1th order roots of limited growth.

C. Mycorrhizae (i) ageotrophic pyramidal roots
(ii) simple mycorrhizal roots not in clusters.

The number of new white fleshy roots in each category was estimated as a percentage of the total number of roots present in that category and given the following ratings :

Rating	Percentage of Total Roots in each Category
0	0
1	1-5
2	6-10
3	11-25
4	26-50
5	51-100

A mean rating with standard errors for each root category was determined at each harvest.

Rainfall data was taken from the nearest field observation station some 3 km southwest of the plots.

RESULTS

Rainfall

Over the 15 month study period the typical pattern of winter rain and summer drought predominated (Fig 2a). This broad pattern experience seasonal variations from year to year. Two of these, which may have influenced root growth, are as follows. Firstly, late spring rains in November were heavy (72 mm). Secondly, of the 78 mm of rain that fell from December to April, 47 mm was due to a single thunderstorm in February. These storms are not annual events and may occur during other summer months. As a result of heavy rains in November and February A_0 and A_1 soil horizons were both warm and moist. It is within these layers that most of the feeder roots of jarrah occur.

Long roots

Long roots (Fig. 1a, b, c) when actively growing had white fleshy tips with large root caps. Three types of long roots, based on the site of origin of the new root and the presence of branches, were recognized. Simple extensions from the dormant ends of suberized long roots, produced in the previous period of growth, were more abundant in spring and late autumn into early winter (Fig. 2b). These two flushes of new root growth were separated by periods of little or no new root initiation in late winter (August) and summer. A rapid response to summer rain occurred with new root production apparent within several days of the soil becoming wet.

The second major site of new long root initiation occurred in roots from one to many years old which ranged in diameter from 1-5 mm. These roots had a well developed suberized and lignified periderm.

New roots were variable in size ranging from fast-growing roots about 1 mm in diameter to slow-growing roots about 2 mm in diameter. New root production (Fig. 2c) followed the same periodicity as linear extensions from long roots (Fig. 2b). However, very few new roots were observed after summer rain.

Long root growth enables jarrah to extend its root system through the soil and litter layers and leads to an increase in the total length of the major suberized roots. As well, initiation of lateral roots on new seasons' fleshy long roots forms the framework on which mycorrhizal and non-mycorrhizal short roots will be produced. Like the parent root, the framework laterals develop secondary growth but differ in that extension growth is often limited. Dell and Wallace (1981) referred to these roots as being of the n - 2th order. Periodicity of growth of long roots with new laterals (Fig. 2d) was similar to long roots without laterals characterized by spring and late autumn flushes as well as growth after summer rain. These roots were consistently more abundant in early winter than spring. Growth during the former period probably provides sites for new short root production the following spring.

Non-mycorrhizal root clusters

The morphology of these roots were described by Dell and Wallace (1981). The fine feeder roots (nth order), unlike the n-1th order roots of limited growth to which they are attached (Fig. 1d), were short lived. New nth order roots arose either from the same season's fleshy n-1th order roots, or from previous seasons' n-1th order roots. In the latter case new roots arose between sites of previous seasons' short roots which may have been shed or were still attached but dead.

Initiation of new n-1th order roots occurred both from new branched long roots as well as from older n-2th order roots within an established framework of root clusters. Timing of new n-1th order roots (Fig. 3a) was almost identical to that of the branched long roots (Fig. 2d). Similarly new white short roots followed the same seasonal trend. However, unlike long root initiation short roots were formed after very light falls of summer rain (< 5 mm).

Mycorrhizal roots

Mycorrhizal roots were divided into two classes, namely, ageotrophic pyramidal roots corresponding to the white mycorrhizae of Malajczuk and Hingston (1981), and simple mycorrhizal roots, mainly the black mycorrhizae of Malajczuk and Hingston (1981). Generally the pyramidal roots were associated with the shallow litter layer whereas the simple mycorrhizae which were unbranched or forked were more common in the sandy laterite.

Though some simple mycorrhizal roots were initiated in spring and after summer rain (Fig. 3c) most new root growth occurred in late autumn and winter rising to a maximum in July. Production of new pyramidal mycorrhizae followed the same seasonal variation as displayed by the simple mycorrhizae, though stimulation of root growth was not as enhanced in winter. Numbers of pyramidal mycorrhizae were generally low due to the small litter load present at the site as a result of control burning. In contrast to new long root initiation which occurred equally in spring and autumn, mycorrhizal root growth was more prevalent in autumn and winter.

DISCUSSION

In this study a qualitative assessment of new root growth was made by examination of root tips. Although no distinction was made between old live and dead roots, and no account of natural mortality was made, the data are adequate for the purpose of following large seasonal trends in root growth. A strong periodicity in root initiation occurred with a spurt of growth following autumn rain (May-June) and in spring (Sept.-Oct.). Little root activity was recorded in late winter and during summer drought. This seasonal pattern of new root growth was broadly similar for long roots, non-mycorrhizal and mycorrhizal root clusters though some differences were observed. For example, new long root growth occurred equally in spring and late autumn whereas the majority of new mycorrhizal roots were formed from May to July.

In jarrah, the initiation of new fine root growth in the spring is consistent with data reported for tree species in the northern temperate latitudes (Lyr and Hoffmann 1967). This growth session follows a quiescent winter period (Zimmermann and Brown 1971) where non-mycorrhizal roots, although not dormant, experience very slow growth rates. In jarrah the period of reduced new root production in August may be due to low soil temperatures, though the role of internal factors such as hormone balances cannot be excluded.

With the exception of Larix and some everygreen conifers, root growth commences before shoot growth in the spring (Lyr and Hoffmann 1967). In jarrah shoot growth commences several months after root activity in the spring. However, in contrast to some tree species (Deans 1979, Head 1967) no decline in fine root production was observed with the onset of shoot growth. Considerable

changes occur in the fine root components of trees during the growing season (Deans 1979; Ford and Deans 1977; Roberts 1976). In certain conifers and deciduous trees, for example, the turn-over rate for small roots can be high (Kummerow et al. 1978, Persson 1978, 1980) with death occurring very quickly in local dry areas (Lyford and Wilson 1966). Moisture probably has a greater influence on fine root development and survival than any other soil factor.

