PROJECT TITLE: Effectiveness of mycorrhizae as protection for eucalypt and pine seedlings in soils infested with *Phytophthora cinnamomi* Rands.

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Introduction:

The objective of the project currently being completed was to determine the identity of fungal symbionts and the function of ectomycorrhizae in jarrah forest in relation to the pathogenicity of Phytophthora cinnamomi. An essential pre-requisite for the study was a survey of the distribution and mode of occurrence of native fungi in a variety of forest environments. Species of epigeous fungi from 25 genera were collected, identified and a collection of 70 cultures including species from 18 genera was established. Hypogeous fungi were collected, identified as being in a further 25 genera, and 77 cultures from 13 genera were set up in the collection. Observation of associations of fungi with roots of forest species and evidence from pure culture synthesis trials in the laboratory indicated that most of the identified species are capable of forming ectomycorrhizae. In addition to their role in improving forest nutrition, ectomycorrhizae are reported to function as protection for eucalypts and pines from infection by root pathogens such as Phytophthora cinnamomi (Bowen 1973, Marx 1972).

Evidence has been obtained to support the hypothesis that ectomycorrhizae provide a barrier to infection of eucalypts and pines by zoospores of P. cinnamomi.

Fungi from the collection of native species and known fungal symbionts have been successfully cultured and shown to form eucalypt and pine mycorrhizae in pure culture, pot trials and nursery trials.

The results of experiments over two years are summarized in the Appendix.

Recent work in USA has also provided evidence that inoculation of pines with specific fungal symbionts forming ectomycorrhizae significantly increased survival of seedlings after out-planting and enhanced nutrient uptake thus improving growth in difficult sites.

These results indicate the potential for developing effective mycorrhizae for various eucalypt species in indigenous forests and on bauxite mined areas, and for pines in sites where infection with root pathogens is a problem.

The justification for persevering with development of mycorrhizae to provide protection from primary infection by P cinnamomi is the very

low levels of the pathogen isolated from soils (Blowes et al 1982) and the short periods during the year when positive isolations can be obtained. Compared with the situation in Eastern States forests this suggests that environmental conditions for P. cinnamomi are either marginal or not suitable in many Western Australian forest sites. Thus all means of manipulating the environment to decrease its suitability for the pathogen and of increasing the resistance of roots to primary infection can be expected to be beneficial in limiting disease spread.

The objective of the study in this proposal is to field-test eucalypt and pine seedlings having effective ectomycorrhizae formed synthetically using known fungal symbionts developed for sites infested with P. cinnamomi.

Experimental Approach:

1. Investigate practical procedures to inoculate nursery seedlings with beneficial fungal symbionts to produce ecto-mycorrhizae.
2. Outplant trials of seedlings with various synthetic ectomycorrhizae on areas requiring rehabilitation, e.g. bauxite pits, woodchip coupes, P. cinnamomi infested sites.
3. Laboratory and glasshouse trials to investigate the effectiveness and processes involved in protection of seedlings by ectomycorrhizae in soils infested with P. cinnamomi and using inoculation with zoospores of the pathogen.

Reference:

Blowes, W.M., W.A. Heather, N. Malajczuk and S.R. Shea (1982). The distribution of Phytophthora cinnamomi Rands at two sites in South Western Australia and at Durras in South-eastern New South Wales. Aust. J. Bot., 30: 139-45.

Financial Support Requested:

	1983/84 $	1984/85 $
Salary (E.O.)	21,900	22,640
Salary On-Costs*	7,665	7,924
Maintenance	2,500	2,750
Equipment	-	-
Travel+	2,500	2,000
Total	34,656	35,314
Estimated Inflation (10%)		38,845

* Provision for superannuation, long service leave, leave loading, workers compensation.

\+ Provision for local travel and interstate to Melbourne per "International Conference on Plant Pathology".

The level of support requested is to employ L. Sanfelieu who is at present engaged in the studies of ectomycorrhizae outlined in the Appendix. His experience will allow this project to progress without interruption.

APPENDIX

Component 1: Fungi Collection, Isolation and Verification of Ectomycorrhizal Status

A list of available cultures and their mycorrhizal status is included as in Table I and II. Many ectomycorrhizal fungi are difficult to maintain in artificial culture and factors influencing their mycorrhizal formation are unknown, therefore, there has not been a large increase in the size of generic diversity of the collection. However, the ecotypic range of a number of species has been increased. Further, with the help of some visiting international fungal taxonomists (Professor H. Thiers, San Francisco State College, and Professor Egon Horak, Swiss Federal Institute of Technology) some of the problems of description and classification have been solved. A number of joint publications in this area are planned. In addition, the collection of fungi has revealed distinct differences in the variety and fruiting times of the fungal floras of the jarrah and karri forests. Although the significance of these differences is not yet understood, the 1982 fungal season will supply added information in this area.

Component 2

The effect of applied P and N on the abundance of epigeous and hypogeous fungal sporocarps and eucalypt mycorrhizae in Jarrah forest areas of varying fire history

The philosophy behind this component is that the litter layer is an important factor in the ectomycorrhizal habit and in areas where the litter cover has been removed, the use of fertilizers may stimulate the development of ectomycorrhizae. Therefore, in 1980 plots were set up to observe the effect of applied N and P in forest areas with varying litter cover. The 1981 fungal season has provided preliminary data in the following areas.

2.1 Patterns of Epigeous Sporocarp Occurrence

The spatial distribution of fungal sporocarps from each genera represented on the plots were mapped for each sampling date. This has provided information on fungal succession and periods of ectomycorrhizal activity during the season. The mapping should also help to locate

areas of ectomycorrhizae within the plots and indicate which genera of fungi may be ectomycorrhizal. (There may be some correlation between fruiting abundance and ectomycorrhizal activity - see section 2.3.) Fungi were collected later into the year from litter sites (Cobiac 1, Amphion, Virgin Jarrah) than from the burnt sites. The extended fruiting season can be attributed to the mulching effect of the litter layer which would assist in prolonging ectomycorrhizal activity.

2.2 Biomass of Fungal Sporocarps

All sporocarps observed on the plots were collected to estimate the biomass of fungal material produced relative to fertilizer treatment. Results are presented in table 3 and indicate that there has been a positive response to applied fertilizer. Phosphorus on its own appears to have depressed fungal fruiting (and ectomycorrhizal activity?) and N/P interaction has stimulated the same, (exception N/P - Cobiac 1.). Plots, previously established in a 1977 karri regeneration site and treated with N and P, were monitored for fungal abundance. The results from these plots follow the same trend as the jarrah sites. In addition, a simple pot trial was set up to test the effect of applied N and P on the initiation and development ectomycorrhizae in unburnt and burnt jarrah litter. Tentative results are presented in table 4 and represent the area (cm^2) in which ectomycorrhizal roots were observed. Phosphorus was again limiting and N/P interaction the most stimulating in initiation. It was estimated that the fresh weight of ectomycorrhizae was doubled (or greater) on the unburnt litter, however, the jarrah seedlings were larger in all treatments on the burnt litter where N in particular stimulated fleshy root production and shoot growth at the expense of ectomycorrhizal development.

2.3 Estimation of Ectomycorrhizal Root Activity

Soil cores were removed from the plots, washed and subject to a set counting procedure. The root counts are now awaiting computer analysis and should provide information on correlation between sporocarp and ectomycorrhizae numbers, fertilizer effect on ectomycorrhizal root activity and the distribution of ectomycorrhizal types within the soil profile.

Component 3

Zoospore Survival and Interaction with Jarrah Ectomycorrhizae

Zoospores have been used as the infective agent in these studies for a number of reasons. Primarily, because they are the major propagule responsible for the spread of P. cinnamomi in the forest and as such represent the initial contact between the root and the pathogen. In addition, they are particularly suited to quantitative studies, allowing control of inoculum size and easy estimation of population sizes. The encystment of a zoospore, prior to germination, is a necessary component of the infection process and the results of these studies are based on the survival of zoospore cysts.

