Report to the

JARRAH DIEBACK RESEARCH

FOUNDATION

For period

1981-82

by

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Note: The material contained in this report is confidential and should not be cited.

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SUMMARY.

1. In the period from 1945 to 1965 extensive areas of jarrah forest were killed by Phytophthora cinnamomi. From 1965 to 1982 P.cinnamomi has infected thousands of hectares of forest but jarrah mortality rates have been very low. A principal concern has been that a change in climatic conditions could cause the resumption of mass jarrah decline. In 1981/82 several areas of forest were located where massive decline and death of jarrah and associated species was observed.

2. Previously two broad site susceptibility categories were recognized:-

a) Lowland moisture gaining sites where the soil environment permits prolonged sporangial production of P.cinnamomi and survival throughout the year.
b) Upland sites where asexual reproduction (sporangial formation) is restricted to periods when the soil was warm and wet and fungal survival in soil over summer is markedly reduced.

3. A third site category has now been identified. Upland sites with a layer of concreted sheet laterite in the surface soil horizons have been shown to be highly conducive to P.cinnamomi spread and intensification. Preliminary surveys indicate that the extensive areas of forest which were subjected to mass decline in the period 1945 - 65 had this site characteristic.

4. Soil population levels in the surface horizons on upland sites vary according to rainfall distribution. However, within even severely diseased areas the recovery rate from random samples of the surface soil is low. This is not due to low rainfall. Even in heavily summer irrigated plots spread and intensification of the fungus was restricted. We conclude that lateral spread of the fungus in the surface of horizons of undisturbed upland sites is almost totally dependent on lateral movement by mycelial growth through the horizontal root system of Banksia grandis or by movement in soil carried by vectors.

5. The literature on Phytophthora cinnamomi and our own studies have suggested that the fungal distribution and the factors favouring reproduction would decrease with depth. However, we have found that on sites where a concreted lateritic layer in the surface horizons is present:-

a) P.cinnamomi can occur at a very high density above the layer of the sheet laterite at depths up to 75cm.
b) The fungus can reproduce at the surface of this layer and in root channels within the layer.
c) Zoospores are laterally transmitted in water running over the surface of the layer.

It has previously not been possible to explain the massive and rapid decline of jarrah forest on upland sites based on the known distribution and reproduction characteristics of P.cinnamomi on upland sites.

We conclude that this specific site characteristic is an essential pre-requisite to rapid spread and intensification of the fungus on undisturbed upland sites.

6. Sporangial formation and zoospore release in the surface of soil horizons is restricted to relatively brief periods of the year. Stimulants need to be present in the soil and a particular sequence of rainfall events during periods of relatively high soil temperature are required to complete the sporangial production cycle. Our preliminary research indicates that the presence of a sheet laterite layer near the surface markedly reduces these constraints because it impedes vertical water transmission. Further research is required to define the effect of abnormally high unseasonal rainfall on the spread and reproduction of the fungus at depth in the soil profile.

7. Preliminary studies indicate that sheet laterite is present in many forest sites. However, on a proportion of the upland sites it is broken or at a considerable depth below the soil surface where it does not permit significant water impedence and lateral transmission of water. Identification of the areas of forest which have this horizon characteristic present and the interaction between rainfall intensity

and distribution and site type is a major research priority.

8. Studies of the stimulatory capacity, zoospore transmission and fungal survival in a range of soil types representing the spectrum of field resistance have shown some significant differences. However, the effect of soil type appears to be relatively minor compared to soil horizon structure.

9. Excavations of trees growing on sites which do not have a concreted lateritic layer present in the surface soil horizons and which have been exposed to Phytophthora cinnamomi for several years indicate that jarrah growing on these sites is relatively resistant to P.cinnamomi. The fungus has been detected in the horizontal roots but its occurrence is low and infections have often been found to be 'compartmentalized'.

10. 41 trees growing on sites which were subject to massive rapid decline and death were excavated. On all sites where mass decline was observed a concreted layer of sheet laterite was present in the surface horizons. P.cinnamomi was consistently recovered from lesioned tissues in 39 of the 41 trees. The fungus was frequently present in the stump and major horizontal roots. Other potentially pathogenic fungi were detected in some trees but the recovery rate was low. We suggest that these organisms are secondary invaders.

11. Detailed excavations of 12 affected trees down to the caprock layer revealed extensive infection of the vertical root systems. The roots were infected in the surface of the layer and within the root channels. We tentatively conclude that the initial attack by Phytophthora cinnamomi on jarrah trees on these sites is on the vertical root system.

12. It had not previously been possible to explain how P.cinnamomi kills jarrah trees. Xylem potential measurements of trees in affected areas indicated that they were under severe stress. We conclude that the death of jarrah results primarily from destruction of the vertical root system and/or the extensive invasion of horizontal root systems and stump by P.cinnamomi which prevents uptake of water from depth in the soil profile. Death results from dehydration.

13. Preliminary results from extensive studies using wound inoculation techniques indicate that lesion extension in jarrah secondary tissue is markedly seasonal. Maximum rates of extension occur in summer.

Temperature has a marked effect on lesion extension rates.

14. Site and vigour do not appear to affect lesion extension rates in any one year. However, it is possible that the ability of the tree to contain the fungus in subsequent years may be affected by vigour.

15. The rates of lesion extension in wound inoculated trees are too low to account for the observed invasion of jarrah trees in sites experiencing mass decline. We are currently testing the hypothesis that jarrah is pre-disposed to invasion of secondary tissue by stress induced by destruction of the vertical root system.

16. We tentatively conclude that the presence of a concreted layer of sheet laterite in the surface horizons is a pre-requisite to the development of massive and rapid decline of jarrah trees.

17. Jarrah is normally very resistant to invasion by P.cinnamomi. The tree has a range of defence mechanisms including the formation of a periderm layer which appears to often terminate lesion extension. We have found situations where the initial lesion, which was terminated by the production of the barrier, was re-activated. We are currently investigating this phenomenon.

18. Research has continued on the potential role of understorey manipulation in reducing disease.

a) Our results continue to indicate that some legume species have a suppressive effect on the fungus. It has been shown that legume species could make a significant contribution of nitrogen in the forest and that soil amended with legume roots has a relatively high concentration of ammonium nitrogen and this is correlated with lower levels of P.cinnamomi.

b) A comprehensive study of the ecology of Banksia grandis has been completed and a computer model which accurately stimulates Banksia grandis growth and development has been developed. Preliminary stimulation studies indicate that the Banksia grandis understorey can be controlled by silvicultural methods.

In our previous report and in published papers we have suggested that understorey manipulation could significantly reduce disease spread and intensification. The results of our current research gives us more

confidence that this approach to control together with intensive hygiene will result in effective control of P.cinnamomi on sites where vertical water transmission is not impeded.

19. Prognosis.

In our previous report we concluded that "our previous assessments of the potential for control of Phytophthora cinnamomi in the jarrah forest has probably been over pessimistic". We are reluctant to be optimistic but the results of our subsequent research lead us now to conclude that it is highly probable that it will be possible to achieve effective control of Phytophthora cinnamomi over a large proportion of the jarrah forest which is currently uninfected.

This assessment is based on our belief that:-

a) Spread and intensification of Phytophthora cinnamomi on upland sites is very limited in the absence of significant disturbance, except on sites with particular horizon characteristics which permit water impedence and lateral transmission.

b) There is circumstantial evidence that the majority of the forest areas with this specific site characteristic have already been infected by P.cinnamomi.

c) Jarrah is normally highly resistant to P.cinnamomi.

d) On sites which are freely drained the impact of P.cinnamomi can be reduced to insignificant levels by minimizing disturbance, maximizing hygiene,reducing the Banksia grandis understorey and promoting a canopy of resistant leguminous species.

20. Future Research.

The research findings contained in this report potentially provide the basis for a large number of research projects. However, we believe that there are two principal lines of research which are required to confirm or refute our prognosis.

a) The precise processes which permit reproduction and lateral transmission of P.cinnamomi at depths in the soil profile need to be defined. This will then permit the delineation in time and space of the relative susceptibility of different jarrah forest sites.

b) The resistance mechanisms in jarrah require further study. We need to confirm or refute our hypothesis that, under normal site conditions, jarrah is resistant to P.cinnamomi invasion. The factors that cause jarrah trees to be predisposed to P.cinnamomi need to be identified to permit extrapolation of our results in time.

We believe that, given adequate resources, we will be able to conclude this research and refute or confirm our prognosis within two years.

Future Funding of Research.

Current Status.

Two research assistants based at Dwellingup are currently being funded by the Foundation. Funding of these assistants will be terminated in August 1982. (Funding for Dr.Tippett and one research assistant will terminate in January and March 1984).

The research carried out which is described in this report has three major components:-

a) Supervision. This is currently being carried out by Dr.Shea, Dr. Shearer and Dr.Tippett.

b) Field work. This is primarily being undertaken by technical support staff and divisional workmen funded by the Forests Department. (Up to 10 field staff have been employed during the past year).

c) Backup laboratory analysis. This is primarily undertaken by assistants funded by the Jarrah Dieback Foundation and constitutes our major bottleneck. We have adequate staff to supervise and undertake field studies. Our major staff deficiency is in the laboratory analysis area. Each field project generates large amounts of work in the laboratory. For example, we estimate that we have analysed in excess of 6,000 soil samples for P.cinnamomi; over 1,000 sporangial mats have been analysed and we have plated more than 5,000 pieces of plant tissue in the previous 12 months. While we are able to train Forests Department staff to undertake field studies the laboratory work requires personnel with special biological research skills. We have been fortunate that we have been able to employ young recent graduates in biological sciences to undertake this work.

To achieve our objective of resolving the major areas of research outstanding within 2 years we believe that it is essential to increase our laboratory assistants to three for a 2 year period (1982-1984). A major component of our field research involves excavation. We have been fortunate to obtain the service of a skilled backhoe operator to assist us with this work over a period of 3 years. Accordingly provision has been made in the budget to contract this operator over the two year period.

The budget estimates are outlined below:-

BUDGET ESTIMATES.

	1982/83	1983/84	TOTAL
Personnel			
3 research assistants	45,000	48,000	93,000
Equipment	1,000	1,000	2,000
Backhoe hire	5,000	5,000	10,000
TOTALS	$51,000	$54,000	$105,000

INTRODUCTION.

This report summarizes the results of investigations into Jarrah Dieback carried out under the supervision of Dr. S.R. Shea, Dr. B. Shearer and Dr. J. Tippett for the period May 1981 - May 1982. Major progress has been made in elucidating the major factors of the environment affecting P.cinnamomi and the interaction between the principal host, jarrah, and the pathogen.

1. Studies of the Pathogen/Environment Interaction.

1. (a) Site - In a previous report we have defined two broad site susceptibility types. Moisture gaining sites on the valley floors maintain relatively high soil moisture levels throughout the year. P.cinnamomi can be consistently recovered from the surface soil horizons in these sites throughout the year. On upland sites the fungus cannot survive the (normally) prolonged period over summer when the soils remain at and below 15 Atmospheres. The fungus survives on these sites in the roots of highly susceptible species. Fungal propagules are generated in the soil by formation of sporangia and the release of zoospores. Sporangial formation can only take place when the soil is warm and wet (spring and autumn) and when stimulatory factors are present.

Results of our recent research indicate that there is a third site category which is highly conducive to the spread and intensification of P.cinnamomi.

This site type is characterized by the presence of a sheet layer of concreted laterite at between 5 - 75cm below the soil surface. Our preliminary studies indicate that, although the surface horizons of these sites are freely drained, water is impeded and laterally transmitted at the surface of the sheet laterite.

1. (b) Factors affecting survival, transmission and reproduction of P.cinnamomi in the surface horizons of upland sites.

1. (b) 1. The effect of seasonal and year to year variation on inoculum levels in the soil.

Soil inoculum levels have been monitored on a range of upland sites over a period of four years. The importance of the timing and sequence of rain for the build-up of inoculum of P.cinnamomi

in the surface soil of free draining sites is illustrated in Fig. 1. In 1979 the opening autumn rains were late and cold and spring rains were intermittent, finishing by mid-December. In 1980 and 1981 autumn rains were much earlier than in 1979. In 1980 spring rains were intermittent, as in 1979, but in 1981 rains fell frequently throughout spring and into the summer of 1982.

Analysis of the results from the four years of data shows:-

- Early autumn rains allow rapid build-up of soil inoculum. This is because a number of factors favouring sporangium production act together (i.e. rainfall falling on warm, highly stimulatory soils.)

- Inoculum produced in the soil in autumn will survive through the winter thereby increasing the chance of spread of the fungus during the winter months and also providing foci for sporangium formation in spring.

- In spring soil inoculum increases are linked to sporangium formation, which is the result of a strong interaction between soil temperatures, moisture and stimulation. Following winter, soil temperature and stimulation must increase to levels optimal for sporangium production. In addition, the sequence of rainfall must allow the soil to remain moist for sufficient periods of time to allow formation and maturation of sporangia and release of zoospores.

The timing and frequency of spring rainfall in 1979 and 1980 was not as favourable as 1981-1982 for the formation of sporangia. Heavy rainfall in January 1982 was sufficient to cause sporangial formation and release and zoospores were generated into the soil. The rainfall pattern in 1981-1982 was such that P.cinnamomi was present in the soil on upland infected sites for a twelve month period Fig. 2.

The impact of rainfall distribution on inoculum presence and survival in the soil has obvious implication to hygiene management strategies.

1. (b) 2. B.grandis as a source of inoculum.

Although there are extensive areas of the forest infected with P.cinnamomi, we have found that it is difficult to recover the fungus from randomly located soil samples. Following the discovery that B.grandis could be invaded by P.cinnamomi and that

Fig. 1. Seasonal and year to year variation in inoculum levels in the surface soils on upland sites.

FIG. 1

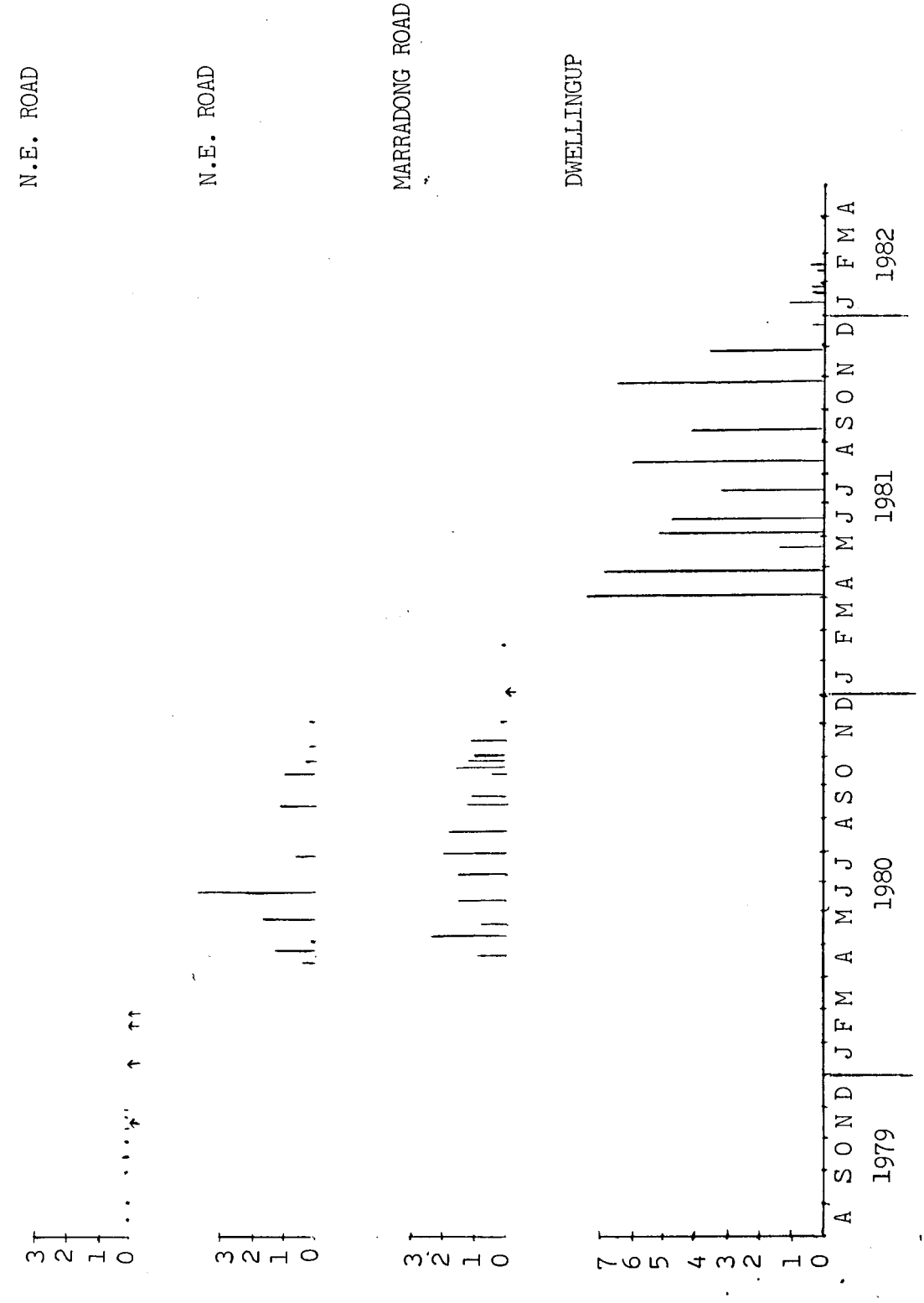
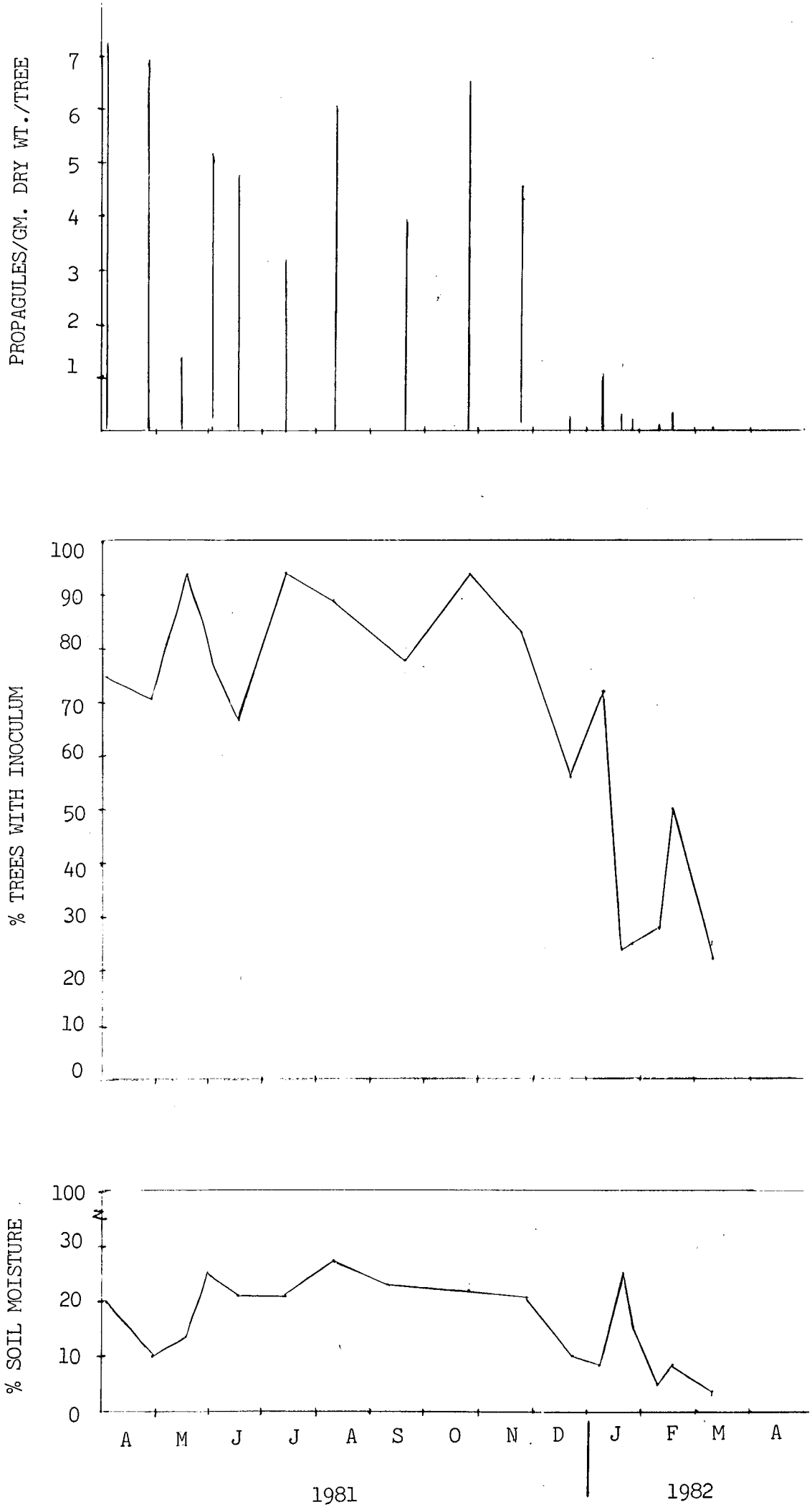

N.E. ROAD
N.E. ROAD
MARRADONG ROAD
DWELLINGUP
PROPAGULES/
GM DRY WT./
TREE
A S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F M A
1979
1980
1981
1982

Fig. 2. Inoculum levels in surface soils during 1981-82. Note presence of P.cinnamomi in soil during the summer of 1981-82.

