The Response of Jarrah Forest Native Legumes to *Phytophthora cinnamomi* Rands and Fertilization

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Abstract

1. Introduction

On freely drained jarrah (*Eucalyptus marginata* Sm) forest sites the periods during which soil temperature and moisture conditions are suitable for *P.cinnamomi* Rands (the causal agent of Jarrah Dieback, Podger 1968) activity can be reduced or eliminated by the promotion of a dense canopy (Shea 1975). It is unlikely that any silvicultural treatment to the overstory would promote a canopy dense enough to significantly reduce site susceptibility but the composition and density of the shrub and understory layers of the forest can be manipulated by changing the intensity and frequency of fire.

Under a moderate intensity control burning regime, native leguminous species occur only as a scattered under-

story component in the forest (Fig. 1) but following intense fire these species become dominant (Fig. 2). For example, following the Dwellingup wildfire of 1961 large areas (approximately 25,000 hectares) regenerated with species of legumes - predominantly Acacia pulchella (R.Br.), A.strigosa (Link.) and Bossiaea aquifolia (Benth.) - forming a dense and continuous canopy. Apparently in many forest areas there is a large store of legume seed in the soil which will germinate following high intensity fire. McCormick (1971) has demonstrated that low intensity burning will cause legume-dominated areas to revert to a proteaceous-dominated shrub and understory.

The effect of legumes on spread and intensification of jarrah dieback, is to a degree dependent on their relative susceptibility to P.cinnamomi. If they are highly susceptible to the fungus then the reduction in P.cinnamomi activity by the creation of a less favourable soil physical environment may be compensated for by the provision of a large, susceptible food base. Thus it is necessary to evaluate their relative susceptibility.

Lange (1959) has reported nodulation of jarrah forest legumes but there has been no attempt to quantify their capacity to fix nitrogen. Significant changes in the nutrient status of the soil and its microbiological character could result from a change to a legume-dominated understory, hence their capacity to fix nitrogen was assessed in this trial.

2. Method

The effect of P.cinnamomi and fertilization on six native species (A.pulchella, B.aquifolia, A.extensa (Lindl.), Mirbelia dilitata (R.Br.), A.strigosa and A.myrtifolia (Willd.)) and the capacity of these species to fix nitrogen was determined in a factorial pot trial: The seed was pretreated by placing it in boiling water and soaking for 24 hours and germinated on blotting paper contained in petri dishes. Four pregerminated seeds were sown in 15 cm pots containing P.cinnamomi-free laterite soil. Each treatment combination was replicated four times. The pots were regularly watered to maintain soil moisture levels at approximately field capacity.

(a) Fertilization

Six months after sowing half the pots were fertilized with Superphosphate, Potassium phosphate, and trace elements at the following rates -

$(H_2PO_4)_2H_2O + x\ (CaSO_4 \cdot 2H_2O)$ = 1000 kg/hectare

K_2SO_4 = 125 kg/hectare

Trace elements (B, Co, Cn, Fe, Mn, Mo, Zn, Mg) = 2.21 kg/hectare per element

Nine months after sowing the pots were refertilized at the same rate.

(b) Inoculation

Inoculations were carried out in half the pots 4 days after the initial fertilization. Each pot was inoculated with 10 ml of sterile water containing 1800 chlamydospores. Soil taken from four cores in each pot was mixed with the solution of chlamydospores and then packed into the holes. Inoculation was repeated one month later using P.cinnamomi infested wheat grain. 5 ml of infested grain were placed in each of four cored holes in each pot.

(c) Assessments

(i) The experiment was terminated 12 months after sowing and six months after the initial inoculation. Health and total height of individual seedlings and total shoot dry matter production was recorded in all pots.

(ii) Total nodule fresh weight was determined in uninoculated pots.

(iii) Samples of nodules were taken from each pot in the control section of the experiment and their ~~$N_2(C_2H_2)$-fixing~~ nitrogenase activity was determined by the acetylene reduction technique. (Dilworth 1966; Schoollhorn and Burns 1966).

(iv) P.cinnamomi assessment

Four cores of soil were taken for each of the inoculated pits and stored in moist, cool conditions prior to determination of the quantity of P.cinnamomi propagules per gram of soil by the imunofluorescent technique (Malajzcuk et al 1975).

