Progress Report on

Phytophthora cinnamomi Research by

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and

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SUMMARY

1. Extensive field measurements of *P.cinnamomi* population densities on freely drained sites associated with natural infections and inoculations have shown that:-

a) the fungus has a very restricted distribution in the soil throughout most of the year.

b) during the summer months survival in soil is negligible.

c) the fungus can occur in the soil after the onset of winter rainfall and persist until the following summer.

d) in years of average or below average rainfall the fungus is confined to the zone immediately adjacent to the host in which it has over summered in.

e) transmission of the fungus from these "reservoirs" of inoculum is dependent on overland flow of water during the period in which zoospores are being produced.

f) a particular sequence of climatic events involving warm wet periods followed by rainfall of sufficient intensity to cause overland flow is required before the fungus can achieve widespread distribution on freely drained sites.

2. The relatively poor capacity of *P.cinnamomi* to survive in soil when it is dry, its dependence on susceptible host tissue to oversummer, and the necessity for overland flow to achieve widespread distribution suggests that the intensity of the disease on free drained sites can be markedly reduced by :-

a) minimizing disturbance which involves transmission of the fungus in soil and minimization of soil disturbances (eg. soil compaction) which favour overland flow.

b) reducing the number of infection reservoirs by reduction of the density of the *B.grandis* understorey (other hosts species do act as reservoirs of inoculum but their density is much less than that of *B.grandis*).

3. On moisture gaining sites within the forest conditions, for fungal survival and reproduction occur for long periods and it is unlikely that any susceptible species will survive in these sites in the long term. The presence of *P.cinnamomi* at relatively high densities throughout the year in soil in these sites identifies them as major sources of inoculum for accidental transmission.

4. Sporangial production on freely drained sites is restricted to autumn and spring. Although sporangia can be produced following relatively short periods when the soil is warm and wet, a particular sequence of climatic events is necessary before zoospores are released and transmitted over significant distances. The factors effecting the production of zoospores are still only partially understood but involve:-

a) soil moisture levels less than 150 mb

b) relatively high (>15°c. ?) soil temperatures for periods exceeding ? hours.

c) the presence of soil stimulants at sufficient density to stimulate sporangial production.

d) the occurrence of rainfall following the formation of sporangia to stimulate release of zoospores.

e) the occurrence of rainfall of sufficient intensity to cause overland flow at the time when sporangia are suitable for the release of zoospores.

Research is currently being carried out which will permit a precise definition of the sequence of climatic events required to generate zoospores. When this information is available it will be possible to relate it to previous seasonal and yearly climatic events.

5. There are a number of site (eg. soil type) and management factors. (eg. ponding of water - baring of soil) which can have a profound effect on sporangial production and zoospore release and transmission. These factors are currently being investigated.

6. There are unknown factors effecting the capacity of the soil to stimulate sporangial production. Identification of these factors and their ecology is a major research priority.

7. Banksia grandis has been identified as a major contributor to the spread and intensification of the disease. Parrallel studies on the ecology of this species have shown that it is possible to reduce its density to required levels using silvicultural techniques.

The identification of other species which have a similar function as B.grandis is an urgent research priority.

8. Studies on the effect of an A.pulchella understorey have confirmed that it does have a suppressive effect on the fungus. It is important to emphasize however, that the promotion of this species as a potential control measure can only form part of the control strategy. The long term effectiveness of a legume understorey in providing control of the disease will only be determined when we have more information on the basic epidemiology of the disease. For example, it is possible that the positive effect of a legume understorey could be over ridden by abnormally high rainfall. The effect of other legume species on spread and intensification of the disease is currently being investigated.

9. P.cinnamomi can invade the large suberized roots and collar region of jarrah. It is possible that the extensive jarrah mortality observed during the 1950's and

early 1960's was caused by P.cinnamomi acting as a collar rot pathogen. It is possible that the variation in degree of invasion observed is due to genetic factors. However, host physiology could be a major factor effecting susceptibility. Identification of the factors affecting the capacity of P.cinnamomi to completely invade jarrah is a major research priority and is currently being investigated.

10. Overall the research carried out suggests that our previous assessments of the potential for control of P.cinnamomi in the jarrah forest have probably been over pessimistic.

Our assessment is that there is an excellent chance that we will be able to maintain jarrah forest on free drained sites over substantial areas with the following qualifications:-

- *a) Disturbance of the soil must be kept to a minimum and intensive hygiene measures sustained.*
- *b) The B.grandis understorey must be reduced.*
- *c) It is possible that abnormally high rainfall may cause severe intensification of the disease but the probability of this happening will be markedly reduced if disturbance is minimized and if the B.grandis density is reduced.*
- *d) The factors which cause P.cinnamomi to act as a collar rot pathogen may not be suject to control by cultural treatments. If this is then management practices which are aimed at reducing the density and distribution of P.cinnamomi in the soil may be ineffective.*

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8. *Infection of B.grandis by P.cinnamomi*

9. *Biology and ecology of B.grandis*

10. *P.cinnamomi on Bauxite pits.*

2. Introduction

The following is a summary of the research carried out on Phytophthora cinnamomi at the Dwellingup and Kelmscott research stations over the period June 1979 - December-1980. Part of the research was conducted jointly by the two stations but a proportion was carried out as seperate projects. Research into chemical aspects of Acacia pulchella which involved considerable imput from the Kelmscott research station, was carried out in co-operation with Dr. Kagi, W.A.I.T. Similarly, a major study of the ecology of Banksia grandis andthe use of Silvicultural technqies to control its density was undertaken in co-operation with Dr. Bell, Unviersity of W.A. Both these studies have been reported by Dr. Kagi and Dr. Bell and are only briefly summarized in this report.

Some of the projects were directly funded by the foundation but in the main the technical assistants provided from Foundation funds worked as part of the existing dieback research program. Hence, apart from two specific projects which were carried out by the two research assistants funded by the Foundation no attempt has been made to seperate "Foundation funded research" from the general research program.

3. Seasonal Studies of Population densities, sporangial formation and Stimulation, and Root Infection, 1979 - 80.

It was hypothesized that P.cinnamomi was an ephemeral soil inhabitant and that the timing of fungal activity in relation to host growth and the physical environment was a major factor affecting the epidemiology of the disease. Previous studies had been restricted to studying a limited number of factors over time because of logistical constraints. The resources of the two stations were combined to study a number of factors at one site in relation to the disease. These studies were supplemented by co-operative studies with a post-graduate student from Murdoch University (Miss D. Schilds) and a specific study on zoospore survival and root infection by Dr. B. Dell. (Murdoch University).

In summary the study involved measurement of the following factors over a period of 18 months at a typical dieback site located 30 km NE of Dwellingup:-

- Rainfall, soil moisture, soil temperature. Soil moisture and temperature was recorded within the "old dead zone", at the "green line" and under a dense jarrah regrowth stand.
- P.cinnamomi soil population levels:-
    - a) in the "old dead zone" (random samples)
    - b) at the "green line" (random samples)
    - c) adjacent to the stumps of dead banksia.

- *Sporangial formation on mycelial discs.*
- *The capacity of soil to stimulate sporangial production*
- *The timing of root growth of Eucalyptus marginata*
- *Chlamydospore survival*
- *Zoospore survival and root infection*
- *Survival of P.cinnamomi in infected Banksia grandis roots over time*
- *Measurement of the capacity of P.cinnamomi to spread from infected B.grandis roots*

Several aspects of this study are being prepared for formal publication and the results are therefore only briefly summarized.

3.1 Population density of P.cinnamomi in soil.

The recovery rate of P.cinnamomi from the soil in both the "old dead zone" and along the "green line" was low throughout the period of measurement. (Table I)

TABLE I

	No. of sample times	Total No. of Samples	Total No Samples positive	% Recovery
OLD DEAD ZONE	17	1360	0	0
GREEN LINE	17	1360	10	.7

It is unlikely that this was a sampling artifact since the recovery of P.cinnamomi in a paired study (using the same techniques) which involved sampling of plots which had been irrigated was high.