FIG. 2 DWELLINGUP 1981 - 82.



it was usually present in the lower stem and stump of this species, a system of sampling around the base of recently killed B.grandis trees was introduced. This technique has been employed over a period of three years and has proved to be a very sensitive method of detecting the fungus.

As part of continuing studies on infected B.grandis as sources of inoculum of P.cinnamomi, inoculum density in the soil at the base of infected B.grandis was monitored in 1981. Figs. 2 and 3.

- Early rain in mid-March and early April falling on warm, highly stimulatory soils resulted in the production of sporangia and build up of high soil inoculum densities in early April.

- Inoculum present in the soil in autumn survived through the winter, increasing the chance for spread of the fungus.

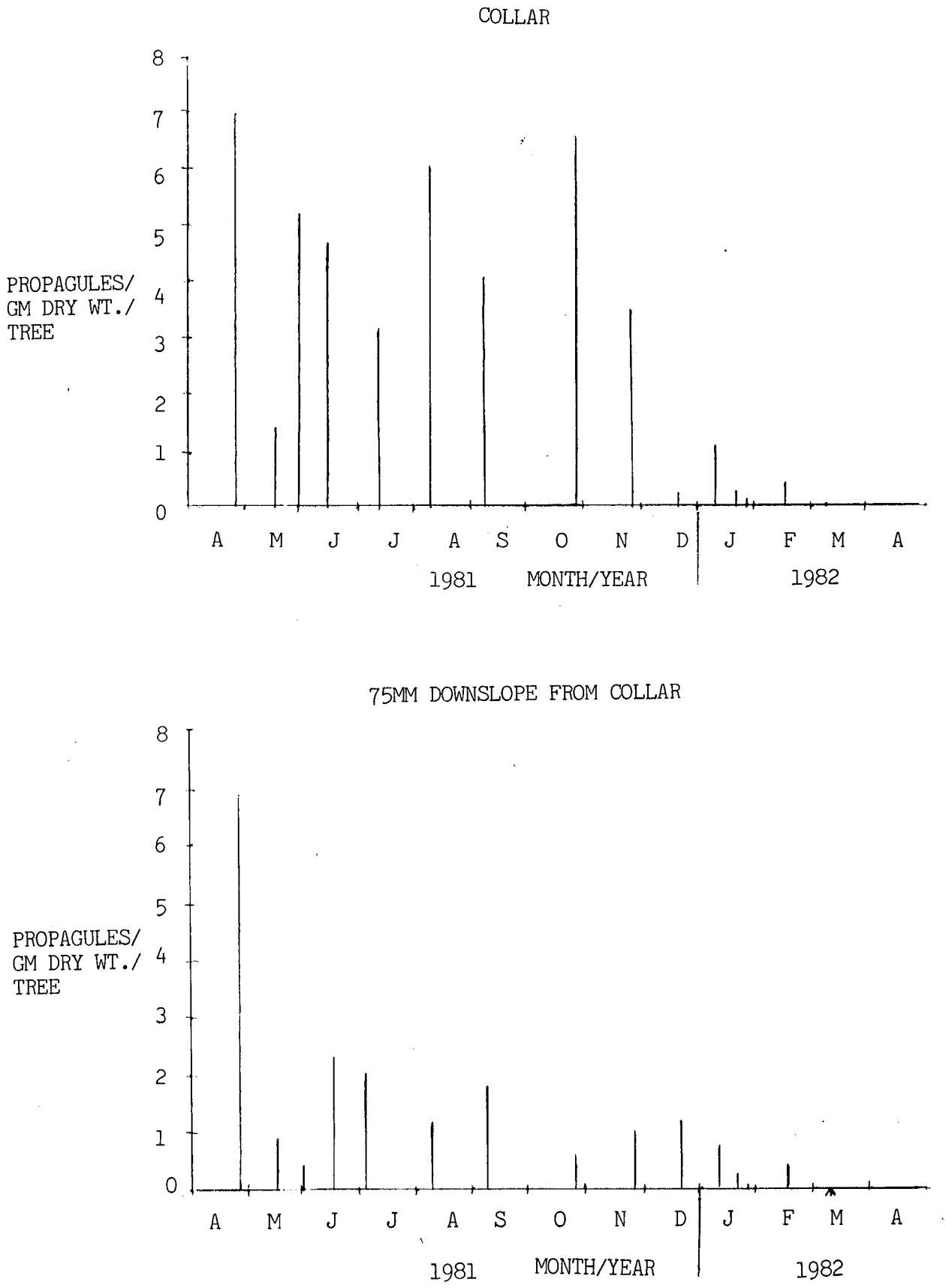
- The frequency and intensity of rain in spring resulted in sporangium formation and build up of soil inoculum.

- Rains in mid and late December and mid-January allowed inoculum to survive into summer and was sufficient to induce sporangial formation and release. This was the first time in the previous four years of monitoring soil inoculum levels, that viable P.cinnamomi inoculum has been consistently found in surface free draining soils in summer. In conjunction with the collar samples the surface soil horizons were also sampled at a distance of 75cm downslope of the tree, in an attempt to measure lateral spread of the pathogen.

The pattern of recovery of soil inoculum 75mm downslope of the Banksia collar was similar to that for the collar samples (Fig. 3) but the levels were about half that for the collar. On pooling samples from all trees there was no correlation between soil inoculum density at the collar and 75mm downslope. The pattern of recovery of soil inoculum suggests that lateral spread of P.cinnamomi inoculum through the surface soil away from the infected collar is limited.

Fig. 3. P.cinnamomi distribution in surface soils in relation to B.grandis.

FIG. 3


COLLAR
PROPAGULES/ GM DRY WT./ TREE
0
1
2
3
4
5
6
7
8
A M J J A S O N D J F M A
1981 MONTH/YEAR 1982
75MM DOWNSLOPE FROM COLLAR
PROPAGULES/ GM DRY WT./ TREE
0
1
2
3
4
5
6
7
8
A M J J A S O N D J F M A
1981 MONTH/YEAR 1982

1. (b) 3. The effect of irrigation on spread and inoculum density in the surface horizons of freely drained sites.

It is possible to explain lateral movement of P.cinnamomi through soils by mycelial extension through the horizontal root system of Banksia trees. However, the generally low recoveries of P.cinnamomi from surface soils was not compatible with the hypothesis that the fungus caused decline and death of jarrah by feeder root attrition. This could only occur if a substantial proportion of the fine root system of jarrah was exposed to P.cinnamomi. It was hypothesized that abnormally high rainfall during warm periods could generate high populations of P.cinnamomi in the soil by mass overland flow of water containing zoospores.

The objective of this investigation was to determine if below average rainfall was responsible for the low recovery rates of P.cinnamomi from infected sites and if inoculum is dispersed in overland flow. A naturally infected area with dead B.grandis trees with leaves still retained was irrigated approximately twice a week during the period from 6 October, 1980 to 6 February 1981. Surface soil was sampled about twice a month on a uniform grid, in order to map the spread and intensification of inoculum. At the end of each irrigation, surface water was sampled for detection of zoospores.

No zoospores were detected in the majority of water samples following irrigation. In the few cases where zoospores were recovered only one or two spores were detected in the sample . Initially spread of inoculum was slow with most inoculum remaining localized within a few foci. No general spread of inoculum occurred until the equivalent of 700 mm of rain had been applied to the plot over about three months in summer. The change in the distribution of inoculum is illustrated in Figs. 4, 5.

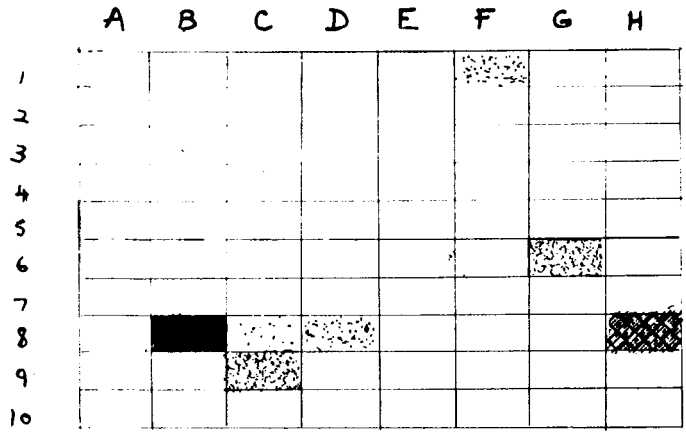
Changes in the ability of the soil to stimulate sporangia may have been a factor in the marked increased in the number of foci of inoculum in February. Stimulation in surface soils declines if the soil is kept continually moist but increases when the soil is alternately wet and dry.

This irrigation experiment, in conjunction with observations of build up and spread of inoculum from infected Banksia, suggests that lateral spread of inoculum in the surface soil of free draining upland sites is

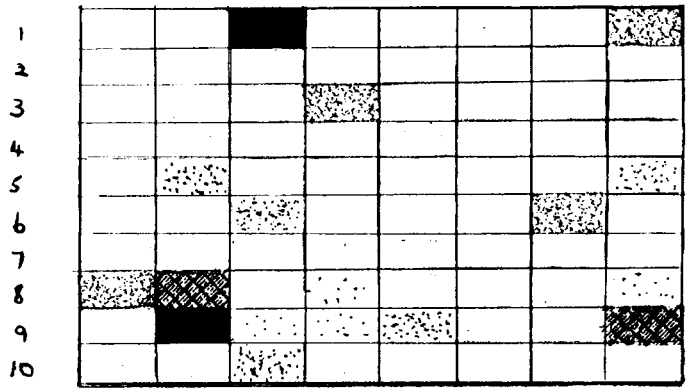
Fig. 4. Spacial distribution of P.cinnamomi inoculum following heavy irrigation.

FIG. 4

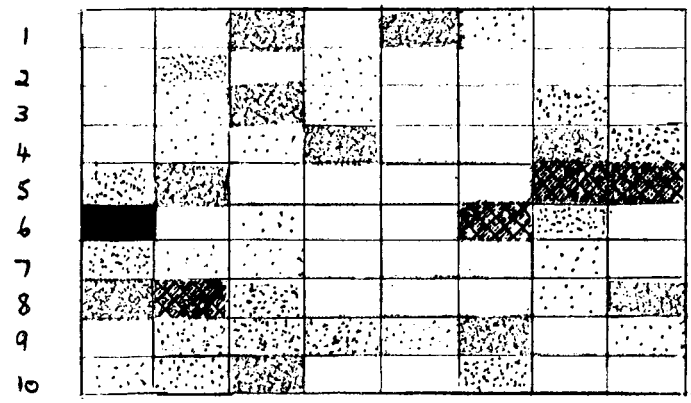
22 SEPTEMBER 1980



26 NOVEMBER 1980



6 FEBRUARY 1981



PROPAGULES/GM. DRY WT.:-

.01 to 1.00

1.01 to 4.00

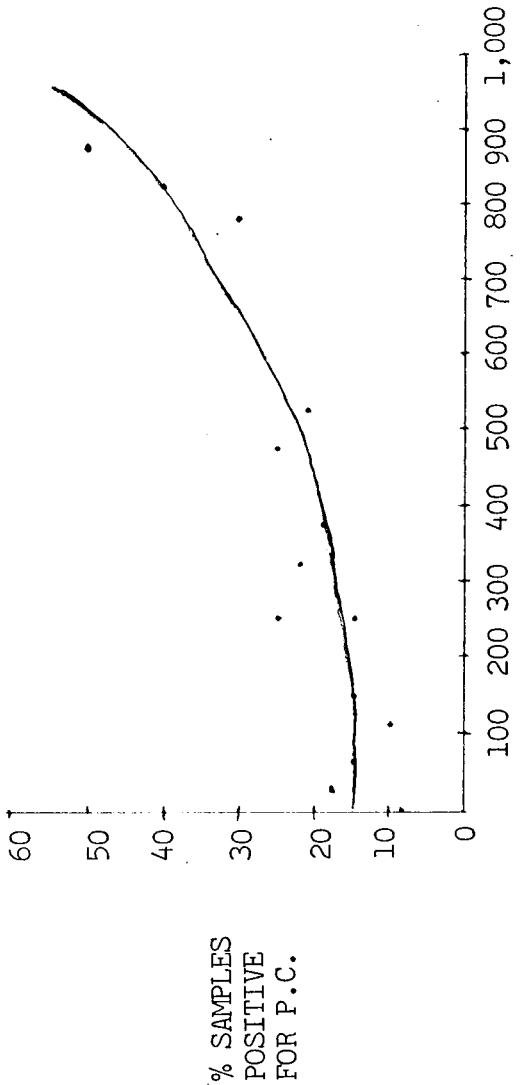
4.01 to 9.00

9.01 to 16.00

>16.00

Fig. 5. Percentage recovery rate in relation to quantity of water applied.

FIG. 5


% SAMPLES POSITIVE FOR P.C.
60
50
40
30
20
10
0
100 200 300 400 500 600 700 800 900 1,000
MM. OF WATER APPLIED
6/10/80 to 6/2/81

limited. In these sites significant movement of inoculum in surface soil can only take place when there is excessive disturbance resulting in the disruption of drainage sufficient to generate overland flow.

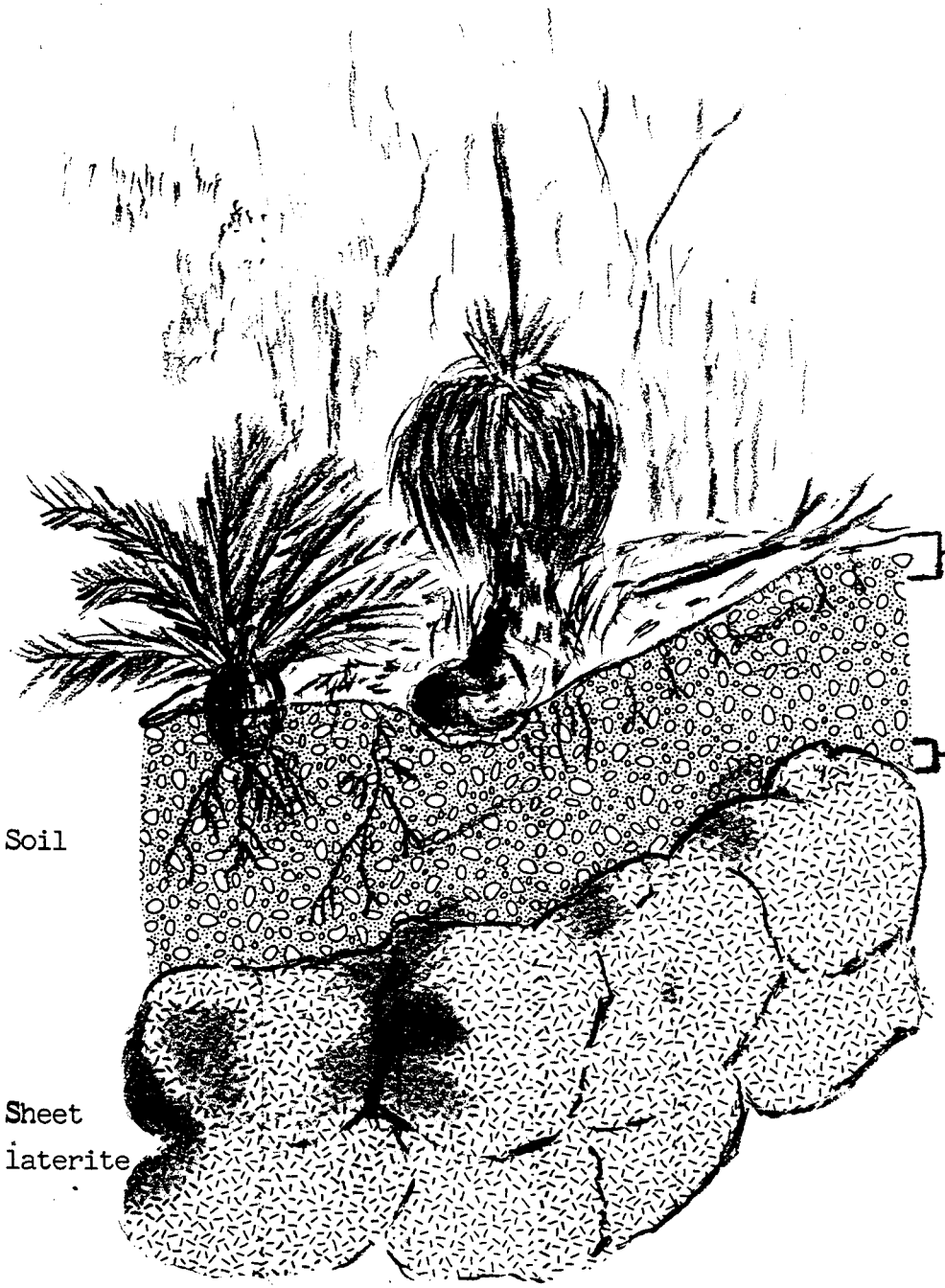
1 (c) Distribution, Reproduction and Transmission of P.cinnamomi on upland sites where a concreted sheet laterite is present in the surface horizons.

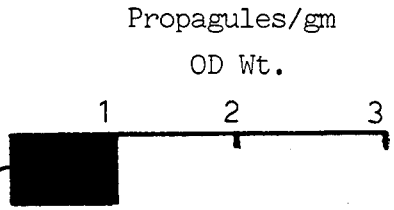
As noted above, we have been unable to explain the massive disease extension and intensification on upland jarrah forest sites which occurred in the period from 1945 - 1965. Our observations, over a number of years, of fungal reproduction and distribution in the surface soils of free drained sites were that reproduction and distribution in soils was extremely limited in time and space. In 1982 we observed several areas where disease was extending rapidly and causing mass rapid death of jarrah (see below). During extensive excavations on these sites, which involved hydraulic excavations of root systems, we observed ponding and lateral transmission of water at the surface of a concreted lateritic layer which was present at all of the sites. Previous studies had shown that P.cinnamomi occurrence and environmental factors favouring spore formation on upland sites decreased with depth. However, we hypothesized that on sites with a concreted layer of caprock near the surface that the fungus could reproduce at depth. To test this hypothesis surface soils and the soil at the interface of the sheet laterite layer were sampled. The results confirm our hypothesis, Fig. 6 . Contrary to all published literature on the fungus, we have found it occurs at a very high density at depths of up to 75cm at the surface of the lateritic layer.

Preliminary studies have shown that the zoospores can be laterally transmitted in running water at the surface of the lateritic layer. Our current research indicates that sporangia form in potholes of saturated soil in the lateritic layer.