Interruption of tree root growth in summer has been reported by many workers (Deans 1979, Lyr and Hoffmann 1967, Roberts 1976). Shortage of available water for root growth is probably the major limiting factor. In the jarrah forest the decline in new surface root production in early summer parallelled a decline in precipitation. With increasing solar radiation the surface soils quickly dry. However, new roots can rapidly form following summer rain. Jarrah has the potential not only to regenerate long and short roots after occasional heavy summer rains but may also produce new fine roots within an established framework following light falls of rain that wet the upper 2-4 cm of soil for several days.

The cessation of growth in midsummer appears to be due to unfavourable environmental conditions, namely drought. Root growth recommences in the autumn following rain. During this growth period much of the framework for fine feeder roots is established. This second peak of root activity in autumn has also been well established for trees in Central Europe (Lyr and Hoffmann 1967) although it is not universal (Deans 1979).

The timing of new root growth in jarrah coincides with those periods when soil moisture and temperature regimes are likely to be favourable for Phytophthora cinnamomi to reach high population levels in the soil. Shea et al. (1980) have shown that these

periods can be very short on well drained upland sites such as the one where this study took place. High soil moistures and soil temperatures following summer rain have been shown to result in rapid root growth and it is likely that considerable infection of roots could occur since high soil densities of P. cinnamomi have been induced by irrigation in summer (Shea and Dell 1981).

ACKNOWLEDGEMENTS

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REFERENCES

1. DEANS, J.D. (1979) Fluctuations of the soil environment and fine root growth in a young Sitka Spruce plantation. Plant and Soil 52, 195-208.

2. DELL, B. and WALLACE, I.M. (1981). Surface root system of Eucalyptus marginata Sm. : Anatomy of non-mycorrhizal roots. Aust. J. Bot. 29. 565-77.

3. FORD, E.D. and DEANS, J.D. (1977) Growth of a Sitka Spruce plantation : spatial distribution and seasonal fluctuations of lengths, weights, and carbohydrate concentrations of fine roots. Plant and Soil 47. 463-85.

4. HEAD, G.C.(1967) Effects of seasonal changes in shoot growth on the amount of unsuberized root on apple and plum trees. J. Hort. Sci. 42, 169-80.

5. KIMBER, P.C. (1974) The root system of jarrah (Eucalyptus marginata) West. Aust. For. Dep. Res. Pap. No. 10.

6. KUMMEROW, J., KRAUSE, D. and JOW, W. (1978) Seasonal changes of fine root density in Southern Californian chaparral. Oecologia 37, 201-12.

7. LYFORD, W.H. and WILSON, B.F. (1966) Controlled growth of forest tree roots : technique and application. Harvard Forest Pap. No. 1

8. LYR, H. and HOFFMANN, G. (1967) Growth rates and growth periodicity of tree roots. Int. Rev. For. Res. 2, 181-236.

9. MALAJCZUK, N. and HINGSTON, F.J. (1981) Ectomycorrhizae associated with jarrah roots. Aust. J. Bot. 29, 437-46.

10. PERSSON, H. (1978) Root dynamics in a young Scots pine stand in Central Sweden. Oikos 30, 508-19.

11. PERSSON, H. (1980) Spatial distribution of fine-root growth, mortality and decomposition in a young Scots pine stand in Central Sweden. Oikos 34, 77-87.

12. ROBERTS, J. (1976) A study of root distribution and growth in a Pinus sylvestris L. (Scots Pine) plantation in Thetford Chase, East Anglia. Plant and Soil 44, 607-21.

13. SHEA, S.R. (1975) Environmental factors of the northern jarrah forest in relation to pathogenicity and survival of Phytophthora cinnamomi. Bulletin, Forests Dept. Western Australia. No. 85.

14. SHEA, S.R. and DELL, B. (1981) Structure of the surface root system of Eucalyptus marginata Sm. and its infection by Phytophthora cinnamomi Rands. Aust. J. Bot. 29, 49-58.

15. SHEA, S.R., GILLEN, K.J. and LEPPARD, W.I. (1980) Seasonal variation in population levels of Phytophthora cinnamomi Rands in soil in diseased, freely drained Eucalyptus marginata Sm. sites in the northern jarrah forest of south-western Australia. Prot. Ecol. 2, 135-56.