The fungus was recovered at relatively high population levels in soil adjacent to Banksia's which were recently killed. (leaves still retained.) There was a distinct seasonal trend in recovery rates in samples taken adjacent to dense and dying B.grandis Fig. 1. The period of high recovery corresponded with a period of increased sporangial production, Fig. 2, and high soil moisture and temperature levels.

The results of this study confirm our hypothesis that P.cinnamomi is in an empheral soil inhabitant on freely drained sites. While it is possible to explain the death of the highly susceptible components of the jarrah forest such as B.grandis it is difficult to account for the death of jarrah by P.cinnamomi at this site. A number of live jarrah trees are still persisting in the "old dead zone" at the experimental site. It is unlikely given that the recovery rate in th's zone was low,that P.cinnamomi could cause significant damage to these trees unless there is a major increase in soil population density induced by abnormally heavy rainfall in spring or autumn.

3.2 Sporangial Production

The pattern of seasonal sporangial production(Fig. 2)corresponded to that observed in previous years and in sites which sporangial production was being monitored under legume stands and adjacent control sites (see section 3).

We are now confident that the capacity of the fungus to produce sporangia is restricted to relatively brief periods in autumn and spring.

During the past 3 years there has been below average rainfall in spring and autumn. We believe, however, that late spring rainfall will generate large quantities of sporangia. This is supported by the results of the research carried out during the current year (see section 4.4.2). The fungus can produce large number of sporangia in autumn but in the years during which sporangial production has been measured autumn rainfall has been late and the period of sporangia production has been short. It is possible however, that early prolonged autumn rainfall could generate large numbers of sporangia over a extended period.

3.3 Stimulation of sporangial production

Jarrah root growth and new root initiation was recorded in 3 zones at the experimental site - "old dead zone","dying Banksia zone"and adjacent healthy forest. Growth and initiation was recorded by observing roots growing om small plastic trays covered by nylon mesh,soil and litter. Forty seven trays were established.
The growth and root initiation pattern was markedly seasonal Fig.3 . Root growth was restricted to the warm wet periods in autumn and spring. There was no significant difference in the pattern of root growth between the three sites. It is possible that the occurrence of root growth during the period when P.cinnamomi has the capacity to produce sporangia is a factor contributing to the disease.

3.5 Root infection

a) Zoospore inoculations of the fine specialized roots of jarrah were carried out in co-operation with Dr. Dell during the spring of 1979. The objective of the trial was to determine:-

1. the components of the surface root system of jarrah which can be invaded by P.cinnamomi
2. the capacity of P.cinnamomi to survive in the soil and roots.
3. the distribution and spread of the fungus in jarrah roots.
4. the effects of root infection on regeneration of roots.

Pods of specialized roots were drenched with a zoospore suspension during the period of root growth in spring.

Inoculation caused significant infection of surface root pads primarily by entry via fine roots. Preliminary evidence suggests that infection of larger roots (3-4 mm) occurs both via infection of the fine root clusters as well as via new lateral roots initiated from the large roots. As soil moisture levels dropped P.cinnamomi could not be isolated from the soil. But, the fungus continued to survive in jarrah roots. The pathogen invaded extraxylary tissues and spread within large roots up to 4mm in diametre.Infection was extensive in some roots and restricted in other. The capacity of P.cinnamomi to destroy the framework of roots which support the extensive and specialized fine root system of jarrah could partially account for the destructive effect of the fungus on this species. However, overall infection frequency, were relatively low.

b) A second zoospore inoculation trial was carried out during the spring of 1980 using the root observation trays which had been established the previous year. Ten trays were inoculated with a zoospore drench. The trays were harvested six weeks after inoculation and roots were surface sterilized and plated and soil was direct plated and baited for the presence of the fungus using lupin radicles. The fungus was detected in soil from 6 of the trays (2 at a high propagule density) but root infection only took place in one tray. In this tray the infection frequency was approximately 1% and only suberized roots were infected. The tray yielding P.cinnamomi had a B.grandis root present which had approximately ten times the frequency of infection of the jarrah root.

The relatively low rate of recovery of P.cinnamomi from jarrah roots following very high inoculum doses during optimum periods for infection suggests that jarrah roots are relatively resistant to the fungus.

3.6 *Chlamydospore survival*

The survival of laboratory produced chlamydospores was measured over a range of sites in addition to the main study area. In general the population density of the fungus declined rapidly after inoculation, even when the soil was wet. The decline was more rapid when inoculations were carried out under dry soil conditions. However, the fungus was detected in the soil in spring 1980 in some plots which were inoculated the previous spring. This suggests that the fungus was able to survive during the summer in roots.

3.7 *Zoospore survival*

The pattern of zoospore survival was similar to that of chlamydospores. However, there is some evidence which suggests that zoospores may survive for longer periods in dry soil if they remain encysted. This phenomenon is currently being investigated.

3.8 *Survival of P.cinnamomi in infected B.grandis roots.*

Results from a study of the capacity of P.cinnamomi to survive in infected B.grandis tissue are shown in Fig. 4. Infected B.grandis root pieces were placed into the soil in August 1979. Root pieces were withdrawn periodically over the following 12 months and plated to determine if P.cinnamomi was present. The results of this study is significant since it not only demonstrates the capacity of the fungus to over summer in B.grandis roots but it also suggests that P.cinnamomi can saprophytically re-invade dead B.grandis when it is rewetted.

3.9 *Measurement of the capacity of P.cinnamomi to spread from infected B.grandis roots.*

Blocks of small replicated field plots, established in a pure jarrah regrowth stand near Dwellingup, W.A., were inoculated with root pieces which had been cut from trees of B.grandis and artificially infested with P.cinnamomi in the laboratory. One block of these plots was treated with agricultural lime. Periodically, soil cores were removed from the plots and seperated into soil and root fractions which were plated onto selective agar medium for the isolation of P.cinnamomi.

The objectives of the trial were:-

1) *Examination of the timing of the process of infection of small jarrah roots under field conditions in relation to seasonal changes in the soil physical environment.*
2) *To quantify the extent of infection of the fine root mass.*

3) *Evaluate the use of lime fertilizer application as a possible means of suppressing infection.*

Results from the platings of the soil core fractions indicated that there was no marked fungal movement and infection of the root mass in the plots over the study period, (July 1979 - May 1980). Inoculum viability was greatly reduced after November 1979. Maximum measured movement of the fungus to November 1979 was 25-40 mm downslope, and in most cases movement was significantly less than this (Table 2).

TABLE 2 ISOLATION OF P.CINNAMOMI IN RELATION TO DISTANCE OF CORES FROM INOCULUM ROOT PIECES IN THE FIELD IN LIMED AND UNLIMED PLOTS

UNLIMED PLOTS			LIMED PLOTS		
Plot	Distance from inoculation plug (mm)	Isolation of P.cinnamomi	Plot	Distance from inoculation plug (mm)	Isolation of P.cinnamomi
7A	40	+	11A	35	-
7B	0	+	11B	0	-
9A	18	-	12A	0	+
9B	30	-	12B	35	-
10A	50	-	13A	25	+
10B	30	-	13B	0	+
12A	45	-	14A	40	-
12B	25	-	14B	30	-
15A	35	-	15A	0	+
15B	27	-	15B	0	+

The results of this study agree with those obtained from a similar study where infected B.grandis roots were placed under legume stands (see section 4). The results suggest that movement of the fungus in surface drainage (which was not frequent during the study period) may be very much more important than any movement through soil. It has been shown that zoospores of P.cinnamomi display negative geotaxis and this may be a significant factor in transfer of zoospores over short distances from roots to surface drainage pathways. The examination of zoospores movement in different jarrah forest sites would appear to be very worthwhile.

Assessment of the viability of inoculum plugs and the presence of P.cinnamomi in soil associated with the plugs, after rain in April 1980, yielded the fungus from 4 out of 5 plots in both unlimed and limed treatments. P.cinnamomi was associated with both soil and inoculum pieces in unlimed plots, but only with inoculum pieces in limed plots.

