The Role of Banksia grandis Willd. in the Survival and Spread of P.cinnamomi in the Jarrah (Eucalyptus marginata Sm.) Forest of south Western Australia.

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Abstract.

Phytophthora cinnamomi was consistently recovered from the major horizontal and vertical roots and lower stem of Banksia grandis. The pattern of uphill extension of the disease can be explained by pathogen growth through the major horizontal roots. P.cinnamomi can exit through B.grandis bark but lateral spread through freely drained soil from infected tissues is limited. P.cinnamomi survived in dead B.grandis root pieces buried in the soil for two years.

It is unlikely that control of P.cinnamomi in the jarrah forest will be achieved in the presence of a dense B.grandis understorey.

Introduction.

An introduced soil borne pathogen Phytophthora cinnamomi (Rands) has been identified as the causal organism of a major disease (Jarrah Dieback) of the Jarrah (Eucalyptus marginata Sm.) forest of south Western Australia. The fungus causes mortality of a number of the understorey and shrub components of a forest and in many areas has caused extensive mortality of jarrah, the principal overstorey species (Podger, 1972).

Banksia grandis (Willd.) is the major component of the understorey on most of the freely drained upland sites in the forest, and commonly occurs in dense thickets. Prior to logging, early reports from forest surveyors indicated that B.grandis occurred at a relatively low density in the virgin forest (Western Australian Forests Department. unpublished). The proliferation of the understorey has been attributed to the decrease in competition caused by logging and soil disturbance which favours seedlings establishment and growth. (Shea unpublished).

B.grandis is highly susceptible to P.cinnamomi and preliminary research has shown that this pathogen can invade the lower stem of this species (Shea, 1979). B.grandis is usually the first species to

be killed following the introduction of the pathogen to uninfected forest. Within one to three years following the establishment of a new infection, mortality occurs, and within five years the perimeter of the infection is clearly marked by dead or dying banksia. The extension of the disease upslope from an infection has been measured by recording Banksia mortality. (Shea and Dillon, 1980).

On upland free drained sites within the forest, the soil physical and microbiological environment is suitable for sporangial formation and release for relatively brief periods in autumn and spring and the capacity of the fungus to survive in soil over summer when soil moisture potentials are less than 15 bar is negligible (Shea, 1975; Shea and Dillon, 1980). Consequently P.cinnamomi is a transient soil inhabitant in freely drained soils in the jarrah forest. It is frequently difficult to recover the pathogen from the surface soil horizons even in severely diseased areas when soil moisture levels are not limiting (Shea and Dillon, 1980). This suggests that a major factor contributing to the spread and intensification of P.cinnamomi is the presence of highly susceptible host whose major root systems can be invaded by the pathogen. In this paper we report on further studies on the role of B.grandis on the survival and spread of

P.cinnamomi in the forest.

Materials and Methods.

1. Distribution of P.cinnamomi in infected B.grandis.

A large (3 m high and 20 cm in diameter) B.grandis tree which had died in the previous 4 - 6 weeks and which was located in the perimeter of an established infection, was excavated in mid winter and bought to the laboratory for dissection. Two centimeter cross sections were removed from the stem at the soil surface, 20 cm and 40 cm above the soil surface, and from all the major roots at intervals of approximately 10 cm to a distance of 60 cm from the base of the stem.

Samples of the bark and wood from each cross section were cut, surface sterilized in 70% ethanol for 30 seconds, washed in distilled water and plated on selective agar (Tsao and Guy, 1977) and incubated at 25°C for two weeks. The samples were examined for the presence of the fungus every two days.

This procedure was repeated for a similar sized tree which had been killed approximately 12 months previously. This tree was sampled in mid summer.

2. Distribution of P.cinnamomi across the perimeter of an infection.

A 10 m by 3 m plot was located across the perimeter of a diseased area to include two banksia which were healthy,two recently killed trees (leaves retained), two trees dead approximately one year, and two trees which had been dead more than one year. Soil samples from the surface 10 cm layer of soil were taken on a 1 m x 1 m grid within the plot in late summer. Samples of the proteoid root system were also collected from around the base of each tree. The plot was then carefully excavated to expose the root systems of all the trees. The position of all roots was mapped and 2 cm thick cross sections of all roots and the stem at ground level were taken at intervals of approximately 20 cm.

Soil samples were sieved through a 2 mm sieve to remove large roots and stones. Fifty mls of distilled water was added to a 25 g sample of sieved soil mixed thoroughly and poured onto a 15 cm diameter petri dish containing selective agar (Tsao and Guy, 1977). A 5 g sample of the sieved soil was taken for dry weight determinations. The plates were incubated for 48 hours at 25°C and then the soil slurry was then washed off the plates. The plates were then incubated for a

further 24 hours and examined for the presence of P.cinnamomi. The root samples were assayed as described above.