The root system and soil fraction of each pot were separated. The soil was baited for the presence of P.cinnamomi using the lupin baiting technique.(Chee and Newhook 1974). Pots not yielding P.cinnamomi were further

tested for the presence of P.cinnamomi using the cotyledon baiting technique (Marks and Kassaby 1972). Twenty five fine (.1 mm), ten medium (.1 - 1 mm) and ten large (1 mm) roots each approximately 2 to 4 cm in length were randomly selected from the root mass in each pot, surface sterilized 3% "Linsley" and plated on $P_{10}VP$ agar (Ocano and Tsao 1966).

3. Results Results are summarized in Fig. 3.

(a) Mortality, Height and Shoot Dry Matter Production

During the establishment phase and prior to inoculation significant mortality occurred in pots sown with B.aquifolia and M.dilitata and these species were excluded from the statistical analysis. One death of one A.strigosa plant in a fertilized, inoculated treatment and four fertilized, inoculated M.dilitata plants were the only mortalities recorded after inoculation.

An analysis of variance of average height and shoot dry matter production was carried out. Inoculation had no significant effect on height growth but fertilization was

highly significant. The height of all of the 4 species analysed was significantly increased (p.01 level) by fertilization. Fertilization significantly increased shoot weight in all species. Inoculation had no significant effect on the shoot weight of A.pulchella and A.strigosa (p.01) but increased the shoot weight of A.extensa (p.01) and A.myrtifolia (p.05).

(b) Recovery of P.cinnamomi

(i) Roots. The fungus was not recovered from the roots of A.pulchella and A.extensa but it was consistantly recovered from the fine and medium roots of fertilized A.myrtifolia and A.strigosa. No A.strigosa roots in the unfertilized pots yielded P.cinnamomi and only one fine root in one pot of unfertilized A.myrtifolia gave a positive recovery. Although there were incomplete numbers of seedlings in B.aquifolia and M.dilitata pots samples of roots of these species were taken. Of eight pots containing B.aquifolia fine roots from only one unfertilized pot yielded the fungus. Fine and medium roots from two (unfertilized) out of five pots of M.dilitata sampled consistantly

yielded P.cinnamomi.

(ii) Soil. Soil from seven of the inoculated eight A.strigosa pots and six of the inoculated eight A.myrtifolia pots yielded P.cinnamomi from either baited lupin roots or E.sieberiana (F. Muell.) cotyledons. Fertilization had no effect on recovery rates. The fungus was recovered by baiting from only one (unfertilized) of the inoculated, eight A.extensa pots and no A.pulchella pots yielded P.cinnamomi. The fungus was recovered from two out of eight B.aquifolia pots and two out of four M.dilitata pots. It was impossible to reliably detect P.cinnamomi propagules using the imunofluorescent technique. Some thick-walled chlamydospores were detected but counts within and between treatments were variable.

(iii) ~~$N_2(C_2H_2)$~~ nitrogenase activity and nodule fresh weight.

An analysis of variance of ~~nodule~~ nitrogenase activity and nodule weight was carried out. The ~~$N_2(C_2H_2)$~~ nitrogenase activity of fertilized and unfertilized A.myrtifolia, A.extensa, A.pulchella and A.strigosa ~~nodule~~ and nodule weights are shown in Fig. 3. There were incomplete samples of M.dilitata and B.aquifolia but the mean ~~$N_2(C_2H_2)$-fixing~~ nitrogenase activity of the nodules

sampled from unfertilized plants was 17.5 and 48.9 and from fertilized plants was 6.7 and 107.4 ~~n.moles/mm/gm~~ n. moles C_2H_4/gm f.wt. nodule/min. ~~fresh weight~~ respectively.

Fertilization had no significant effect on the ~~$N_2(C_2H_2)$-fixing~~ nitrogenase activity of A.pulchella, A.strigosa and A.myrtifolia nodules, but depressed the ~~$N_2(C_2H_2)$-fixing~~ nitrogenase activity of A.extensa (p.05) nodules.

Fertilization significantly increased the nodule fresh weight of A.pulchella (p.05), A.extensa (p.01) and A.myrtifolia (p.01), but had no significant effect on A.strigosa nodule weight.

Discussion

B.aquifolia and M.dilitata seedlings appear relatively resistant to P.cinnamomi but further testing is required because of the occurrence of pre-inoculation mortalities. The ability of the remaining species tested to survive and grow in the presence of P.cinnamomi in soil physical environmental conditions which were highly favourable for fungal activity in comparison to the environmental conditions occurring on upland jarrah forest sites indicates that they would survive on these sites in the presence of

the fungus. The small and medium (1 mm) roots of some species were infected by P.cinnamomi but the larger roots were not invaded. Thus the beneficial effect of dense legume stands of the species tested on the soil physical environment would not be compensated for by an increase in the susceptible food base for the fungus. In fact, as the burning regime necessary to promote native legumes is unfavourable for the highly susceptible proteaceous species, there would be far less susceptible host tissue on legume-dominated sites.