This result could be attributed to site factors and experimental methods, however, it may also indicate that the lime treatment has affected sporangia production, zoospores release, mortality or survival. Other work carried out by the group has indicated that sporangia production should not have reduced by the lime application. Investigation of these points is continuing.

We tentatively conclude from this study that , in addition to the sequence of climatic events necessary to induce sporangial formation and release, high intensity rainfall sufficient to cause overland flow is necessary to transmit the pathogen on free drained sites.

4. Studies of the effect of a legume understorey on fungal development

4.1 Development under legume and Banksia

During the 1979 season the below average rainfall prevented a rigorous assessment of the effect of a legume understorey on P.cinnamomi. However, there was evidence that sporangial production was suppressed under A.pulchella stands. The results of studies of the capacity of P.cinnamomi to survive in inoculated B.grandis roots and extend into the soil under legume and now legume sites were presented at the APPS conference and are summarized below in abstract form :-

" The capacity of Phytophthora cinnamomi to cause mass destruction of the jarrah (Eucalyptus marginata) forest vegetation on freely drained sites can be attributed primarily to :1) the ability of the pathogen to rapidly increase soil population levels, and 2) the presence of a highly susceptible B.grandis understorey which provides a large food base and reservoir of inoculum. The object of this work was to determine if replacement of the susceptible understorey with one dominated by legumes, would inhibit survival and reproduction of the fungus. Open sites were used as controls.

Pieces of B.grandis stem (ca. 1.5 cm diam.) were inoculated with Phytophthora cinnamomi in the laboratory and used to infect plots in the open or under A.pulchella and B.grandis canopies. With regular sampling during the period from September to November there was no consistent difference between canopy types on the development of Phytophthora cinnamomi in soil sampled greater than 3 cm from the infected stem pieces; P.cinnamomi was randomly detected in soil at distances greater than 1 cm from the inoculum. When soil 3 cm on either side of the stems and stem pieces were sampled in mid-December, there was consistantly less Phytophthora cinnamomi in soil from open sites than that under B.grandis. Development of the pathogen under A.pulchella varied with site, being less than under B.grandis at one site, but not significantly different at another. The greater survival of Phytophthora cinnamomi in stem pieces occurred under B.grandis and the poorest survival in the open or under A.pulchella. Soil temperature and moisture flunctuated greatly in the open and was cooler and dryer under A.pulchella than B.grandis

4.2 Legume ammendments

The effect of legume root ammendments on P.cinnamomi survival and pathogenicity were also studied. The results of preliminary trials were presented to the APPS conference and are summarized here in abstract form. "Pathogenicity of Phytophthora cinnamomi to Eucalyptus marginata seedling was reduced in inoculated soils ammended with Acacia pulchella roots, relative to soil ammended with Banksia grandis roots or which were not ammended. Although overall mortality of seedling was low ($<5\%$) and there was no significance difference in mortality between treatments, seedling height and shoot dry weight was greatly reduced in soil ammended with B.grandis compared to those ammended with A.pulchella. There was greater root rot of E.marginata seedlings growing in soil ammended with B.grandis than in soil ammended with A.pulchella. Root development of seedlings in soil ammended with A.pulchella was not significantly different to that in non-inoculated, non-ammended controls. The fungus was recovered from 23% of the root system from soil ammended with B.grandis, compared to only 2% of roots from A.pulchella ammended soil.

Survival of P.cinnamomi was better in B.grandis than A.pulchella ammended soil. The fungus was recovered from soil from only 17% of inoculated pots ammended with A.pulchella and at low density (0.1 propagules/gm dry wt.) This compared with recoveries from 100% and 84% of inoculated pots not ammended or ammended with B.grandis with densities of 53.4 and 3.9 propagules/gm dry wt., respectively.

4.3 The Effect of Decomposing Legume Roots in Soil Nitrogen and the Effect on Development and Survival of P.cinnamomi

It has been demonstrated that a number of Acacia sp. have the ability to fix nitrogen at high rates. Following fire most literature suggests that there is a loss of nitrogen to the ecosystem through volatilization of the shoot. It is possible the roots of the legume spp. could contain high amounts of nitrogen which would not be lost from burning but would be released during decomposition into the Jarrah forest system.

It has been shown that some Acacia Sp. have an inhibitory effect on the survival of P.cinnamomi. The inhibition could be due to a chemical phytoalexin produced in roots or the microbes associated with the roots or high levels of nitrogen. There is some evidence that nitrogen in the ammonium form has an inhibitory effect on the survival of P.cinnamomi.

The aims of this study are to quantify the input of nitrogen (total nitrogen & inorganic nitrogen) into the Jarrah forest from decomposing roots of Acacia Sp. and their effect on the survival of P.cinnamomi.

4.3.1 Glasshouse experiment.

The main objective for using a controlled environment was to give a controlled rate of decomposition from known root ammendments.

The treatments were: 3 root types - resistant Acacia pulchella (Sp), Acacia lateriticola (Al) and susceptible Banksia grandis (Bg); 2 ammendment levels of 5 and 20% by weight; 3 decomposition times of 5, 10 and 15 months and 2 inoculation treatments, inoculated and a non-inoculated control. The treatments were replicated 3 times. During each decomposition time the treatments were sampled 3 times to determine inorganic nitrogen and P.cinnamomi levels.

After the first 5 months the inorganic nitrogen was highest in Al 20% and Al 5% (6.75 and 5.37 ppm respectively) slightly less in Ap 20% and Ap 5% (4.95 and 4.32 ppm respect.) with the lowest values in Bg 20% and Bg 5% (2.14 and 3.25 ppm respect.).

There appears to be no effect at this stage of the treatments on P.cinnamomi

4.3.2 Field Trials

4.3.2.1 Nitrogen Budget

6 soil litter/shoot and root samples from each plot were analysed for nitrogen (total) before and following burning to determine the effect of burning on the nitrogen budget.

Preliminary results suggest that burning had no effect on soil nitrogen however, there was a high degree of variability within treatments.

4.3.2.2 Rate of Decomposition & Release of Nitrogen from Acacia Roots .

The treatments were: 2 roots - A.pulchella and A.lateriticola; 2 size classes - 7.45 mm and 0.5 - 4.5 mm diametre; 2 root quantities - The roots were added to plastic mesh bags and placed in the cut/burnt A.pulchella and A.lateriticola plots. There were enough mesh bags to give 12 replicates and 6 sampling times over 1-2 year period. The total nitrogen and weight of the roots will be measured at each sampling time.

After 3 months in the soil the Ap and Al roots had the same weight loss of 36% in the smaller roots and a 14% weight loss in the larger roots. After 7 months the Al smaller roots had the largest weight loss of 46% with a 40% weight loss for Ap. The larger Ap and Al roots had a 33% and 32% weight loss respect. Total nitrogen currently being analysed.

4.3.2.3 Inorganic Nitrogen & the Survival of P.cinnamomi
Both cut/burnt and living plots had 4 inoculated subplots (Inoculation was by P.cinnamomi infected banksia plugs). There was 5 soil sampling times from around the plugs over the spring/summer period. Soil sampling for nitrogen (inorganic) consisted of 27 random cores/plot. This was (where possible) correlated with P.cinnamomi soil sampling times.

Although there were 4 sampling times for nitrogen to date only data from one sampling time is available. This shows the highest nitrogen in Ap and Al cut/burnt plots (4.56, 4.42 ppm respect) compared to Ap and Al living (1.87, 3.25 ppm respect.). and Bg cut/burnt and living plots (2.15, 1.67 ppm respect.)

Preliminary results suggest that there is a decrease in P.cinnamomi density in all cut/burnt plots. This could be due to a drying out effect, as these plots are all open sites. There does not seem to be a correlation between inorganic nitrogen levels and the survival of P.cinnamomi although statistical analysis is in progress.