3. Distribution of P.cinnamomi in the vertical root system.

The capacity of P.cinnamomi to invade the vertical root system of B.grandis was determined by excavating eleven recently killed trees and one tree showing no symptoms on five infected sites in August 1982. A backhoe was used to dig a trench on one side of the tree to a depth of between 1.5 to 3 m. The vertical roots immediately below the stump were then carefully exposed with trowels. Soil samples were taken from the surface soil surrounding the collar of each tree at 10 cm intervals immediately adjacent to each partially exposed vertical root to the bottom of the excavation. Soil samples were also taken at 10 cm depth intervals at a distance of 5 cm and 30 cm from major vertical roots. The samples were assayed for P.cinnamomi as described above. Two centimeter thick sections were taken from all exposed vertical roots and any horizontal lateral roots which were adjacent to the face of the excavation at intervals of 10 cm. Vertical roots were sampled to a depth of between 100 to 200 cm. Eighteen vertical roots and nineteen lateral roots were sampled and assayed for P.cinnamomi as described above.

4. Survival of P.cinnamomi in B.grandis roots.

The root systems of several healthy B.grandis trees were excavated and approximately 500 root sections, varying in diameter from 3.5 cm to 1.5, and 2 cm long were obtained. A 1 mm diameter hole was drilled through the centre of each root piece. The pieces were autoclaved twice at 121°C for 15 minutes. Each root piece was inoculated under sterile conditions by placing agar, on which actively growing mycelium was present, in the drilled hole. Three different Western Australian P.cinnamomi isolates were used (Isolate numbers - Sc. 22, Sc. 90, Sc. 72, CSIRO Division of Forestry Research, Kelmscott). The root pieces were then placed in glass petri dishes and incubated for four weeks at 25°C.

Five 10 m x 10 m plots were established in an area of healthy forest located on a freely drained site with typical lateritic podzolic soils. Within each plot three 2 m x 1 m sub plots were established. Fifty two pieces of each isolate were placed at a depth of 6 cm on a 20 cm x 20 cm grid to form a subplot within each major plot. The plots were positioned across the slope to prevent cross contamination.

The trial was established on the 15th August, 1979. Two root pieces were randomly removed from each sub plot (10 per isolate) every two weeks over the following 50 weeks and assayed for P.cinnamomi as described above. Fifty weeks after the establishment of the experiment, the number of samples was reduced to one per plot (5 per isolate). This sampling continued for the next eight weeks.

At the initiation of the trial two additional subplots of isolate number Sc. 72 were established. Forty eight root plugs and the soil surrounding each plug were removed and assayed for P.cinnamomi as described above in August 1981 two years after the trial had been established.

5. Transmission of P.cinnamomi from B.grandis tissue to soil.

The time taken for P.cinnamomi to vent from infected B.grandis tissue and the capacity of the pathogen to be transmitted from infected tissue to soil was investigated as follows:-

a) two 1 cm thick cross sections of the lower stem were cut from three recently killed B.grandis trees which were located in the perimeter of an infected area in late summer,

and returned to the laboratory for dissection. Samples 1 x 2 x 3 cm were cut from each cross section so as to include bark and wood along the longitudinal axis of the section. A sample of each cross section was plated to determine if P.cinnamomi was present. The cross sections which gave low recovery rates were discarded. The remaining samples were then dipped into paraffin wax to seal all the cut surfaces. The bark end of each section was unsealed.

Each section was placed with the bark face downwards into a 3 cm diameter open ended plastic vial containing uninfected sieved lateritic soil. The vials were then placed into either glass petri dishes into which a water level of 1 cm was maintained, or in scintered glass buchner funnel containing soil which was maintained at a soil moisture level equivalent to .02 bar by the method described by Duniway (1976). Twelve vials were removed randomly from each container at 3, 4, 9, 10, 12, 14 days after the trial was established. At each sampling time the moisture content of 3 g of soil from each vial was determined by gravimetric

methods and the remaining soil was plated directly on selected agar as described above. The wax was removed from each wood section and half the section was plated on selected agar.

b) Over a period of 12 months, several recently killed (leaves retained) B.grandis were excavated from the field and after removal of the upper stem and major roots to within 1 metre of the stump they were placed in sloped water tight boxes containing a sandy loam collected from an uninfected upland lateritic site. The boxes were placed in a glasshouse, maintained at 25°C and irrigated. Soil samples were taken at varying distances from the stump and at varying intervals after the initiation of the experiment and were assayed for P.cinnamomi as described above. Water collected from the lower bases of the boxes were also assayed for P.cinnamomi by direct plating.

c) In the mid summer of 1981 4 soil samples were taken with a 2 cm diameter modified King tube from a 20 cm diameter circle around the stumps of ten B.grandis trees which had been

recently killed, and 10 Banksia grandis trees which had been dead approximately 1 year located around the perimeter of an active infection. The samples from each tree were bulked and assayed for P.cinnamomi by the direct plating method described above. Soil removed from the sample holes were replaced with uninfected soil. The soil immediately surrounding each tree was then irrigated daily with 5000 mls of water. Four soil samples were taken from the area immediately adjacent to the stumps of all trees, at 4, 9, 13, 18, 23, and 28 days after the initiation of irrigation. Following each sampling the soil was replaced with uninfected soil. The soil from each tree was bulked and assayed for P.cinnamomi by direct plating.

