Beverley

REPORT OF THE DISCUSSIONS OF A WORKING GROUP ON JARRAH DIEBACK, HELD IN THE INSTITUTE OF AGRICULTURE, UNIVERSITY OF WESTERN

AUSTRALIA 29/6/77

Present: Dr. E.R. Hopkins, Dr. S. Shea* and Mr. J. Havel (Forests Department);
Dr. M.J. Mulcahy (E.P.A.); Professor J.F. Loneragan, Professor
M.J. Dilworth, Dr. Andrew Glenn*, Mr. H. Nesbitt* and Miss B. Reynolds
(Murdoch University); Dr. J. Majer and Dr. R. Kagi (W.A.I.T.);
Dr. F.J. Hingston, Dr. N. Malajczuk*, Mr. Jan Titze* and
Mr. Mark Dudzinski* (C.S.I.R.O.); Mr. R. Hilton*, Dr. D. Keast*,
Miss L. Walsh, Dr. Inez Tommerup, Dr. I. Abbott, Mr. K. Sivasithamparam,
Mr. T. Boughton and Professor C.A. Parker (University of Western
Australia).

Convenor: C.A. Parker, with assistance from S. Shea, N. Malajczuk and J. Titze.

*Asterisks indicate persons who presented invited "discussion papers" setting out main findings from research completed, indicating research presently being undertaken, and areas of research contemplated.

Main Issues Arising from the Discussion Papers

Dr. S. Shea: an overview of Jarrah Dieback.

- 1. Dieback syndrome known for many years before Podger, Doepel and Zentmyer found the causative agent in 1965.

 Estimated that about 200,000 ha are affected by dieback, caused by *Phytophthora cinnamomi*, and that the present amount of spread is about 20,000 ha per annum.
- 2. It is difficult to calculate the <u>rate</u> of spread, but it is hoped to estimate this from intensive aerial photography.
- 3. The disease is advancing mainly along the western scarp and moving eastwards. At present most is found within a zone between the scarp and 10-15 km east of the scarp. But there is evidence that this distribution is partly fortuitous, i.e. logging was mainly in this zone.
- 4. The disease spreads rapidly downhill, probably because of zoospores borne by surface-water, but possibly by lateral water-movement below the surface. Chlamydospores could be involved in subsurface movement.
- 5. The disease spreads slowly uphill. This can be accounted for primarily by movement along the roots of susceptible plants.

 Mycelial movement through the soil is possible but slow, based on average movement of 1-5cm, only, per year.
- 6. Very few new outbreaks of P. cinnamomi in disease-free areas are not associated with human activity.
- 7. There has been much more human activity in the jarrah forest in the central and eastern zone recently than ever before e.g. road

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building, logging, prospecting, mining, recreation, power-lines. It is known that *P. cinnamomi* can be established in new areas by movement of even small amounts of infected soil.

- 8. The present quarantine restrictions were imposed in an attempt to halt or slow the dissemination of the disease by human activity. They will also give the Forests Department time to evaluate rates and mechanisms of spread. Then, when the symptoms have been expressed, and aerial photography done, it should be possible to locate most of the new infection.
- 9. No known tree species can replace jarrah on the upland lateritic sites, but alternatives are available for the lowlands. It is doubtful whether any replacement tree can perform the hydrological function of jarrah in upland lateritic soils.
- 10. The attempt should therefore be made either to make *P. cinnamoni* less pathogenic to jarrah, or to suppress it. There are perhaps only 10-20 years remaining in which to discover a realistic system of control. Once the disease has passed into the stage of infection of the understorey it will not be possible to control it.
- 11. The best hope at present lies in forest management. Banksias and a number of other understorey shrubs are very susceptible to P. cinnamomi. Present firing regimes, worked out to protect the forest from damaging and dangerous wildfires, appear to be favouring the growth of banksias. Hotter fires could be used to favour leguminous understorey shrubs, some of which are resistant to P. cinnamomi, e.g. Acacia pulchella. Some legumes also appear to make the soils on which they grow 'suppressive' to P. cinnamomi (with Dr. Malajczuk).