16. ZIMMERMANN, M.H. and BROWN, C.L. (1971) Trees Structure and Function (Springer-Verlag : New York)

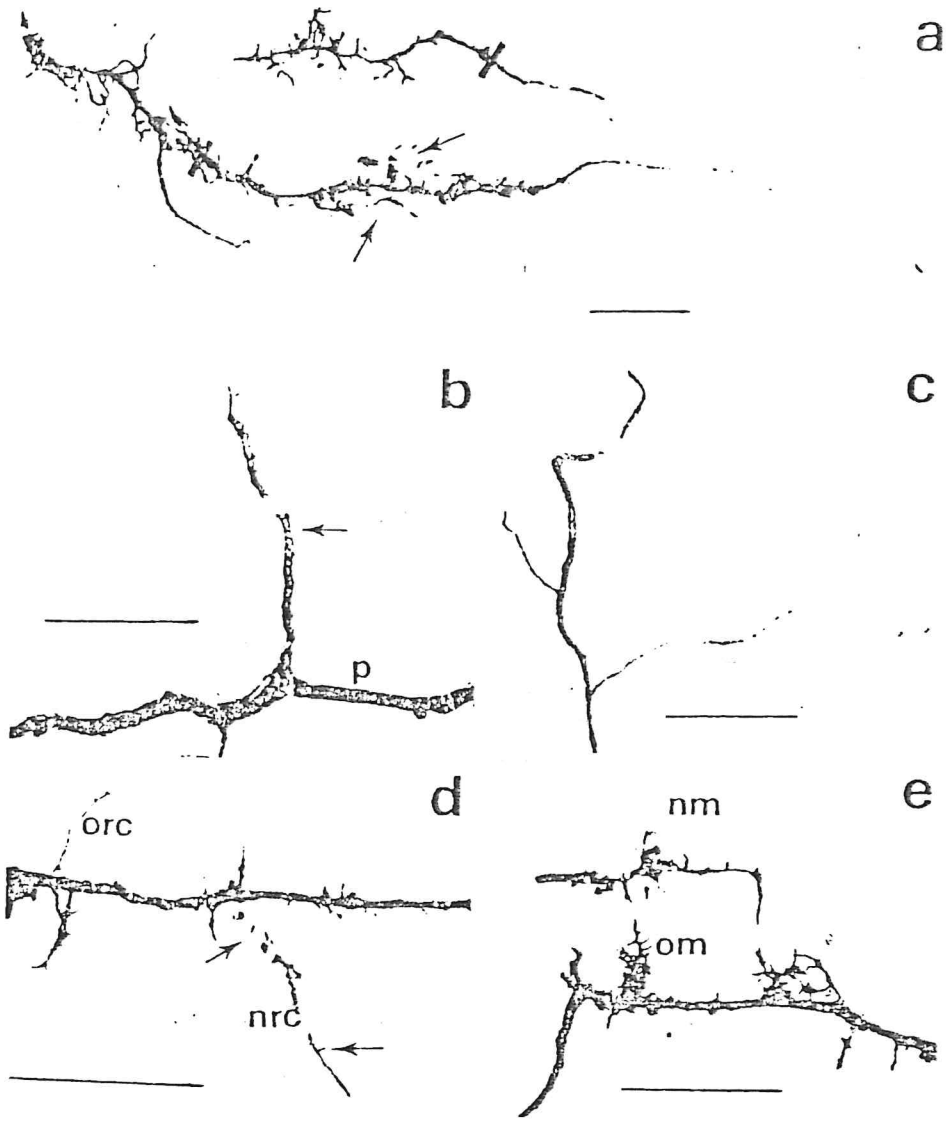

Fig. 1. Components of surface root system of jarrah showing the new root categories referred to in the text.

a. long roots initiated as linear extensions from dormant long root tips upper - unbranched, lower - branched (arrows), bar shows the site of origin of current season's growth.

b. long root (arrow) initiated as a lateral from parent root (p) with periderm.

c. branched new long roots.

d. non-mycorrhizal root clusters showing new fleshy roots (nrc) and old dead roots (orc), nth order short roots are arrowed.

e. new (nm) and old (om) pyramidal mycorrhizal roots.

Length of bar = 1 cm.

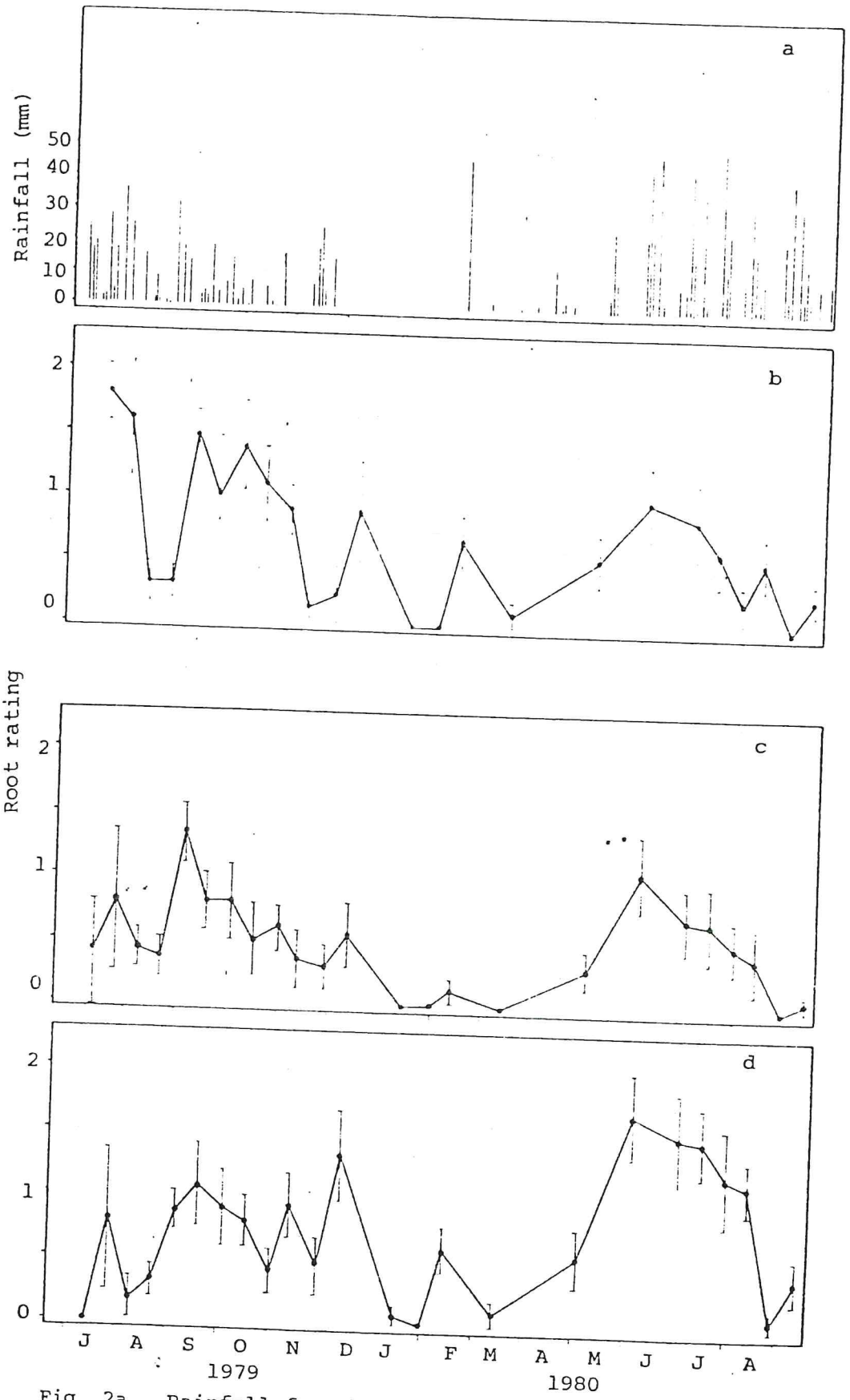

Fig. 2a. Rainfall for the period June 1979 to September 1980
2 b,c,d. Effect of time of the year on new long root production in jarrah. b = unbranched linear extensions from dormant long root tips. c = unbranched laterals from parent roots with periderms. d = branched long roots.