Results.

1. Distribution of P.cinnamomi in B.grandis.

The distribution of P.cinnamomi in the large roots and lower stem of a recently killed B.grandis tree is shown in Figure 1. P.cinnamomi was recovered from both bark and wood samples throughout the major root system and lower stems of both of the trees sampled. Fungus was

detected at 20 cm from the soil surface in the trunk of the tree which had recently been killed but could not be detected from trunk samples taken above the soil surface in the tree which had been dead for approximately one year.

The distribution of P.cinnamomi in the roots and trunks of B.grandis trees located at the perimeter of an advanced infection is shown in Figure 2. The fungus was not detected in the soil, healthy trees, or the trees which had been dead more than one year. It was detected in most of the large roots and the lower stems of the recently killed trees and trees which had been killed within 12 months of sampling and from proteiod root samples taken adjacent to the collar of all dead trees.

P.cinnamomi was consistently recovered from the vertical roots of infected B.grandis. Recovery rates were higher in vertical roots than from surface lateral roots. In the tree which was sampled which was not exhibiting symptoms the fungus was recovered from the vertical root but was not recovered from the lateral roots or stump. P.cinnamomi was recovered consistently and at a high density from soil sampled immediately adjacent to infected vertical roots. The fungus

was recovered from soil sampled 5 cm and 30 cm from infected vertical roots but at a lower rate and density. The distribution of P.cinnamomi in B.grandis vertical roots and the soil adjacent to 5 cm and 30 cm from the vertical roots for one site is shown in Fig. 3. The distribution of P.cinnamomi was similar in each of the five sites.

2. Survival of P.cinnamomi in B.grandis roots.

The percentage recovery of P.cinnamomi over time from inoculated B.grandis roots is shown together with soil moisture data (Shea and Shearer, unpublished) derived from an adjacent site, is shown in Fig. 4. There was no significant difference ($p = .05$) in the recovery rates from root inoculated with different isolates. While the soil remained moist, the recovery rates were 100% but when rainfall ceased and the soil dried, the percentage recovery dropped to negligible levels. Following onset of rain in autumn the recovery rate increased to approximately 30% and remained at this level throughout winter and spring. There was a significant ($p = .01$) difference in the recovery rate over time (the recovery rates for weeks 26 - 30 were significantly lower). Four of the 48 root pieces sampled 2 years after the establishment of the experiment yielded P.cinnamomi and the fungus was recovery from the soil

surrounding 8 of the plugs.

3. Transmission of P.cinnamomi from B.grandis tissue to soil.

P.cinnamomi was detected in soils surrounding waxed infected B.grandis wood sections at 11 and 3 days after the sections had been placed in saturated soil and soil maintained at a moisture content equivalent to .02 bar respectively. The density of P.cinnamomi remained at a relatively low level in the saturated soil throughout the experiment but increased to .69 propagules per gram O.D.W. in the soil maintained at .02 bar. The fungus was consistently recovered from the Banksia tissue sections throughout the experiment.

P cinnamomi was detected in soil surrounding infected Banksia grandis stumps which were irrigated and maintained in the glasshouse at 25°C seven days after irrigation commenced. The density of P.cinnamomi in the soil surrounding the stumps increased in time, but the fungus was not detected at distances greater than approximately 10 cm from the stump unless the soil was disturbed. The fungus was not detected in runoff water until the soil was disturbed by creating a trench from the stumps to the base of the

sloping container. However, once the soil stabilized after the formation of the trench and soil movement in the runoff water ceased, no further recoveries were obtained.

P.cinnamomi was not detected in soil samples taken around the base of infected Banksias prior to irrigation. Nine days after irrigation commenced the fungus was detected in soil from three trees. However, it was only after 28 days of daily irrigation that recovery rates increased. (6 out of 10 recently killed, and 1 out of 10 trees which had been dead approximately one year). The density of P.cinnamomi propagules in soil surrounding the trees which had been dead 1 year remained low throughout the sampling period (< 3 propagules per gram O.D.W.) and propagule density in soil surrounding the recently killed trees remained relatively low until the last sampling date (28 days) when it averaged 47 propagules per gm. O.D.W.

Discussion.

Banksia grandis has a well developed tap root which accounts for its ability to maintain relatively high levels of transpiration (Shea unpublished) during the summer months when the surface

(< 1 meter) soil horizons are at soil potentials less than 15 bar. Vertical roots were not observed forming from horizontal roots but in some trees lateral roots were observed to change from the horizontal to the vertical at distances of one to two meters from the stump. Frequently the main vertical root formed beneath the stump bifurcated at depth. Numerous horizontal roots were formed from the vertical root at depths to 1 - 1.5 m. These roots were less than 1 cm in diameter and no major horizontal roots were observed at depths greater than 40 cm. The trees have a well developed horizontal root system. Typically average sized trees have from 2 to 5 major horizontal roots which are formed at the base of the stem at approximately 10 to 30 cm below the soil surface. The extent of the horizontal root system varies with the size of the tree but medium size trees' horizontal roots may extend from between 2 to 3 m from the stump. In dense B.grandis thickets there is a continuous layer of proteiod roots which occurs at the interface of the soil/litter layer .