Further, the legumes will enrich the soil in nitrogen which should improve the growth and resistance of jarrah.

12. There is therefore an urgent need to evaluate different management practices as an aid to containing *P. cinnanomi*.

Mr. Jan Titze: Microbiology and Plant Pathology at the Kelmscott Research Station.

Early work by Dr. Chris Palzer on the pathology of P.c. is now available as a Ph.D. thesis. Other unpublished reports and notes of Palzer's work as well as other W.A. work for the past 10 years, are being collated by Jan Titze and will also be available to interested persons.

In general, Dr. Palzer's work dealt with the role of oospores, zoospores, the physiology of infection by P.c. and its spread in the forest.

2. Present work at Kelmscott is concerned with the role of the chlamydospores of P.c. - their longevity, infectivity and survival in soil.

The L.D.50/unit of infected soil is much higher under jarrah than under the susceptible *Banksia* spp.

Differences have been observed in the pathogenicity of *P.c.* chlamy-dospores from different isolates, and they are more infective between January and June. On the whole they make a good, reliable source of quantifiable inoculum. They survive better in soils with higher than lower organic matter contents.

- 3. Both cross-protection and synergistic interactions have been observed between *P.c.* and *Pythium* spp. in *Banksia* (Jan Titze) and in jarrah (Mark Dudzinski). It may be possible to develop cross protection interactions for the protection of jarrah against *P.c.* Those *Pythium* isolates giving cross protection did not show any antagonism to *P.c.* on agar plates.
- 4. Soil leachates had variable effects on *P.c.* in the laboratory. Sporangia are produced more abundantly when the leachates are diluted. Eventually, they were usually lysed by the microorganisms in the leachate.
- 5. General Soil Microbiology: P.c. chlamydospores have been found in soil and in banksia roots. P.c. may grow in soil in the absence of host roots, and has been reported to persist for up to 3 years in very dry soil, most probably surviving on small particles of organic matter.

A few actinomycetes were found to be moderately antagonistic to P.c. but appear to be active only in rather dry soils when P.c. has already suffered setback from climatic effects of summer. Soil microorganisms have been classified into 9 groups by selective dilution-plating. Using this classification, it can be shown that the microbial population in unburnt soils differs from that in burnt soils, and the soil population in healthy jarrah forest differs from that following dieback. In general, there are rather low numbers of antagonists in jarrah forest soils.

- 6. The Casuarina N₂ fixing symbiosis is being studied because although this species is moderately susceptible to dieback, it nevertheless delays or suppresses dieback in the field; the symbiotic association is still not fully documented.
- Mr. Mark Dudzinski (Kelmscott) has been studying the impact of the disease in the jarrah forest, following work initiated by Dr. Frank Podger.

 Over 10 years, on several sites, jarrah numbers have decreased more than other eucalypts (up to 75% decrease). There is some evidence of jarrah recruitment to areas formerly lower in numbers.

Transects have been set up across dieback areas embracing a range of ecological types, often embracing unaffected sectors, and changes in eucalypt and other tree/shrub populations are being followed.

Observations relate to periods of 11-13 years.

- 1. Slow decline of jarrah trees on most sites, with confirmation of field tolerance of E. calophylla, E. Wandoo, E. patens, E. laeliae, E. megacarpa and E. nudis.
- 2. Eucalypt advanced growth population is dynamic, with an overall decline for jarrah being greater than that for other species.

 Survival of jarrah advanced growth is better in higher topographic positions, whilst Marri shows better survival in lower topographic positions.
- 3. Small amount of recruitment of jarrah from advanced growth class to tree class on all sites.
- 4. Revegetation and regeneration reaches a maximum on sites which have been subject to hot fires, particularly in moist locations.