3.1 Effect of Soil Type on Zoospore Survival

Previous work has indicated that zoospore survival is influenced by soil type; karri loam significantly reduced survival of cysts when compared to controls and Northern jarrah (NJ) lateritic soils. Further studies have shown that jarrah litter has the same effect on encysted zoospores as karri loam. Results are shown in figure 2. The litter extract reduced all cysts inviable prior to testing germination, while the NJ extract increased zoospore numbers on days 8 and 12. This increase can be attributed to sporangial production (and release of viable zoospores) as a survival response by P. cinnamomi to microbial lysis of mycelium from previously germinated cysts in the NJ extract. A summary of zoospore behaviour in these trials is present in the table also in figure 2. Some cyst germination was evident in the litter extract but the hyphae was rapidly lysed by microbial action and no viable zoospores were produced. Figures 6-8 demonstrate bacterial lysis of zoospores and hyphae. The sterile soil extracts demonstrate the above to a result of microbial action. The microbiological suppression of P. cinnamomi by litter may play an important role in the health of the forest and should be considered in future forest management policies.

3.2 Effect of Fungal Exudates on Zoospore Survival:

Ectomycorrhizae suppress P. cinnamomi by any one or all of the following methods. By providing a physical barrier to infection (see

section 3.3), by supporting an antagonistic microflora, by reducing root exudates and/or by releasing toxic exudates from the sheathing mantle. To test the last factor, motile zoospores were exposed to pure culture solutions of isolated ectomycorrhizal fungi. Results are presented in figure 3. A number of fungal cultures significantly reduced cyst survival - H59. (Scleroderma) sp, H16. (Mesophelia trabalis), and others produced a marked decrease in viable cysts - Castorium camphoratum), H68 (Scleroderma varicosum). In another trial, zoospores were exposed to sterilized extracts of "white" and "black" jarrah ectomycorrhizae. Figure 4 shows that the "white" extract reduced zoospore survival while the black extract had little effect. These studies indicate that ectomycorrhizal fungi can produce substances toxic to zoospores of P. cinnamomi and that they may act in nature. More work is required to define the role of these exudates in the interaction between P. cinnamomi and ectomycorrhizae.

3.3 Interaction of zoospores with Jarrah ectomycorrhizae and non-mycorrhizal roots

The role of ectomycorrhizae in the protection of jarrah from P. cinnamomi has been investigated by "point inoculation" of roots with zoospores. A series of scanning electron micrographs, with notes, is presented as results and a publication is planned in this area. "White" ectomycorrhizae were shown to act as a physical barrier to infection by zoospores. This is particularly evident by comparison zoospore behaviour on ectomycorrhizae (Figures 14-19) and non-mycorrhizal roots (Figure 9-13). Large numbers of zoospores were attracted to damaged parts of the non mycorrhizal roots, e.g. areas of lateral root emergence (Figure 12) and physical abrasion (Figure 13), and infection occurred. The ectomycorrhizal mantle limits the possibility of physical root damage, reduces sections of chemotaxic exudates from the root in general and covers the emerging lateral roots with fungal tissue. Potential infection sites are therefore reduced to a minimum.

The protective role of ectomycorrhizae was tested on jarrah seedlings grown in a number of soil types. Results are shown in figure 6. Infection was greatest in the NJ soil and least in unburnt jarrah litter. There appears to be little difference in the effectiveness of

"black" and "white" mycorrhizae against P. cinnamomi, however more work is required to confirm this observation. Few mycorrhizae were produced in the karri loam or burnt jarrah litter where fleshy root development was similar to that in the NJ soil. The reduction in infection rate may, in part, be due to microbial action, however, the major parameter appears to be the degree of ectomycorrhizal development.

Component 4: The Production of Ectomycorrhizal Seedlings, Outplanting Survival and P. cinnamomi Challenge Experiments

Figures 20 and 21 demonstrate the stimulating effect of artificially induced mycorrhizae of the fungus Laccaria laccata on the growth of jarrah and pine. (See also figures 22 and 23). These techniques have been applied in pot trials and in the field at the nursery level of production. The trials, using a selection of known ectomycorrhizal cultures, have just begun and results are unavailable. Further pot experiments are planned during 1982 using additional fungi proven to be ectomycorrhizal in pure culture. The mycorrhizal seedlings produced in these trials will be used for outplant survival studies and P. cinnamomi challenge experiments. The latter, apart from providing some general information on the role of ectomycorrhizae in protection against P. cinnamomi, may indicate whether there are degrees of resistance imported by specific fungi.

Figure 1. Structure of the Programme

OVERALL AIM

BIOLOGICAL CONTROL OF PHYTOPHTHORA CINNAMOMI BY ECTOMYCORRHIZAE

POTENTIAL METHODS

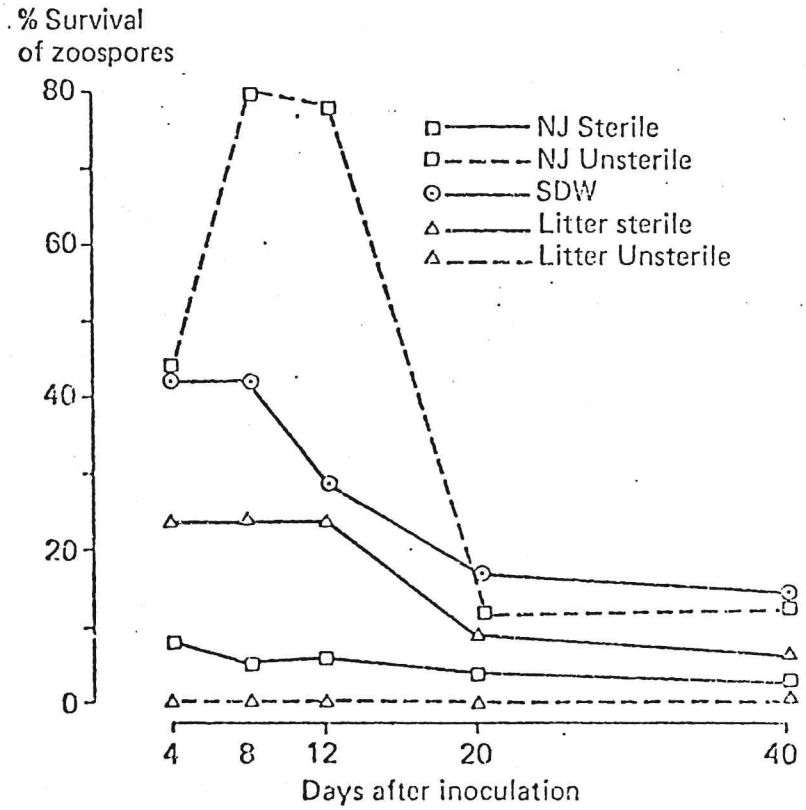
MANIPULATION OF ENVIRONMENT TO PROMOTE ECTOMYCORRHIZAL DEVELOPMENT

- USE OF NUTRIENTS (P/N)
- ESTABLISH VIABLE LITTER/MULCH LAYER
- PRE INOCULATED SEEDLINGS

AREAS OF INVESTIGATION

1. MYCORRHIZAL STATUS OF JARRAH AND KARRI	2. ROLE OF MYCORRHIZAE IN THE PATHOGENICITY OF P. CINNAMOMI	3. FORMATION OF ECTOMYCORRHIZAL SEEDLINGS IN POTS AND NURSERY
- QUALITATIVE AND QUANTITATIVE STUDIES OF ECTOMYCORRHIZAE IN THE FOREST	- ZOOSPORE SURVIVAL STUDIES - SOIL TYPE	- NURSERY INOCULATION OF KARRI SEEDLINGS
- FUNGI COLLECTION	- ECTOMYCORRHZAE AS A BARRIER TO INFECTION	- GLASSHOUSE INOCULATION OF JARRAH AND OTHER EUCALYPT SEEDLINGS
- PURE CULTURE SYTNEHSIS	- SOIL TYPE/ECTOMYCORRHIZAE INTERACTION ON INFECTION	

Figure: 2 The effect of jarrah litter and northern jarrah laterite(NJ) extracts on the survival of motile zoospores. (SWD—sterile distilled water control).