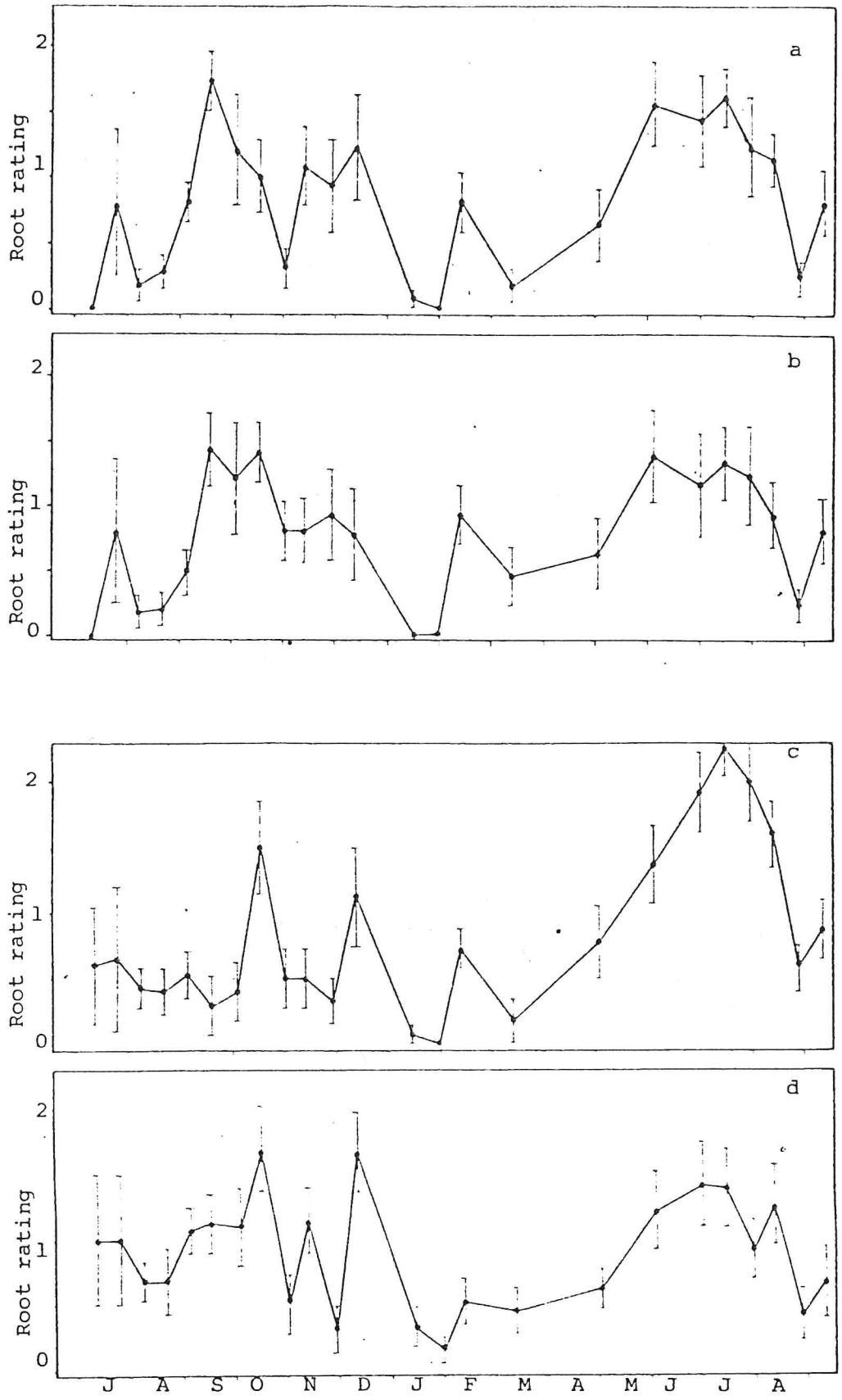

Fig. 3. Effect of time of the year on non-mycorrhizal (a,b) and mycorrhizal fine roots (c,d). a = nth order short roots. b = n-1th order roots of limited growth. c = ageotrophic pyramidal roots. d = simple mycorrhizal roots not in clusters.

C. INFECTION AND SURVIVAL OF PHYTOPHTHORA CINNAMOMI

(i) Inoculation of Root Pads

Infection and survival of Phytophthora cinnamomi in jarrah roots

Progress report presented at May 1980 Perth meeting of Australian Society of Plant Pathologists with Dr. S. R. Shea and Dr. B. L. Shearer, Forests Department Research Station, Dwellingup.

Abstract

A field trial was established in the jarrah forest near Dwellingup to provide information on : (1) the components of the surface root system of jarrah which can be invaded by Phytophthora cinnamomi following a zoospore drench, (2) the survival of the fungus in the soil and jarrah roots, (3) the distribution and spread of the fungus in jarrah roots, and (4) the effects of the pathogen on surface fine root maintenance and development.

Preliminary data is provided for the period October 1979-April 1980. Inoculation during spring flush of root growth led to significant infection of surface root pads primarily by entry via fine roots. Evidence suggests that infection of larger roots (3-4 mm) occurs both via infection of the fine root clusters as well as via new lateral roots initiated from the large roots. As soil moisture levels dropped P. cinnamomi could no longer be isolated from the soil in contrast to the continued survival of the fungus in jarrah roots. The pathogen invades extraxylary tissues and spread within large roots up to 4 mm in diameter was extensive in some roots and restricted in others. It is hypothesized that P. cinnamomi can have very destructive effects on the surface fine feeder root system of jarrah by attacking the framework to which the short feeder roots are attached.

Introduction

Phytophthora cinnamomi is widespread throughout the jarrah forest and is frequently isolated from understorey species. However, despite considerable death of jarrah (*Eucalyptus marginata*) the pathogen has been only isolated occasionally from this species. Little is known about the infection of jarrah roots by *Phytophthora cinnamomi* or about the survival of the pathogen in jarrah roots. This trial was therefore established to provide data on the infection and survival of the pathogen in jarrah roots in the field.