The results of this study demonstrates the capacity of P.cinnamomi to totally invade the root and stem tissue of B.grandis. The typical pattern of disease extension which has been observed

by recording symptom development in the Banksia grandis understorey around the perimeter of infections, can be explained by pathogen movement by mycelial extension through the major horizontal roots of this species. (This has been demonstrated in a separate series of experiments Shea and Deegan, unpublished). The presence of an extensive proteoid root mat maximizes the probability of root contact between the adjoining trees. Hence the opportunity for pathogen transfers from tree to tree is high.

Under favourable soil physical conditions P.cinnamomi can exit from the bark of infected Banksia from between 3 to 11 days. There is circumstantial evidence from this study that the pathogen extends mycelium into the soil. We were able to detect P.cinnamomi in soil surrounding infected B.grandis tissue which was maintained at a soil potential of .02 bar. It is highly improbable that zoospores would have been released at this soil moisture level (Duniway 1975).

The results of this study indicate that while B.grandis acts as a high reservoir of inoculum, extension of the pathogen from infected tissue into the soil is localized. Significant increases in

inoculum density only occurred after prolonged irrigation under optimum temperature conditions for zoospore formation. It is possible that zoospores could be carried in water over the soil surface although we could only demonstrate this when the soils were freshly disturbed. We have rarely observed overland flow of water on undisturbed jarrah forest sites. In a number of field studies conducted over several years downslope extension in the surface layer of soil of P.cinnamomi from infected B.grandis stem and root pieces was negligible (Shea and Shearer unpublished). Thus lateral spread of P.cinnamomi on undisturbed free drained sites appears to be dependent on mycelial extension within the roots of highly susceptible species.

In a more recent study it has been shown that P.cinnamomi can occur at a high density and be transmitted laterally at depths down to 1 m on sites where a concreted lateritic layer is present (Shea et al 1982; Shea et al 1983). Vertical transmission of P.cinnamomi zoospores may occur in soils provided the pore size is not limiting (Duniway 1976). However, this study demonstrates that B.grandis vertical roots can act as major channels for P.cinnamomi vertical transmission. Although the

fungus was detected at depth in soil removed from vertical roots, recovery rates were highest in the soil immediately surrounding the infected vertical roots. In a separate study we consistently observed B.grandis roots occupying the same root channels in a concreted layer of laterite as E.marginata roots, (Shea et al 1983), and we have also observed, in this study, a close association between jarrah and banksia vertical roots, It has been proposed that the destruction of the vertical roots of jarrah trees is the major process resulting in rapid death of this species on sites where P.cinnamomi can reproduce and is transmitted at depth because of the presence of a layer of concreted laterite which impedes vertical water movement (Shea et al 1982). It is likely that the presence of B.grandis on these highly susceptible sites contributes to the intensification of the disease by permitting vertical transmission of the fungus through vertical roots to the root channels within the concreted laterite. It is also possible even on sites where vertical water movement is not impeded that the presence of numerous infected B.grandis vertical roots in close association with jarrah roots may cause significant damage to jarrah's vertical root system.

In a number of studies (Shea 1975; Shea et al 1980) it has been shown that P.cinnamomi has a limited capacity to survive in soil on free drained sites in the forest through the summer months when soil moisture levels in the surface horizons remain below 15 bar for prolonged periods. This study shows that infected B.grandis roots and stumps provide a major reservoir of inoculum which enables the fungus to survive through the summer months. Survival in Banksia tissue over the dry summer may in part result from the maintenance of higher moisture levels in large roots and stumps. However, it is unlikely that the moisture level in the root pieces in which P.cinnamomi survived for two summers would not have reached a point at which they were at equilibrium with the soil moisture levels. It is possible that the difference in survival capacity of P.cinnamomi in roots and soils results from the difference in the rate of drying. Soil moisture levels in the surface horizons deplete rapidly once rain ceases, and it is possible that they reach critical levels for survival of the fungus before resistant spores are formed. Chlamydospores have rarely been detected in soil samples from infected free drained sites in the

forest (Shea and Shearer, unpublished) but have been observed in infected B.grandis tissue (Tippett - unpublished).

The increase in recovery rates in the Banksia root pieces following the onset of autumn rains could be attributed to recolonization following rewetting of the root tissue. The ability of P.cinnamomi to recolonize dead Banksia tissue in the presence of other soil microorganisms however, has not been demonstrated.

We suggest that it is unlikely that any control procedure would be successful in the long term while B.grandis remains a dense component of the understorey of the forest. We are currently investigating the role of other shrub and understorey components of the forest in disease spread and intensification. However, although other shrub and understorey species in the forest are susceptible to P.cinnamomi we have as yet not identified any species which has the combination of root structure, density of occurrence and high susceptibility which makes B.grandis one of the major factors contributing to the spread and intensification of the pathogen.

Acknowledgements.

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Literature Cited.