4. 1980 Field Studies of the Effect of Legumes on P.cinnamomi.

A major study was initiated in 1980 to further test the effect of understorey litter type and litter removal by fire on P.cinnamomi. The following combination of treatments were studied:-

1. Fertilized A.pulchella

2. Fertilized non-legume sites
3. Unfertilized B.grandis sites 4 year litter.
4. Unfertilized B.grandis sites burnt in Autumn
5. Unfertilized B.grandis sites burnt in spring.

the study involved measurement of the following factors during 1980:-

- Rainfall, soil moisture, soil temperature under each vegetation type
- Sporangial production in the field
- Sporangial stimulation
- Zoospore survival
- Survival and transmission of spores from infected B.grandis roots
- Root infection

The results from this trial are currently being evaluated and only presented in outline here.

4.4.1 Temperature measurement

There was a marked depression of temperatures in legume sites which had not been burnt. Temperature difference of up to 10°c. between recently burnt sites and legume sites were recorded during the spring period.

4.4.2 Sporangium production

Sporangia were produced on all sites during the autum but at moderate levels. There were dramatic increases in sporangial production (> 700 sporangia per sample) following spring rainfall during warm days. The preliminary analysis of data suggests that sporangial production under legume stands was suppressed. This is the first year since sporangial production studies were initiated that there has been signficant rainfall in late spring. The results from this years study indicate that the fungus requires higher soil temperature levels than was previously assumed to produce significant quantities of sporangia. In addition to the studies of sporangial production, the effect of stage of development on sporangial release was investigated. The preliminary results suggest that a specific sequence of climatic events are required for sporangial formation, release and zoospore transmission. It should be possible, when the analysis of this data is completed to define accurately the meterological conditions required to cause and transmission of P.cinnamomi in the field. This will permit analysis of long time climatic records in relation to disease spread and intensification.

4.4.3 Stimulation of Sporangium production

The capacity for soil in different treatments to stimulate sporangial production was determined using intact cores rather than soil extracts as has previously been used. The preliminary results indicate that sporangial stimulation is markedly influenced by season. Tests of sporangial stimulation capacity using the soil extract method are too artificial to detect real differences. When intact soil cores taken from the field in winter and early spring were used to test stimulation capacity, sporangial formation could not be induced even when the cores were placed under optimum environmental conditions. Abundent sporangia were produced in soil extracts from the same soil. Soil cores which were taken in late spring and early summer, after a period when the soil had dried in the field, stimulated sporangial production.

The results of this study suggest that although the factors causing stimulation of sporangia are present in the soil throughout the year they require a specific sequence of rainfall and temperature regimes before they occur at densities sufficient to induce sporangial production. Further analysis of the data is required to determine if there are differences between treatments.

This discovery is significant as it suggests that the stimulating factor/s is effected by factors associated with site and season and therefore may be subject to manipulation.

Studies of the effect of soil type on stimulation are also being carried out using the intact core technique (see 5.3). It is proposed to intensify research into this aspect of disease epidemiology as a consequence of this study.

4.4.4 Zoospore survival

Zoospore survival under legume stands was markedly reduced.

TABLE 3

Propagule No/ 100 gm of soil

	8 weeks after Inoc.
Legume	19
Fertilized (non legume)	98
Banksia canopy (non legume)	73

4.4.5 Root Infection

Root infection was markedly suppressed under legume stands. Table 4.

TABLE 4

	Jarrah Root infection	
	4 weeks after inoculation	8 weeks after inoculation
Legume	11%	11%
Fertilized (Non legume)	77%	55%
Banksia canopy	66%	55%

4.4.6 The Effect of Species Composition and Litter type on Population Density

The increase in P.cinnamomi population density within a matrix of infected B.grandis roots was determined throughout the year under the difference treatments. The results suggest that increases in population density are correlated with periods of sporangial production. Preliminary results suggest that the density of P.cinnamomi under the legume stands was less than under the other treatments.

4.5 Susceptiblity of Legume Species.

Following the regeneration of a large number of different legume species which occurred following a large scale experimental moderate intensity burn, a study was initiated to determine the relative susceptibilty of different legume species. 12 different species are currently being tested in a glasshouse pot trial. Although, A.pulchella is known to be resistant it is possible that some "fireweed species" are highly susceptible.

5. The Effect of Site on P.cinnamomi soil Population Levels

Jarrah dieback is particularly severe in low lying moisutre gaining sites in the Darling Plateau. Previous studies have shown that the moisture and temperature regimes on these sites were favourable for P.cinnamomi survival and reproduction for long periods of the year. In contrast the soil physical environment on free drained sites was found to be only marginally favourable for survival of the pathogen.

5.1 Population levels of P.cinnamomi in a freely drained water gaining site.

Phytophthora cinnamomi population levels were measured over a 12 month period in an infected moisture gaining site and on adjacent free drained sites, Fig. 5. The fungus was consistantly recovered at a high population density throughout the year in the moisture gaining site. However, P.cinnamomi population levels in free drained sites were low relative to the moisture gaining site throughout the 12 month period. This study provides direct quantitative evidence of the effect of site on the P.cinnamomi survival and reproduction.

5.2 Variation in P.cinnamomi Population Levels during 1980 on free drained sites

Extensive measurement of P.cinnamomi population levels were carried out during 1980 on 4 different freely drained sites. In two of the sites spring and autumn burn treatments were imposed to determine if there was an effect of this management procedure on population levels. At each site samples were taken adjacent to B.grandis initially downslope and in random plots. At all sites soil moisture and temperature regimes were monitored.

The data from this study, which involved processing of approximately 5,800 soil samples are still being analysed.

The following are preliminary conclusions from the study:-

1) Relatively high population densities were recorded in samples taken adjacent to recently killed B.grandis throughout the year when the soil was moist.

2) The recovery rate from samples taken immediately downslope of recently killed B.grandis was low and the pattern of recovery was erratic.

3) The recovery rate from random samples in actively diseased areas was very low througnout the period of measurement.

In the two previous years we have not been able to detect P.cinnamomi at a high density in the soil in autumn. However, rainfall in autumn of 1978 and 1979 was abnormally low. We attribute the relative high recovery rate in autumn 1980 to the occurrence of average to above average rainfall. However, even though the fungus was detected adjacent to recently killed B.grandis apparently environmental conditions were not suitable for intensification and transmission. In some sites there was an increase in soil population density in spring but the measure was not marked. This could be attributed to the fact that although sporangia were produced there was insufficient following rainfall to cause release of zoospores and transmission.

5.3 Suppressive and conducive soils

In January 1980 work commenced on trials investigating observed suppressive and conducive soil.

Four soils are being studied

- suppressive red loam
- conducive black gravel
- an intermmediate soil type
- typical laterite

5.3.1 Glasshouse experiment

Soils from the four forest sites have been used in a pot trial to measure suppressive or conducive effects of these soils on A) inoculum level and survival of P.cinnamomi in the soil, B) infection of Eucalyptus marginata and Banksia grandis. One month following inoculation with infected Banksia stems there was no consistent difference in P.cinnamomi population density between the suppressive red loam and the conducive black gravel and laterite. Banksia grandis and Eucalyptus marginata mortality was consistently greater in the conducive black gravel and laterite than in the suppressive red loam.

5.3.2 Field experiment

In a field trial the effect of suppressive red loam and a conducive black gravel on A) survival of P.cinnamomi and B) sporangia production was examined over the 1980-81 spring/summer season.

Five field sites of each type were inoculated with 50 inoculated banksia stem pieces. Subsequent soil samples were taken at random around five stems at each site. Eight samples were taken over a period of four months. Soil and corresponding stems were plated to give some indication of the effect of these soils on P.cinnamomi activity and survival in the field environment. Results to date are yet to be statistically processed, but trends show that the fungus may be more favoured in the black gravel soil type.