Materials and Methods

Study site

An upland jarrah pole stand on a well-drained laterite was chosen near Dwellingup, W.A. (32^{o} 34'S, 116^{o} 14'E). Two plots each 6 x 6 m were established in an area of dense regrowth. The inoculated plot was immediately down slope from the control plot. The site was last burnt in 1977, two years prior to the commencement of this study. Here the surface root system of jarrah was concentrated into dense pads in the upper 5 cm of soil.

Inoculation

Fifty galvanized collars 23-26 cm in diameter and 5.5 cm deep were placed over root pads after removal of litter in each plot. The collars were anchored into place with two spikes. After inoculation the litter was replaced over the bare earth. Each pad was inoculated with 200 mls of zoospore suspension (20 ml concentrated suspension diluted with 2% soil extract) containing 1×10^{6} encysted zoospores. The suspension was

poured evenly over the surface of each bare pad within the confines of the collar. On corn meal agar 90% of encysted zoospores germinated after 18h at 20°C. Each control pad was supplied with 200 ml of 2% soil extract.

Sampling

Five root pads from each of the inoculated and control plots were selected at random at each harvest date. The litter was removed and the collars driven into the ground until the top was at ground level. Six cores 3 cm deep x 2.2 cm diameter were taken from within each collar. Two cores were placed in separate plastic bags for measurements of root tips. Four cores were bulked for determination of soil moisture and levels of P. cin omi by soil plating. The rest of the sample within the llar was removed with a spade and placed in a plastic g.

Root plating

Soil, leaf litter and other organic matter was washed by hand from roots in the bulk samples. Roots were sorted into two categories :

(a) components of the mat of fine surface roots or fine root clusters (similar to Fig. 3 of Shea and Dell 1981).

(b) woody roots of ≧ 1 mm diameter with a periderm.

Roots were surface sterilized in 70% ethanol for 30 sec. and plated on PVPH agar (Tsao and Guy 1977). The pieces were cut at intervals of 5-10 mm after they had been pressed into the agar. On average 28 root clusters and 8 woody roots were plated from each collar. The plates were incubated for 48 h at 25°C.

Soil plating

Each bulked soil core sample was passed through a 2 mm sieve. Ten grams of sieved soil was mixed with 50 g of water and poured on to a plate of PVPH agar (Tsao and Guy 1977). After inoculating at 25°C for 48 hours soil was washed from the plates. The number of colonies of P. cinnamomi on each plate was scored.

Number of new root tips

Each core was placed in a beaker, flooded with water and organic matter separated. Roots were decanted several times from the sand and gravel into a fine sieve. They were then washed into a dish 25 x 18 cm divided into 20 rectangles each 5 x 4.5 cm and spread evenly. Two rectangles were chosen at random and the number of new root tips scored under the microscope.

(ii) Single root inoculation

Project Aim

To examine the susceptibility to infection and subsequent spread by Phytophthora cinnamomi in long roots and fine feeder roots of jarrah in the field. Microdroplets of motile zoospores will be applied to individual roots, following which, germination, penetration of roots and spread within roots will be monitored.

Progress Report

A preliminary field inoculation trial was undertaken in 1981. Early results indicate major differences between roots with respect to root infection. A further trial is planned for spring 1982 where the spread of the pathogen from fine into large roots will also be studied.

(iii) Movement of Phytophthora cinnamomi from fine to large roots

INTRODUCTION

It is now clear that the pathogen not only invades fine roots but can also infect and kill large jarrah roots. An early report on isolation of the pathogen from woody jarrah roots follows below.

RESEARCH NOTES

Recovery of *Phytophthora cinnamomi* from Naturally Infected Jarrah Roots

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Jarrah (*Eucalyptus marginata* Sm.), an important commercial timber tree of south-western Australia, is susceptible to a disease called jarrah dieback caused by the soil-borne pathogen *Phytophthora cinnamomi* Rands (6, 8). Symptoms vary from sudden and lethal wilt of apparently healthy trees to chronic dieback over many years prior to death (7).

Very low rates have been recorded for direct recovery of *P. cinnamomi* from roots of diseased jarrah trees (4, 5). The pathogen is generally considered to invade the fibrous surface roots (9, 10) although wound inoculation can lead to infection of larger jarrah roots (4). More recently (11) it has been hypothesized that infection of some of the perennial components of the surface jarrah root pads could cause decline and death of jarrah. This paper reports recent recoveries of *P. cinnamomi* from the main lateral roots of jarrah on upland lateritic podsolics in the northern jarrah forest near Mundaring Weir and Kalamunda.

On 10 July 1980 *P. cinnamomi* was isolated from woody roots of a recently-dead jarrah pole. Roots were washed with water, surface sterilized in 70% ethanol for 1 min., rinsed in distilled water, cut into pieces about 1 cm long and plated onto selective agar (1). Plates were incubated at 25°C for 2-3 days. Colonies of coralloid hyphae with clusters of spherical swellings were transferred to corn meal agar and 2-3 days later discs of fungi were transferred to petri dishes and flooded with non-sterile soil extract. Abundant sporangia were produced in 1-2 days. Sporangia were ellipsoid (48-60 μm x 30 μm) and non-papillate. The colonies were identified using mycelial and sporangial characters as *P. cinnamomi* (3, 12).

Excavation of roots to a depth of 30 cm revealed that a considerable proportion of the jarrah roots were infected (Fig. 1). Many of these roots were in close contact with infected roots of *Dryandra sessilis* (R.Br.) Druce. *P. cinnamomi* was also isolated from two live jarrah roots which passed between infected dead roots and were joined to adjacent healthy trees. Water runoff from a nearby road at this site could have assisted movement of propagules from host to host.

The pathogen was recorded mainly from the bark but also from the xylem of jarrah roots up to 2.5 cm/diam. Hand sections revealed the presence of coralloid hyphae in the secondary phloem and some xylem vessels. Xylem vessels of infected roots had numerous tyloses and there was considerable deposition of polyphenols in the secondary phloem.

Samples of surface fine and woody roots were taken from a further six recently dead jarrah trees with attached leaves on non water gaining sites with symptoms of dieback in the understorey. *P. cinnamomi* was recovered from the larger roots of two trees which were extensively rotted. The pathogen was also recovered from live jarrah roots close to a dead *Banksia grandis* Willd. at another site.