DUNIWAY, J. M., (1975) Water relations in safflower during witting induced by Phytophthora root rot. Phytopathology 65; 886-891.

DUNIWAY, J. M., (1976) Movement of Zoospores of Phytophthora cryptogea in soils of varying textures and matric potentials Phytopathology, 66; 877-882.

PODGER, F. D. (1972) Phytophthora cinnamomi, a cause of lethal disease in indigenous plant communities in Western Australia. Phytopathology 62: 972-981.

SHEA, S. R . (1975) Environmental factors of the northern jarrah forest in relation to pathogenicity and survival of Phytophthora cinnamomi. West. Aust. For. Dep. Bull. 85 83pp.

SHEA, S. R. (1979) Phytophthora cinnamomi (Rands). A collar rot pathogen of Banksia grandis Willd. Aust. Plant Pathol. 8: 33-34.

SHEA, S. R. and DILLON, M. J. (1980) Rate of Spread of Phytophthora cinnamomi Rand. infections in the Jarrah (Eucalyptus marginata Sm.) Forest. Forests Department of Western Australia, Research Paper 65.

SHEA, S. R., SHEARER, B. L. and TIPPETT, J. (1982) Recovery of Phytophthora cinnamomi Rands from vertical roots of Jarrah (Eucalyptus marginata Sm.) Australas. Plant. Pathol. 11 (3): 25-28.

SHEA, S. R., SHEARER, B. L., TIPPETT, J. T. and DEEGAN. P. M. (1983) Distribution, Reproduction and Movement of Phytophthora cinnamomi at various depths of soil on sites highly conducive to Jarrah Dieback in south Western Australia. (To be published in Plant Disease).

TSAO, P. H. and GUY, S. O. (1977) Inhibition of Mortierella and Pythium in a Phytophthora - Isolation medium containing hymexazol. Phytopathology 67: 796 - 801.

Fig. 1 Distribution of *Phytophthora cinnamomi* in recently killed *Banksia grandis* (*P.cinnamomi* was recovered in shaded areas).

Fig. 1. Distribution of Phytophthora cinnamomi in recently killed Banksia grandis (P.cinnamomi was recovered in shaded areas).