Summary of the results of soil treatment and zoospore survival.

Treatment	Germination in soil suspension	Viable in mycelium suspension	Viable zoospores on P_{10}UPH
SDW	–	–	YES
NJ	+	YES	YES
Litter	+++	NO	NO
Litter Sterile	+	YES	YES
NJ Sterile	++	YES	YES

Figure: 3 . Effect of various Fungal Metabolites on the survival of motile zoospores.

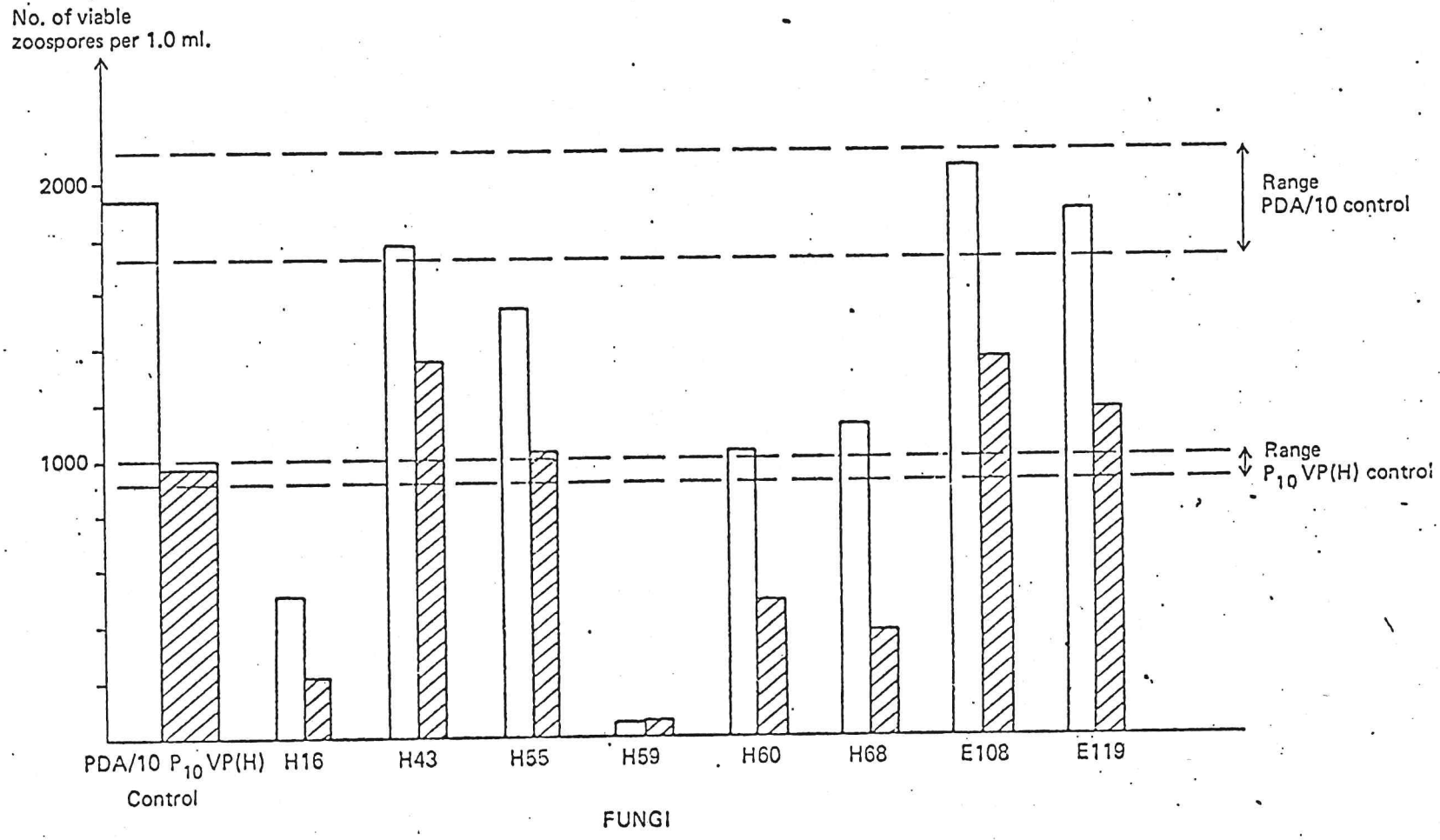


Figure: 4 Effect of sterile root and soil extracts on the survival of motile zoospores.