- Ground flora species numbers increased on some sites as a result of colonisation by several species; however density of many individual species is markedly reduced.
- Assessment of plantings of pregerminated seed (comparable to natural rejuvenation) indicated virtual total loss of Banksia grandis and jarrah in old dieback zones after 13 years, whilst Marri showed approximately 50% mortality. Banksia grandis and jarrah showed slightly poorer survival in healthy forest than Marri, which showed similar mortality to that in the old dieback
- 7. Controlled, spring burning of one transect carrying 6-8 years accummulation of fuel did not significantly influence uphill disease spread or disease expression during the next 13 months in comparison to the adjacent unburnt transect.

Mr. Roger Hilton:

- 1. Dr. C. Palzer's Ph.D. thesis is now available in the Reid Library, U.W.A. and he will be sending copies to libraries in W.A. concerned with forestry. It is principally a fundamental study of zoospore behaviour but includes a chapter on the inoculation of banksias in the field. Summary of main points of thesis: Palzer was able to establish the high infectivity of the individual zoospore, both in the presence of other organisms and in axenic conditions. Synergism was not a requirement. Reduced infection probability under non-sterile conditions was quantified. zoospores were shown to display negative geotaxis. Patterns of direct/indirect germination of sporangia were shown to be under purely physical influence and not dependant on associated organisms. The reliable methods developed to produce quantities of inoculum were used to study infection in laboratory, glasshouse, and field. In the field, discovery of the ability of P.c. to grow into the
- Mr. Hilton's own work has been mainly on the larger fungi, which have some potential for predicting and possibly controlling disease. The mycorrhizal fungi particularly are likely to be useful in these ways. Little is known about the types of mycorrhizal fungi in the jarrah forest, and even less on their specificity with host plants.

woody roots of banksia was important.

- The general state of knowledge respecting the microfungi of jarrah forest soils is also very poor, although some knowledge has been built up on the fungi of agricultural soils, especially in connection with takeall. There is little doubt that burning influences the larger fungi and probably the microfungi as well.
- The most promising line of research is to gather data on the constant association of fruit-bodies with forest trees, then to isolate candidate species in culture and attempt synthesis of mycorrhiza with plants grown in sterile conditions. To this end, ability to identify fruit-bodies is fundamental. The genera known to be important mycorrhiza-formers all over the world are common in our forests, with the odd exception of Lactarius spp. Species and/or individuals of Amanita, Boletus, Russula, Cortinarius, Rhizopogon, Ramaria, etc. are abundant in season but the species themselves have yet to be

clearly defined. Visit by an expert familiar with the Northern Hemisphere forms would be invaluable. Brief visits by Dr. Roy Watling, Dr. Derek Reid, and Mr. Jasper Daams have already been most helpful, but a person resident for some months, perhaps even a year, is needed. Dr. Egon Horak of the Swiss Federal Institute of Technology would be ideal. He is a world authority, has already spent 1-2 years in New Zealand and New Guinea, and is interested in coming. Mr. Hilton is preparing a memorandum on this for the Jarrah Dieback Committee.

Question: Did Dr. Palzer infect larger jarrah roots with *P.c.*?

No, the *P.c.* could not penetrate suberised jarrah roots unless they were wounded. He did note that lesions from infected smaller roots could not colonise larger jarrah roots, whereas they could in Banksia.

Comment: When P.c. is occupying nearby susceptible roots such as banksia, the fungus may strike more powerfully and effectively at young jarrah roots.

Question: Does jarrah die because of water stress or nutrient starvation or both?

Answer: (E.R. Hopkins) Weakened by nutrient starvation and water stress, it is probable that death is finally caused by water stress. Knowledge of the physiology of the disease is lacking.

<u>Comment:</u> (J.F. Loneragan) Where a plant is dying of root-disease-induced nutrient starvation, in a soil with certain marked deficiencies, it should be possible to devise diagnostic techniques to provide early warning signals about the onset of disease. Plant nutritionists could hope to do this.

Mr. Hilton informed the meeting that a new forest ecologist, Dr. David Bell, had joined the Botany Department to lecture in ecology and to work on reafforestation of mined sites. Research student, Mr. Ian Colhoun, is working on the nutrition of trees under different burning regimes.