Preliminary research indicates that, although caprock is present in many jarrah forest sites, it is often broken and does not cause impedence of water. We hypothesize that, in the absence of severe disturbance to drainage, on upland sites where a concreted lateritic layer is not present the reproduction and lateral transmission of P.cinnamomi in soil at the surface or at depths will be very limited .

Fig. 6. Distribution of P.cinnamomi on sites where a concreted layer of sheet laterite is present.


Soil
Sheet
laterite


Propagules/gm
OD Wt.
1
2
3

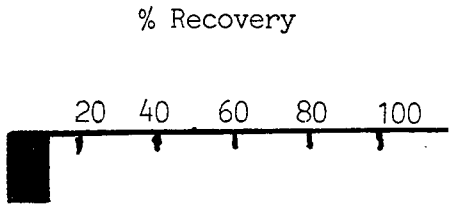

% Recovery
20
40
60
80
100

FIG. 6

The details of the site characteristics which permit reproduction and transmission at depths is currently being investigated. Definition of these site characteristics, which preliminary studies have shown to be consistently present in the areas of forest which were subjected to massive disease spread and intensification in the period from 1945 - 1965, has obvious major implications to the management of the disease.

1. (d) Factors Affecting Sporangial Formation.

The major mechanism by which P.cinnamomi achieves high density in the soil is its asexual reproduction cycle. The process of sporangial stimulation, formation and release is complex and very sensitive to physical and microbiological factors in the soil environment. One of the major pre-requisites to defining the effects of site and climatic factors on disease spread and intensification is the elucidation of these mechanisms.

1 (d) 1. Effect of soil stimulation.

P.cinnamomi will not produce sporangia if stimulatory factors are not present in the soil. Work with undisturbed soil cores has shown that the ability of surface soil to stimulate sporangium production is markedly seasonal with soils highly stimulatory in summer. Stimulatory capacity declines abruptly following the onset of winter conditions. However, if the soil is diluted they remain stimulatory throughout the year. Saturation and puddling of the soil and alternate wetting and drying seems to increase the stimulatory capacity of the soil to induce sporangia. Work is in progress to determine the effect of the interaction between temperature and moisture on the ability of soils to stimulate sporangia.

A detailed investigation was carried out to determine the effect of season and soil type on sporangial formation and release. Four soil types were tested:-

1) Loam - highly resistant field soil
2) Yellow sandy loam - moderately resistant
3) Sandy loam - " "
4) Black gravel - highly susceptible.

Sporulation at -1.0 kPa soil water potential varied seasonally, with stimulation of sporulation in all soil types being greatest

in summer, intermediate in spring and low in autumn and winter (Fig. 7).

This seasonal pattern of the ability of soil to stimulate sporangium formation highlights the importance of early rains in autumn for the production of inoculum. Not only do the early rains fall on warm soil, but they also fall on soil that is highly stimulatory for sporangium formation.

For summer and spring samples, sporulation and zoospore release followed the same pattern with soil type; highest levels in yellow sandy gravel, intermediate levels in sandy loam and black gravel and low levels for red loam. Sporulation and zoospore release were significantly less in red loam than the other three soil types. Although there were significant differences in the response of the fungus to the soils the differences were not sufficient to explain the marked effect of these soils on the disease occurrence in the field

1 (d) 2. Effect of soil type on movement of zoospores.

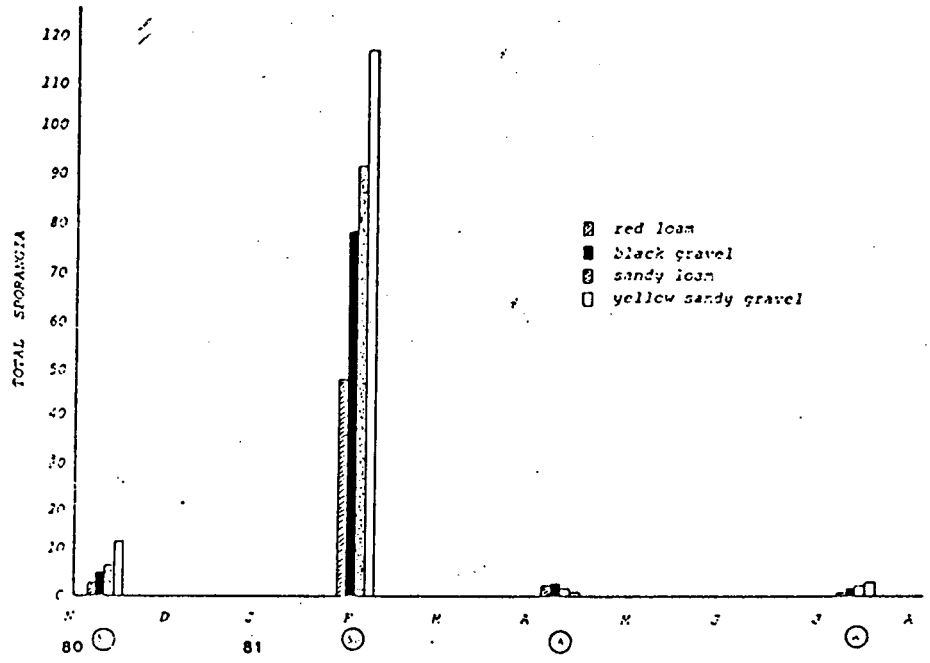
In a glasshouse experiment motile zoospores were placed in the centre of soil cores of red loam or black gravel held at -0.5 kPa in Buchner funnels with fitted glass plates. *Phytophthora cinnamomi* was detected 2cm from the inoculation point in the red loam and greater than 3cm in the black gravel (Fig. 8). The shorter dispersal distance in the red loam compared to black gravel may be due to soil texture differences between the two soils. Soil chemical and microbiological factors may also affect spore encystment and survival.

1 (d) 3. Effect of temperature on sporangium formation.

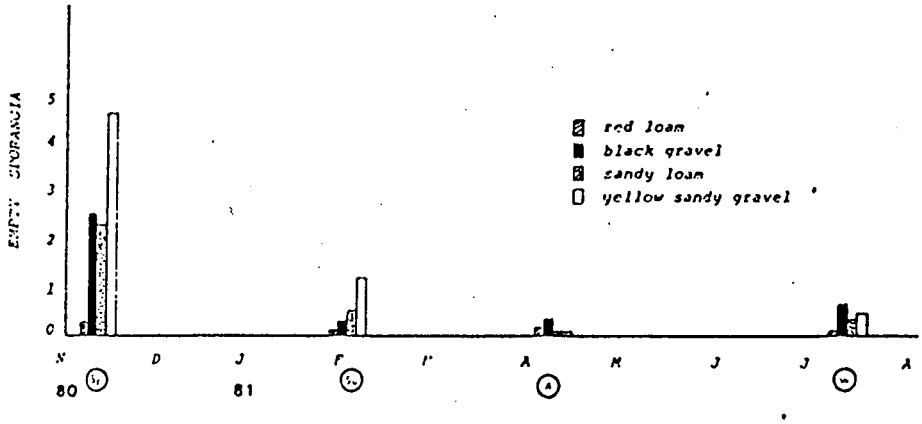
The relationship between temperature and sporangium production in soil leachates is very skewed towards high temperatures, with optimal sporangium production in the temperature range 23 - 28° C (Fig. 9). Further work is currently being carried out to determine the effect of fluctuating temperatures and the interaction between soil temperature and moisture and stimulatory capacity on sporangium production.

Figure 7. Summation of A) Total (viable and empty) and B) empty sporangia of Phytophthora cinnamomi. For each four day sporulation period in spring, summer, autumn and winter for red loam, black gravel, sandy loam and yellow sandy gravel.

FIG. 7


TOTAL SPORANGIA
120
110
100
90
80
70
60
50
40
30
20
10
0
red loam
black gravel
sandy loam
yellow sandy gravel
N
D
J
F
M
A
M
J
J
A
80
81

a)


5
4
3
2
1
0
red loam
black gravel
sandy loam
yellow sandy gravel
N
D
J
F
M
A
M
J
J
A
80
81

b)

Fig. 8. Number of infected Eucalyptus sieberi cotyledons by Phytophthora cinnamomi over 5, 11 and 17 days at 1 , 2 and 3 cm from inoculated point in A) red loam and B) black gravel.

FIG. 8

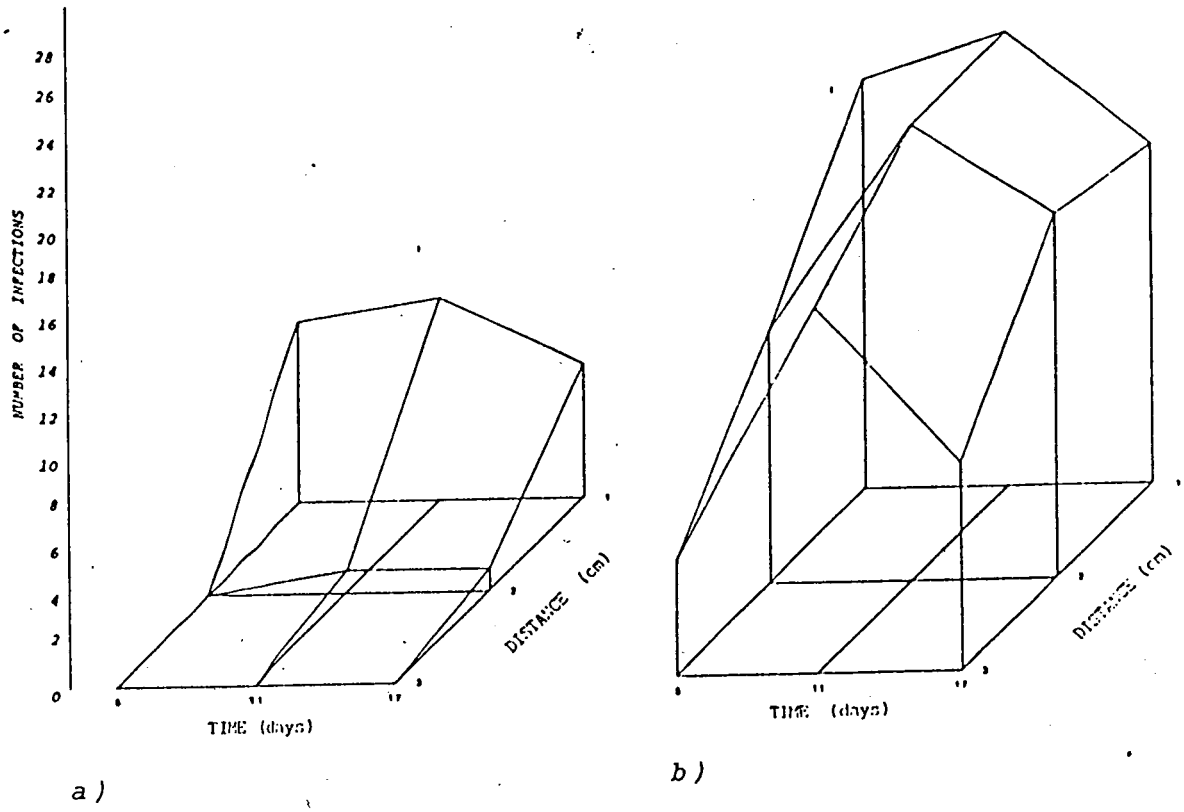
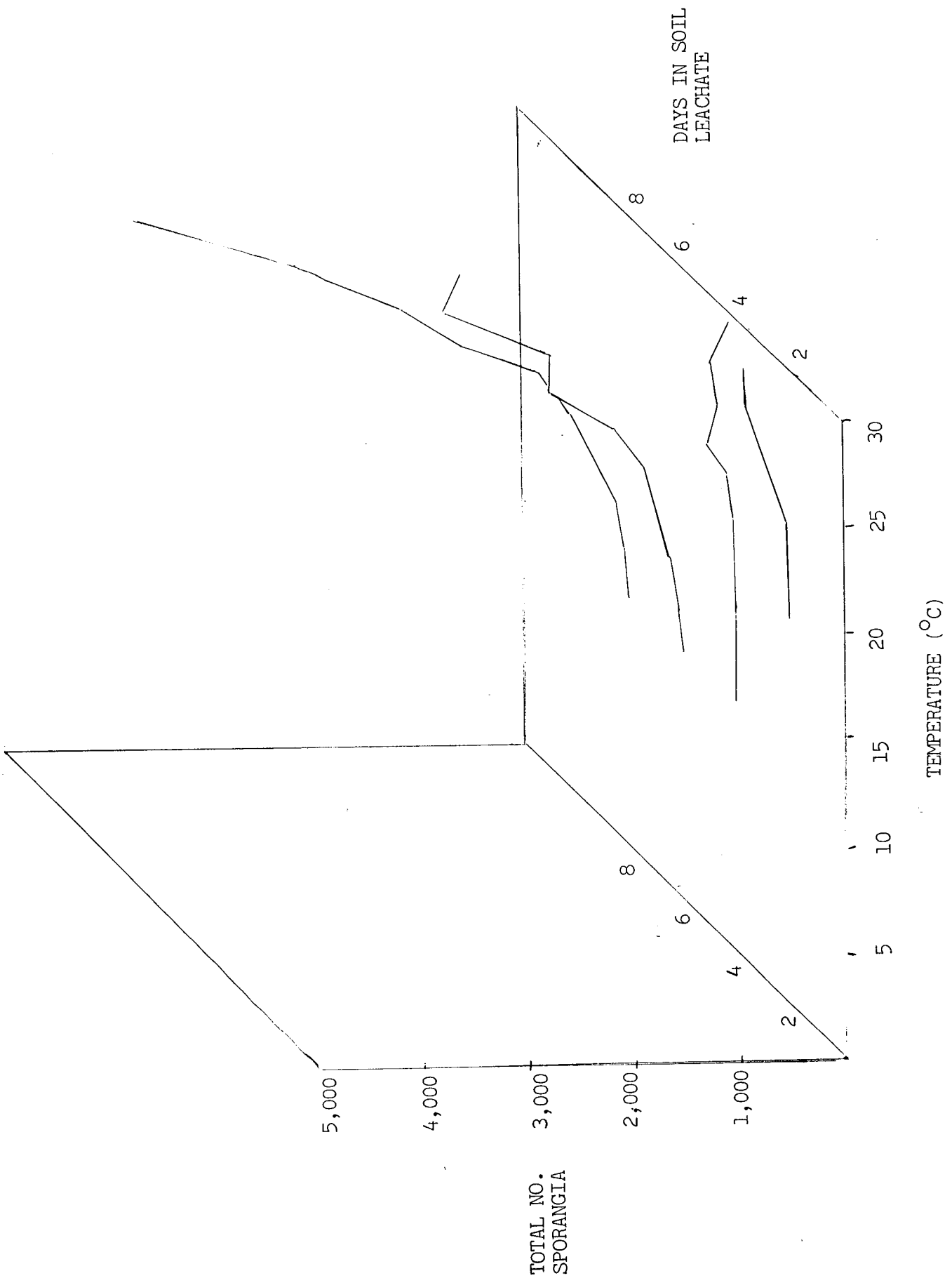

NUMBER OF INFECTIONS
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
TIME (days)
DISTANCE (cm)
a)
TIME (days)
DISTANCE (cm)
b)

Fig. 9. The effect of Temperature on Sporangial Production in soil leachates.

FIG. 9


TOTAL NO. SPORANGIA
5,000
4,000
3,000
2,000
1,000
DAYS IN SOIL LEACHATE
2
4
6
8
TEMPERATURE (°C)
5
10
15
20
25
30

1 (d) 4. Effect of soil moisture on sporangium formation.

Although sporangia can form when the soil is at field capacity, release only occurs under conditions of saturation. Saturated soil conditions such as that which occurs in the root channels formed in sheet laterite are required for significant sporangial formation and release.

1 (e) Survival and Reproduction on Resistant and Highly Susceptible Sites.

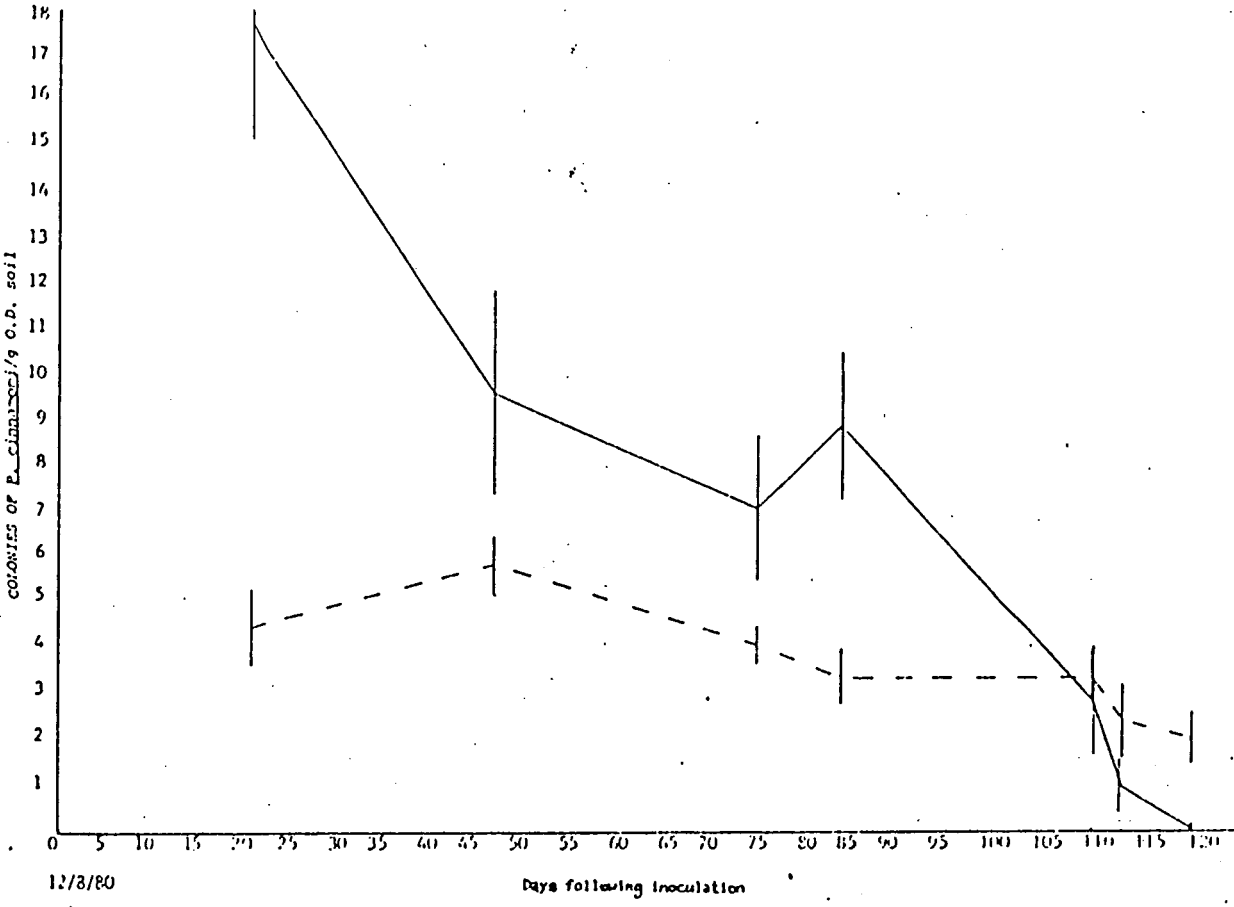
Sites which are characterized by having black gravel in the surface horizons have been recognized from field observations to be highly susceptible to the disease. These sites typically formed the bulk of the area of forest which was subjected to extension disease spread and intensification in the period 1945-65. Conversely, the red loam soils formed in the incised river valleys are highly resistant. The surface soil horizon of black gravel and red loam site types were inoculated in the field with infected pieces of B.grandis tissue and the development of P.cinnamomi followed in surface soil (top 3cm). In the first 110 of the trial, P.cinnamomi soil population levels were greater for the black gravel than the red loam (Fig. 10). The differences between the soil types in soil population levels could not be related simply to soil moisture and temperature. Although significant differences were observed in the surface soil, they were not sufficient to explain the major differences in field susceptibility which have been observed.