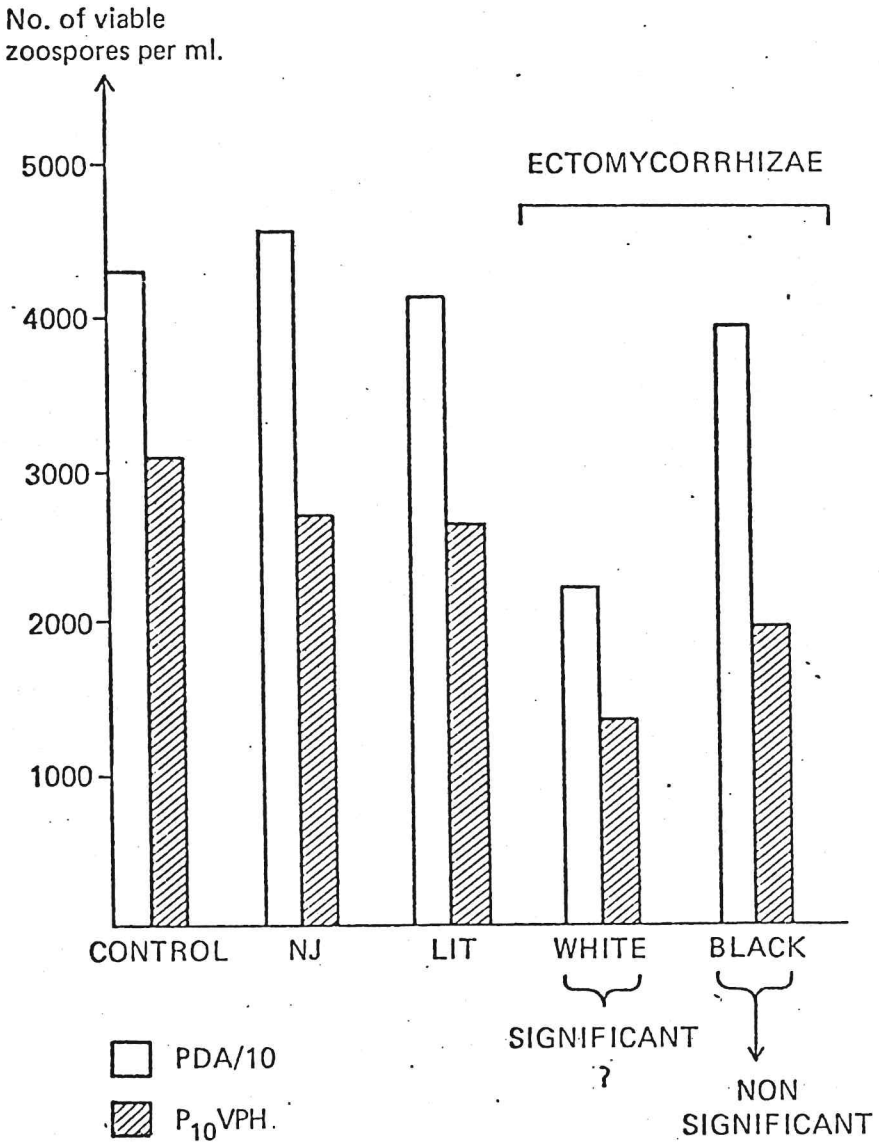
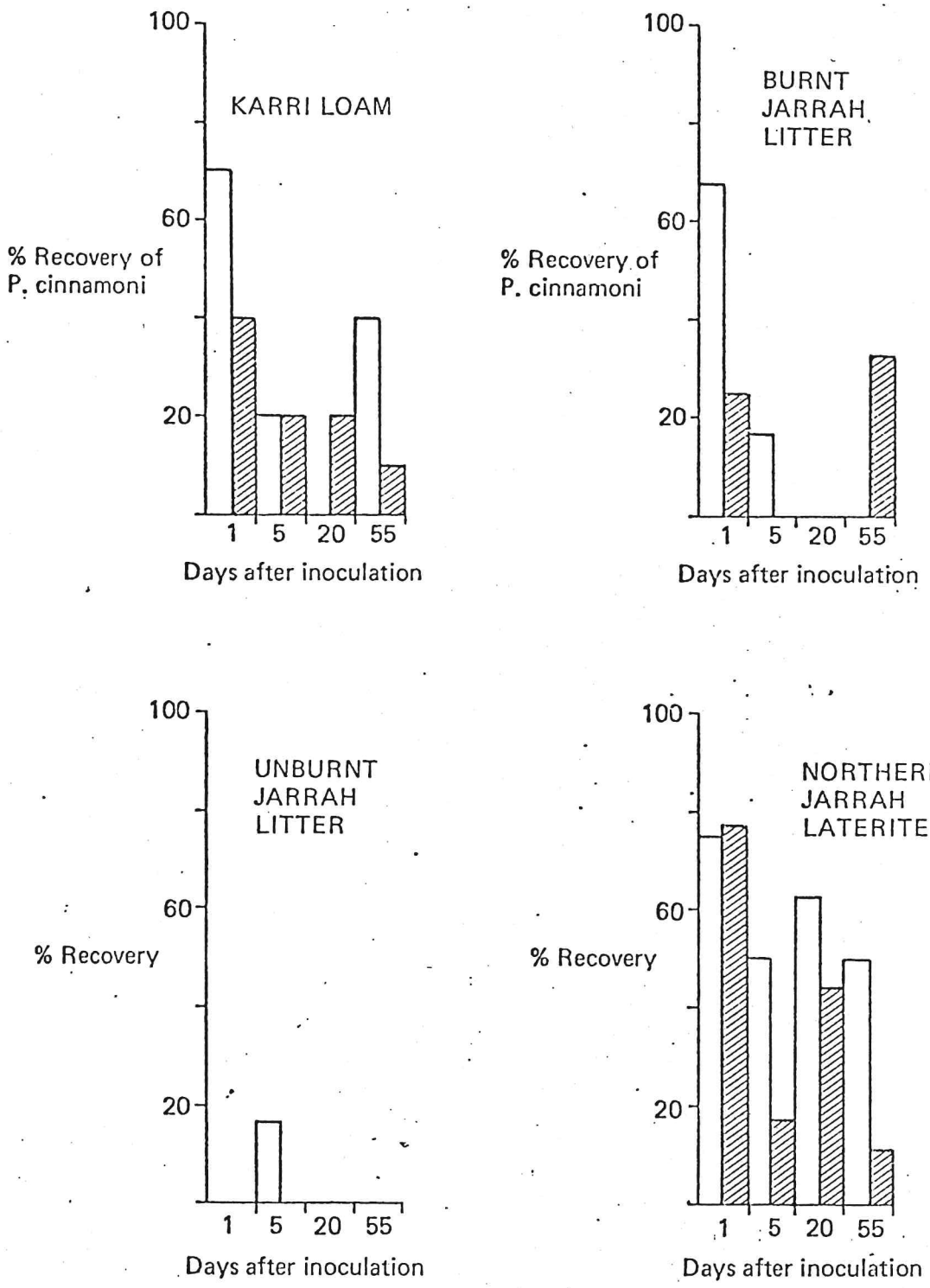


Figure: 5 Effect of soil type on the recovery of Pc from non mycorrhizal ☐ and ectomycorrhizal roots ▨ of jarrah.



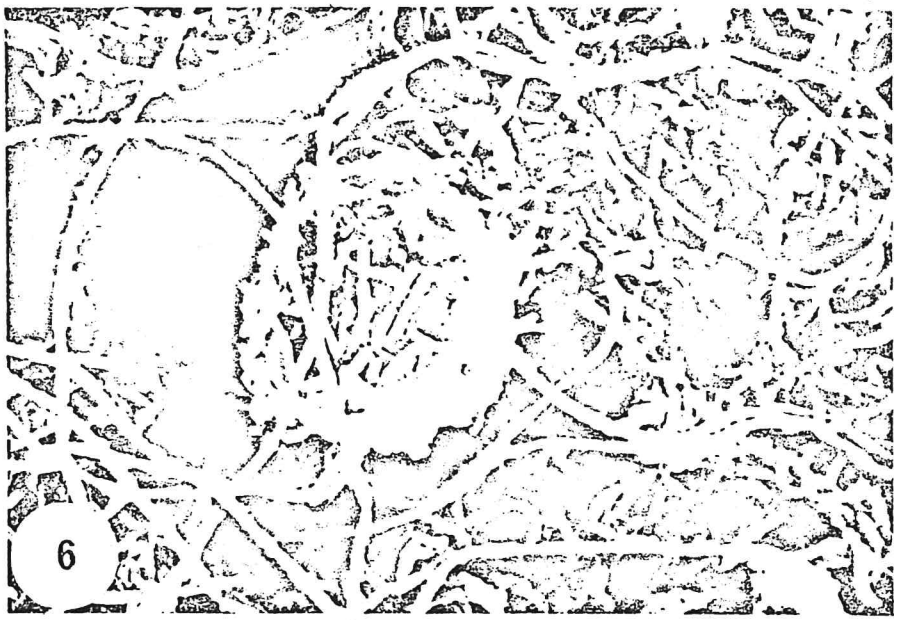
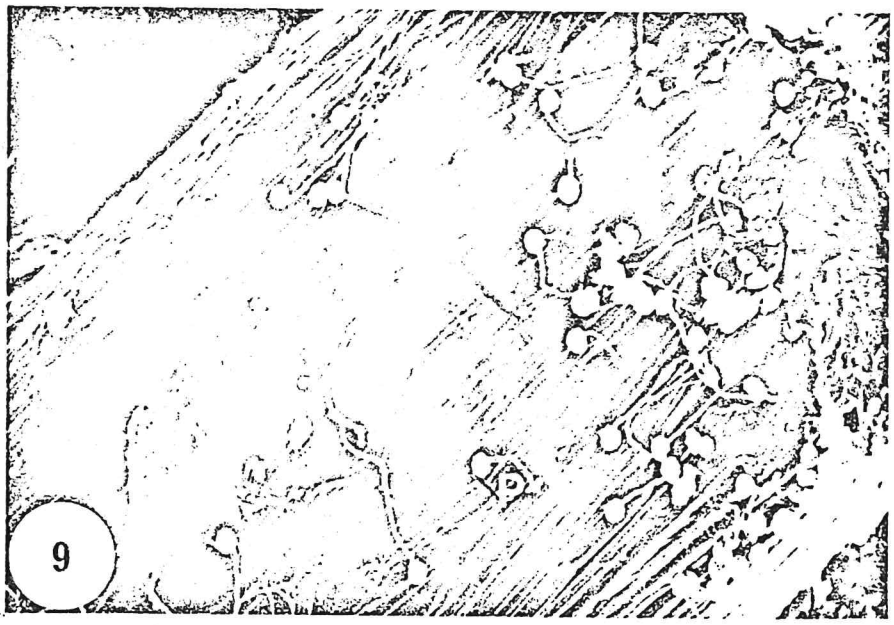


Fig. 6 An encysted zoospore on a non mycorrhizal Jarrah root showing rhizosphere bacteria on the surface of the cyst. It is not known whether the zoospore has germinated and penetrated the root or if it is inviable.

Fig. 7 An encysted zoospore that has germinated and shows bacteria on the surface and lysis of the germ tube.

Fig. 8 A germinated zoospore that has been lysed by bacteria, the cyst and germ tube have undergone complete lysis and been rendered inviable.