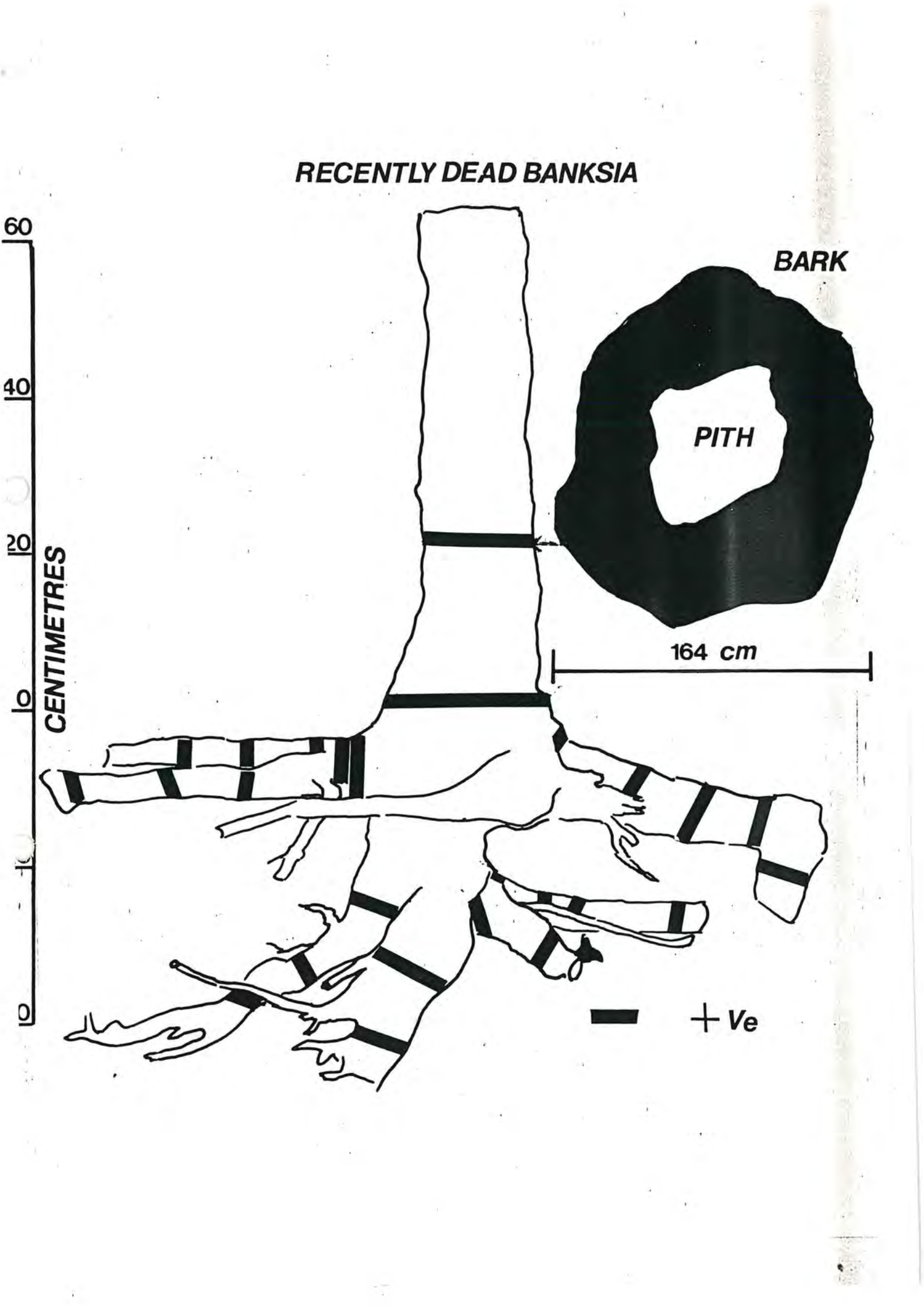

RECENTLY DEAD BANKSIA
60
40
20
0
CENTIMETRES
BARK
PITH
164 cm
+Ve

Fig. 2 Upslope of spread of *Phytophthora cinnamomi* infection measured by symptom development in the *Banksia grandis* understorey (After Shea and Dillon 1980).

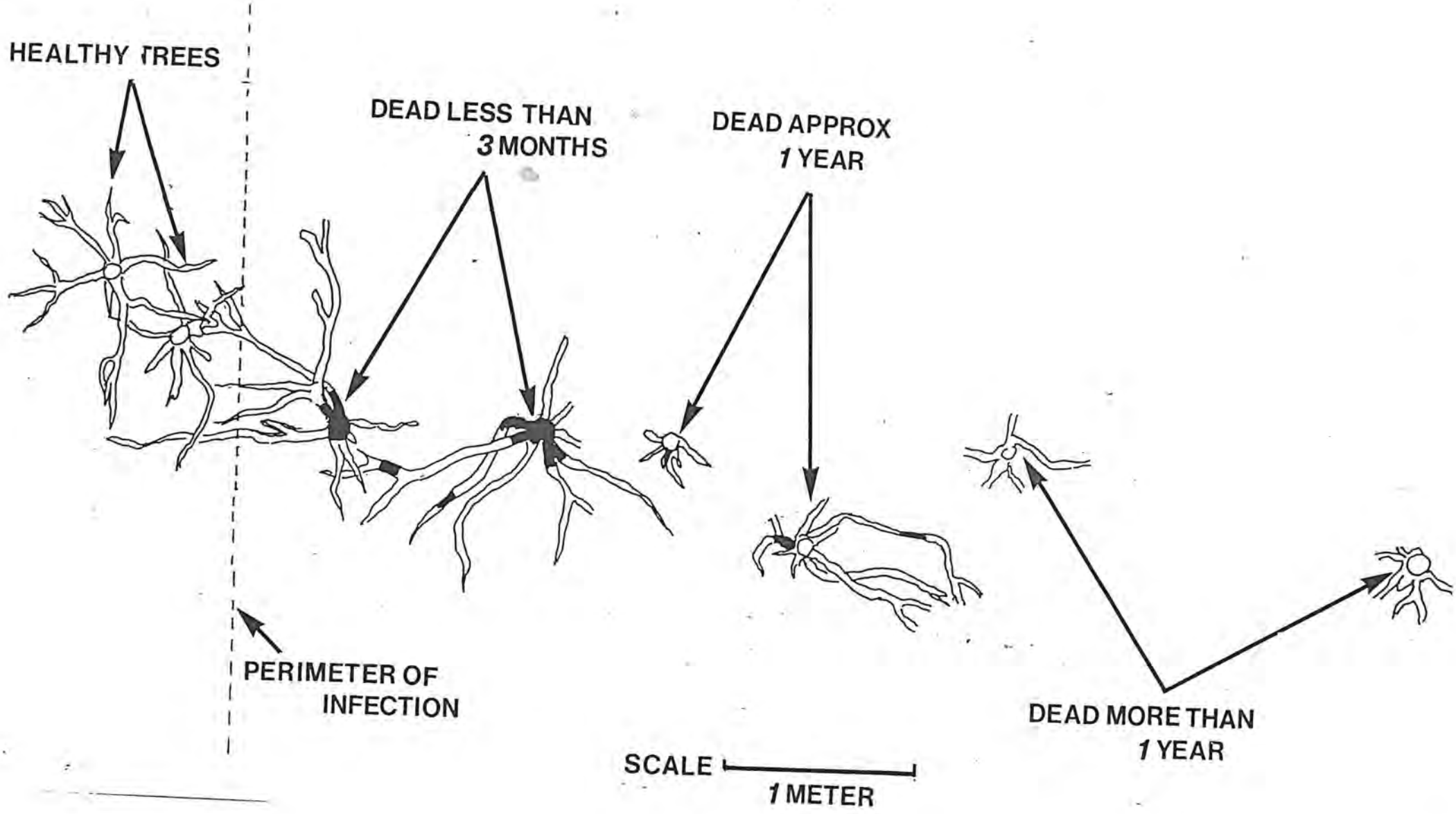

HEALTHY TREES
DEAD LESS THAN
3 MONTHS
DEAD APPROX
1 YEAR
PERIMETER OF
INFECTION
SCALE
1 METER
DEAD MORE THAN
1 YEAR

Fig. 3 Vertical distribution of *Phytophthora cinnamomi* in *Banksia grandis* and soil. (*P.cinnamomi* was recovered from shaded areas).