2. Studies of the Host/Pathogen Interaction.

Various hypotheses have been proposed to explain decline and death of jarrah in P.cinnamomi infected areas. However, there has been no satisfactory rational hypothesis to explain the mass collapse of jarrah stands observed during the period from 1945-65. A principal obstacle has been the absence of mass death of jarrah in forest areas over the last 15 years. For example the jarrah death rate of less than .06 trees per hectare in 1980/81 was recorded by detailed analysis of aerial colour photography over 3000 hectares of affected forest. The rate of death of jarrah that has occurred over the past 15 years does not constitute a significant management problem. However, there has been concern that there could be a major return to the conditions

Fig. 10. Number of colonies of Phytophthora cinnamomi recovered by soil plating of red loam (_____) and black gravel (_____) . Each point is the mean (± S.E.) of 25 samples.

FIG. 10


COLONIES OF B. cinarasi/g O.D. soil
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
105
110
115
120
12/8/80
Days following inoculation

that led to the mass decline in the jarrah forest which was observed in the forest from 1945-65.

A survey of trees which were exhibiting a rapid death syndrome demonstrated that P.cinnamomi could be recovered from large suberized roots and in some cases the collar regions of affected trees. This finding was contrary to the assumption made by all research workers that P.cinnamomi was a fine feeder root pathogen. (see previous report).

Following the discovery that P.cinnamomi could invade secondary tissue a major research effort has been directed towards determining if this was the mechanism causing death of jarrah and the factors affecting the capacity of jarrah to withstand invasion by P.cinnamomi.

2 (a) Distribution of P.cinnamomi in Jarrah Trees in Infected Areas where Jarrah Mortality is not occurring.

In the period since 1965 large areas of jarrah forest have been infected by P.cinnamomi , as indicated by the death of the B.grandis understorey, but overstorey mortality has been negligible. Since it was possible that these trees could have latent infections which could reactivate, an attempt was made to determine if the fungus was present in the root systems. Twelve trees have been excavated and their root systems have been intensively sampled. In the majority of the trees which were excavated there was no evidence of lesions and no recovery of P.cinnamomi obtained from the root system. Two trees in the middle of an old infection had very poor crowns and a proportion of the major horizontal roots systems were dead. There was evidence that the fungus had killed part of the horizontal roots but that the infection had been blocked by the tree. In three trees dissection of the root system revealed evidence of lesions and the fungus was recovered from the same tissue.

2 (b) Distribution of P.cinnamomi in Trees Exhibiting a rapid death syndrome.

In 1981 and 1982 several areas of P.cinnamomi infected forest were located where there were large numbers of dying jarrah trees and extensive mortality of the shrub and understorey layer (Fig. 11.)

Fig. 11. Mass collapse of Jarrah Forest on site with a concreted layer of sheet laterite present in the surface horizon.
Note: Rapid deaths.

Fig. 11.

Following is a summary of the preliminary results from an intensive study of the distribution of P.cinnamomi within the root systems and lower stem of affected trees in these areas.

A total of 41 trees were excavated from 7 different areas in the forest located within a 60km radius of Dwellingup. Three of the sites were located downslope of a major site disturbance (bauxite mine or major road) which caused excessive runoff. The trees in the infected areas, which varied in size from 5 to 20 hectares, were exhibiting varying degrees of crown decline (Fig. 11). The first observable symptom was slight chlorosis and slight wilting followed by complete death of the crown. In some sites trees, whose crown had been killed in the previous year, formed epicormic shoots on the lower part of the bole but these invariably died. A number of trees were observed to pass from a stage of apparently unaffected green crowns to complete death of the crowns within a period of six weeks, during the late summer and early autumn of 1982. Jarrah mortality in some sites within the affected areas occurred within two to three months of Banksia grandis deaths.

The trees sampled varied in size from small saplings to large veterans and trees in all phases of crown decline were sampled. The root systems and lower stump of affected trees were excavated by a combination of mechanical and hydraulic methods. The completeness of the excavation varied and it was impossible to excavate the trees below a concreted lateritic layer, which was present in all sites, at depths varying from 5 - 75cm. Following excavations the root systems were labelled and photographed and the stumps and root systems returned to the laboratory for analysis.

Phytophthora cinnamomi was consistently recovered from the bark and wood of the root samples of all but two trees on selective and non-selective agar. Phytophthora cinnamomi was more frequently isolated from fresh lesions, but it was also recovered from the dead tissue and, occasionally, healthy tissue above the lesion front. Typically P.cinnamomi grew vigorously from all of the root pieces plated from freshly lesioned roots. In a number of trees lesions were observed in the lower stump and P.cinnamomi was recovered from this tissue. But, in a number of the trees, there were no lesions in the stump. A number of other potential plant pathogens were recovered from lesioned and dead tissue, but the rate of recovery of these fungi was relatively low in comparison to Phytophthora cinnamomi. It is probable that these fungi are secondary invaders. That is, they are only able to invade the tissue after the trees have been attacked by P.cinnamomi (Table 1). During

TABLE 1. Recovery of *P.cinnamomi* from affected trees.

TABLE 1

Number of Trees sampled.	No. of Trees from which P.cinnamomi was recovered	No. of Trees from which was recovered from the stump.	No. of Trees from which Cytospora eucalypticola was recovered	No. of Trees from which Endothia havanensis Bruner was recovered	No.of Trees from which Pestalotia spp was recovered
41	39	13	7	6	3

Total number of roots plated.	Total Number of roots yielding P.cinnamomi.	% recovery of P.cinnamomi from horizontal roots	% recovery of P.cinnamomi from vertical roots	% recovery of P.cinnamomi from lesioned tissue	% recovery of P.cinnamomi from discoloured phloem	% recovery of P.cinnamomi from tissue with no visible symptoms
626	275	30%	66%	82%	49%	34%

the initial phase of this survey sampling was concentrated on the surface root system, and the lower stem. However, at one of the sites where large numbers of jarrah trees had died, intensive sampling of the horizontal root systems and the lower stem of ten trees in various stages of crown decline failed to give any isolations of Phytophthora cinnamomi and no lesions were detected. Further excavations of the root system of two trees to a depth of approximately 50 - 75cm below the soil surface, where a concreted lateritic layer occurred, revealed numerous vertical roots penetrating the layer through distinct 'pot holes' in an otherwise solid sheet of concreted laterite. It was only possible to obtain samples of the vertical roots penetrating these holes to depths of between 5 - 50cm below the lateritic layer, because of its concreted nature. However, at these depths the majority of vertical roots were found to be lesioned or dead, with active lesions extending upwards. Phytophthora cinnamomi was consistently recovered from these roots on selective and non-selective agar (Fig. 12).

Following this observation ten trees on several other sites, where extensive and rapid mortality of jarrah was occurring, were excavated so that the vertical root system of the trees could be sampled. It was impossible to obtain samples of vertical roots to depths exceeding 50cm below the surface of the layer of sheet laterite and often the vertical roots could only be sampled to a depth of 5 - 10cm within the layer, even with the aid of pneumatic hammers and explosives. However, P.cinnamomi was consistently recovered from the vertical roots sampled. For example, 26 vertical roots were sampled from one trees which had recently died (Fig.13A). The majority of the roots had lesions present and frequently the point at which the root had broken off was dead. P.cinnamomi was recovered from all of these roots. The length of lesions in the vertical roots varied between sites and within trees. In some of the roots the lesion extended from the point where it had broken off in the concreted lateritic layer to where it joined the horizontal root. However, there were numerous roots where the lesion was contained to a zone within 5 - 10cm where it had broken off in the layer. A number of the vertical roots which were excavated had small (< 2mm in diam.) suberized roots and fine feeder roots were proliferating from the main suberized root within the root channel in the concreted lateritic layer. These roots were invariably dead and consistently yielded P.cinnamomi on selective and non-selective agar (Fig. 13) B.grandis roots were often present in the same root channels as the jarrah roots and were infected with P.cinnamomi. In all of the sites where rapid and extensive mortality of jarrah trees has been observed a concreted lateritic layer was present at depths

Fig. 12. P.cinnamomi distribution in vertical roots
Blackened areas infected with P.cinnamomi
Hatched areas - dead tissue.

FIG. 12(a)

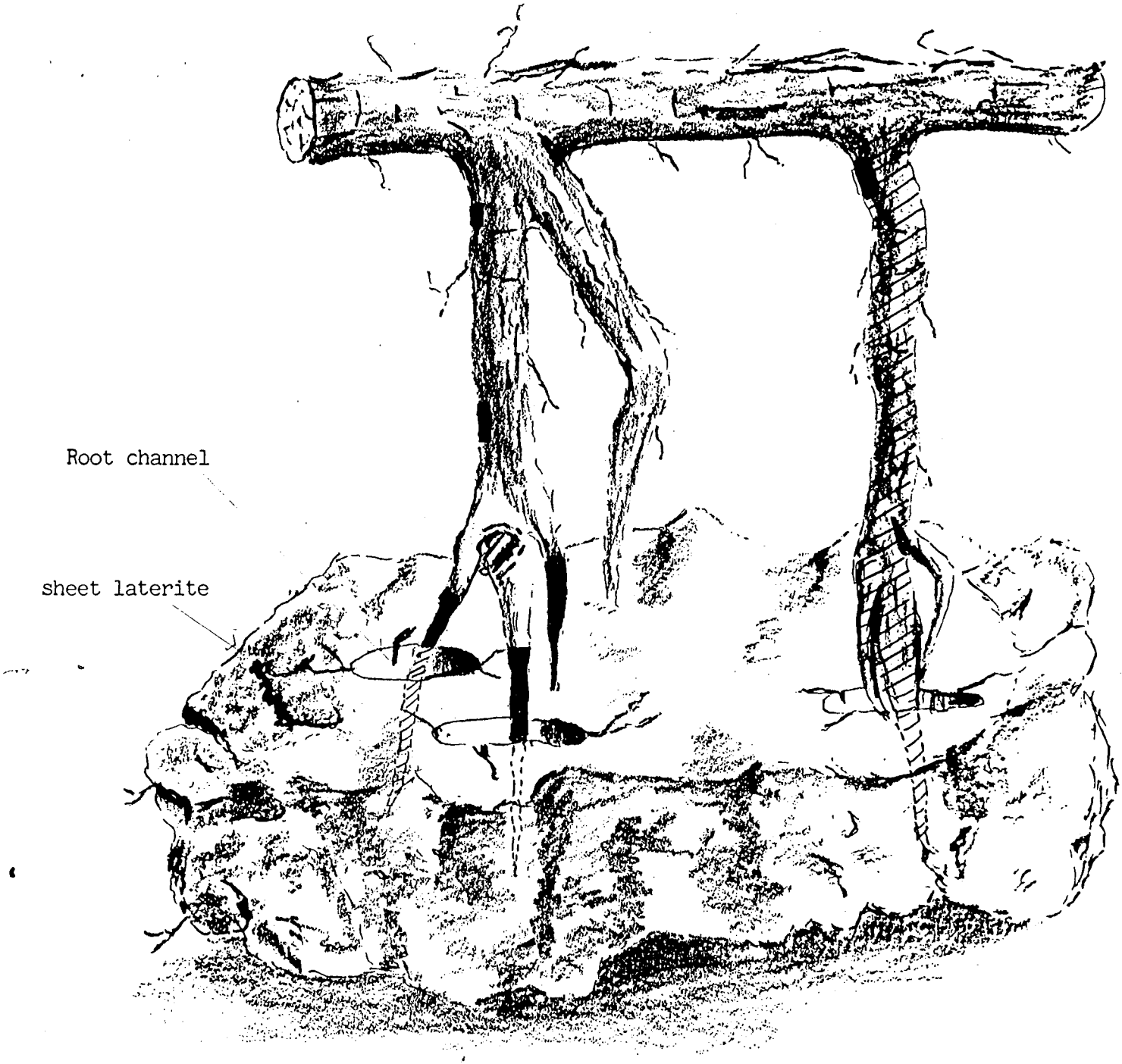


FIG. 12(b)

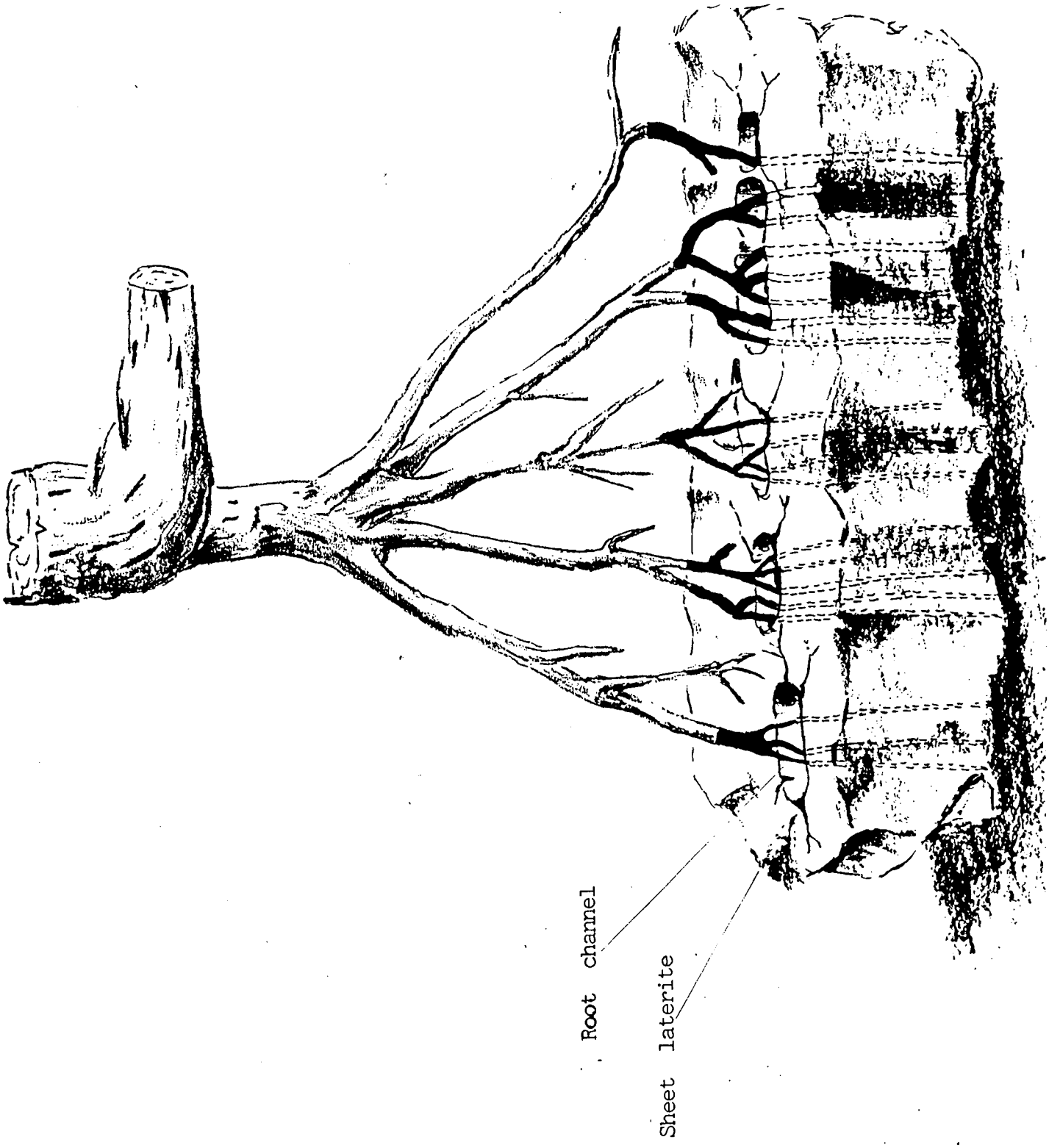


Fig. 13. Typical pattern of P.cinnamomi distribution in tree undergoing rapid decline. Blackened areas infected with P.cinnamomi.

Fig. 13.

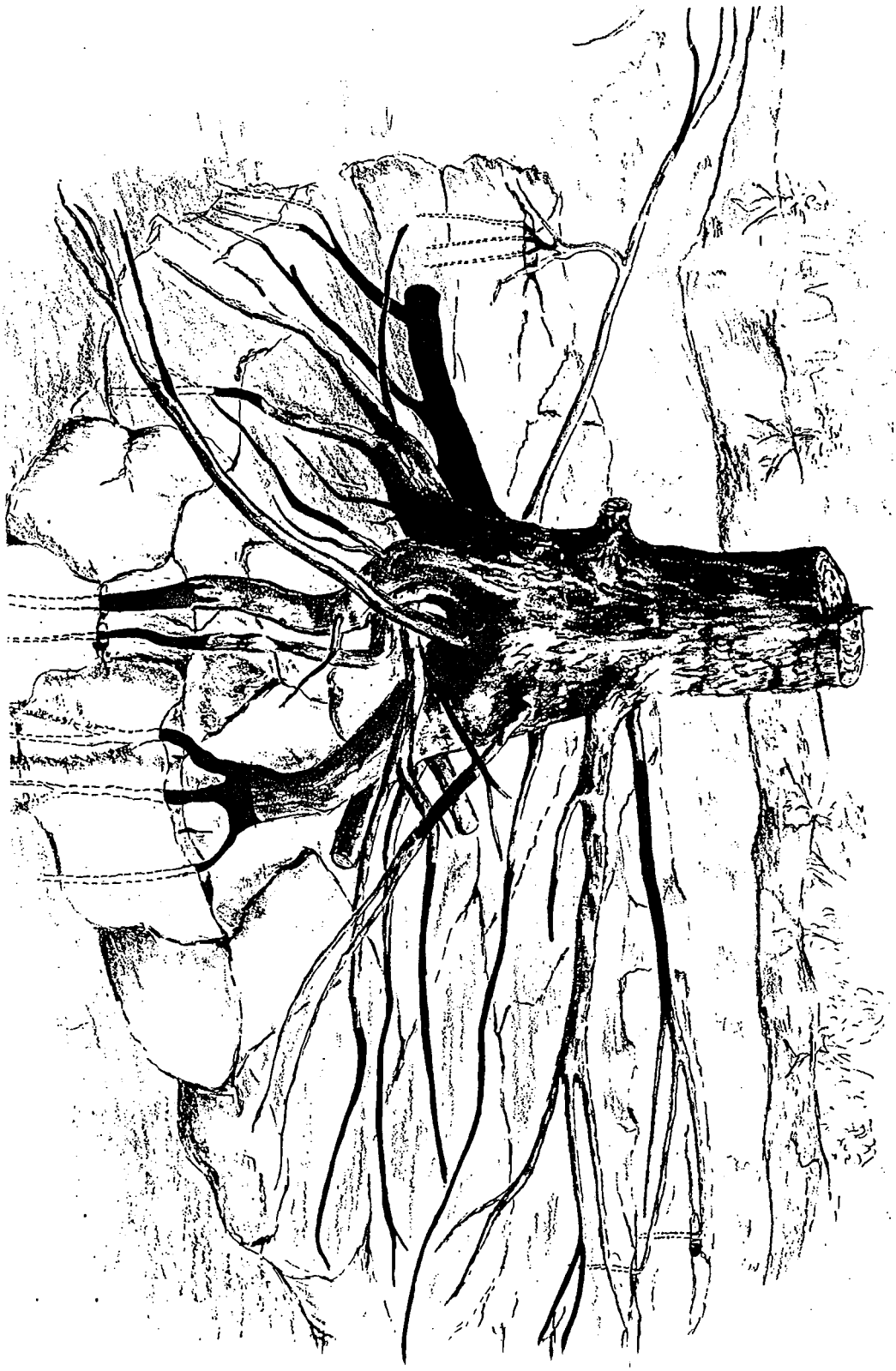


Fig. 13A. Excavated rapid death tree. 26 vertical roots were obtained from this tree. All yielded P.cinnamomi from the zone when the root passed through the root channels in the laterite layer.

varying from 5 - 75cm. During the hydraulic excavation we observed ponding of water within the root channels in the lateritic layer (in some sites free water remained in the root channels for 48 hours before draining away) and lateral movement of water on the surface of the concreted lateritic layer. For some of the trees it is possible that P.cinnamomi could have invaded the vertical roots via the horizontal root system. But there were numerous examples where either the horizontal root system was not infected, or where there was a zone of uninfected vertical roots between the infection in the vertical roots within the sheet lateritic layer and the horizontal root. We conclude that infection of the vertical roots occurred at the surface of the concreted lateritic layer or within the root channels in the layer.