Fig. 9 A surface view of a non-mycorrhizal Jarrah root with a larger number germinated zoospores. Penetration of the root surface and infection has occurred. (P)



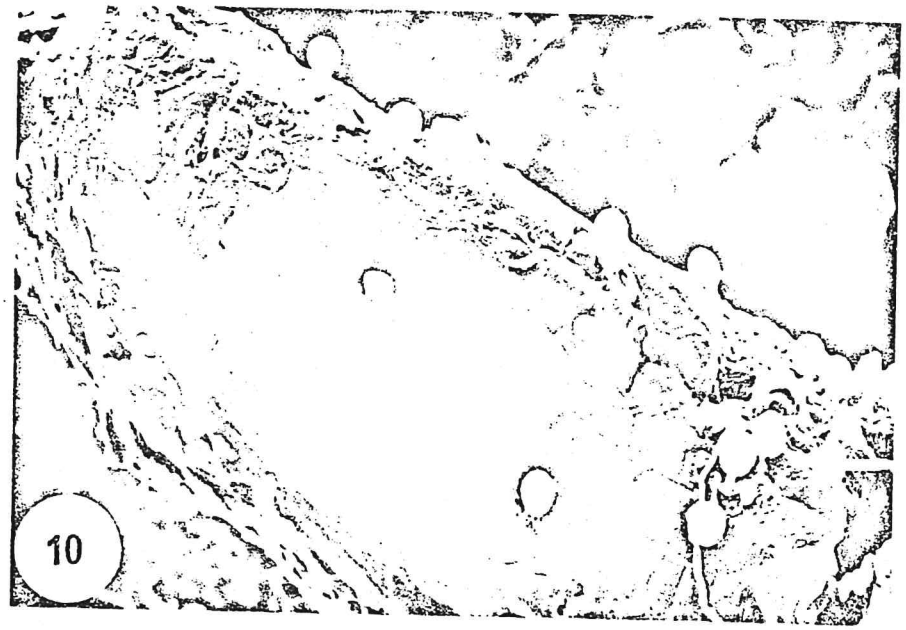
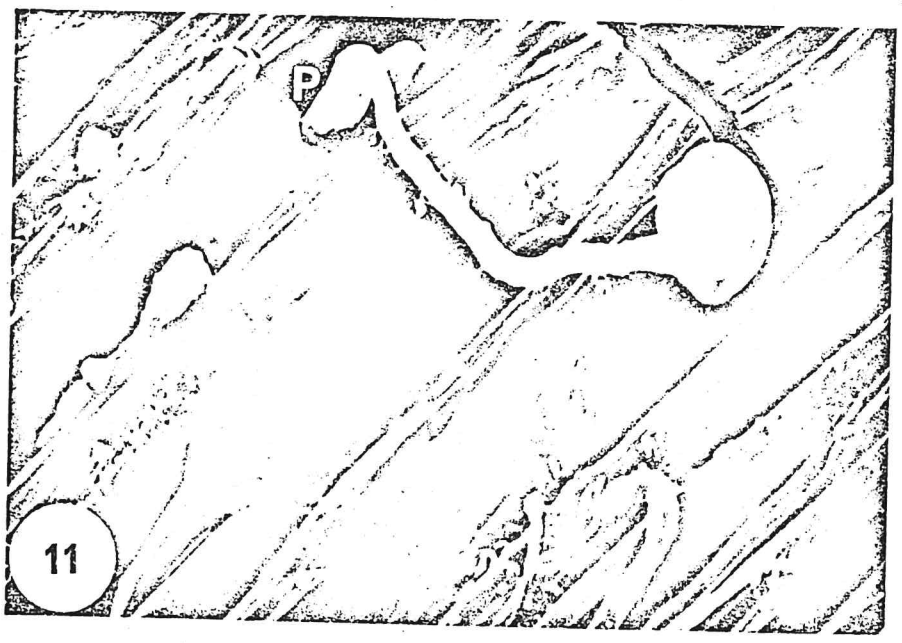


Fig. 10 Zoospores on the surface of a young, fleshy Jarrah root. Most zoospores are located at the junction of epidermal cells and have penetrated the root.

Fig. 11 An enlarged view of the marked zoospore (P) in figure 9. The germ tube has penetrated the root at the junction of two epidermal cells.



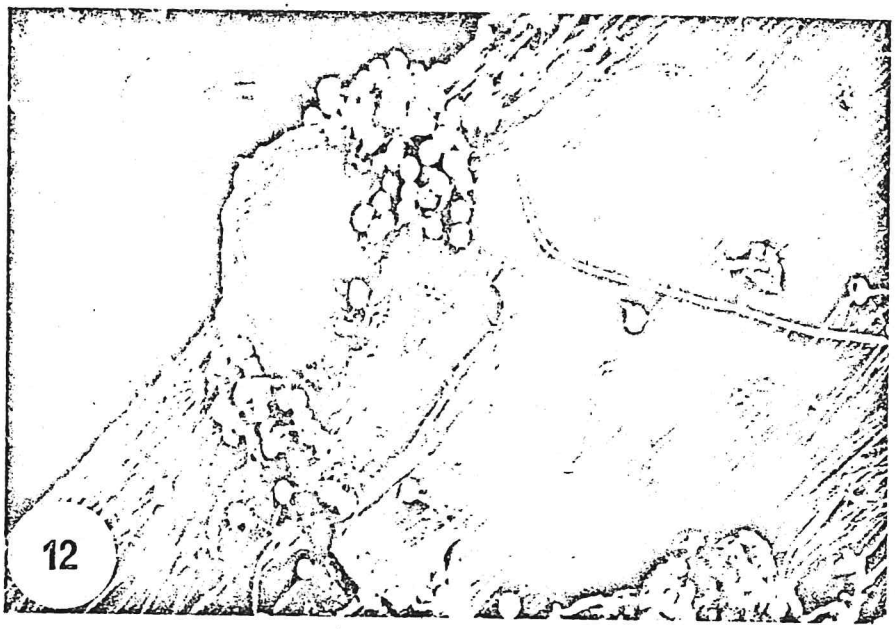


Fig. 12 Zoospores aggregated about an emerging lateral root on a non-mycorrhizal Jarrah root. Infection has occurred.

Fig. 13 Zoospores attracted and attached to a damaged area on a non mycorrhizal Jarrah root. The zoospores have germinated and penetrated the root.

Fig. 14 A surface view of an ectomycorrhizal Jarrah root showing the fungal mantle covering the whole root and enveloping the lateral root.

Fig. 15 An enlarged view of the dense, interwoven net of fungal tissue that forms the ectomycorrhizal mantle. It is this mantle that provides part of the physical barrier to infection by P. cinnamomi.