Seasonal stimulation of sporangia production was examined using the four soils. Four soils samples were taken during spring, further sampling is to be undertaken in, summer, autumn and winter. Undisturbed soil cores were placed in buchner funnels for moisture regulation. Funnels were equilibrated to 10 millibars suction and remained at this level for the experiment. Sporangial production on leaf discs infected with P.cinnamomi were used to assess stimulating capacity.

Results to date are not complete but indicate the ability of both suppressive and conducive soils to stimulate sporangia formation is very seasonal. During the period in spring when the soil becomes stimulating to sporangia production there *appears to be some suppressive factors operating in the red loam soil.*

6. *Stimulation of the Effect of a Wet Spring on P.cinnamomi Spread and Intensification*

Research on the epidemiology of P.cinnamomi in the Jarrah Forest has been handicapped over the previous 5 years by the occurrence of below average rainfall. The studies that have been carried out strongly suggest that the spread an intensification of P.cinnamomi in soil (as distinct from the fungal invasion of B.grandis) is very restricted unless there is above average rainfall in either spring or autumn.

In anticipation of another year of average or below average rainfall a study was initiated in which a diseased site was irrigated to simulate the occurrence of above average spring rainfall.

A 20 x 20 m plot was established in an infected area where there was a number of B.grandis trees in various stages of decline. Soils samples were taken at 80 systematic location points in the plot over the period September to December 1980. The plots were irrigated to maintain high soil moisture levels. Periodically the irrigation rate was increased to cause temporary overland flow and ponding. The preliminary results of this study clearly demonstrate the capacity for the fungus to increase its density in the soil given optimum environmental conditions. But extension of the fungus from the initial points of infection (principally B.grandis trees) is dependent on overland flow of water from the infected area. Thus even though moderate rainfall during periods of warm weather in late spring will cause the fungus to rapidly increase its density in the soil adjacent to infection reservoirs it does not achieve broadscale distribution unless it is carried in water flowing overland. Our studies suggest that this will only occur in steep slopes or where the soil has been disturbed during normal rainfall events.

The ability of the fungus to achieve broadscale distribution could be markedly reduced by:-

a) *Reducing the number of infection points. That is reduction of the species (primarily B.grandis) which permit over summer survival.*

b) *Minimizing the opportunities for overland flow.*

7. *Infection of Jarrah by P.cinnamomi*

During the past 15 years mortality of jarrah in dieback effected areas has been relatively low. Decline and death of jarrah typically has been slow and the deterioration of crowns in infected areas has been difficult to distinguish from the generally poor crown vigour exhibited by jarrah in healthy areas.

There have been exceptions to this general pattern. Isolated rapid deaths of jarrah has been observed and in some areas where there has been extensive disturbance (for example downslope of bauxite pits) there has been a dramatic collapse of the jarrah overstorey. The generally low rate of jarrah mortality over the previous 15 years contrasts with reports of rapid and extensive mortality during the period 1945-1960.

It has been proposed that the slow decline and eventual death of jarrah results from destruction of the specialized fine feeder root system of this species. However, it is difficult to explain rapid death of jarrah by the process of fine feeder root attrition.

A number of studies had been carried out with the aim of determining the degree of invasion of P.cinnamomi into jarrah roots. In all cases it was not possible to recover P.cinnamomi from large suberized roots although the fungus was recovered from the suberized roots which formed the framework of the specialized fine feeder root system. A further study was carried out in 1980 to further investigate the capacity of P.cinnamomi to invade large suberized roots.

P.cinnamomi had extensively invaded the large root system and the collar of the rapid death syndrome trees, Table 5. In one tree the fungus was recovered 2 metres above the ground in the stem. In trees not exhibiting the rapid death syndrome the degree of invasion was markedly reduced.

Table 5. Isolation of *Phytophthora cinnamomi* from the stem and large roots of dead and live *Eucalyptus marginata* at seven sites.

Tree	Site	Stem(1		Root diameter class					
				1-5cm		5-10cm		≥10cm	
		Bark(2	Wood(3	Bark	Wood	Bark	Wood	Bark	Wood
		(4		(5					
Dead	1	7/8	7/8	10/23	11/23	1/3	1/3	-	-
	2	2/4	0/4	0/1	0/1	3/5	0/5	3/4	3/4
	3	1/8	0/8	-	-	0/2	0/2	-	-
		1/8	1/8	-	-	0/10	0/10	-	-
	4	1/8	0/8	0/2	0/2	0/3	0/3	0/1	0/1
	5	4/11	3/11	0/7	0/7	5/25	2/25	0/4	0/4
	6	3/5	3/5	8/8	7/8	3/4	3/4	-	-
		1/6	0/6	1/8	1/8	0/4	0/4	-	-
	7	1/6	0/6	3/15	0/15	0/2	0/2	-	-
		2/6	2/6	3/5	3/5	1/3	1/3	-	-
		0/4	0/4	4/10	4/10	-	-	-	-
Live	2	0/8	0/8	0/10	0/10	0/17	1/17	0/2	0/2
	3	0/8	0/8	-	-	0/4	0/4	0/1	0/1
	3(6	0/8	0/8	-	-	0/5	0/5	0/1	0/1
	4(6	0/8	0/8	0/3	0/3	0/7	0/7	-	-
	5(6	0/8	0/8	0/5	0/5	0/6	1/6	0/2	0/2
	6	0/6	0/6	7/17	2/17	0/5	0/5	-	-

(1 Base of stem just below soil surface

(2 Tissue external to the cambium

(3 Adjacent to the bark

(4 Number samples infected/total number collected

(5 Number of roots infected/number of roots in each diameter class

(6 Tree close to *Banksia grandis* which had died more than 1 year ago.

The difference in the response to *P. cinnamomi* of the two classes of trees sampled could be attributed to :-

a) Genetic differences which effect resistance.

b) Different levels of exposure to the pathogen which were not evident during the study

c) Differences in host physiology induced by subtle site factors

d) Various combinations of the above.

The discovery that P.cinnamomi can, under certain conditions, totally invade the root system of jarrah has profound implications to the jarrah dieback research and management program. It is probable, given the observations of total and rapid overstorey collapse around bauxite pits, that under certain environmental conditions that markedly favour the fungus,jarrah can be as susceptible as the B.grandis. Can the extensive and rapid decline of jarrah during the period 1945-1960 be attributed to the occurrence of environmental conditions which resulted in P.cinnamomi totally invading the root system and collar region of jarrah ? Why do some jarrah trees resist infection whereas others are subject to total invasion ? Can the relative susceptiblity of Jarrah be manipulated by cultural techniques ?

This research has led to the initiation of a number of studies on the effect of host physiology on susceptiblity and was the basis for our submission to employ a research scientist to investigate this aspect of the disease.

7. Infection of B.grandis by P.cinnamomi

The discovery that P.cinnamomi is a collar rot pathogen in B.grandis confirmed our observations of the important role this species plays in the spread and intensification of the disease. This research has also provided the basis for a rapid and reliable field assay system that has been employed extensively to map the location of the disease in the forest.

Further studies of B.grandis have been carried out in co-operation with Miss D. Schilds (Murdoch University). The preliminary results from these studies were presented to the APPS conference and are summarized here in abstract form:-

"Eight B.grandis trees in different stages of death, across a disease front were excavated in late summer and intensively sampled for the presence of P.cinnamomi.

The fungus was detected in recently killed trees and in those that had been dead for over two years. P.cinnamomi was present in the fine roots, large roots, and the stump and lower stem region of the trees but was not recovered from soil samples taken in the diseased site.

A recently killed Banksia and a Banksia that had been dead for approximately one year were excavated from other diseased areas and were dissected .In recently killed

Banksia, P.cinnamomi can invade the stump and stem except for the pith, and all of the large roots. In the older dead Banksia, P.cinnamomi was detected in the large roots and the stump but not in the stem region. The presence of the fungus was less extensive in the older dead Banksia, than in the recently killed Banksia. Infected root and stump pieces were sectioned and scanned under the microscope and no chlamdospores were observed. The results indicate that B.grandis acts as a reservoir for P.cinnamomi over the harsh summer months, enabling the fungus to re-infect the soil when conditions become conducive.