2(c) Causes of Jarrah Deaths.

Ever since P.cinnamomi was identified as the causal organism numerous attempts have been made to explain how this pathogen causes death of jarrah trees. It has been suggested that jarrah death could be caused by attrition of the surface fine feeder root system by P.cinnamomi. This may be the mechanism by which the fungus kills some trees, but the number of trees killed following an extended period of crown decline, which is the only pattern of deaths that can be explained by feeder root attrition, is low and does not constitute a significant management problem. Diameter growth rates of trees in these areas is not different from trees in uninfected sites (see previous report). We conclude from the results of this study that the extensive and rapid mortality of jarrah that has been observed in the past results from invasion of the major root systems of the trees, preventing water uptake and that this is the principal mechanism by which Phytophthora cinnamomi kills jarrah trees. Our hypothesis that death results from dehydration is supported by measurements of xylem pressure potential of trees in areas subject to massive rapid death. (Fig. 14). (The research described in this section has been accepted for publication in Australasian Plant Pathology).

2(d) Factors affecting P.cinnamomi capacity to Invade Secondary Tissue.

2 (d) 1. Seasonal changes in rate of invasion of secondary tissue.

The rate of lesion extension in jarrah coppice is

Fig. 14. Xylem potential of affected trees in relation to controlled trees and trees in infected areas not exhibiting decline.

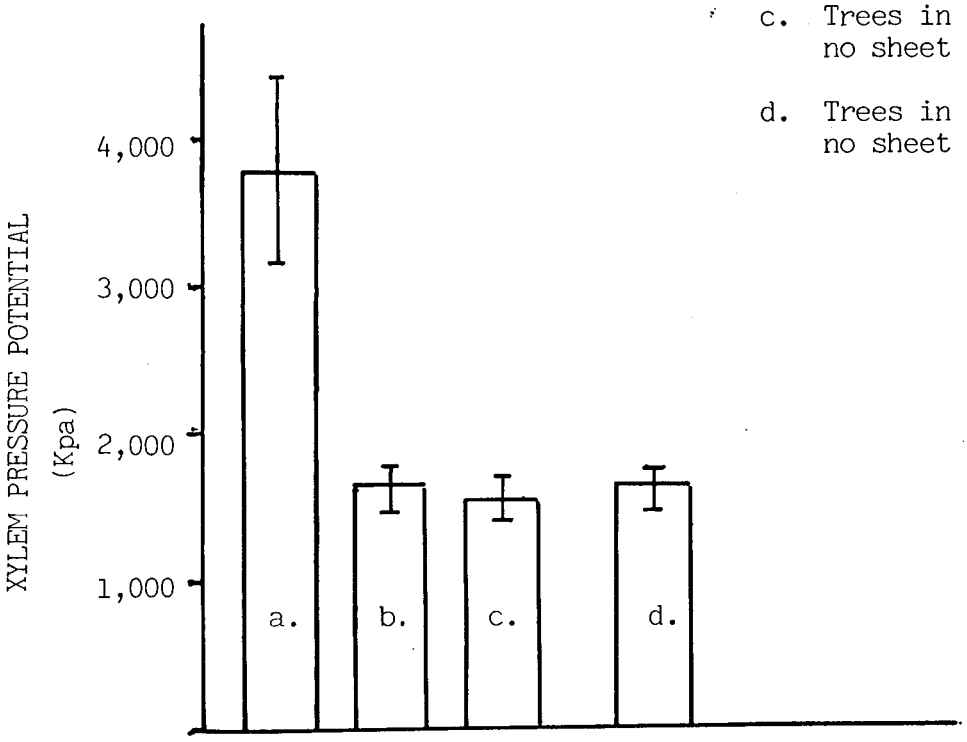
FIG. 14

a. Trees in infected site with sheet laterite present.

b. Trees in un-infected site with sheet laterite present.

c. Trees in infected site with no sheet laterite.

d. Trees in un-infected site with no sheet laterite present.



markedly seasonal with maximum lesion extension occurring in summer and early autumn (Fig. 15). Lesion extension in roots was similar to that observed for coppice, although the rate of lesion extension was less in roots. Whether or not the seasonal variation in lesion extension is due to physical or host physiological factors is currently under investigation. Our current investigations indicate that the rate of lesion extension is a major factor affecting the capacity of the host to halt lesion extension by barrier formation (see below).

2 (d) 2. Effect of temperature on lesion extension.

The rate of lesion extension in excised roots was very temperature dependent with maximum lesion extension in the temperature range 25 to 30° C. Fig 15A. This suggests that significant lesion extension can only take place during summer.

2 (d) 3. The effect of Site and Host Vigor on Susceptibility.

2 (d) 3. (1) Pot Trials - It was possible that the observed major variation in field susceptibility of jarrah to P.cinnamomi could have resulted from a inherent site characteristic such as fertility. A pot trial was carried out to test this hypothesis -

In this trial intact soil cores were removed from red loam, black gravel, sandy loam and yellow sandy gravel. Intact cores were used to retain the structural features of the soils.

Growth of B.grandis seedlings was similar for the four soil types. Growth of E.marginata seedlings was most vigorous in the red loam and poorest in the sandy loam. Following inoculation, the rate of death of B.grandis seedlings was greater in the black gravel, sandy loam and yellow sandy gravel than in the red loam (Fig. 16). Survival of B.grandis was marginally greater in red loam than the other three soils.

In comparison to the pattern of deaths in B.grandis seedlings, survival of E.marginata seedlings was highest in sandy loam followed by black gravel then yellow sandy gravel, with greatest number of deaths in the red loam.

Fig. 15. Lesion extension in relation to season (coppice stems).

FIG. 15

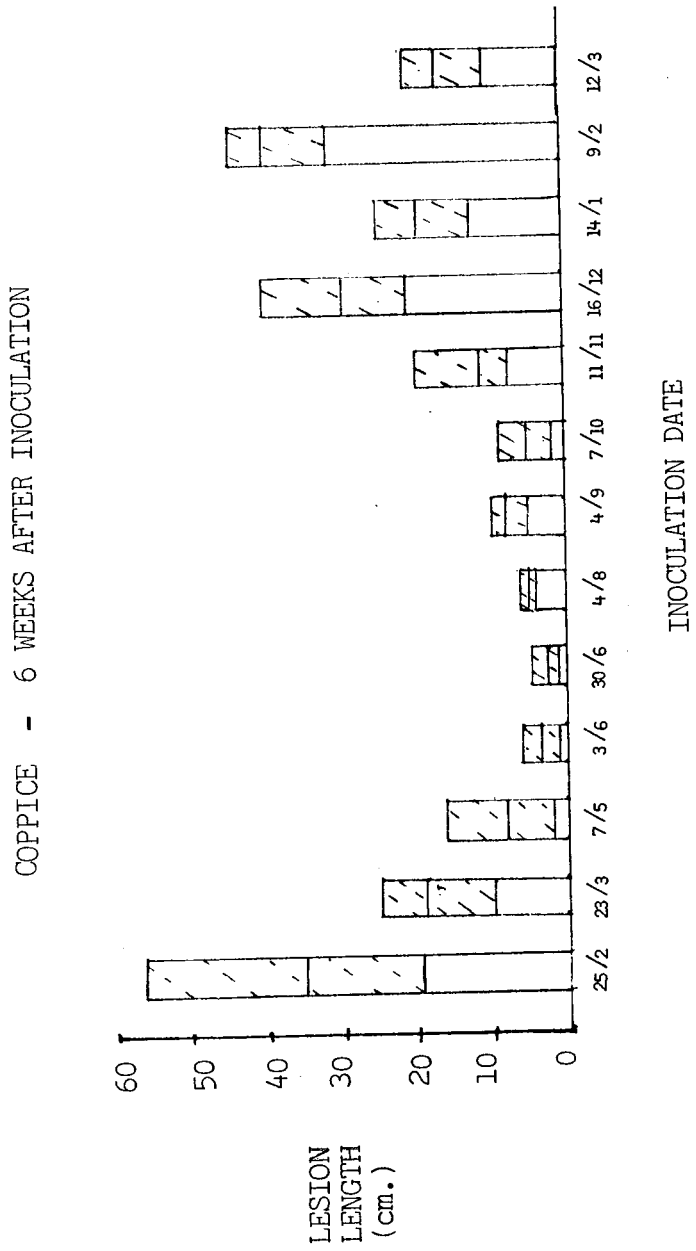

COPPICE - 6 WEEKS AFTER INOCULATION
LESION LENGTH (cm.)
60
50
40
30
20
10
0
25/2
23/3
7/5
3/6
30/6
4/8
4/9
7/10
11/11
16/12
14/1
9/2
12/3
INOCULATION DATE

Fig. 15A The effect of temperature on lesion extension in excised jarrah roots.

FIG. 15A

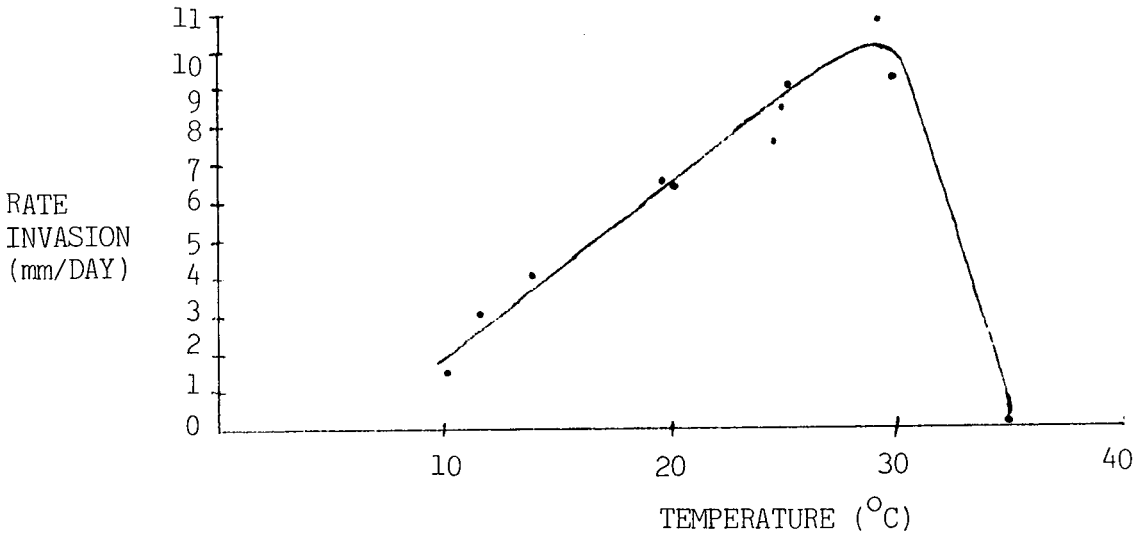
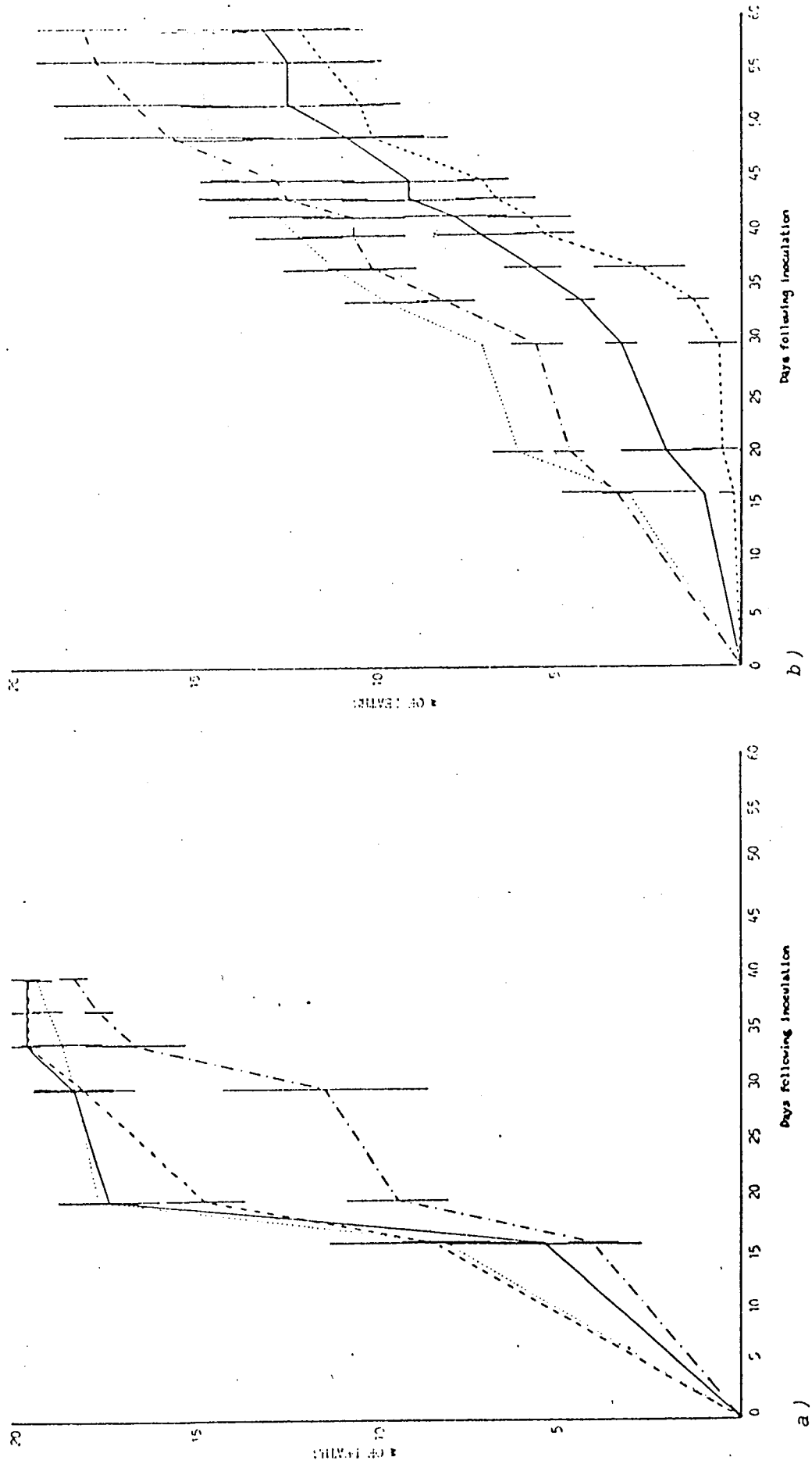

RATE
INVASION
(mm/DAY)
11
10
9
8
7
6
5
4
3
2
1
0
10
20
30
40
TEMPERATURE (°C)

Fig. 16. Mortality of B.grandis and Jarrah grown in different soils and inoculated with P.cinnamomi.

FIG. 16


Days following inoculation
b)
Days following inoculation
a)

Development of P.cinnamomi was least in the red loam. Greatest increase occurred in the black gravel early in the experiment followed by a sharp decline correlated with an increase in soil water potential. Soil population levels of P.cinnamomi for the sandy loam and yellow gravelly loam gradually increased to a maximum, similar to that for the black gravel, then slowly declined.

Although P.cinnamomi population levels and B.grandis deaths were least for the red loam, the greatest number of E.marginata deaths observed in this soil type does not reflect the disease situation in the field. This suggests that high soil fertility does not have a major effect on the susceptibility of jarrah.

2 (d) 4. The effect of site and host vigor on lesion extension.

The effect of these factors was investigated by wound inoculation studies of over 200 trees in various sites and with different degrees of vigour.

Factors such as flowering, seeding and site, etc., which may affect the rate of lesion extension have been studied. There was no difference in the rate of lesion extension between flowering and non-flowering trees. There was no consistent difference in rates of lesion extension in trees from areas subject to moderate or low intensity burn and trees growing on highly susceptible (black gravel) and resistant sites. The rates of lesion extension observed in these studies do not adequately explain the mass death of jarrah and the extension and rapid invasion of secondary tissue observed on the highly susceptible sites. We are currently testing the hypothesis that accelerated lesion extension results from stress induced by destruction of the vertical root system.

2 (e) Histological Studies of P.cinnamomi invasion of Secondary tissue.

Detailed histological studies of the process of invasion and lesion development by P.cinnamomi in jarrah and other susceptible and resistant species have been carried out. Following is a summary of this work:-

2 (e) 1. P.cinnamomi invades the phloem of susceptible plant species.

The phloem in fine roots, woody roots and stems may be vulnerable to invasion. In large roots and stems, phloem constitutes the inner bark. In jarrah the phloem is pinkish in colour when healthy, but brown and discoloured if invaded by P.cinnamomi (Fig.17 a, b, c).
Lesion extension in roots is lateral rather than tangential. Columns of discoloured phloem result. None of the inoculated roots had been completely girdled by P.cinnamomi . Roots express greater resistance than coppice stems to P.cinnamomi. Alternatively, the roots may be a less favourable food base for the fungus. This may be related to polyphenol composition of bark as well as available carbohydrates.

2 (e) 2. Wound periderm formation is an important resistance mechanism in eucalyptus to P.cinnamomi. (Fig. 17 d).

Lesion extension in jarrah is stopped by wound periderm formation in roots and stems; except when hyphal growth is particularly rapid, as in summer. (Wound periderms are layers of suberized and lignified cells which 'wall-off' areas of necrotic bark). P.cinnamomi lesions may develop in marri after wound inoculation, but these lesions are contained. The lesions are bounded by wound periderms. Wound periderm formation takes approximately 4 weeks in jarrah roots, but speed of development is dependent on the season. All jarrah roots and stems, inoculated or not, form new layers of bark in spring (October, in 1981). A new layer of bark is produced annually and formation of the subsequent periderm (spring bark layer) can isolate peripheral areas of infected bark and small lesions. Formation of wound and subsequent periderms, deeper and deeper in phloem of diseased roots, results in the symptom of 'flaky' bark. Roots which had suffered invasion or were in an 'unhealthy' condition often exhibited 'flaky' bark.

2 (e) 3. Phloem necrosis caused by P.cinnamomi, is associated with kino vein formation in jarrah as well as in marri. (Fig. 17 e, f).

The vascular cambium reacts to phloem necrosis with formation of a 'gum' vein. Kino can impregnate the invaded bark and turns yellow on drying. Kino vein formation, like wound periderm

Fig. 17 (a). Lesion extension of P.cinnamomi in jarrah coppice stem. Lesion took 26 days to form.