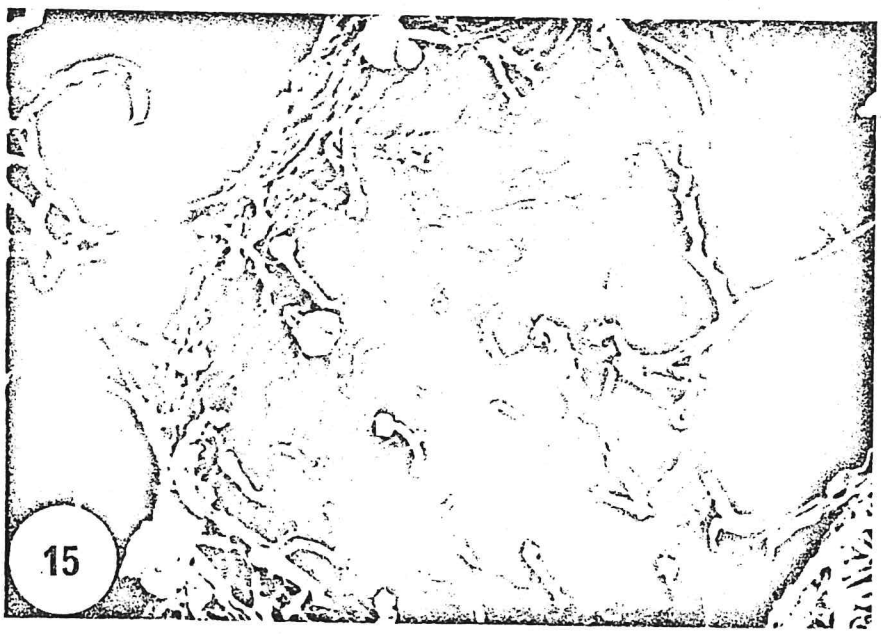
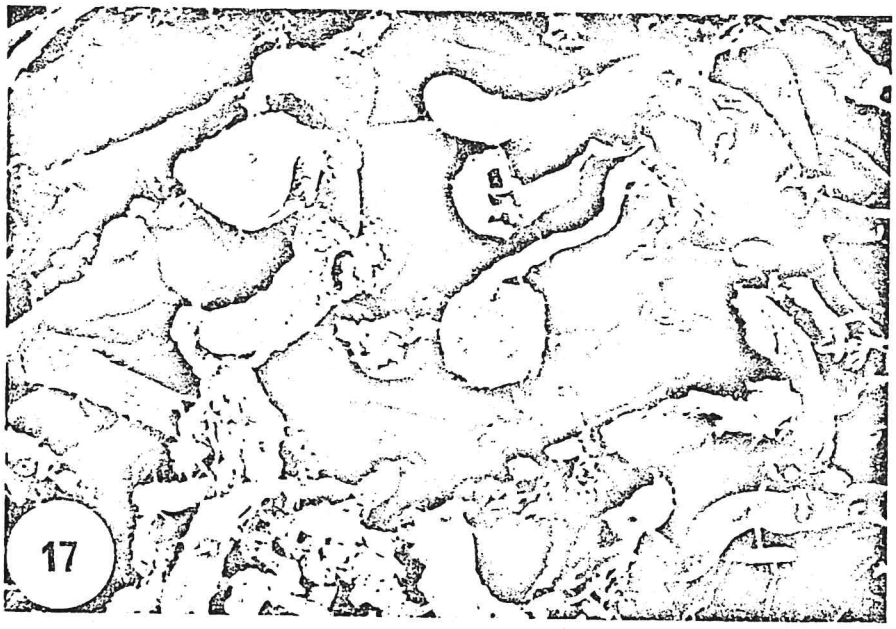


Fig. 16 A surface view of an ectomycorrhizal root of Jarrah that shows some features that may be of use in differentiating mycorrhizal types. These features are easily confused with germinated zoospores.

Fig. 17 A germinated zoospore on the ecotmycorrhizal mantle of a Jarrah root. Penetration of the mantle may have occurred but the hartig net between root cells may have prevented infection of the root tissue.



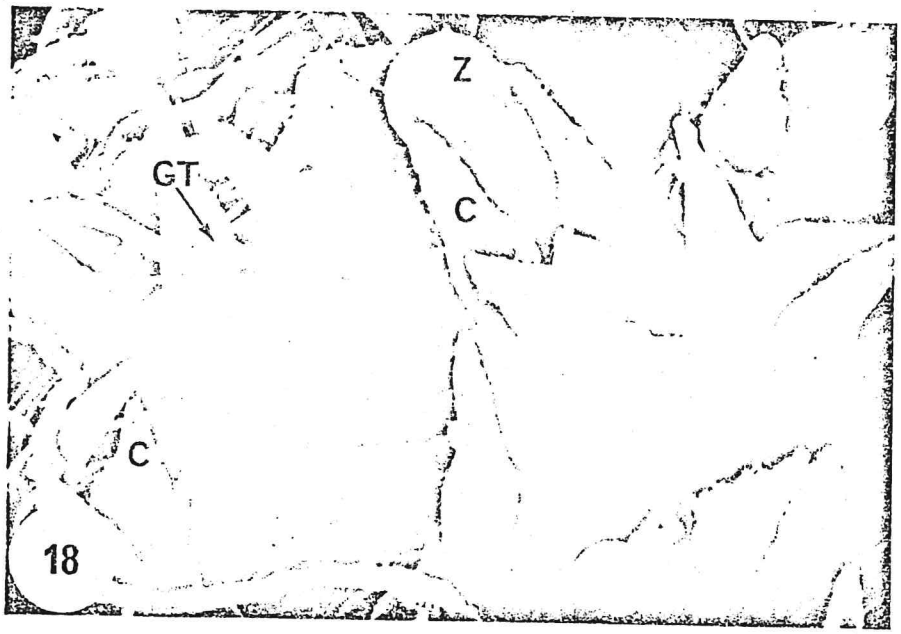
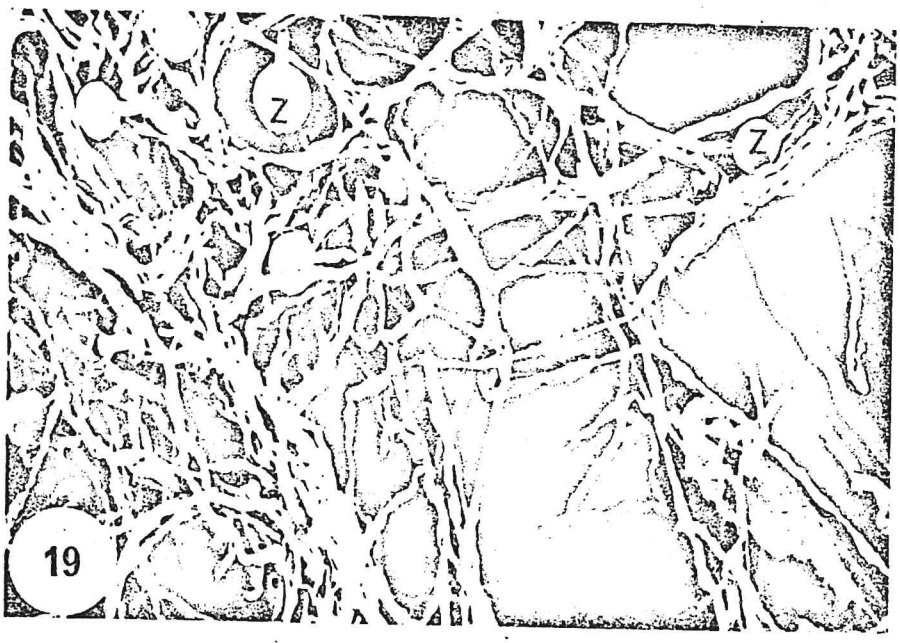


Fig. 19 A dense mat of mycelium from germinated zoospores (Z) on the surface of a non mycorrhizal root of Jarrah grown in Karri loam. The hyphae is lysed and it is uknown whether infection has occurred.

Fig. 18 A germinated zoospore (Z) on the surface of a "typical" white jarrah ectomycorrhizal root. The germ tube (GT) has grown through the cystidia (C) of the ectomycorrhizal fungal tissue but has not penetrated the mantle.