These results show that *P. cinnamomi* is capable of invading large jarrah roots. Since the fungus can survive in jarrah roots at least over one summer these roots may act as inoculum reservoirs for subsequent infection of roots of adjacent trees. Further work is required to establish whether total invasion of the larger roots occurs before or after tree death. It is interesting that the pathogen invaded woody jarrah tissue in which starch reserves are low. High starch reserves occur in the xylem parenchyma of large roots of *Banksia* and *Dryandra* which are systemically invaded by *P. cinnamomi* (2).

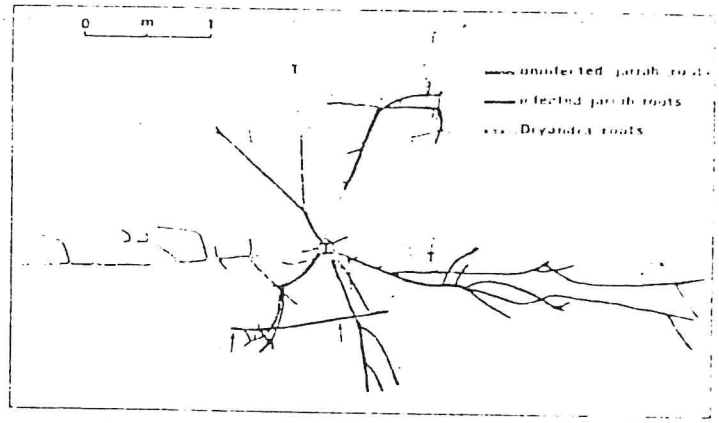

Figure 1. Roots of a small dead jarrah tree exposed to a depth of 30 cm showing distribution of infected and non infected roots. Two adjacent infected shrubs of *Dryandra sessilis* are shown. Infected jarrah roots from adjacent healthy trees are arrowed. T = trunk.

ACKNOWLEDGEMENTS

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REFERENCES

(1) Masago, H., Yoshikawa, M., Fukara, M., and Nakanishi, N. (1977) — Selective inhibition of *Pythium* spp. on a medium for direct isolation of *Phytophthora* spp. from soils and plants. *Phytopathology* **67**: 425-428.

Project Aims

(i) To determine whether the phloem of large woody roots can become infected directly through the periderm, or through root junctions.

(ii) To determine whether woody roots become infected after invasion of fine feeder roots.

PROGRESS REPORT

A 12-15 month trial was established in the field in August 1982. No results are available at this stage.

EXPERIMENT 1 - GROWTH OF JARRAH AND INFECTION BY PHYTOPHTHORA CINNAMOMI

The aims of the first experiment undertaken were :

(1) To observe differences in growth of Eucalyptus marginata (jarrah) seedlings in different jarrah forest soils.

(ii) To observe the effect on growth of inoculating jarrah with Phytophthora cinnamomi.

(iii) To observe the effect of soil type on infection of jarrah roots.

(iv) In corollary; to test the selected forest soils for natural P. cinnamomi reserves.

EXPERIMENTAL DESIGN.

Single seedlings of jarrah were grown in pots of soil. Treatments consisted of four soil types each with and without Phytophthora cinnamomi inoculation. Each treatment was replicated six times.

Soils : Four soils were collected from the jarrah forest (Table 1). Two (Kelmscott laterite and Dwellingup laterite) were collected adjacent to sites of matched soil type where jarrah dieback due to P. cinnamomi was present. The black gravel was from an old jarrah dieback "graveyard" site. These three soil types were taken to be susceptible soils. The other soil was a suppressive type (Malajczuk et al., 1977).

Inoculation : In the 'inoculated' pots two P. cinnamomi impregnated banksia plugs (20 mm long) were buried near the soil surface equidistant from the plant. In the 'uninoculated' pots, 2 sterile banksia plugs were similarly placed.

Harvest : Shoots and roots were harvested from each pot 74 days after inoculation. Roots were plated on to selective agar

TABLE 1. DESCRIPTION OF SOIL TYPES USED IN THE EXPERIMENTS

OIL TYPE	LOCATION	NATURAL VEGETATION	DESCRIPTION	NORTHCOTE* CLASSIFICATION	COMMENTS
Red Loam 'suppressive"	Off Nanga Road Park Block Murray Land district steep valley slope	Marri/blackbutt overstorey some jarrah blackboy & bracken dominant in understorey	Red earth; light sandy clay pH 5.0***	Gn 2.11	Similar to Loam soil in Malajczuk et al., 1977 Erosional Landform** S1 Murray Landform Unit
elmscott aterite 'susceptible"	Off Douglas Road Victoria Block. Cockburn Sound Land District gentle upper slope	jarrah/Casuarina fraseriana understorey: Banksia grandis, blackboy	Yellow sandy gravel pH 4.9***	KS-Uc4-21	Finer gravel than Dwellingup Laterite
wellingup aterite susceptible"	next to Murray Road Young Block Murray Land district on slope of a minor valley	jarrah/marri overstorey understorey: Banksia grandis, blackboy	Yellow sandy gravel pH 5.0***	KS-Uc4-21	Laterite Landform** L1. Yarragil Landform Unit
lack Gravel susceptible"	Off Stawell Road, Park Block, Murray Land district gentle upper slope	Sparse jarrah/marri/ Casuarina fraseriana extensive Dryandra sessilis some blackboy	sandy gravel (black) overlying duricrust at depth of 30 cm pH 4.3***	KS-Uc4-21	Laterite Landform** L1(b) Dwellingup Landform Unit.

* Northcote (1979)

** From McArthur et al. (1977)

*** soil pH determined in 1/5 W/V 0.01M Calcium chloride, shaken for 30 mins.

(Tsao and Guy, 1977) and P. cinnamomi infection was scored.

Results

Growth Response in the Absence of Phytophthora cinnamomi

Table 2a and Fig. 1 show the effect of soil type on root and shoot growth of jarrah seedlings after 169 days. Plants grown in black gravel had the largest roots and shoots. However after 379 days root and shoot dry weights were not significantly different from plants grown in red loam (Table 2b). From this growth response it was assumed that black gravel and red loam had the higher soil fertilities. In contrast growth of seedlings on Kelmscott laterite was very slow and plants at both harvests had small shoot and root systems. Dwellingup laterite was intermediate in fertility, with plants producing yields between those in Kelmscott laterite and the two fertile soils.