FIG. 17 (a)

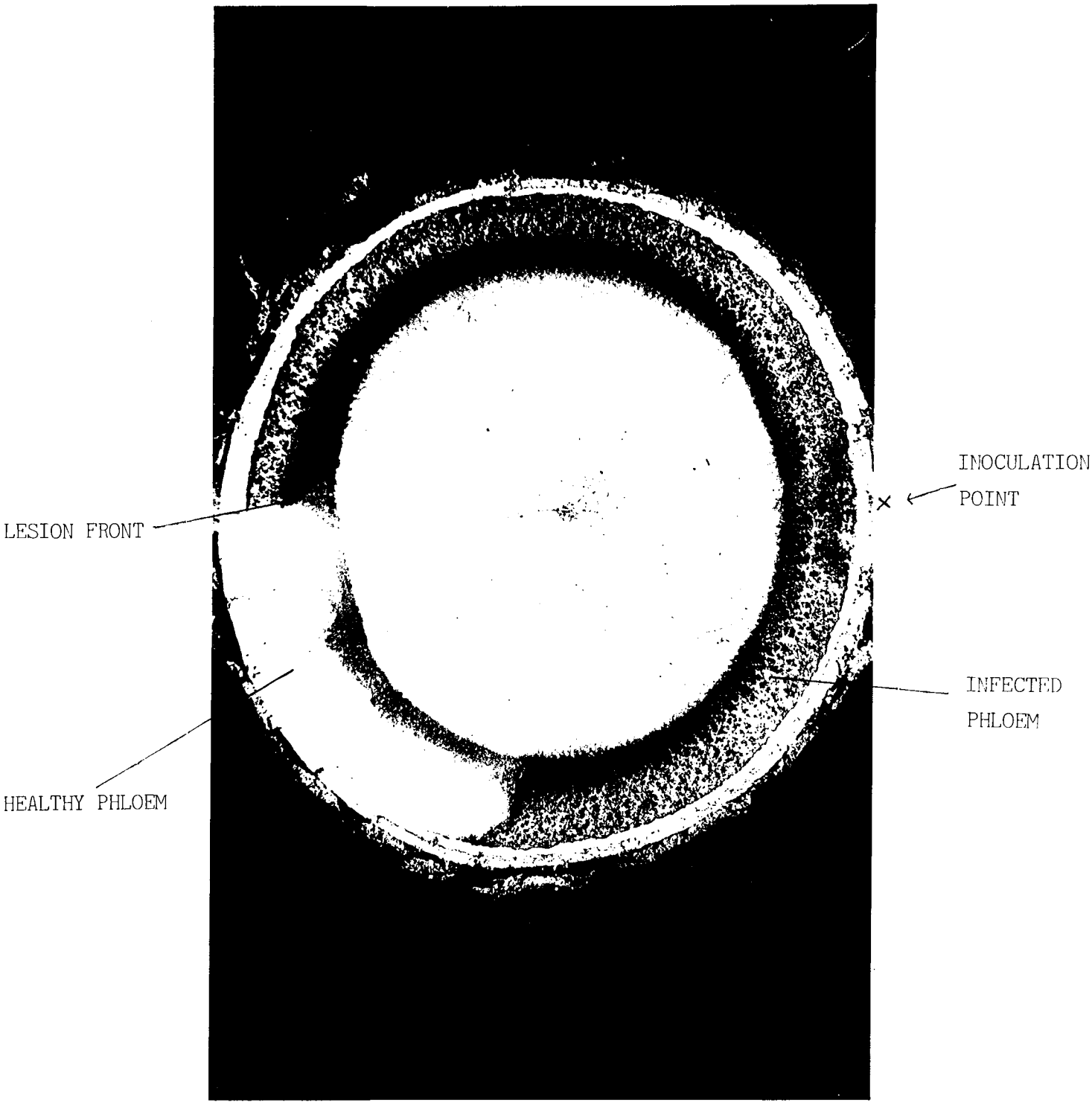

INOCULATION
POINT
LESION FRONT
INFECTED
PHLOEM
HEALTHY PHLOEM

Fig. 17 (b). Lesion formation in coppice stem following inoculation. Dark tissue is infected with P.cinnamomi.

FIG. 17 (b)

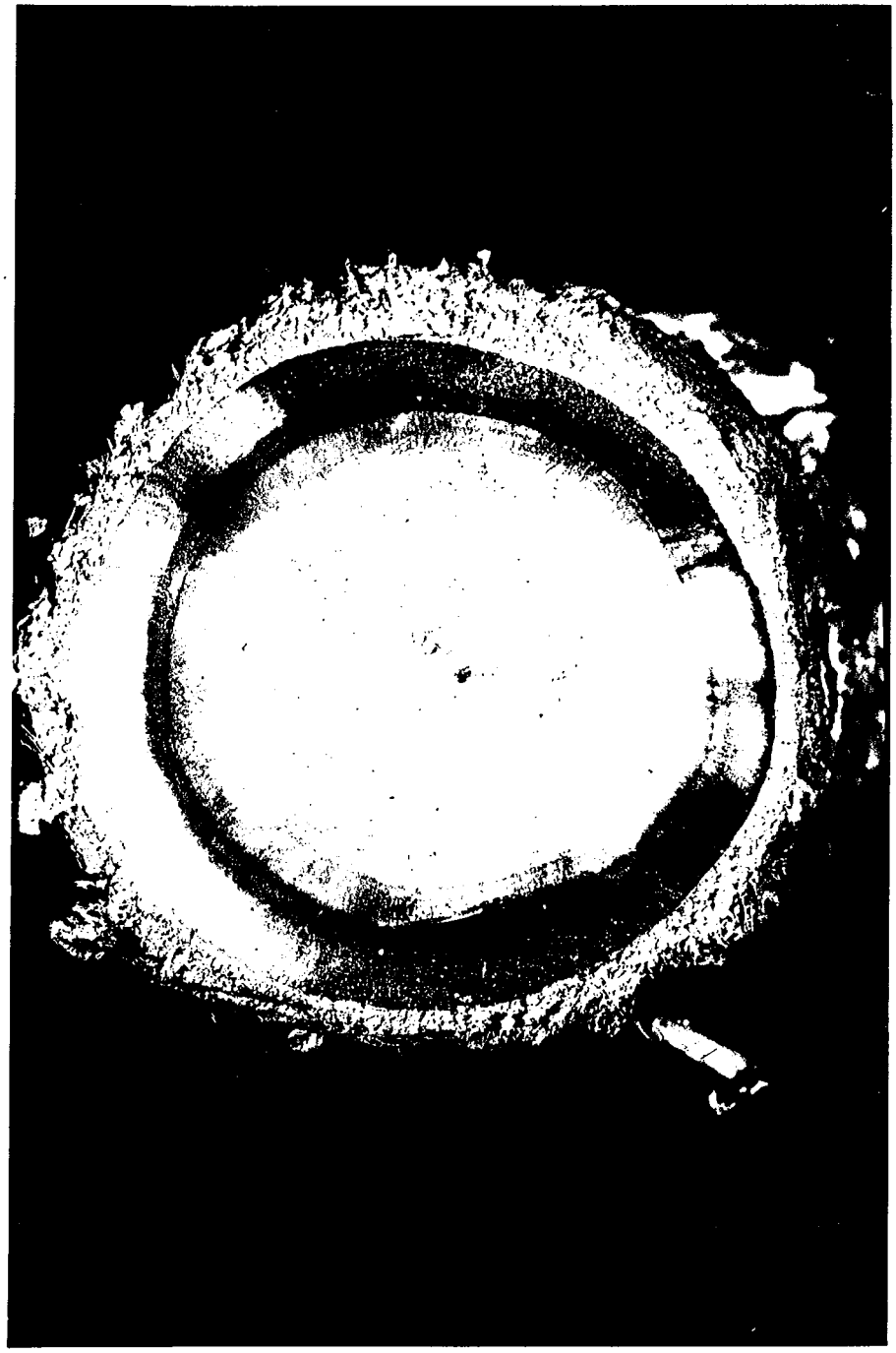


Fig. 17 (c). Jarrah root naturally infected with *P.cinnamomi*. Dark tissue is the lesion.

FIG. 17 (c)

Fig. 17 (d). Wound periderm formed as a barrier to *P.cinnamomi* extension.

FIG. 17 (d)

Fig. 17 (e) Kino response to P.cinnamomi invasion in coppice stems

FIG. 17 (e)

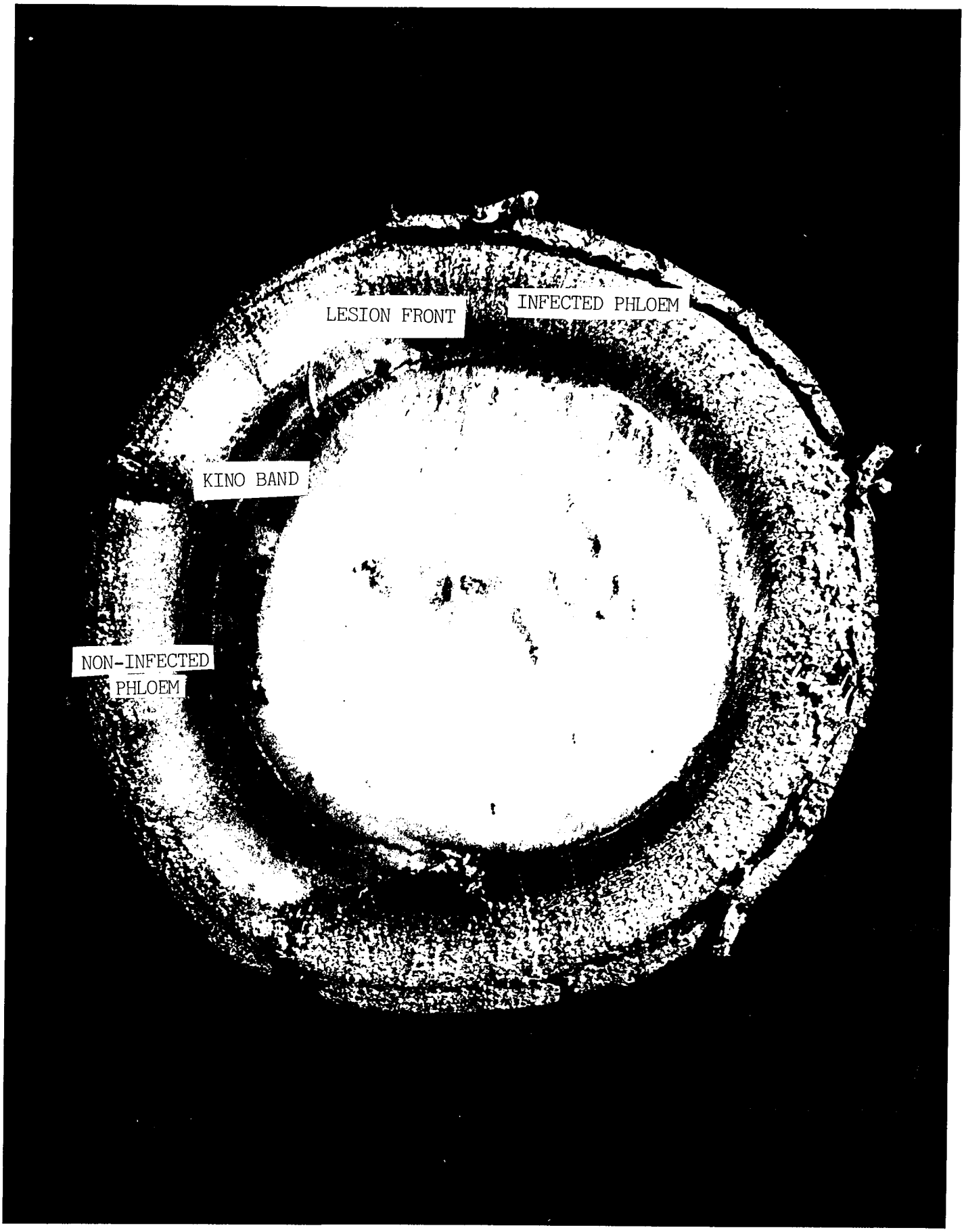
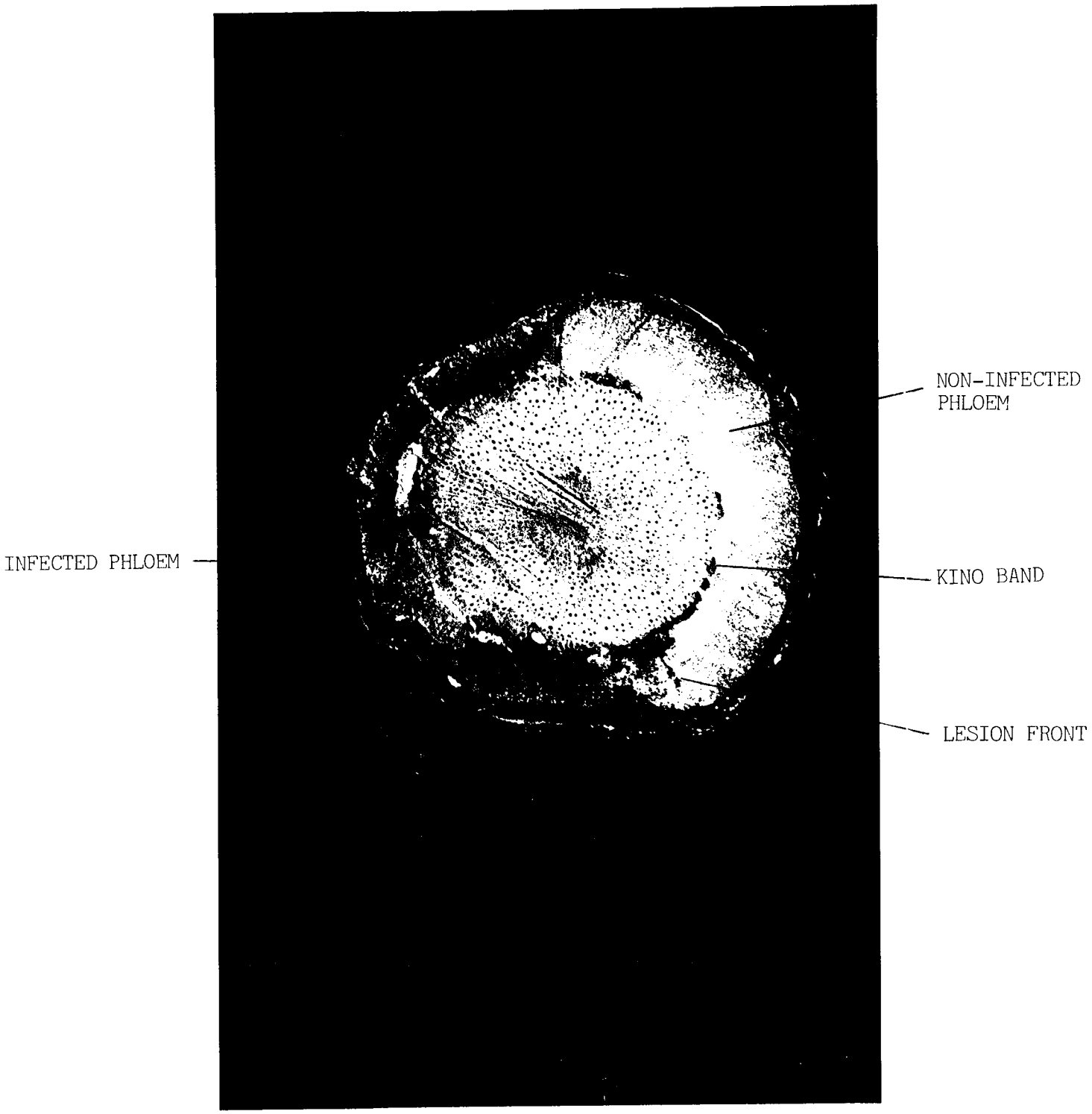

LESION FRONT
INFECTED PHLOEM
KINO BAND
NON-INFECTED
PHLOEM

Fig. 17 (f). Naturally infected root showing kino responses.

FIG. 17 (f)


NON-INFECTED PHLOEM
INFECTED PHLOEM
KINO BAND
LESION FRONT

formation, is a 'non-specific resistance mechanism' to pathogens in eucalypts. Kino has been shown to inhibit hyphal growth of P.cinnamomi in the laboratory. (Marri kino may be more effective than jarrah kino). (Effect on chlamydospore viability or sporulation of the fungus is not known yet). Kino vein formation is a seasonal reaction. The most extensive veins formed in trees inoculated during summer and autumn. Kino veins form in roots as well as stems. P.cinnamomi invasion of phloem is most rapid in summer and autumn which coincides with the time of most rapid kino vein formation.

2 (e) 4. Phloem invasion of Banksia grandis and Dryandra sessilis is much more rapid than in E.marginata and P.pinaster. (Fig. 17 g).

P.cinnamomi hyphae proliferate rapidly in phloem and ray parenchyma of banksia roots and stems. Banksia and Dryandra seedlings (three months old), died two weeks after inoculation with P.cinnamomi, and phloem at the collar was invaded. Extensive ramification by the fungus of the lower order jarrah roots was not observed. P.cinnamomi hyphae, vesicles and chlamydospores are easily 'spotted' in infected Banksia parts but density of propagules is low in jarrah, and tissues must be scanned to observe propagules.

2 (e) 5. Chlamydospores can form in infected bark.

Chlamydospores have been observed in infected jarrah phloem but they are more common in infected banksia and rays.

2 (e) 6. P.cinnamomi hydrolyses primary walls in the inner bark but can not delignify tissue. (Fig. 17 h).

Sieve tubes and parenchyma in the phloem collapse and disintegrate after invasion; fibres are uninfected. Reisolation of the fungus becomes increasingly difficult as lesions age (Reisolation may be dependent on presence of chlamydospores). Hyphae and vesicles are most easily observed in the first two weeks after phloem inoculation. (Once the fungus has exhausted available food reserves its growth on the tissue ceases). In roots, secondary invaders follow the pathogen in. P.cinnamomi can be rapidly displaced as the dominant organism in a lesion and P.cinnamomi's behaviour is typical of a primary pathogen; and the term 'facultative saprophyte' is less appropriate, to describe it.

Fig. 17 (g). P.cinnamomi in phloem and vessels of Banksia grandis.

FIG. 17 (g)