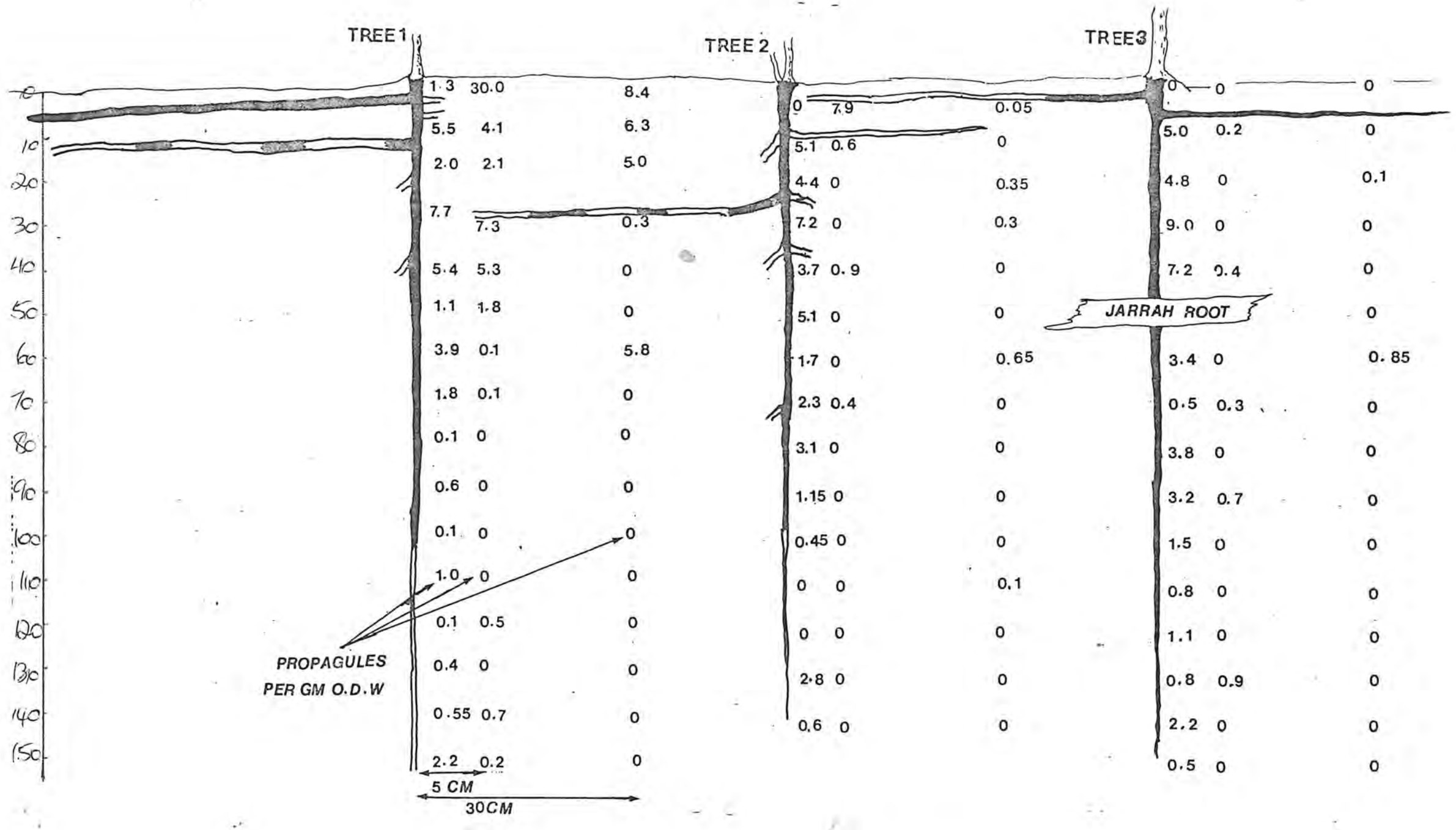
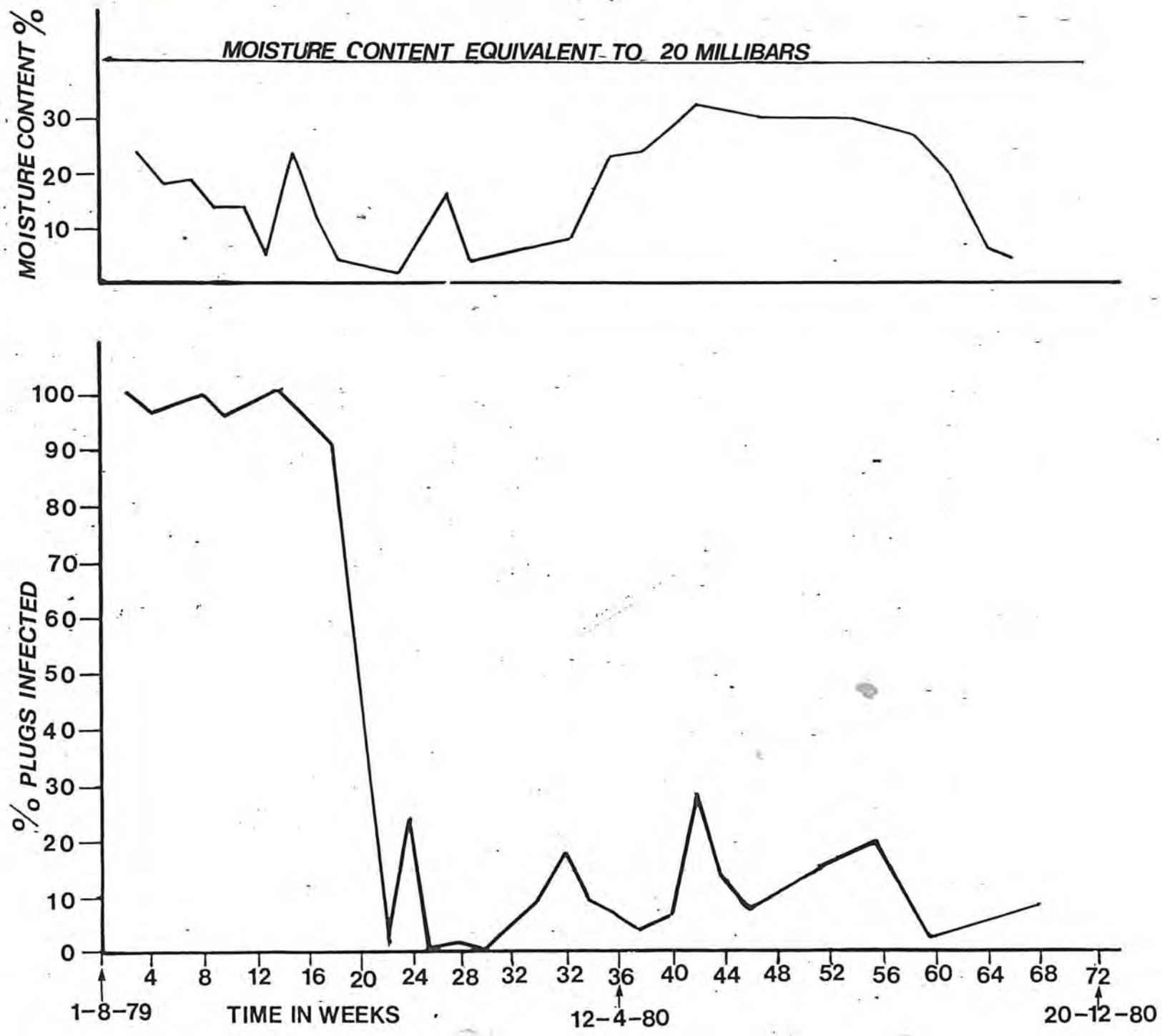