In field studies the movement of P.cinnamomi from naturally infected Banksia stumps into the soil has been shown to occur primarily in spring. This phenomenon has been simulated in glasshouse studies and these indicate that movement into the soil around infected stumps is localized, and spread downslope is dependent on movement of propagules carried in overland or subsurface water-flows. Further studies regarding this aspect are being continued."

Preliminary studies have been made to determine the rate of other shrub and understorey species in the spread and intensification of the disease. Preliminary results suggest that the stem of Xanthorrea pressii is not invaded by P.cinnamomi. The fungus however, was recovered from the suberized roots of this species.

8. Biology and Ecology of B.grandis.

Studies of the biology and ecology of B.grandis were undertaken in co-operation with Dr. D. Bell (University of Western Australia) to determine if it was possible to develop silvicultural technqiues to reduce the density of this species. Results from this study were presented to a conference on Banksia and are summarized in the following abstract.

"The density of B.grandis in the E.marginata forest has increased markedly following exploitation of the forest for timber. This increase is attributed to soil disturbance and reduced competition resulting from logging and the imposition of a firing regime which does not disfavour this species. Reduction and maintenance of the B.grandis component of the forest to a low density is an essential pre-requsite to control of Phytophthora cinnamomi the soil borne pathogen causing Jarrah dieback.

Control of the B.grandis understorey cannot be achieved by single even treatments, for example high intensity fire or herbicides, because regrowth to pre-treatment densities occurrs within 10 to 20 years. Hence, the biology and population dynamics of the species have been studies in an attempt to devise a management regime which would maintain the density of the B.grandis understorey at a disireable level.

Growth rates of B.grandis in stands of different densities were determined by dating trees using a combination of internode and growth ring counts. The relationship between cone production and tree size/age, seed production per cone, seedling survival and the structure of the B.grandis understorey in jarrah forest stands with different burning and silvicultural treatments has been determined.

The effect of fire of different intensities on survival of B.grandis seedlings, lignotubers, and trees in 1 cm. size classes was determined from field plots which had been subjected to a range of fire intensities.

The data has been incorporated in an interactive Fortran stand computor model which is being calibrated by comparing simulated stand structures at different stages of development with comparible natural stands. The model has the capacity to simulate the effect of different burning regimes alone and in combination with other silvicultural treatments on the density, structure and development of the B.grandis understorey."

9. P.cinnamomi in Bauxite Pits.

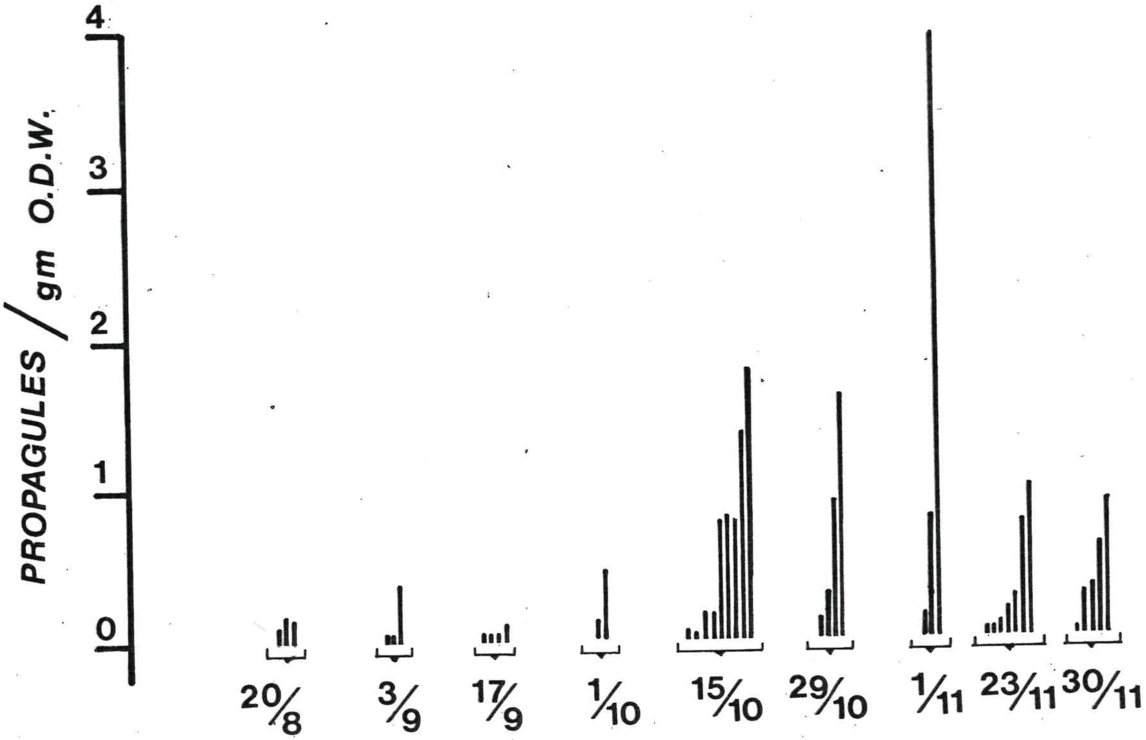
Preliminary studies of the incidence of P.cinnamomi on bauxite pits have been carried out. The fungus has been recovered from dying jarrah at the Del Park and Jarrahdale sites and from dying Banksia at Jarrahdale No. 1 site. P.cinnamomi was recovered from large suberized roots and the collar of jarrah which had been planted on bauxite sites.

Previous studies had shown that P.cinnamomi's capacity to sporulate was markedly enhanced in water ponded in ripped lines. The disruption of drainage which results from mining and the transmission of water in ripped lines and drains in bauxite pits will create ideal conditions for asexual reproduction and transmission of species on bauxite pits. Thus, unless the fungus can be eradicated from bauxite pits and its re-introduction prevented it is probable that susceptible species restablished on bauxite pits over a period of time will succumb to the disease. It is highly unlikely given the amount of disturbance associated with mining that it will be possible to prevent the introduction of the fungus in overburden soil. However, practice such as "double stripping" will markedly increase the probability that this will occur since this practice involves movement of soil back into the pits without stockpiling. Evaluation of the fungus is not a practical

Hence, attempts to restablish species which are susceptible to P.cinnamomi on bauxite pits are not likely to be successful in the long term.

The large expenditures which are currently being incurred in an attempt to restablish pit mining diversity of species on pits should be viewed in this perspective.

