REPORT OF WORK DONE

JANUARY 1981 - FEBRUARY 1982

E.M. Davison

A. GENERAL MYCOLOGICAL SUPPORT FOR INVESTIGATORS IN DIEBACK RESEARCH

1. Culture collection

The culture collection, which at the moment contains 240 cultures of fungi, is used for reference purposes. It contains authenticated cultures of many species of *Phytophthora*, many isolates of *P. cinnamomi* from the jarrah forest, recent isolates of *Phytophthora* spp. from the Donnybrook sunklands, and cultures of fungi from jarrah. The culture collection will continue to be expanded.

2. The isolation medium

The antibiotic medium which is routinely used for the isolation of *Phytophthora* spp. from soil was assessed to determine whether it could be used in a quantitative way to measure the number of viable *Phytophthora* propagules in soil. Growth rates of 60 isolates of *P.* spp., including 25 isolates of *P. cinnamomi*, were compared on the basal medium with and without antibiotics. Mycelial growth rates of the same isolate on the two media were not the same, in some cases the inclusion of antibiotics stimulated mycelial growth, while in other cases mycelial growth was inhibited. These effects were not consistent between isolates of the same species.

The effect of the antibiotic medium on zoospore germination and germ tube growth was also investigated. The antibiotic medium inhibited zoospore germination and subsequent germ tube development; the effect being most pronounced in unencysted zoospores. There was no correlation between the effect of the antibiotic medium on mycelial growth and on zoospore germination and germ tube growth.

If the antibiotic isolation agar is to be used in quantitative work, the behaviour of the particular *Phytophthora* isolates to be used should be determined beforehand so that correction factors can be applied if necessary. It is not possible to predict the growth response of any isolate on this medium.

This work has been completed, and is being prepared for publication.

3. Identification of fungi

Assistance has been given to the Forests Department in the identification of *Pythium* and *Phytophthora* spp. isolated from the Donnybrook sunklands and from the jarrah forest, and to Alcoa in the identification of fungi from the rehabilitated minesites. In addition, fungi other than *P. cinnamomi* have been assessed in a large CSIRO Division of Forest Research - W.A.I.T. pot trial.

A joint plant anatomy/mycology workshop was run with Dr. J. Tippett in July 1981 for people involved in dieback research.

Assistance with identification of fungi will be given whenever requested.

B. INVESTIGATION OF THE ROLE OF *PHYTOPHTHORA CINNAMOMI* IN THE DECLINE AND DEATH OF JARRAH

1. The effect of *P. cinnamomi* on jarrah growth

Growth rates of paired jarrah trees in healthy, dieback affected, and along the margin of a dieback zone have been compared during the growing season December 1980 - December 1981; the results are shown in Table 1.

Table 1. Growth (increase in girth) of jarrah, December 1980 - December 1981

	Healthy	Dieback affected	Dieback margin
Mean growth (girth, mm)	4.83^{a}	8.47^{ab}	10.56^{b}
s	4.20	6.45	4.60
n	12	12	12

Similar suffixes indicate results which are not significantly different ($\alpha = 0.05$)

Xylem pressure deficits and nutrient analyses of leaves* were carried out in April and October, there were no significant differences between trees in the three groups. **

Tree density affects jarrah growth. As the trees in the growth trial are not evenly spaced, tree density may have a greater effect on growth than *P. cinnamomi* in upland lateritic sites.

The growth trial is being continued.

CSIRO Division of Forest Research have started a similar but larger growth trial.

2. Infection of jarrah by *P. cinnamomi*

This project is being carried out in collaboration with Dr. J. McComb and Dr. I. Tommerup.

It is hoped that jarrah plantlets produced by tissue culture will be more uniform than jarrah seedlings, and if the plantlets are comparable with seedlings they will be ideal material in which to study the early stages of infection by *Phytophthora cinnamomi*. So far this investigation has

* By kind arrangement with Professor J. Loneragan.

** I am grateful to the Forests Department for shooting down twigs.

been concerned with developing suitable techniques for producing the fungus, and staining and examining it in the host.

3. Pathology of jarrah

As very little is known about the pathology of jarrah, a start has been made on identifying many of the fungi associated with twig, branch and trunk cankers. Unfortunately, jarrah's rough bark makes it impossible to see the sunken lesions associated with developing cankers, so it is only possible to find these cankers when damage such as dead twigs, branches and trunks indicates their presence. When bark is removed, necrotic phloem is exposed, and isolations have been made from the margin of the dead tissue. The fungi most frequently isolated from these cankers are *Cytospora eucalypticola*, *Endothia havanensis*, *Discosporium* sp. and *Botryosphaeria ribis*.