TREE 1
TREE 2
TREE 3
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
1.3 30.0 8.4
5.5 4.1 6.3
2.0 2.1 5.0
7.7 7.3 0.3
5.4 5.3 0
1.1 1.8 0
3.9 0.1 5.8
1.8 0.1 0
0.1 0 0
0.6 0 0
0.1 0 0
1.0 0 0
0.1 0.5 0
0.4 0 0
0.55 0.7 0
2.2 0.2 0
0 7.9 0.05
5.1 0.6 0
4.4 0 0.35
7.2 0 0.3
3.7 0.9 0
5.1 0 0
1.7 0 0.65
2.3 0.4 0
3.1 0 0
1.15 0 0
0.45 0 0
0 0 0.1
0 0 0
2.8 0 0
0.6 0 0
0 0 0
5.0 0.2 0
4.8 0 0.1
9.0 0 0
7.2 0.4 0
JARRAH ROOT
3.4 0 0.85
0.5 0.3 0
3.8 0 0
3.2 0.7 0
1.5 0 0
0.8 0 0
1.1 0 0
0.8 0.9 0
2.2 0 0
0.5 0 0
PROPAGULES
PER GM O.D.W
5 CM
30 CM

Fig. 4. Survival of *Phytophthora cinnamomi* in infected *Banksia grandis* root pieces over time.

FIG 4


MOISTURE CONTENT %
MOISTURE CONTENT EQUIVALENT TO 20 MILLIBARS
30
20
10
% PLUGS INFECTED
100
90
80
70
60
50
40
30
20
10
0
4
8
12
16
20
24
28
32
32
36
40
44
48
52
56
60
64
68
72
1-8-79
TIME IN WEEKS
12-4-80
20-12-80