Dr. Malajczuk

- The rhizosphere population (including mycorrhizal fungi) of jarrah roots gives considerable protection to jarrah seedlings against *P.c.* However, with *E. calophylla*, the rhizosphere population gave full protection. Thus *E. marginata* (jarrah) and *E. calophylla* (Marri) are equally susceptible when axenically-grown seedlings are challenged by an inoculum of *P.c.*
- 2. Krasnozem soil carries a high population of actinomycetes, many of which are antagonistic to P.c., so that jarrah seedlings growing on these soils are resistant to dieback. Unfortunately, not much of the jarrah forest grows on this soil. Even a small proportion of Krasnozem soil mixed with a typical sandy lateritic soil will give protection against P.c. at 6-25% addition.
- 3. Acacia and other leguminous species are frequently resistant to P. cinnamomi, and may also carry an antagonistic rhizosphere population. From these results, and from experiments in collaboration with Dr. Shea, there seems a real possibility of achieving biological control of P. cinnamomi by fostering leguminous under-storey shrubs.

- 4. There are few mycorrhizas on jarrah roots on burnt soils, but many on unburnt soils. Thus both the nutrition of the jarrah tree and its susceptibility to P.c. could be adversely influenced by controlled burning, which regularly destroys the protective, and biologically active litter layer.
- 5. Studies on microbial populations of soils show that while some soils (e.g. the Krasnozems) are suppressive to *P. cinnamomi*, most jarrah forest soils are conducive. Soils with high organic matter content are generally more suppressive than those with low organic matter, and soils growing *Acacia pulchella* are very suppressive to dieback in pot experiments (with S. Shea).
- 6. How important are the mycorrhizal fungi in conferring resistance to P.c. in Marri, and to a lesser extent in jarrah? These fungi have not yet been isolated in pure culture, so that soil inoculum, containing a host of different microorganisms, must be used to provide the mycorrhizal fungi. It is important that these fungi be isolated (if possible) and grown for use in inoculation experiments. It would seem, on the basis of their sheathing habit and also of their abundant growth within the root cortex, that they are likely to exert major influence against invasion by P. cinnomomi.

Comments: The litter layer is important as it allows the plant roots to feed on the soil surface where the highest concentration of the available nutrients are. Moreover, a well developed litter layer supports a large and diverse population of soil fauna (many of which are fungal feeders) and of microorganisms, particularly the microfungi.

Dr. Glenn: Briefly reviewed the literature on the effects of other organisms on P. cinnamomi. Dr. Glenn is working in collaboration with Dr. N. Malajczuk and Mr. Nesbitt and Ms. Reynolds.

This group is examining the lysis of the hyphae of P.c. and the induction of abortive sporangia by bacteria. A range of soil bacteria are involved and they hope to arrive at suggestions for practical means of manipulating the forest environment to encourage the antagonistic micro-organisms and so effect some control of the disease. Ms. Reynolds is studying the microorganisms involved in antagonism and Mr. Nesbitt at the effect of soil conditions on survival of the pathogen.

Mr. Nesbitt

- Studying the effect of different soil conditions on the persistence of P. cinnomomi in the soil as a saprophyte.
 Because it is not conceivable to enrich jarrah forest soils by externally produced organic amendments, special attention is being paid to the natural litter on the forest floor.
- 2. Organic matter. Lysis of P.c. hyphae increases with increasing amounts of litter added to a carrier soil. Abortive sporangia are formed in the high litter treatments.
- 3. Water. Maximal lysis occurs at water contents between 50% Field Capacity and Field Capacity. No sporangia are produced at 50% F.C. but maximum occurs around and above F.C.