Cytospora eucalypticola was described from South Africa in 1965 where it was associated with the death of drought stressed *Eucalyptus saligna*. It is also associated with a bleeding canker in *Angophora costata* in Eastern Australia. *Endothia havanensis* was described on several eucalypts in Cuba in 1916 where it was thought to be a saprophyte, it has not previously been recorded in Australia. The *Discosporium* is an undescribed species. *Botryosphaeria ribis* is a widespread canker-causing pathogen of woody plants which is common in the Americas, Southern Europe, and Australia. Although it has been recorded from eucalypts in the U.S.A and Argentina it has not been recorded in eucalypts in Australia.

Although these fungi were sometimes isolated in pure culture from the margin of lesions, they were more often isolated in combination. In dieback sites, *Phytophthora cinnamomi* and *P. citricola* were sometimes isolated in association with other fungi from the lesion margin of girdled trunks.

The main fungi isolated from twig, branch, and trunk cankers are shown in Table 2.

Table 2. Fungi isolated from twig, branch, and trunk cankers of jarrah

	Twig cankers (<5mm diam)	Branch cankers (>5mm diam)	Trunk cankers (<1m girth)	
			Dieback sites	Healthy sites
Cytospora eucalypticola	4	26	21	2
Endothia havanensis	10	7	8	0
Discosporium sp.	1	21	16	1
Botryosphaeria ribis	0	0	2	2
Phytophthora cinnamomi	0	0	7	0
P. citricola	0	0	2	0
Number of specimens examined	12	45	45	5

Table 3 gives an estimate of the probability of recovering these fungi from cankers, based on their isolation frequency.

Tables 2 and 3 show that *Endothia havanensis* is most frequently associated with twig cankers, while *Cytospora eucalypticola*, *Discosporium* sp. and *Botryosphaeria ribis* are more commonly isolated from trunks. Although *Phytophthora cinnamomi* and *P. citricola* were isolated from 20% of the trees in dieback sites, they were only isolated from 4% of all the pieces of tissue plated. Therefore they may not be as important as originally thought.

Pathogenicity testing has shown that *Botryosphaeria ribis* is a vigorous pathogen of jarrah.

Table 3. Isolation frequency of fungi from twig, branch, and trunk cankers of jarrah

	Twig cankers (<5mm diam)	Branch cankers (>5mm diam)	Trunk cankers (<1m girth)	
			Dieback sites	Healthy sites
Cytospora eucalypticola	26%	42%	20.5%*	15%*
Endothia havanensis	72%	5%	4%	0
Discosporium sp.	1%	19%	13%	21%
Botryosphaeria ribis	0	0	2%	21%
Phytophthora cinnamomi	0	0	3%	0
P. citricola	0	0	<1%	0
Number of pieces plated	68	453	800	96
Number of specimens examined	12	45	45	5

* A quarter of all platings were made into P_{10}VPH agar, selective for *Phytophthora* spp.

A nematode has sometimes been isolated with *Cytospora eucalypticola*. It is an undescribed species of *Deladenus*, a genus whose members have a complex life cycle which includes insect parasitic and fungus feeding stages. The nematode is in culture and feeds readily on *C. eucalypticola*. Infective female nematodes have been found in wood, but the insect vector is unknown. *Cytospora eucalypticola* may also be carried by the vector.

This work is continuing.

4. Pathology of eucalypts used in rehabilitation plantings

This work has just been started with Mr. Gardner, Alcoa. Results of preliminary isolations are shown in Table 4.

Table 4. Isolation of fungi from lesions in eucalypts other than jarrah

	Cytospora eucalypticola	*Endothia havanensis*	*Botryosphaeria ribis*	*Discosporium* sp.
Eucalyptus calophylla		+		+
E. diversicolor			+	
E. globulus	+			
E. megacarpa	+			
E. resinifera	+			
E. saligna	+	+		
E. wandoo	+	+	+	

Mr. Gardner will be surveying the rehabilitation areas to assess the extent of trunk cankers in rehabilitated mine sites.

An honours student will be working on *Cytospora eucalypticola* on *E. saligna* in 1982.

5. The effect of water on jarrah growth

Widespread death of jarrah in dieback sites occurs during or following wet years. The deaths are attributed to *Phytophthora cinnamomi*, but even during 1965 which was a wet year, Podger was only able to isolate *P. cinnamomi* from 5 out of 45 jarrah trees sampled.