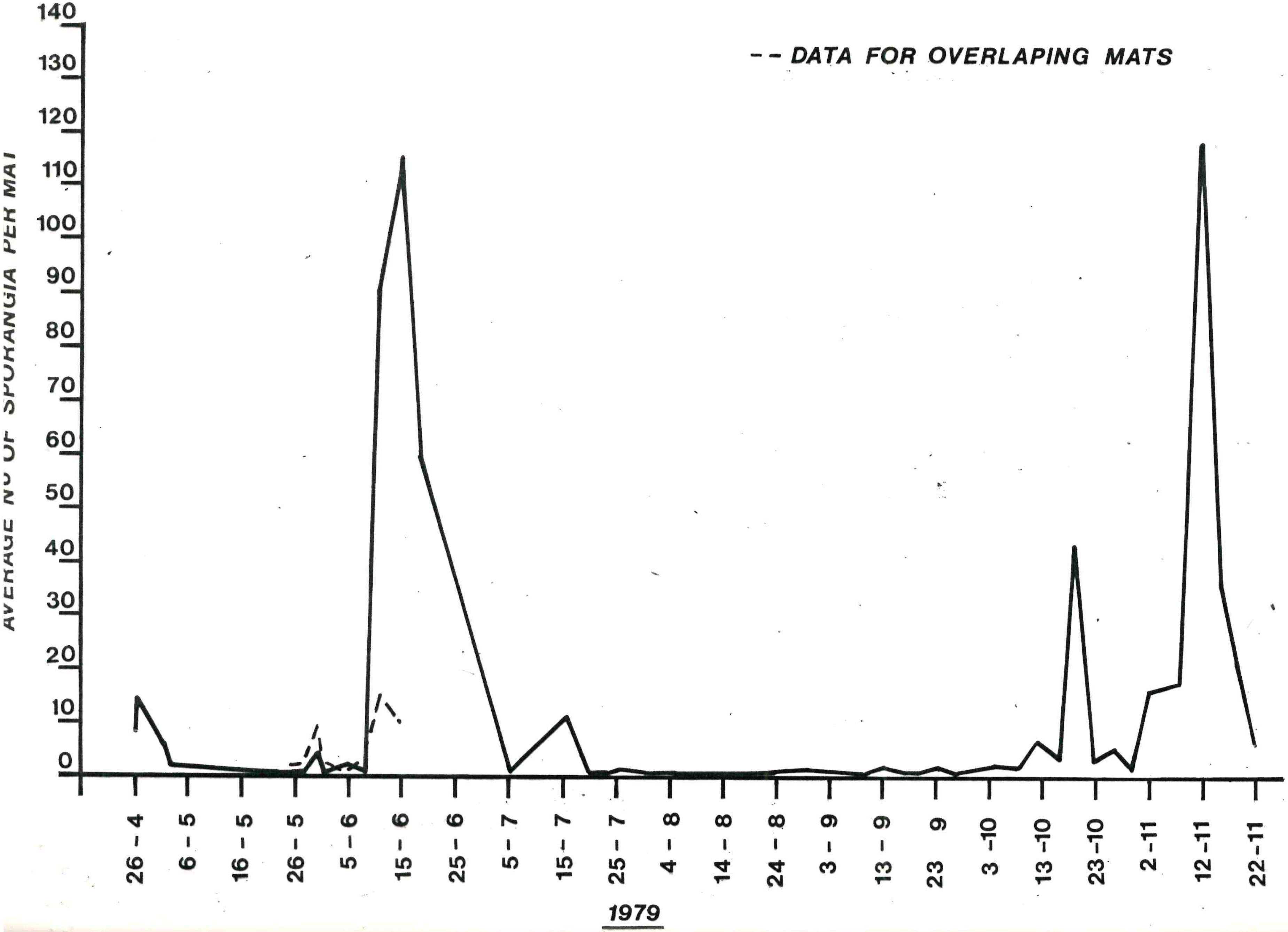
<u>Fig.1.</u> *Recovery of <u>P</u>.<u>cinnamomi</u> from soil adjacent to dead <u>B</u>.<u>grandis</u> collars.*



PROPAGULES /gm O.D.W.
4
3
2
1
0
20/8
3/9
17/9
1/10
15/10
29/10
1/11
23/11
30/11
SAMPLE DATE
(1979)