- 4. Soil temperature. Survival of the pathogen studied over range 7-36° Hyphal lysis maximal at 27° (similar to optimum growth temperature of P.c.). The highest levels of sporangia were formed between 20-25°. At 36° there is hyphal autolysis in sterile soils comparable to the lysis observed in non-sterile soils.
- 5. Effects of pH. The pH of the 100% litter is 4.5 compared to 6.0 in the lateritic soil. The effect of variation in pH was examined in soil leachates derived from 100% organic matter. pH adjusted to 4.5, 5.0, 5.5 and 6.0. There was 15% more lysis in pH 6.0 leachate compared with pH 4.5 leachate.
- 6. Overall factors conducive to lysis of hyphae are:
 - 1. Temperature 27º
 - 2. Water content slightly less than Pc.
 - 3. High organic matter
 - 4. Soil pH around 6.0

7. Future plans:

- 1. To examine the effects of varying litter material on survival of P.c.
- 2. To extend the studies to look at survival of P.c. in infected tissues, particularly with respect to infection potential.
- 3. To examine more closely the effects of litter on zoospores and chlamydospores.

Dr. Keast

- 1. Early work has involved the search for alternative vectors for Phytophthora cinnamomi besides man, such as birds or other animals.
- 2. Has sampled insects and attempted to isolate *P. cinnomomi* chlamy-dospores which might pass through the gut of insects or birds. To this end is making spores radioactive so that when fed to birds they might be identified in the faeces.
- 3. Certain soil microbes (fungi and nocardia species) have caused deformities and antibiosis to *P. cinnanomi*.
- 4. The work in general has suggested gross limitations in quantitive detection of *P. cinnamomi* and general knowledge of biochemistry of the organism. Qualitative methods are also required for adequate differential diagnosis of *P. cinnamomi* within samples before any knowledge of control mechanisms can be achieved. It is intended that this area will be developed along with a search for lytic viruses to *P. cinnamomi*.
- 5. Is commencing a study of antibiotic producing micro-organisms in forest soils in another study; there could be some spin off in the area of *P. cinnamomi* control.

General Discussion

There was general concensus that:

1. Jarrah Dieback is indeed the most serious problem in the jarrah

forest. It not only destroys valuable timber trees, but pauperises the range of plant species. This in turn will affect the wild-life and almost certainly the hydrology of the region.

- 2. Much of the groundwater in this region is highly saline, so there is a real danger of increasing salinity in the streams flowing through the jarrah forest if p c. destroys the deep-rooted jarrah.
- 3. The spread of the disease is mainly due to man's activities: logging, mining, recreation, road building.
- 4) Quarantine regulations must be maintained, at least until more is known about the rate and mechanisms of spread of P.c.

More research support is urgently needed. Research strategies should be target-oriented, examining (a) soil and forest management for dieback control and (b) the ecology and biology of *P. cinnamomi* in relation to the litter layer, the surface soil and the mycorrhizal association(s) of jarrah.

Research Priorities

- 1. Strengthen the research into forest management, but especially into the effects of different controlled burning cycles upon under-storey shrubs, leaf litter and the biological functions of its population of soil and litter biota.
- 2. study the fungus *P. cinnamomi* itself, its ecotypes, its ability to infest and spread in soils of different moisture contents, its hypovirulent or avirulent variants and the possibility of employing these as control agents.
- $\sqrt{3}$. Make a detailed study of the relationship between P.c. and the dieback disease.
- 4. Develop quantitative diagnostic techniques to provide "early warning" systems for recognising subclinical disease in plants, and for soil colonisation by P.c.
- 5. Isolate the common mycorrhizal fungi of jarrah and marri, so that their nutrient functions and effects on P.c. infection may be better understood.
 - 6. Study the soil fauna and the microorganisms of the jarrah forest litter, and of the surface soil.
 - 7. Make a detailed study of the root inhabiting fungi of soils and roots in the jarrah forest, with a view to detecting other root—infecting fungi, and to look for cross-protection against P.c.

A committee was formed to make recommendations from the meeting. Members are: J. Havell, S. Shea, M. Mulcahy, J. Loneragan, C. Parker (Convenor).

(Mulchella)