Phytophthora cinnamomi was not recovered from the roots of these plants nor from the soils using several techniques of soil plating and baiting.

The classification of jarrah forest soils as suppressive or conducive is thus not directly related to overall soil fertility. However soil fertility cannot be simply dismissed as a factor in jarrah dieback as different elements may be limiting at different sites.

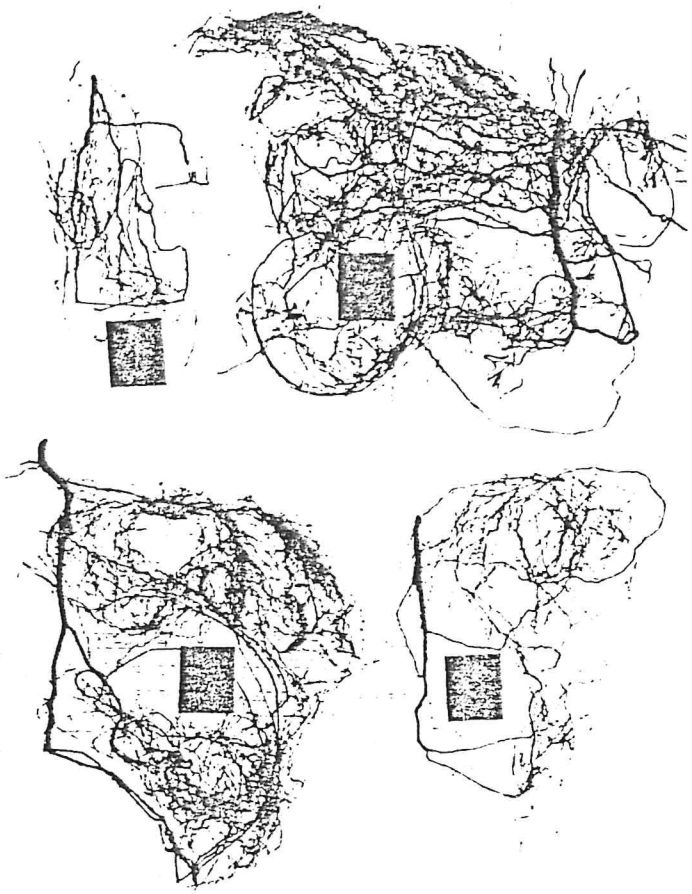
Growth Response in the Presence of Phytophthora cinnamomi.

Inoculating with P. cinnamomi did not significantly reduce the growth response of jarrah seedlings grown in the four soils (Table 2b).

Root Infection

The amount of root infection was very low for plants grown in Kelmscott laterite (Table 3). This may have been due to the small size of the root system. Root infection values for the other three soils were higher but not significantly different from each other.

Fig. 1. Fresh roots of 169 day old jarrah seedlings grown in pots containing 2.5 kg of four forest soils.



1 = Kelmscott Laterite

2 = Black Gravel

3 = Dwellingup Laterite

4 = Red Loam

TABLE 2a

Effect of soil type on root and shoot dry weights of jarrah seedlings grown for 169 days in unfertilized jarrah forest soils.

Soil Type*	Shoot Dry Weight** (mg/plant)	Root Dry Weight** (mg/plant)
RL	3730 (370)	1460 (169)
KL	570 (20)	327 (94)
DWL	1430 (130)	499 (62)
BG	5280 (530)	1494 (125)

* RL = Red Loam KL = Kelmscott Laterite

DWL = Dwellingup Laterite BG = Black Gravel

** Values are means of 4 reps. Standard errors in parenthesis.

TABLE 2b

Effect of soil type and infection by *Phytophthora cinnamomi* on shoot dry weights of jarrah seedlings grown for 379 days in unfertilized jarrah forest soils. Plants were harvested 72 days after inoculation.

Soil Type*	Shoot Dry Weight** (mg/plant)	
	- P.cinnamomi	+ P. cinnamomi
RL	9746 (464)	9735 (790)
KL	1630 (80)	1698 (105)
DWL	6756 (515)	6392 (592)
BG	8977 (925)	9682 (568)

* RL = Red Loam KL = Kelmscott Laterite
DWL = Dwellingup Laterite BG = Black Gravel.

** Values are means of 6 reps. Standard errors in parenthesis.

TABLE 3

Phytophthora cinnamomi infection of inoculated and uninoculated jarrah seedlings grown in 4 soil types. Plants were harvested 72 days after inoculation

Soil Type	Root Infection* (%)	
	Inoculated	Uninoculated
RL	10.7 (7)	0
KL	0.8 (1)	0
DWL	22.5 (12)	0
BG	15.0 (9)	0

* Values are means of 6 reps. Standard errors in parenthesis

EXPERIMENT 2 - FERTILIZATION AND THE INFECTION OF JARRAH BY PHYTOPHTHORA CINNAMOMI

Clearly, major differences in soil fertility occur in the jarrah forest (Experiment 1). Fertilization is also a management tool that can be used by foresters to change the forest environment. It is generally recognised that nitrogen and phosphorus are the major limiting nutrients in natural forests. Thus this experiment was designed to test the effect of the addition of macronutrients on the infection of jarrah roots by P. cinnamomi. Lime was added as a third variable because of reports in the literature that application of calcium carbonate can suppress P. cinnamomi root rot of jarrah (Boughton et al., 1978).

EXPERIMENTAL DESIGN

Jarrah seedlings were grown in a 2 x 2 factorial combination of with and without lime ($CaCO_3$) and with and without macronutrients. Each treatment was replicated four times.

Soils : The same four soil types described in Table 1 were used.

Inoculation : The soils were inoculated by the P. cinnamomi impregnated banksia plug method used in Experiment 1.

Harvest : Plants were harvested 87 days after inoculation. Roots were plated on to selective agar. Shoots were divided into new growth, mature leaves and stems. These were analysed for six essential elements to establish whether leaf symptoms that developed could be related to nutrient status of the plants. The results for nutrient analysis will not be presented here as they are still being processed.

RESULTS

The addition of macronutrients increased the number of plant deaths, the number of infected plants and the level of infection in infected plants (Table 4, 5 and 6 respectively). The addition of lime

TABLE 4

Deaths of jarrah seedlings growing in 4 soil types with fertilizer applications after inoculation with P. cinnamomi. Plants harvested 87 days after inoculation.