The marked reduction in P.cinnamomi population levels in pots planted with A.pulchella and A.extensa is significant. The relatively high recovery rates from A.myrtifolia and A.strigosa pots confirms that the inoculation was viable. There were thick-walled chlamydospores present in some A.pulchella and A.extensa pots but it was impossible to quantify them and the results from baiting indicate that these propagules were dormant. The mechanism by which fungal population levels were reduced is unknown. Broadbent and Baker (1974) attribute the P.cinnamomi suppressive characteristics of some soils to the presence of a micro-

biological factor. In these suppressive soils exchangeable calcium and magnesium, nitrogen and organic matter levels were higher in comparison to the conducive soils. It is possible that A.pulchella and A.extensa created soil environmental conditions which favoured micro-organisms antagonistic to P.cinnamomi. The control mechanism may be associated with the specific Rhizobium-Legume relationship. Drapeau et al (1973) have demonstrated inhibition of Phytophthora cactorum (Lebert and Cohn) Schroet by three Rhizobium strains.

There is some circumstantial evidence legume-dominated sites have a less favourable soil microbiological environment for P.cinnamomi activity than non-legume sites. Soil organic matter levels on legume-dominated soils is higher than on adjacent non-legume sites (Hatch and Shea - unpublished data) and legumes are frequently a dominant component on the P.cinnamomi resistant soils which are found on the incised river valleys within the Jarrah Forest.

The widespread and dense regeneration of legume species, in areas where previously their occurrence was sparse,

following the Dwellingup wildfire indicates that these species are an important, if often latent component of the forest vegetation. The mean ~~$N_2(C_2H_2)$-fixing~~ nitrogenase activity of A.pulchella, the most widespread of the native legumes (63.9 n.moles C_2H_4/gm fresh wt/min.) compares reasonably with the ~~$N_2(C_2H_2)$-fixing~~ nitrogenase activity of a number of legumes recorded by Hardy et al (1971). It is impossible to extrapolate the data from this trial to the field situation, but the capacity of at least one native legume to fix nitrogen at a relatively high rate gives weight to the hypothesis that before fire was controlled in the forest periodic high intensity fires caused the regeneration of dense legume stands which contributed significant amounts of nitrogen to the ecosystem. It is difficult to explain the relatively high dry matter production of virgin Jarrah in an ecosystem from which Nitrogen must be continually withdrawn as a consequence of frequent controlled or wildfire (Ovington 1968; Vines et al 1971), without the presence of a mechanism for significant inputs of nitrogen.

Fertilized A.myrtifolia and A.strigosa were more susceptible to P.cinnamomi but this could be attributed to the

larger root mass in the fertilized pots. Fertilization had no effect on P.cinnamomi survival in the soil. The response of these legumes to fertilizer application was not unexpected because of the poor nutrient status of the laterite soils. Although fertilization significantly depressed the ~~$N_2(C_2H_2)$ fixing~~ nitrogenase activity of one species this effect is likely to be more than compensated by the increase in nodule fresh weight in response to fertilization. Thus the positive effects of legumes could be increased by beneficial fertilization.

A major constraint on the development of a technique to control P.cinnamomi in the forests of S.W. of Western Australia is the widespread occurrence of the disease in a forest which has a relatively low value per unit area. Over a period of years low cost control burning techniques have been developed in the Jarrah Forest (Peet 1967) and currently the forest is burnt on a 5 tc 7 year cycle. Current research suggests that it may be possible to regenerate legume seed which is dormant in the soil by a modification of the existing burning regime without a major increase in the current estimated cost of 40 cents per

hectare (Peet pers. comm.).

Controlled field inoculation trials, currently in progress, are the only methods by which the effect of dense legume stands on the spread and intensification of Jarrah dieback can be determined conclusively. Current evidence suggests, however, that the promotion of dense legume stands will at least reduce P.cinnamomi activity by creating a less favourable soil physical and micro-biological environment and reducing the density of highly susceptible host tissue. It is not unlikely that the promotion of dense legume stands will also improve the general health status of the forest by causing significant additions of nitrogen to the ecosystem.

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Fig. 1

Shrub and understory dominated by proteaceous species. Prior to a moderate intensity fire the predominant understory species was A.pulchella.

Fig. 2

A. pulchella understory originating after intense fire.