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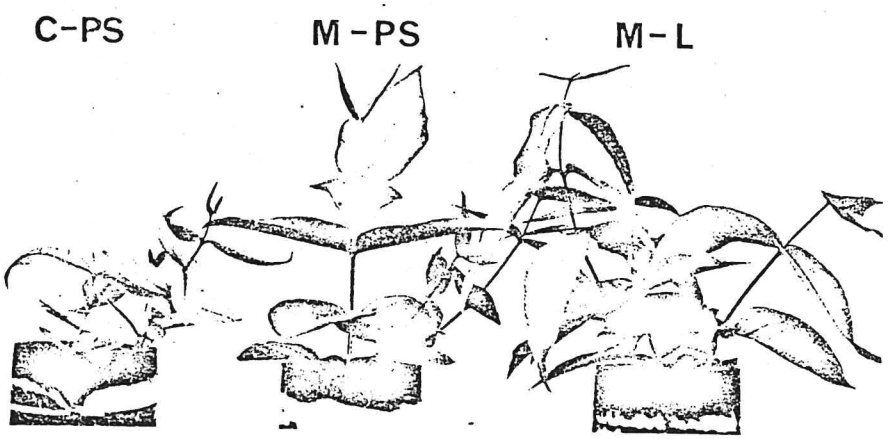
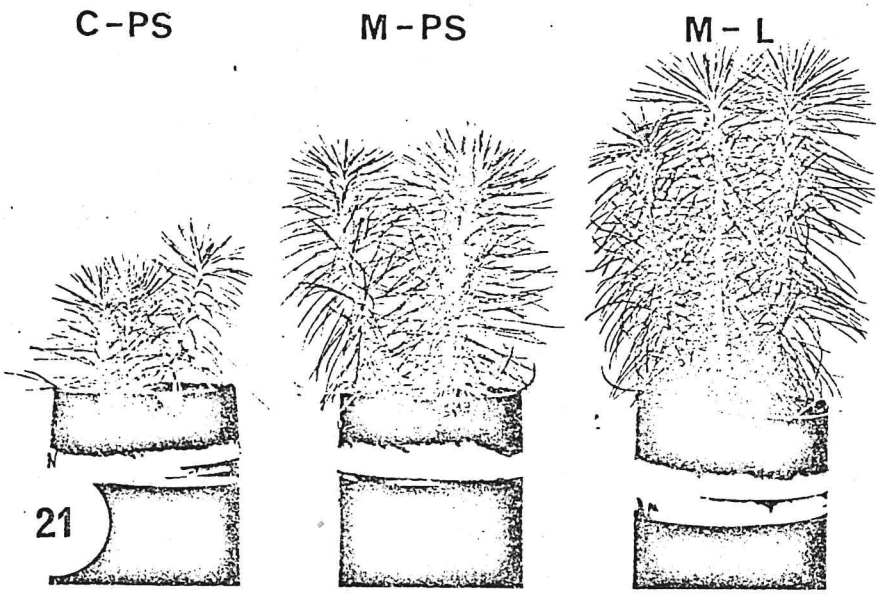


Fig. 20 Growth response of Jarrah seedlings inoculated with the ectomycorrhizal fungus Laccaria laccata. C-PS is a non mycorrhizal control in peat and sand. M-PS is mycorrhizal in peat and sand. M-L is mycorrhizal in sterile jarrah litter.

Fig. 21 Growth response of Pinus radiata seedlings inoculated with Laccaria laccata. The key to the treatments is the same as Fig. 20.



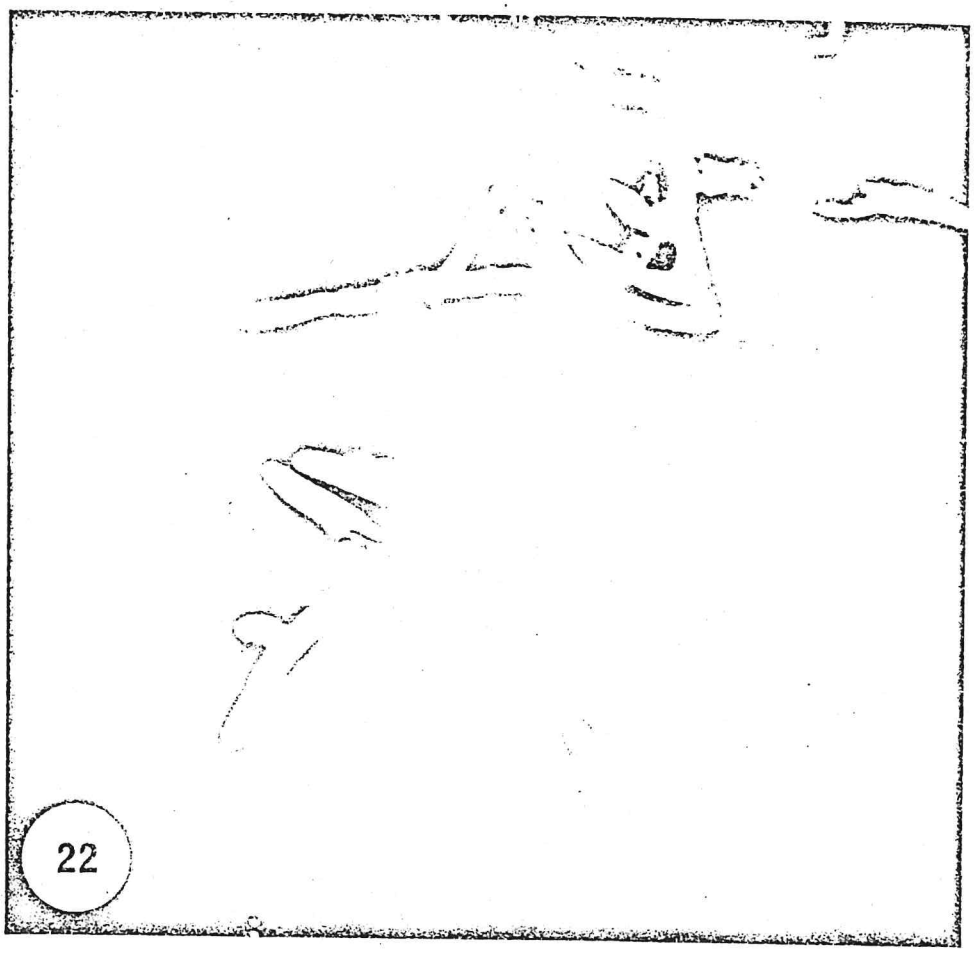


Fig. 22 Jarrah ectomycorrhizae formed in sterile conditions by Laccaria laccata in pure culture synthesis tubes.

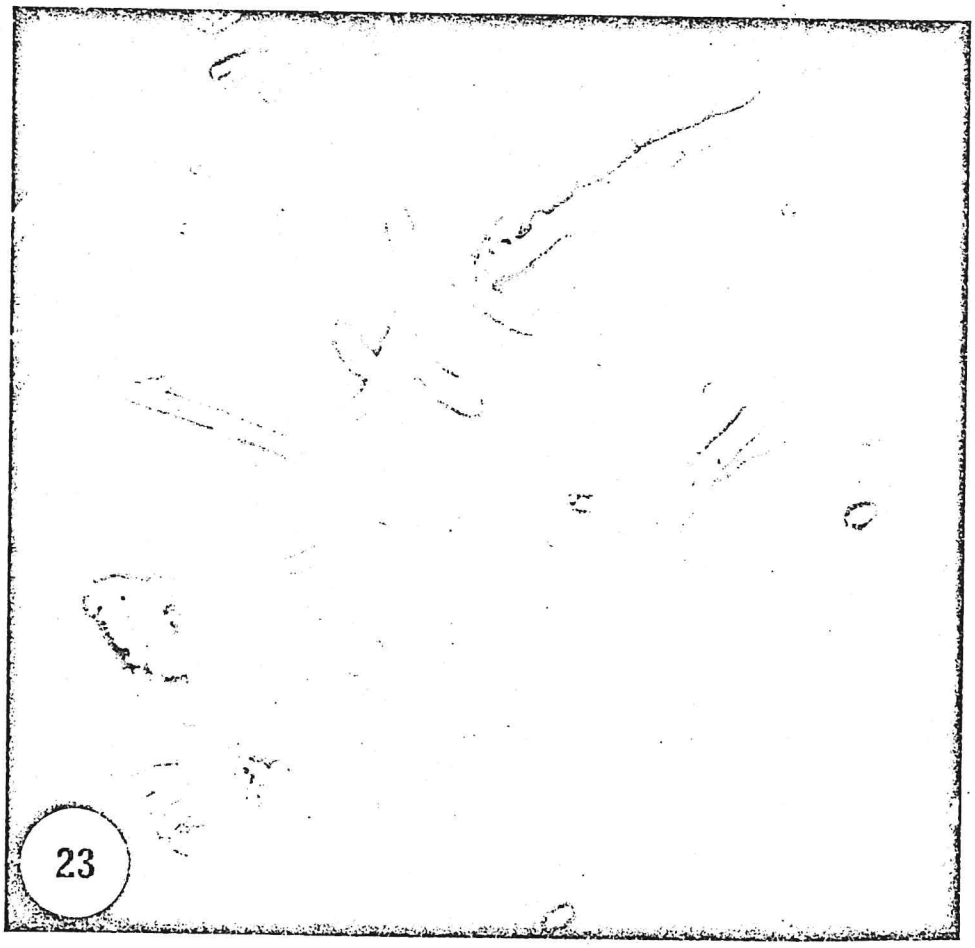


Fig. 23 Jarrah ectomycorrhizae formed in soil inoculated with Laccaria laccata.