Soil Type	Percentage Plant Deaths*			
	Fertilizer Treatment			
	- Lime		+ Lime	
	- Macronutrients	+Macronutrients	-Macronutrients	+Macronut
RL	0	6.2	6.2	6.2
KL	0	50	0	18.8
DWL	0	29	0	0
BG	12.5	29	9.1	0

* Values are means of 8 reps.

TABLE 5

P. cinnamomi infection of jarrah seedlings growing in 4 soil types with fertilizer applications. Plants were harvested 87 days after inoculation.

Soil Type	Percentage Plant Infection* Fertilizer Treatment			
	- Lime		+ Lime	
	- Macronutrients	+ Macronutrients	- Macronutrients	+ Macronutri
RL	6.2	28.5	26.5	14.0
KL	6.2	100	0	56.5
DWL	32	71	44	33
BG	50	67	45	0

* Values are means of 8 reps.

TABLE 6

Level of P. cinnamomi infection of infected jarrah seedlings growing in 4 soil types with fertilizer applications. Plants were harvested 87 days after inoculation

Soil Type	Percentage infection of roots with P. cinnamomi present			
	Fertilizer treatment*			
	- Lime		+ Lime	
	-Macronutrients	+Macronutrients	-Macronutrients	+Macronutr
RL	10	54	30	80
KL	5	90	0	68
DWL	24	50	18	42
BG	59	63	54	0

* Values are means of 8 reps.

partially reversed this trend.

Of the unfertilized and "plus lime" treatments the Kelmscott laterite showed the lowest infection (Table 4 and 5); the same trend as in Experiment 1. However it showed the highest infection when macronutrients were added in the presence or absence of lime.

The 'suppressive' Red Loam soil showed least number of infected plants when macronutrients with and without lime were added (Table 5). However it was the only soil to show a large increase in infection when lime was applied in the absence of macronutrients. This was reflected in the level of infection (Table 6).

The differences in percentage root infection achieved in Experiments 1 and 2 may have been related to the time of year when they were undertaken. The results parallel those recorded by Marks et al. (1973) for field grown plants in the Eastern States. The level of lime used in this experiment to achieve some reduction in root infection was similar to that used by other workers (Boughton et al., 1978). However these levels are unrealistic because on some soils severe chlorosis occurred in our experiment.

EXPERIMENT 3 - SOIL TRACE ELEMENT DEFICIENCIES

Many Western Australian agricultural soils are well known to be deficient in one or more trace elements. Also, it has been reported several times in the literature that trace-element deficiencies may result in increased root rot by susceptible plants.

Experiment 2 indicated that improving soil fertility with the addition of macronutrients could lead to greater root infection. In many of these plants (those with micronutrients absent) nutrient disorders were observed.

Consequently a trace element omission trial was set up to determine which trace elements could be deficient in soils of the jarrah forest.

EXPERIMENTAL DESIGN

Jarrah seedlings were grown in pots of Dwellingup laterite jarrah forest soil previously described (Table 1) and on a trace element deficient sand from Lancelin. Dwellingup laterite was chosen as representative of a typical upland soil. The Lancelin sand was chosen because it has been extensively used in studies on plant nutrition and hence would make a useful comparison with forest soils.

Treatments consisted of adding adequate macronutrients and micronutrients with the elimination of a single micronutrient from each. Each treatment was replicated four times.

RESULTS

In both soils only the treatments with zinc absent or all trace elements absent showed variation from the treatment with all macro- and micronutrients (Table 7). After just five weeks' growth zinc deficiency symptoms were observed.

After finding that zinc was limiting to growth of jarrah seedlings in the two soils a zinc response experiment was undertaken (Experiment 4).

EXPERIMENT 4. ZINC RESPONSE OF JARRAH

This experiment was designed to provide information on the response of jarrah seedlings to zinc. This information is needed before the effect of the zinc status of the soil on the infection of jarrah roots can be examined (Experiment 5).

TABLE 7

Effect on shoot dry weight of jarrah seedlings by omitting a trace element from a full fertilizer treatment. Plants grown in Dwellingup laterite for 84 days.

Fertilizer Treatment*	Shoot Dry Weight** (mg/plant)	
All	2122	(112)
All + Fe	1803	(410)
All -B, Mo, Co	1917	(277)
All - Cu	2030	(167)
All - Zn	1217	(157)
All - all trace elements	810	(42)
Nil	142	(5)

* All = N, P, S, Ca, K, Mg, B, Co, Cu, Mn, Mo, Zn

** Values are means of 4 reps. Standard Errors in parenthesis.

EXPERIMENTAL DESIGN

Jarrah seedlings were grown in pots of Lancelin Sand with adequate macro and micronutrients. Treatments consisted of seven levels of added zinc ranging from 0 to very high levels; 4000 µg/kg soil.

RESULTS

There was a good response to applied zinc (Fig. 2). Maximum dry matter was produced at a zinc level of 133 µg Zn/kg soil. This response is equivalent to that for wheat plants (Loneragan, pers. comm.). The response is so strong that zinc deficiency probably occurs in our forests. This is likely to occur when macronutrients become non-limiting such as after fire, fertilization with nitrogen and phosphorus, etcetera. It may also occur more readily in older trees.

EXPERIMENT 5 INCREASED INFECTION OF JARRAH BY PHYTOPHTHORA CINNAMOMI DUE TO ZINC DEFICIENCY

Recent experiments at Murdoch University (J.F. Loneragan and colleagues) have shown that zinc is needed to maintain the membrane integrity of roots. Also there is some evidence to suggest that fairly high levels of zinc may be required in the soil to promote normal root growth.

Experiment 5 was undertaken to test the hypothesis that zinc deficiency may cause increased susceptibility of roots to P. cinnamomi. This experiment is due for completion by November 1982.

A second hypothesis, that zinc deficiency reduces the ability of jarrah roots to regenerate and thus may be the whole or partial cause of increased susceptibility of zinc deficient jarrah to P. cinnamomi is suggested for future work.

Fig. 2. Effect of zinc application on root and shoot dry matter production of 84 day old jarrah seedlings. Values are means of 4 reps. Bars represent standard errors.