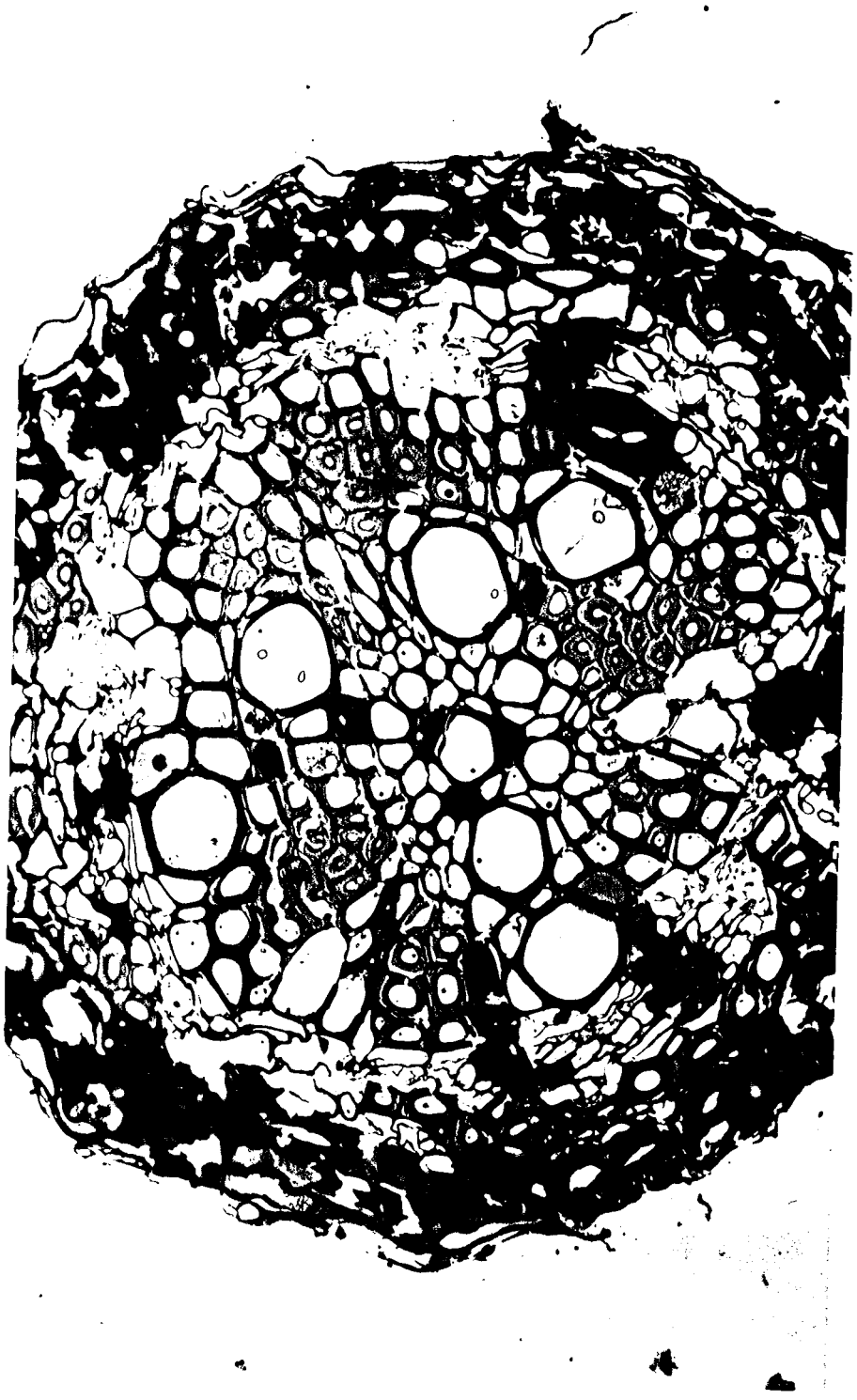
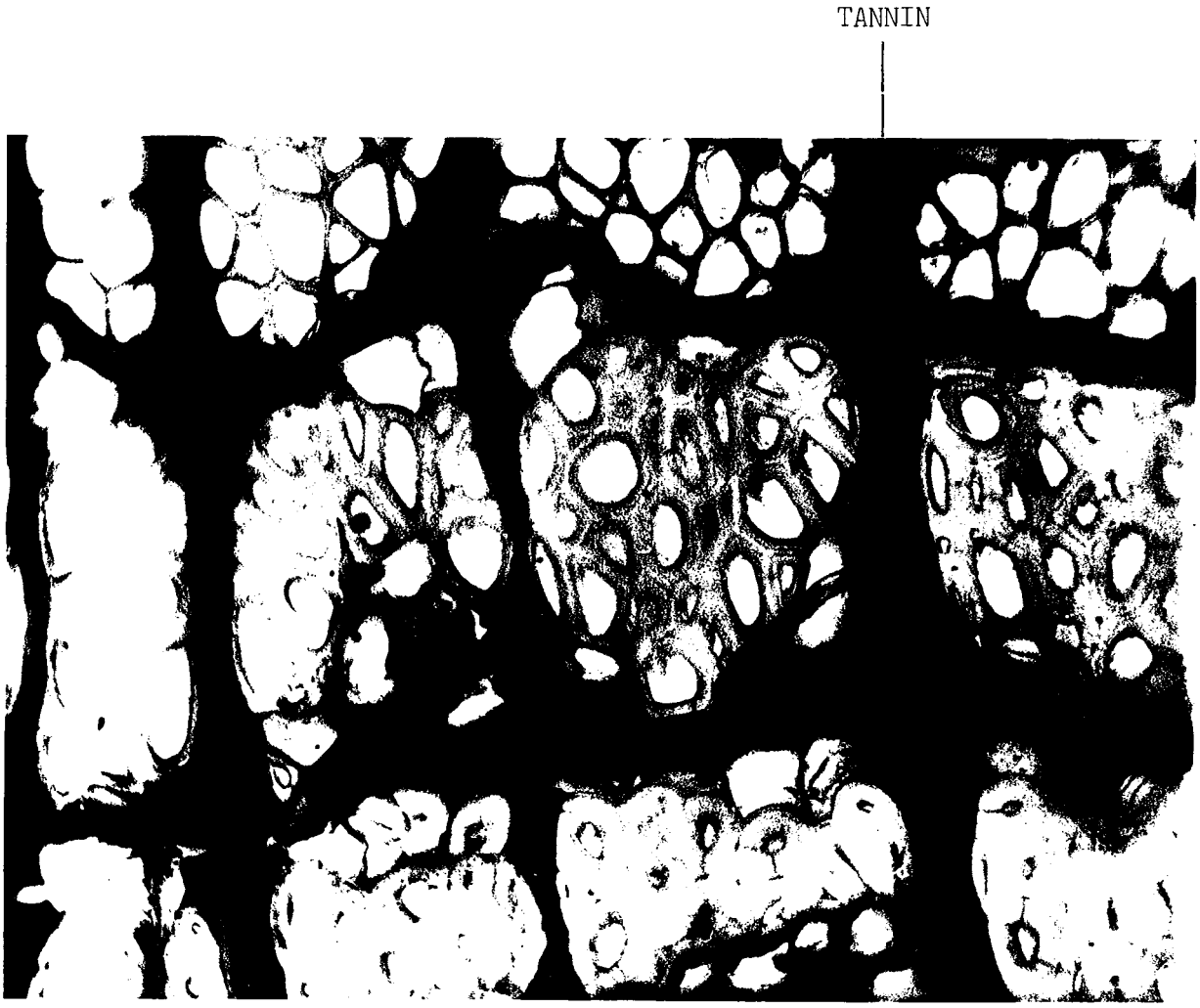


Fig. 17 (h). Tannin accumulation in ray and axial parenchyma of P.cinnamomi invaded tissue.

FIG. 17 (h)



3. The effect of Changing Understorey Composition on Susceptibility.

Studies have continued on the potential to control P.cinnamomi by manipulation of the Jarrah Forest understorey. The philosophy underlying this approach has been outlined in a previous report and numerous published papers. The results of our most recent research described above gives more confidence that this approach to control on sites having specific site characteristics which cause impedence of water flow will markedly reduce disease spread and intensification. Following is a summary of research into the potential effect of increased nitrogen inputs via legume species on the disease and the most recent results from a study of the ecology of B.grandis.

3 (a) A comparison between the effect of Acacia pulchella, A.lateriticola and Banksia grandis on the input of nitrogen into the jarrah forest ecosystem and on the development and survival of Phytophthora cinnamomi.

This study examined:-

- The effect of burning on release of nitrogen from A.pulchella, A.lateriticola and B.grandis stands.
- Nitrogen storage in A.pulchella and A.lateriticola roots and the release of nitrogen by decomposition
- Inorganic nitrogen and survival of P.cinnamomi in soil under burnt and unburnt A.pulchella, A.lateriticola and B.grandis stands.
- Sporangial formation in soil from burnt and unburnt A.pulchella, A.lateriticola and B.grandis stands in a controlled environment.
- Relationship between inorganic nitrogen and survival of P.cinnamomi in soil amended with A.pulchella, A.lateriticola and B.grandis roots in a controlled environment.

3 (a) 1. The effect of burning on release of nitrogen from Acacia pulchella, A.lateriticola and Banksia grandis stands.

The aim of this study was to determine the above and below ground distribution of nitrogen in A.pulchella, A.lateriticola and B.grandis stands prior to and immediately following burning in order to obtain an estimate of nitrogen input and loss to the system.

Prior to burning the largest nitrogen store for all treatments was in the soil, with lower levels in the above ground biomass and roots respectively (Fig. 18). Soil from beneath the A.pulchella canopy had a greater nitrogen store than soil from beneath both B.grandis and A.lateriticola. The difference in soil nitrogen between A.pulchella and B.grandis stands could not be explained by nitrogen formed by the three year old A.pulchella stand alone. The differences between the stands may partly be due to the difficulty of quantifying the nitrogen store in the soil. A large loss of total nitrogen occurred from soil with burning, even though ash from the above ground biomass was incorporated in the soil sample. The large loss in soil total nitrogen has not been observed by other workers and could in part be due to the difficulty of estimating soil nitrogen store.

3 (a) 2. Nitrogen storage in A.pulchella and A.lateriticola roots and the release of nitrogen by decomposition.

The aims of this study were to determine the density, nitrogen content, rate of decomposition and rate of nitrogen release from roots of A.pulchella and A.lateriticola. These results provide data for further studies using legume roots as soil amendments and their effect on P.cinnamomi.

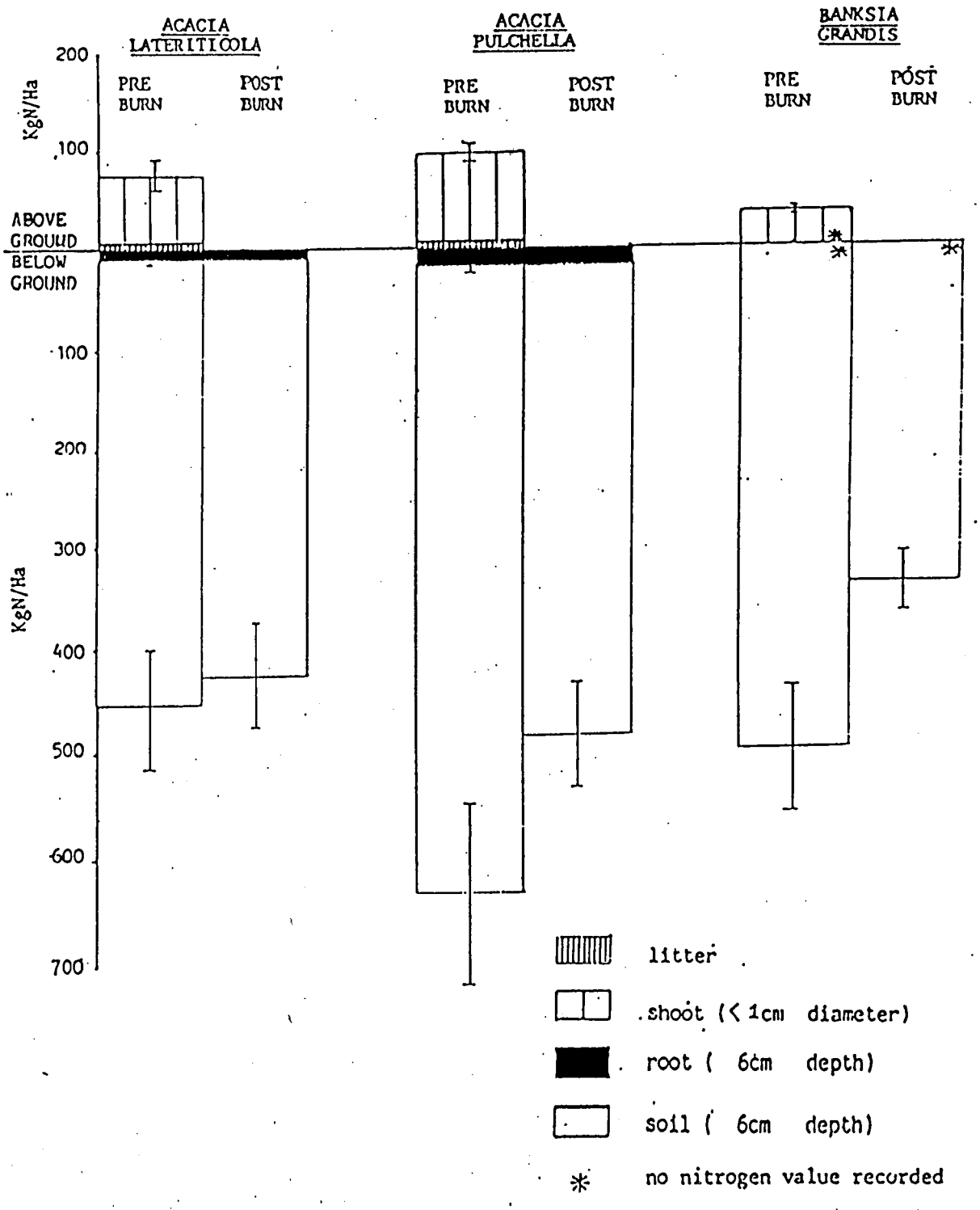
Roots of A.pulchella and A.lateriticola extended up to 2m from the stem of A.pulchella and 1m from the stem of A.lateriticola. The majority of the larger roots were in the top 20cm of the soil profile with the fine roots in the top 20cm.

A.pulchella stands had a greater root mass to a depth of 6cm, (2,092 kg/ha) than A.lateriticola stands (1,329 kg/ha). The root:soil ratio (dry weight) for A.pulchella of 0.6% was about twice that for A.lateriticola. However, there was a high degree of spatial variability.

Both A.lateriticola and A.pulchella showed similar nitrogen concentrations for each of the root size classes. The fine roots had the highest nitrogen

Fig. 18. Total Nitrogen (kg/ha) of Shoots, Litter Root (6cm Depth) and Soil (6cm Depth) for Acacia pulchella, A.lateriticola and Banksia grandis. Plots prior to and following burning. Standard errors are indicated by vertical bars.

FIG. 18


ACACIA LATERITICOLA
ACACIA PULCHELLA
BANKSIA GRANDIS
PRE BURN
POST BURN
PRE BURN
POST BURN
PRE BURN
POST BURN
KgN/Ha
200
100
ABOVE GROUND
BELOW GROUND
100
200
300
KgN/Ha
400
500
600
700
litter
shoot (< 1cm diameter)
root (6cm depth)
soil (6cm depth)
no nitrogen value recorded

concentrations and hence the lowest C:N ratio (39.4 for A.pulchella and 42.0 for A.lateriticola) and the largest roots had the lowest nitrogen concentrations and the highest C:N ratios (88.4 for A.pulchella and 78.1 for A.lateriticola). There were significant differences in the distribution of dry weight (Fig. 19a) and total nitrogen (Fig. 19b) between all root size classes for both Acacia species. A.pulchella and A.lateriticola fine roots had the highest weights (1,312 and 1,122 kg/ha respectively), and the highest nitrogen content (14.8 and 12 kg N/ha respectively). Using the weight of nitrogen per hectare in each size class and the percentage release of nitrogen (difference in root nitrogen content before and following decomposition as a percent) from the roots the weight of nitrogen released from the roots over time was calculated. All the treatments showed a gradual increase in the weight of nitrogen released over time, however, this was not significant. All the treatments had a large error due mainly to the high degree of spatial variability in the root distribution of each size class, although this was more evident in the largest roots.

After 500 days of decomposition the greatest release of nitrogen was from the largest A.pulchella roots (611 gN/ha) followed by size class 2 (294 gN/ha) and size class 3 (250 gN/ha). (Table 2). The greatest release of nitrogen from A.lateriticola roots was from intermediate size classes 2 and 3 (158 and 156 gN/ha), with the largest roots, 4.5mm diameter having the smallest release of nitrogen (110 gN/ha). (Fig. 20).

The termite Heterotermes ferox (Froggatt) played an important role in the breakdown of legume roots. The termite mainly consumed the woody tissue although it appeared that they would also ingest bark to reach the wood. The wood of legume root contained approximately 40% of the total root nitrogen per hectare.

3 (a) 3. Inorganic nitrogen and survival of P.cinnamomi in soil under burnt and unburnt A.pulchella, A.lateriticola and B.grandis stands.

The previous study showed that the root system of three year old A.pulchella and A.lateriticola stands, with 19 and 13 kgN/ha to a 6cm depth respectively are an important nitrogen store in the jarrah forest. This legume nitrogen store is larger than that from 4 year shrubs < 1.5m (11.6 kgN/ha) and understorey trees

Fig. 19. (a) Weight (kg) of root and (b) weight (kg) of nitrogen per hectare of four root size classes of three year old Acacia pulchella and A.lateriticola to a depth of 6cm. Each value is the mean of 15 replicates ± standard error. Size classes, 1. > 4.5 mm diameter
> 2.5 < 4.5 mm
> 0.5 < 2.5 mm
< 0.5mm

FIG. 19

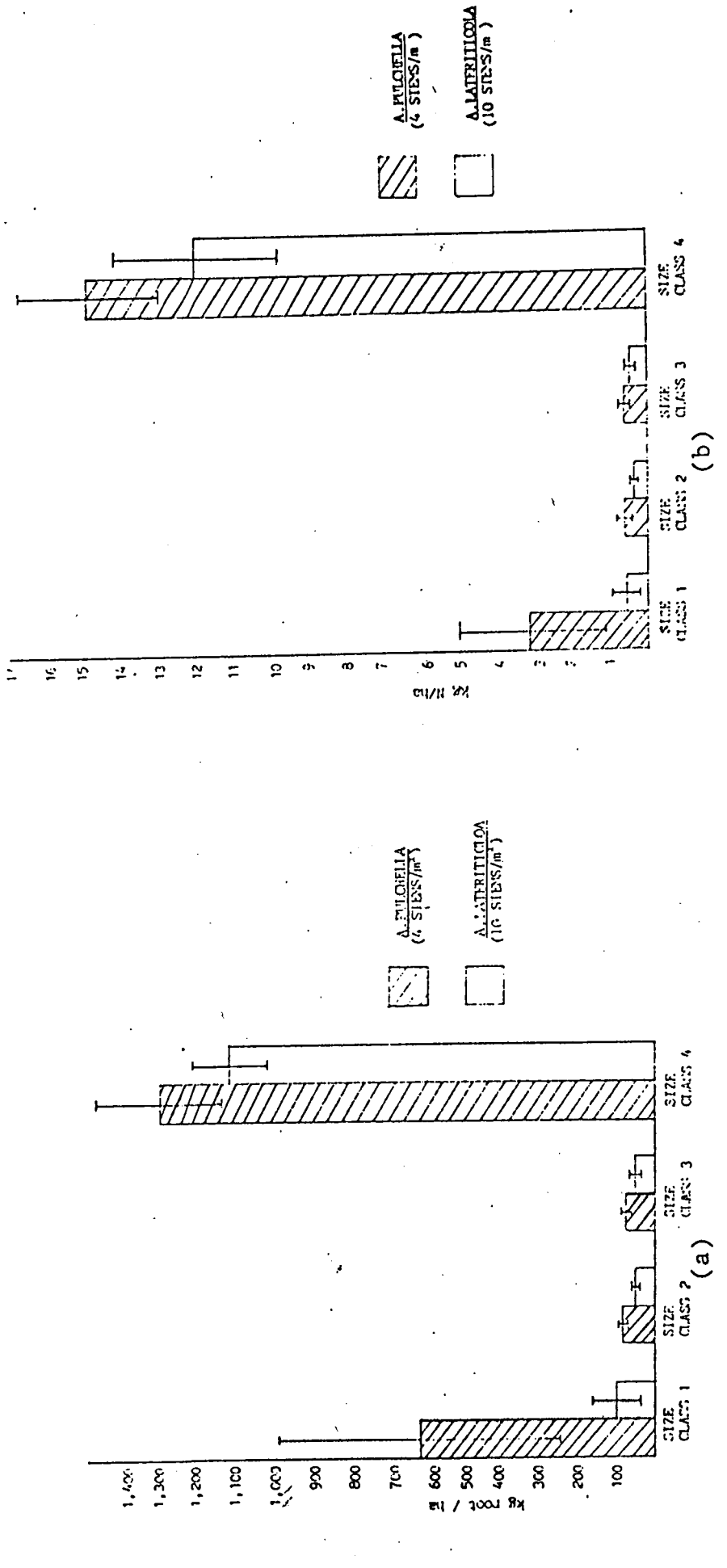

A.FULGIELLA (4 SITES/m²)
A.LATERITICOLA (10 SITES/m²)
SIZE CLASS 1
SIZE CLASS 2
SIZE CLASS 3
SIZE CLASS 4
(a)
(b)

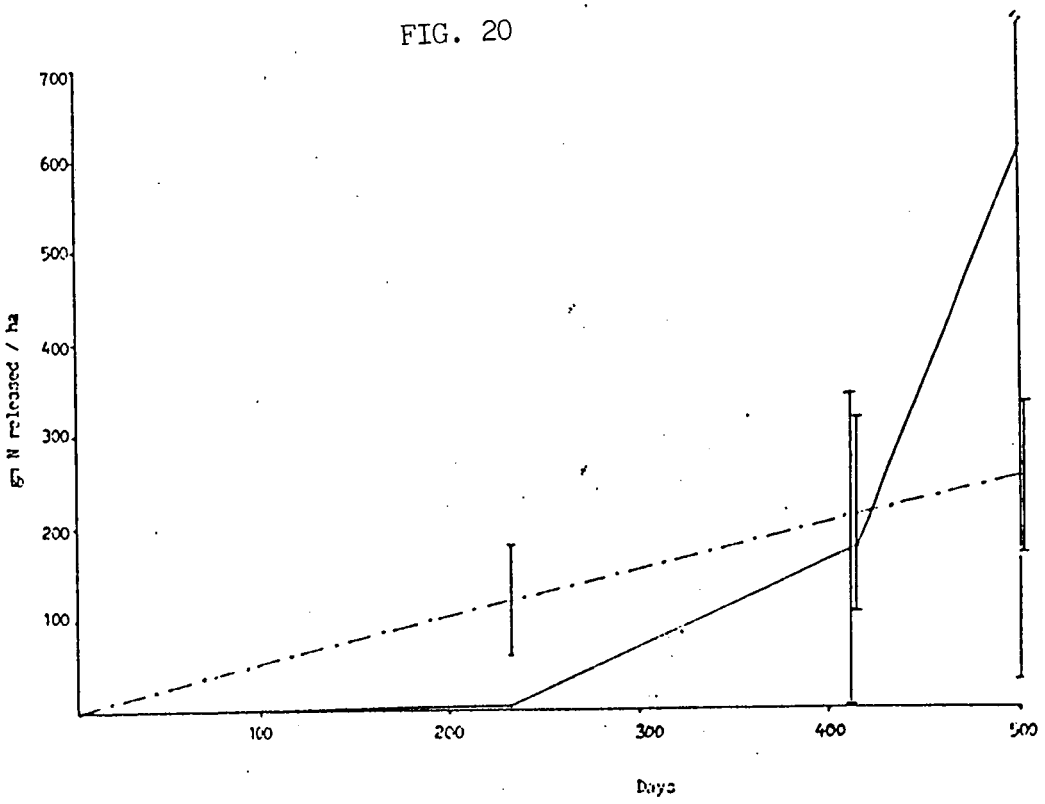
TABLE 2. Weight of nitrogen (gm) released per hectare for roots of Acacia pulchella, A.lateriticola Size classes 1, 2 & 3 in the top 6cm of soil, after 500 days decomposition.
Each figure is the mean of 12 replicates ± standard error.