Table 1.

CULTURE COLLECTION 1981 - EPIGEOUS FUNGI (AGARICOIDE OR AERIAL-FREE SPORED)

Summary: 25+ genera represented in the specimen collection.
70+ cultures from 18 genera (excluding several unknown genera).

Genus	Species	Ecotype No/Location	Positive - Mycorrhizal			
			Jarrah	Tuart	Karri	*P. radiata*
AGARICUS	sp. (>1)					
AMANITA	*conicobulbosa*		(-)	(-)	1	
	priessii		1	1	(1)	
	umbrinella				1	
	xanthocephala		1		1	
	sp. (>1)		1	1	1	
ARMILLARIA	sp.					
BOLETELUS	*obsuricoccineus*		1		1	
BOLETUS	sp. (>1)		1	1	1	
CLITOCYBE	sp.					
CORTINARIUS	*"phlegmacium"*		1		1	
	sp. (H. Thiers.)	1. Dwellingup	+	+	+	?
	sp. (>1)		1	1	1	
HEBELOMA	*crustuliniforme*	2. Nannup/Gnangara	+	?	+	1
LACCARIA	*laccata*	2. Dwellingup/Jarrahdale	+	+	+	+
LACRYMARIA	sp.					
LACTARIUS	sp.	2. Dwellingup/Jarrahdale	1(+)	(-)	(-)	
LEPIOTA	sp. (>1)					
PAXILLUS	*muelleri*		1			
	sp.		1		1	
PHYLLOPORUS	sp.	2. Ludlow/Perth	(1)	1		
ROZITES	*australiensis*		1		1	
RUSSULA	sp.		1		1	
SUILLUS	*granulatus*		-	-	-	1/2
	luteus		-	-	-	1/2
TRICHOLOMA	sp. (>1)		1	1	1	

...../contd.

CULTURE COLLECTION 1981 - EPIGEOUS FUNGI (Continued)

Genus	Species	No. of Cultures	Positive - Mycorrhizal			
			Jarrah	Tuart	Karri	*P. radiata*
AMERICAN CULTURES						
AMANITA	*muscaria*	1	(+)	(+)	(+)	
	pantherina	1				
BOLETUS	*edulus*	1				
CORTINARIUS	*elegantor*	1	(+)			
HEBELOMA	*crustuliniforme*	2	(+)	(+)	(+)	
LACCARIA	*laccata*	4				
SUILLUS	*albidipes*	1				
	brevipes	1				
	brunnescens	1				
	grevillei	1				
	lakei	1				
	ponderosus	1	* All are positive on various species of pine.			
	subolivaceous	1				
	tomentosus	1				

KEY:

\+ Culture positive in pure culture synthesis (PCS) trials.

Blank – Unknown and/or PCS not tried.

1 Culture deemed positive by association - observed in forest.

2 Same genus proven positive by other workers.

? No result obtained yet in PCS trial.

( ) Possible result

\- Definitely not mycorrhizal - fungi specific for set group of plants.

Table 2. CULTURE COLLECTION 1981

HYPOGEOUS FUNGI (SECOTIOID OR UNDERGROUND "ENCLOSED SPORED")

SUMMARY: - 25 genera represented in the specimen collection.

- 77^{+} cultures from 13 genera (excluding several unknown genera).

GENUS	SPECIES	ECOTYPE NO/LOCATION	POSITIVE - MYCORRHIZAL			
			Jarrah	Tuart	Karri	P.Radiata
AUSTROGAUTIERIA	sp.					
CASTORIUM	camphoratum				1	
GYMNOMYCES	sp.				1	
HYMENOGASTER	sp. (>1)				1	
HYSTERANGIUM	sp. (>1)		1	(1)	1	
ILYEODICTYON	sp.		PCS negative			
MARTELLIA	sp.					
MESOPHELIA	labyrinthomyces				1	
	trabalis		1		1	
OCTAVIANINA	sp.					
PISOLITHUS	microcarups	2 Ludlow/Quinninup	1	(+)		
PISOLITHUS	tinctorius	H35 - Dwellingup	+	+	+	?
		H37 - Nannup	(+)	+	+	?
		H38 - Nannup	(+)	+	+	?
		H43 - Amphion	+	+	+	?
		H46 - South Australia	+	+	+	?
		H53 - Agroforestry	+	(+)	(+)	?
		H70 - Jandakot				
		H77 - Nannup				
		H80 - CSIRO (Floreat)	+	+		
		H93 - Mundaring				
		H95 - Dwellingup				
		H98 - Dunsborough	+	+	+	?
		H99 - Cobiac				
		H107 - Gnangara				
		H215 - Boddington				
RHIZOPOGON	luteus		-	-	-	1/2
	roseolus		-	-	-	1/2

.../2

CULTURE COLLECTION 1981 - HYPOGEOUS FUNGI (CONTD)

GENUS	SPECIES	ECOTYPE NO/LOCATION	POSITIVE - MYCORRHIZAL			
			Jarrah	Tuart	Karri	P.Radiata
SCLERODERMA	cepa	Dwellingup/Manjimup	+	+	+	
	varicosum	Dwellingup/Ludlow	+	+	+	
UNKNOWN		H75 - Pemberton	+		(+)	
SELLEROMYCES	sp.		1		1	
AMERICAN CULTURES:		NO. OF CULTURES				
CENOCOCCUM	geophilum	1				
	graniforme	2				
CLAVATIA	fumosa	1				
LYCOPERDON	pyriforme	1				
PISOLITHUS	tinctorius	7				1/2
		Code No.H234(0.125)	+	?	+	(+)
		H235(0.138)	?	+	+	?
		H237(0.183)	+	(+)	+	?
RHIZOPOGON	sp. (15 different species - 1 rep.each)		-		-	1/2

KEY: As per EPIGEOUS sheet.

Table 3. The effect of fertilizer application and fire history on the total biomass (kg(FW)/ha) of fungi found on treated plots in the forest (Year No 1 - 1981)

FERTILIZER TREATMENT 50 kg/Ha	PLOT LOCATION AND FIRE HISTORY					
	COBIAC 1 UNBURNT	COBIAC 2 PB(1)	AMPHION UNBURNT	PLAVINS PB(<7)	VIRGIN JARRAH PB(?)	KARRI REGEN 1977
CONTROL - O/O	33.3	18.1	17.5	10.8	50.6	36.7
PLUS N - O/N	56.7	14.4	10.5	19.6	63.6	37.2
PLUS P - O/P	40.2	10.9	1.7	9.2	26.5	33.5
PLUS N/P	7.4	47.5	78.4	35.6	71.2	181.9

N - Nitrogen as urea

P - Phosphorus as triple superhphosphate

PB( ) - Years since area was prescribed burnt

Table 4. The effect of fertilizer application on the initiation and development of ectomycorrhizae (white pyramidal) in two jarrah litter types in root boxes.

FERTILIZER TREATMENT	AREA OF ECTOMYCORRHIZAL INITIATION (cm^2)	
	UNBURNT LITTER	BURNT LITTER/SOIL
CONTROL - O/O	55	44
PLUS P	20	26
PLUS N	20	26
PLUS N/P	111	70
FRESH WT ESTIMATED	$x2^{\oplus}$	1

TOTAL AREA OF ROOT BOX - 336 cm^2

N - Nitrogen as urea

P - Phosphorus as triple superphosphate
(Both at equivalent rates to field applications)