Two preliminary pot experiments have been carried out to measure the effect of waterlogging on jarrah seedling growth. The results confirm Podger's findings that waterlogging reduces seedling growth.
This work will be continued.

GENERAL DISCUSSION

Phytophthora cinnamomi is one of the few fungi which can be readily identified in its mycelial stage. As a result, other species of *Phytophthora* have been largely ignored, even though they may have similar effects to *P. cinnamomi*. In the past two years, five species of *Phytophthora* from pines in the Sunklands have been identified for the Forests Department, and four *P.* spp. have been identified from other forested areas.

Apart from Harding's work in 1949 and Shearer's recent survey of *Armillaria*, there has been no survey of pathogens of jarrah, consequently it is impossible to assess the role of *P. cinnamomi* in the decline and death of jarrah if other important pathogens are ignored.

The survey of jarrah pathology has shown the constant association of four species of fungi with twig, branch and trunk cankers and trials are being undertaken to assess the pathogenicity of these fungi to jarrah. If they are pathogenic, some of the symptoms included in the complex disorder Jarrah Dieback, will be explained.

Wallace and Hatch, in 1953, discussed the disorder Crown Deterioration in jarrah, and commented that "an associated problem which may or may not be connected with this general crown deterioration is the occurrence, on poorer site qualities, of dying jarrah patches". In subsequent years, both these disorders became known as Jarrah

Dieback. There are four groups of symptoms which are included in this complex disorder: patch death of the understory, decline in the jarrah crowns, sudden death of jarrah, and death of jarrah in wet years. All these symptoms are generally considered to be due to *P. cinnamomi*, however, only the death of the understory has been shown to be due to this organism. The decline in jarrah crowns is widespread throughout the forest, and if the fungi associated with cankers are pathogenic, then they are probably responsible for much of the dead wood in jarrah crowns. Similarly the "sudden death of jarrah" is not confined to dieback sites, although in "healthy" forest these deaths are called drought deaths. If the fungi associated with cankers are pathogenic, these deaths are readily explained.

Although it may be argued that the crowns of jarrah trees in dieback sites decline because of chronic root infection by *P. cinnamomi*, the dendrometer band data presented above does not support this, as in 1981 the healthy trees grew more slowly than dieback affected ones, and tree growth is proportional to functional crown size. One year's data however is insufficient, and measurements in different sites may give different results.

So far, the death of jarrah in wet years has hardly been considered in the research programme. Podger showed that jarrah was the least tolerant of the jarrah forest eucalypts to waterlogging, and our results have confirmed the poor growth of jarrah seedlings in waterlogged conditions. We hope to pursue this area of research more actively during 1982 to determine whether waterlogging by itself, or waterlogging with *P. cinnamomi* are required for the death of jarrah.

The working hypothesis, outlined above, which is based on the concept of jarrah dieback being a complex disorder is summarised in Table 5.

Table 5. A working hypothesis for the complex disorder "Jarrah Dieback"

Symptoms	Confined to dieback site	Cause	Authorities	Confirmed
Patch death of understory	yes	*P. cinnamomi*	Podger Shea, etc.	yes
Decline of jarrah crowns	no	canker causing fungi	Davison et al.	no
Sudden death of jarrah	no	canker causing fungi	Davison et al.	no
Death of jarrah in wet years	?	?waterlogging ?waterlogging + *P. cinnamomi*		

PERSONNEL

Mr. F.C.S. Tay, B.Sc.(Hons) Professional Officer. July 1980 - present

Duties: General assistance with isolation work, particular responsibility for glasshouse work.

Position financed until June 1981 by D.C.E., and by the Interim Foundation for Dieback Research from July 1981 - June 1983.

Mrs. R. Stynes, B.Sc.(Hons) Professional Officer (part-time). April 1981 - present.

Duties: Isolation and identification of plant-parasitic nematodes.

Financed by D.C.E.

Mrs. E. Carter, B.Sc.(Hons) Professional Officer. July 1981 - January 1982.

Duties: Study of infection of jarrah by *Phytophthora cinnamomi* and host's response to infection.

She will be replaced by Mrs. C. Tonkin, B.Sc. (Graduate Research Assistant) from March 1, 1982.

Financed by the Interim Foundation for Dieback Research.

Mr. D. Fraser, B.App.Sc. Honours Student. March - November 1982.

Project: "The importance of *Cytospora eucalypticola* on *Eucalyptus saligna* in W.A."

PUBLICATIONS

E.M. Davison: *Endothia havanensis* on jarrah. Australasian Plant Pathology (in press).