Fig. 2. *Seasonal variation in Sporangial Production.*

SEASONAL SPORANGIA PRODUCTION NORTH EAST ROAD



<u>Fig. 3</u>. Seasonal variation in

Root Growth

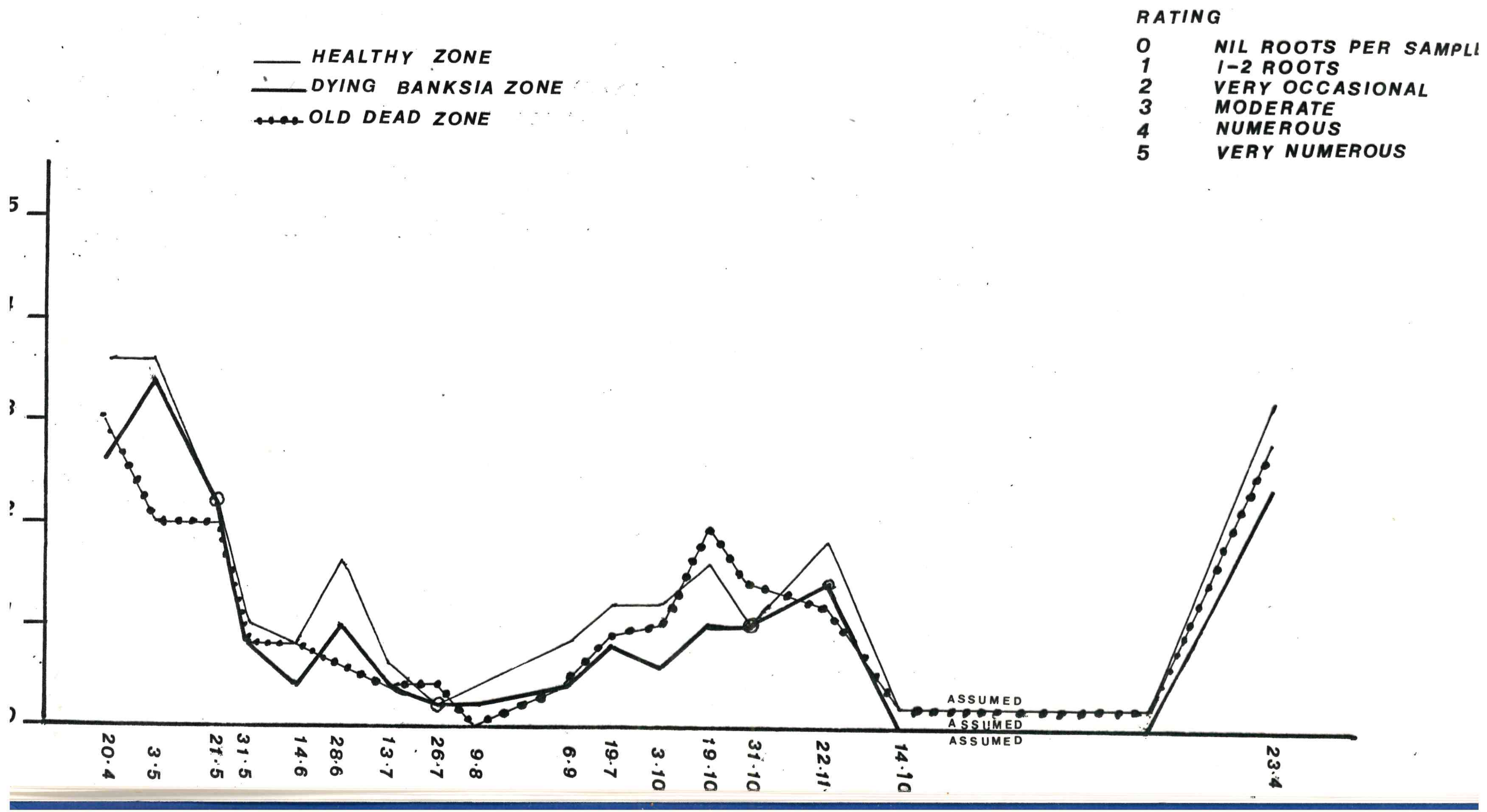
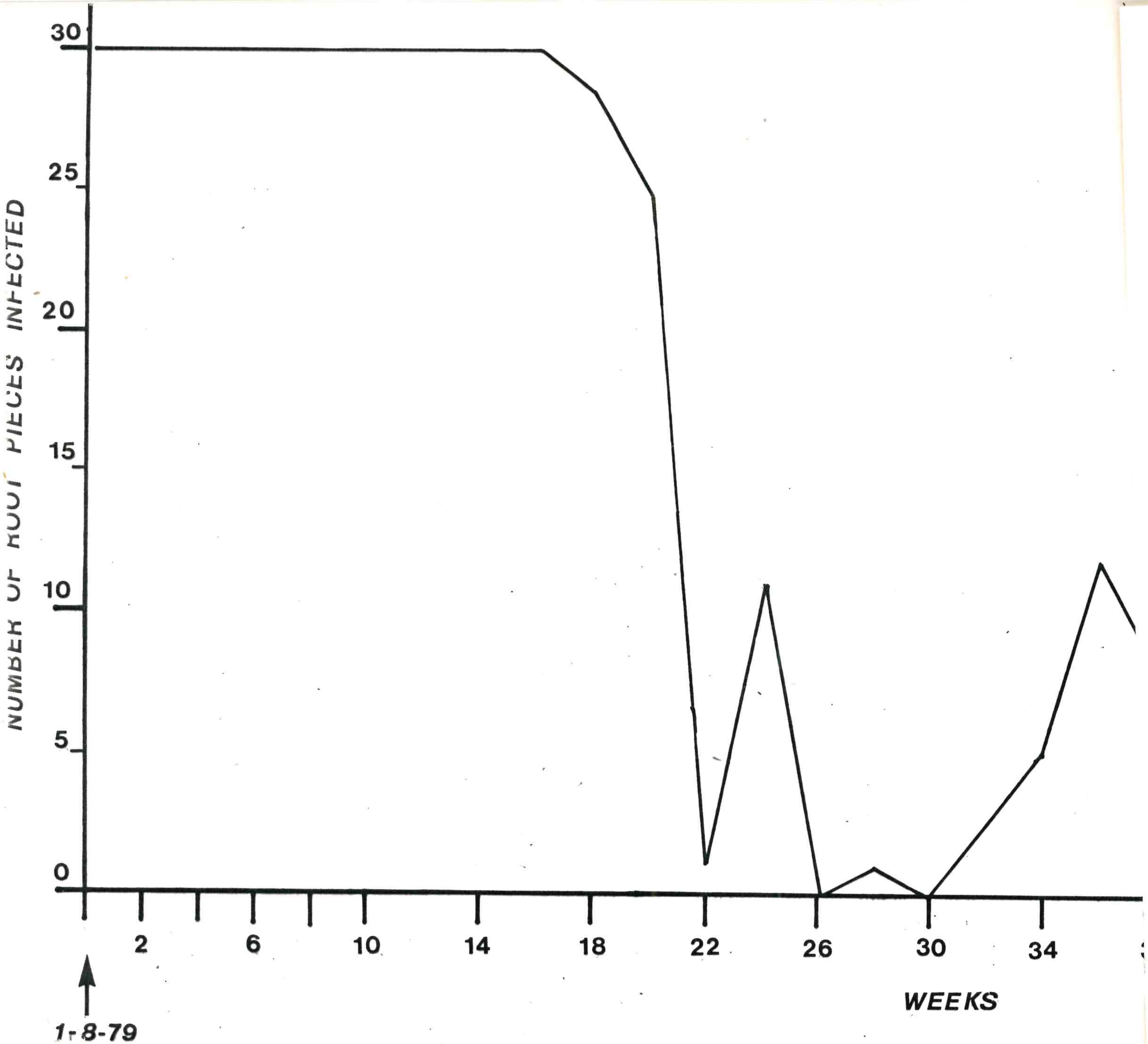
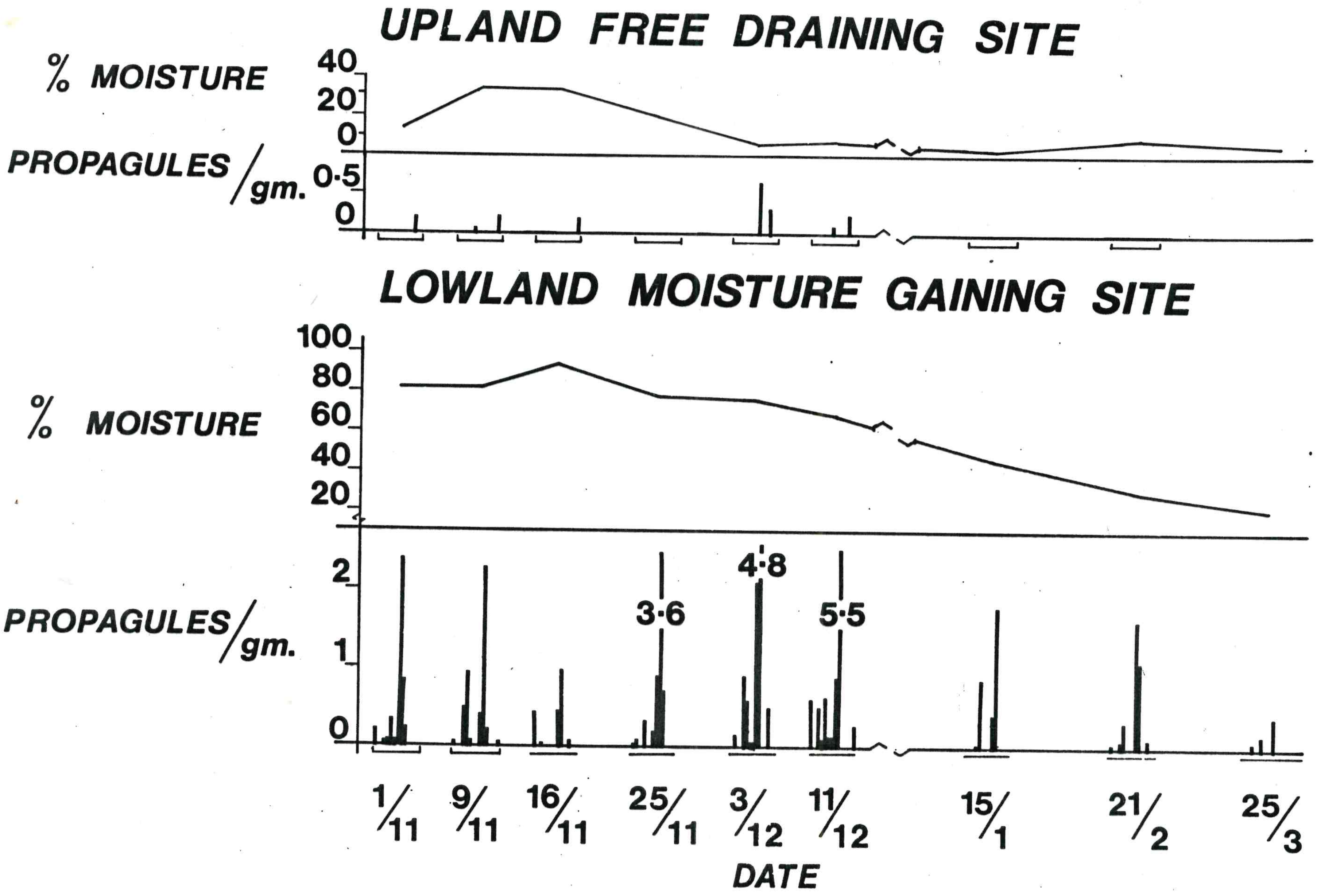

HEALTHY ZONE
DYING BANKSIA ZONE
OLD DEAD ZONE
RATING
0 NIL ROOTS PER SAMPL
1 1-2 ROOTS
2 VERY OCCASIONAL
3 MODERATE
4 NUMEROUS
5 VERY NUMEROUS
ASSUMED
ASSUMED
ASSUMED
20·4
3·5
21·5
31·5
14·6
28·6
13·7
26·7
9·8
6·9
19·7
3·10
19·10
31·10
22·11
14·10
23·4

Fig. 4. Seasonal pattern of survival of *P. cinnamomi* in inoculated *B. grandis* roots.



NUMBER OF ROOT PIECES INFECTED
30
25
20
15
10
5
0
2
6
10
14
18
22
26
30
34
WEEKS
1-8-79

Fig. 5. *Seasonal variation in P. cinnamomi population density on free drained and water gaining sites.*



UPLAND FREE DRAINING SITE
% MOISTURE
40
20
0
PROPAGULES/gm.
0·5
0
LOWLAND MOISTURE GAINING SITE
100
80
% MOISTURE
60
40
20
PROPAGULES/gm.
2
1
0
3·6
4·8
5·5
1/11
9/11
16/11
25/11
3/12
11/12
15/1
21/2
25/3
DATE