SPECIES	SIZE CLASS 1	2	3	Total
A.pulchella gm N/ha	611 ± 579	294*	250 ± 135	1,155
A.lateriticola gm N/ha	110*	158 ± 85	156 ± 106	425

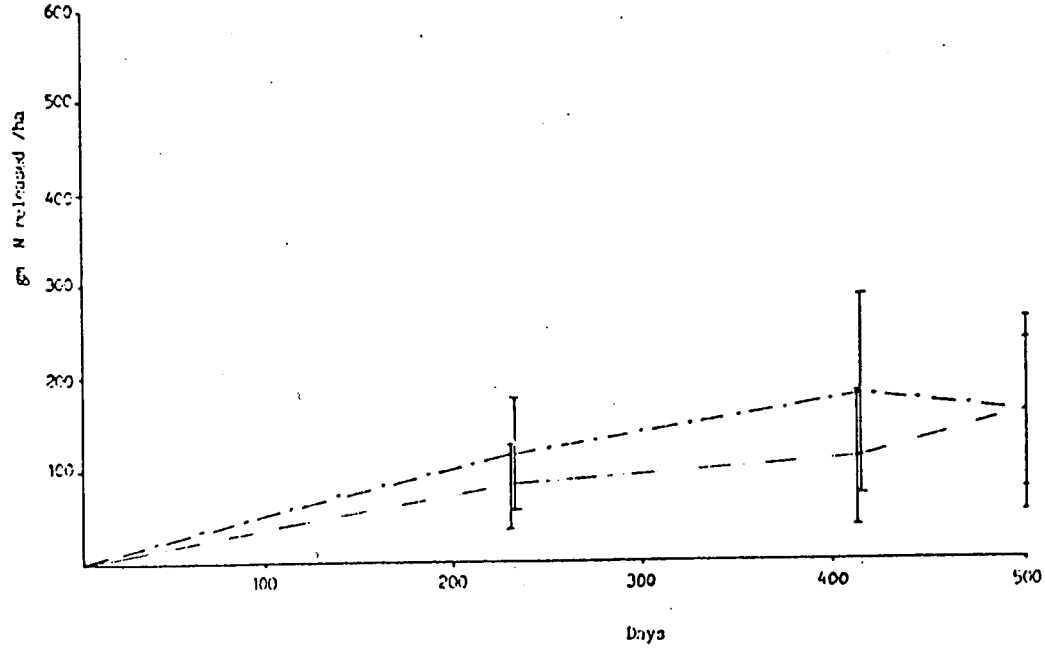
* Calculated using rate of weight loss from the other species of the same size class.

FIG. 20. Weight of Nitrogen released per hectare from (a) Acacia pulchella roots of size classes 1 and 3 (b) A.lateriticola roots of size classes 2 and 3, at three sample times over 500 days decomposition. Each value is the mean of 12 replicates ± standard error._____= Size class 1, _____ = size class 2, _._. = size class 3.

FIG. 20


700
600
500
400
300
200
100
gm N released / ha
100
200
300
400
500
Days

(a)


600
500
400
300
200
100
gm N released /ha
100
200
300
400
500
Days

(b)

> 1.5m (9.9 kgN/ha) but lower than the litter layer (51 kg/ha). The effect on P.cinnamomi, of nitrogen released from legume roots was investigated in a field experiment.

Soil ammonium levels in spring varied with sampling time and year. In 1980 ammonium levels increased from 2 - 4ppm to 3 - 10ppm but decreased from 3 - 10 to 2 - 5ppm in 1981. In both years ammonium levels in burnt and unburnt A.pulchella stands was greater than burnt or unburnt B.grandis. Nitrate levels were low in both years (0 - 1ppm). In 1980 burnt and unburnt legume plots had higher nitrate levels than Banksia plots, but in 1981 there was no significant difference between plots. There was no significant difference in pH between plots with the mean reading being 6 $\pm$ 0.5. In both years survival of P.cinnamomi in soil and inoculated B.grandis wood was greater under B.grandis in the light intensity burn area, than under B.grandis or legume in the medium intensity burn area. In the medium intensity burn area there was no consistent difference in survival of P.cinnamomi between legume or Banksia plots.

In 1980 and 1981 P.cinnamomi survival was not correlated with soil inorganic nitrogen (NH_3 or NO_3) or temperature. However, in both years the overriding factor determining P.cinnamomi survival was moisture; there was a significant positive correlation between soil moisture and colonies of P.cinnamomi/gm dry wt. of soil.

3 (a) 4. Sporangium formation in soil from burnt and unburnt A.pulchella A.lateriticola and B.grandis stands in a controlled environment.

Undisturbed soil samples were removed from burnt and unburnt legume and B.grandis plots in early Autumn, 1981. The top five centimeters of core was transferred to Buchner funnels and held at -1 kPa suction and 22 - 26°C.

There were significantly lower numbers of sporangia formed after four days in cores from beneath unburnt A.pulchella than B.grandis. For both legumes and B.grandis fewer sporangia formed in cores from burnt

than unburnt areas. There was no effect of canopy type or burning on zoospore release of hyphal lysis.

3 (a) 5. Relationship between inorganic nitrogen and survival of P.cinnamomi in soil amended with A.pulchella, A.lateriticola and B.grandis roots in a controlled environment.

The effect of soil amendments of legume and banksia roots on soil inorganic nitrogen and on the survival of P.cinnamomi and jarrah seedlings was investigated in a glasshouse experiment. Mulched roots of A.pulchella, A.lateriticola and B.grandis were added to a conducive Murray road soil, a yellow sandy gravel of the Dwellingup unit. Two amendment levels, 5% and 20% (on dry weight basis) were used for each of the three species. The control was soil with no root amendments. Each treatment was divided into three decomposition stages ; 2 - 7 months, 8 - 14 and 17 - 20 months.

The 20% legume root amended soils had the highest ammonium levels and the lowest P.cinnamomi levels. Levels of P.cinnamomi were suppressed in soils with ammonium levels as low as 2.5ppm (Fig. 21). In contrast, B.grandis root amended soils had the lowest levels of ammonia and highest levels of P.cinnamomi. Ammonium levels was significantly negatively correlated with log of P.cinnamomi colonies/gm dry weight for 5 of the 7 sampling times (Fig. 21). The lowest number of jarrah deaths occurred in the 20% legume root amended soil.

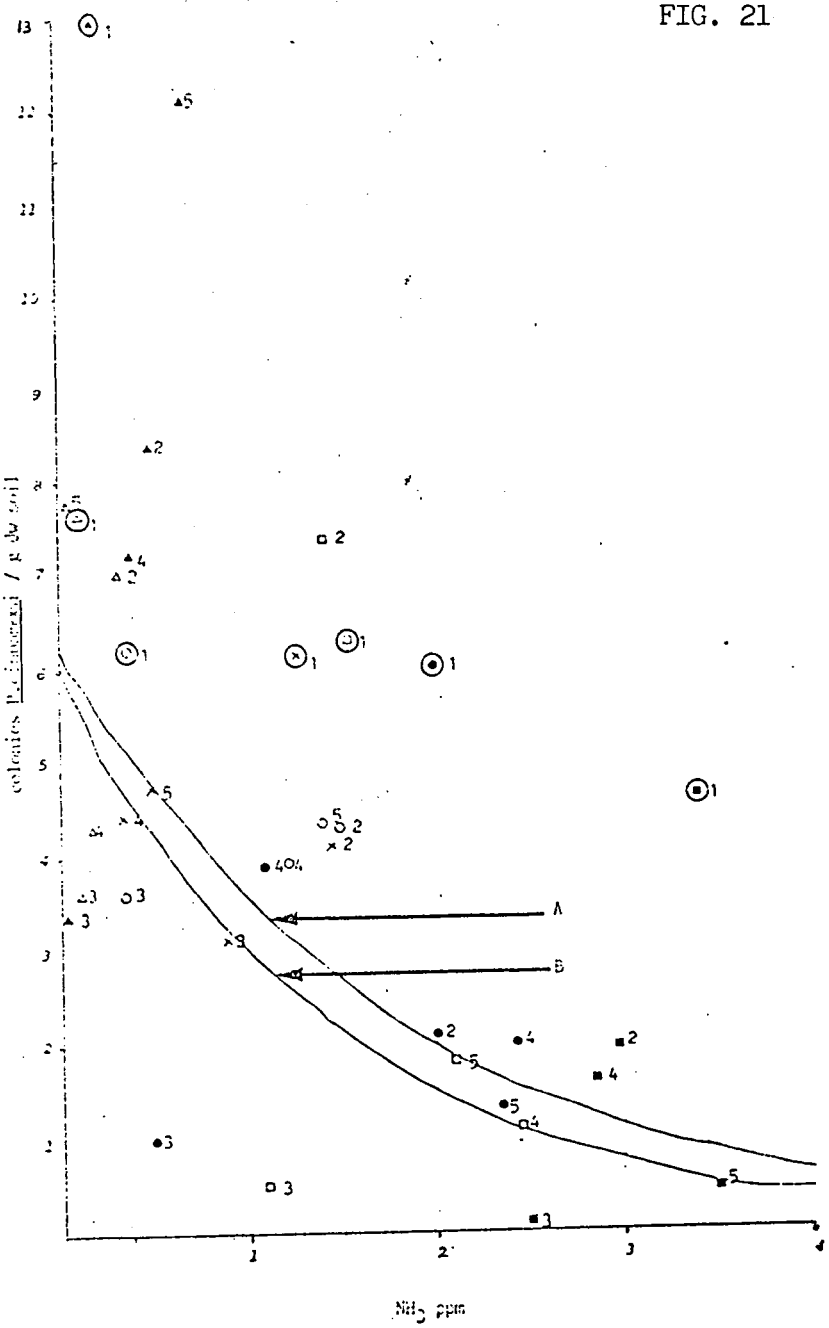
3 (b) Banksia grandis Ecology.

B.grandis is a major factor contributing to the spread and intensification of the disease. The results of our most recent research described above indicate that, on free drained upland sites which do not have sheet laterite near the surface, movement of the fungus through soil is almost totally dependent on the presence of Banksia.

Over a period of two years a detailed study of the ecology of this species has been carried out. Six equations which describe Banksia growth reproduction and survival have been obtained from field measurements. This data has been incorporated into a computer model developed in co-operation with Dr.D. Bell. The model is now complete and we believe accurately simulates the growth and development of the B.grandis understorey in the Jarrah Forest.

Fig. 21. Relationship between the independant variable -NH_3, with the dependant variable - colonies of Phytophthora cinnamomi g dw soil.
A = LOGY = a + bX, using pooled results of all 5 significant sample times r = 0.509.
B = LOGY = a + bX using pooled results of 4 significant sample times. Sample 1 was excluded as it had higher levels of P.cinnamomi compared to other sample times. r = 0.606.
1 = sample 2 of stage 1, 2 = sample 2 of stage 2, 3 = sample 1 of stage 1, 4 = sample 3 of stage 3, 5 = sample 3 of stage 3.

FIG. 21



The rationale behind the development of the model is that it permits rapid evaluation of various approaches to controlling the B.grandis understorey in the forest.

Various management options (e.g. burning, herbicide application, etc.) are now being tested.

In Fig. 22 a simulation of the development of a B.grandis stand following cutting from the virgin forest condition is illustrated. In Fig. 23 the effect of burning at a low intensity and moderate intensity at a six year interval is shown.

This work in conjunction with further field studies indicates that it will be possible to maintain the B.grandis understorey at a low level by practical silvicultural methods.

Fig. 22. Simulated development of the Banksia grandis understorey from typical virgin stand structure to typical stand formed five years after initial logging took place. In this simulation the forest was burnt every seven years with a low intensity fire.

FIG. 22

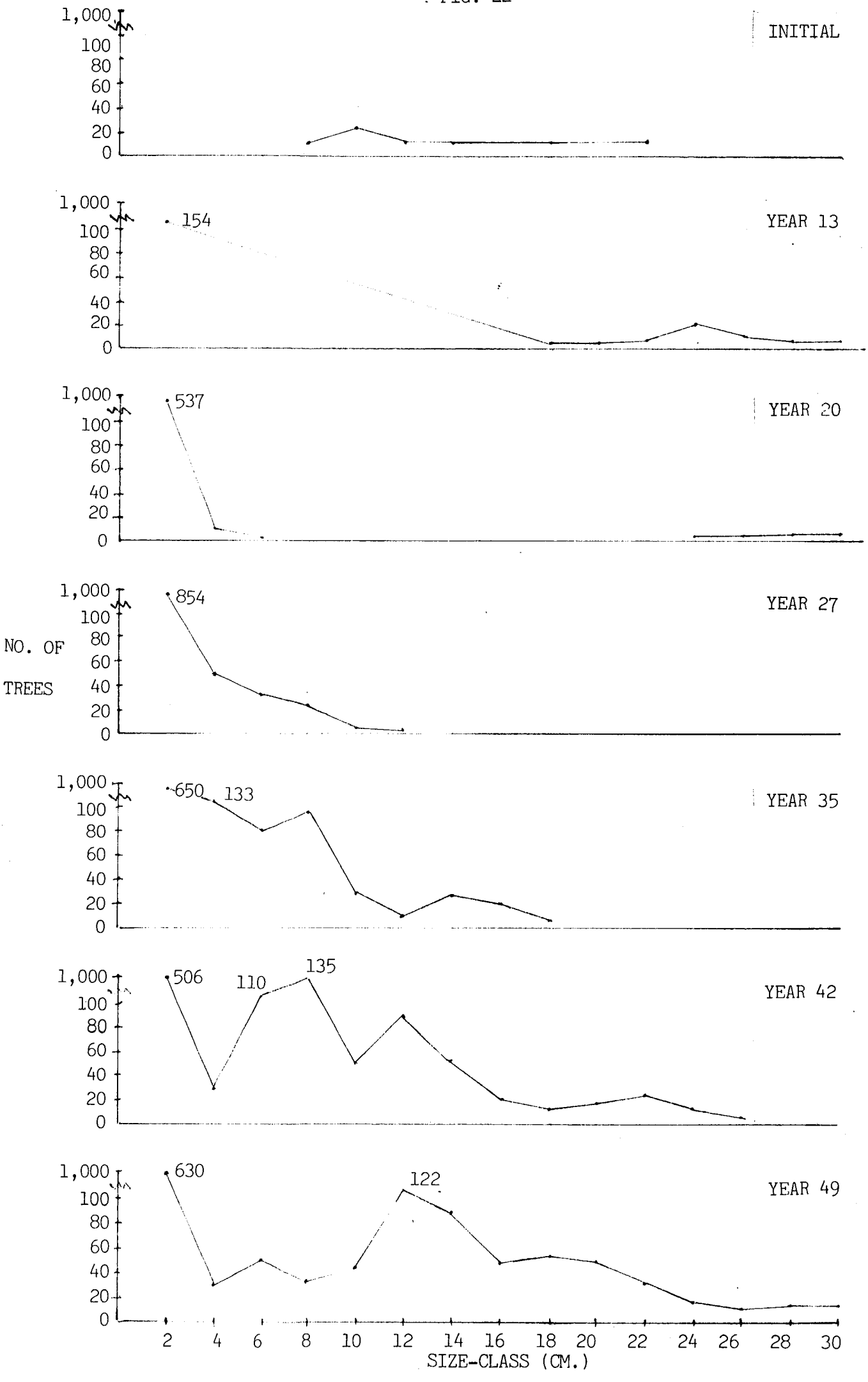

INITIAL
YEAR 13
154
YEAR 20
537
YEAR 27
854
YEAR 35
650
133
YEAR 42
506
110
135
YEAR 49
630
122
1,000
100
80
60
40
20
0
NO. OF TREES
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30
SIZE-CLASS (CM.)

Fig. 23. Simulation of the effect of a low intensity and moderate intensity burning regime on the maintenance and development of the B.grandis understorey.

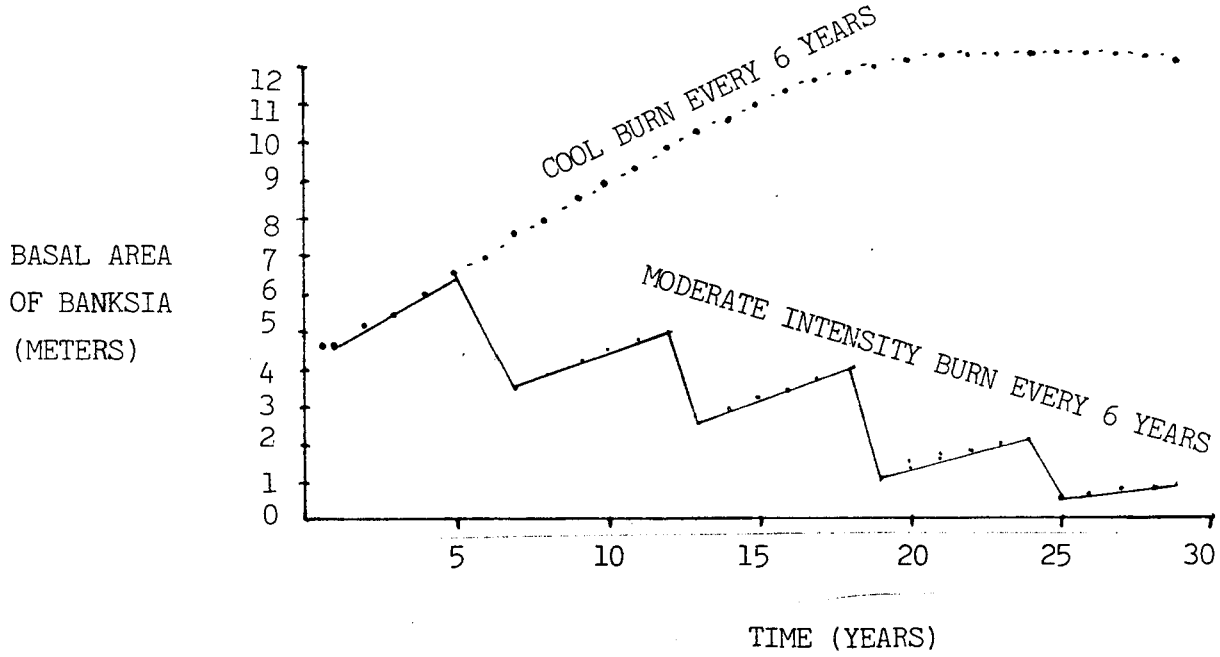

BASAL AREA
OF BANKSIA
(METERS)
12
11
10
9
8
7
6
5
4
3
2
1
0
COOL BURN EVERY 6 YEARS
MODERATE INTENSITY BURN EVERY 6 YEARS
5
10
15
20
25
30
TIME (YEARS)

FIG. 23