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Evaluation of resistance to *Phytophthora cinnamomi* in seed-grown trees and clonal lines of *Eucalyptus marginata* inoculated in lateral branches and roots

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Seed-grown trees and six clonal lines of 3·5–4·5-year-old *Eucalyptus marginata* (jarrah) growing in a rehabilitated bauxite mine site in the jarrah forest were underbark-inoculated on lateral branches (1995) or simultaneously on lateral branches and lateral roots (1996) with isolates of *Phytophthora cinnamomi* in late autumn. Individual seedlings from which the clonal lines were derived had previously been assessed as either resistant (RR) or susceptible (SS) to *P. cinnamomi*. At harvest, the acropetal lesion and colonization lengths were measured. Overall, the length of colonization in roots and branches was more consistent as a measure of resistance than lesion length, because colonization length recorded the recovery of *P. cinnamomi* from macroscopically symptomless tissue ahead of the lesion which, on some occasions, was up to 6 cm. In both trials, one RR clonal line was able to contain the *P. cinnamomi* isolates consistently, as determined by small lesion and colonization lengths in branches and roots. In contrast, the remaining two RR clonal lines used in both trials were no different from the SS line in their ability to contain lesions or colonization. These latter two RR lines may therefore not be suitable for use in rehabilitation of *P. cinnamomi*-infested areas. Differences in lesion and colonization lengths among *P. cinnamomi* isolates occurred only in the 1995 trial. Colonization and lesion lengths in branches were up to eight times greater in 1996 than in 1995, but the relative rankings of clonal lines were consistent between trials. Although colonization was always greater in branches than roots, the relative rankings of the lines were similar between branch and root inoculations. Branch inoculations are a valid option for testing the resistance and susceptibility of young jarrah trees to *P. cinnamomi*.

Keywords: collar rot, disease assessment, jarrah dieback, oomycete

Introduction

Phytophthora cinnamomi is a major soilborne phytopathogen that has had a devastating impact on the *Eucalyptus marginata* (jarrah) forest in Western Australia (WA), to which it has been introduced (Shearer & Tippett, 1989). Most noticeable are the large numbers of dominant trees of the important hardwood-timber species jarrah which have been killed in sites favourable to disease. While jarrah is one of the most susceptible of the *Eucalyptus* spp., some individual trees have survived on infested sites. These trees were used as parents for progeny, which were screened for resistance to *P. cinnamomi* in underbark stem inoculations of 14-month-old seedlings in the glasshouse (McComb *et al*., 1990; Stukely & Crane, 1994). Individual seedlings of families that developed small lesions were designated resistant (RR; resistant lines from resistant families), while those with large lesions were designated susceptible (SS; susceptible lines from susceptible families). The rankings of resistance to *P. cinnamomi* of these half-sib families were validated in glasshouse and field trials (Stukely & Crane, 1994). Seedlings varying in resistance have been micropropagated to produce resistant clonal lines for use in the rehabilitation of infested mine sites and other jarrah forest sites (McComb *et al*., 1990) and the production of clonal jarrah seed orchards (Colquhoun & Hardy, 2000). Some susceptible lines have also been micropropagated.

One of the main criticisms of the initial stem inoculations of jarrah seedlings and subsequent studies using this inoculation method was that resistance was tested on stems and not roots, which are generally the principal site of colonization. Studies with unselected trees have shown

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that stem and root inoculations are strongly correlated in terms of the relative resistance rankings of species of *Banksia* (Dixon & Thinlay Sivasithamparam, 1984); *Eucalyptus* (Tippett *et al*., 1985); and oak (Robin & Desprez-Loustau, 1998). Stem inoculations are preferred over root or soil inoculations, as they provide a convenient and consistent means of evaluating large numbers of hosts and isolates. Furthermore, roots often have large irregularities in morphology (Dixon *et al*., 1984; Tippett *et al*., 1985). No published work has directly compared root with stem inoculation of jarrah clonal lines. Cahill *et al*. (1993) found that there was a good relationship in the relative resistance of five 10-month-old clonal lines when roots were inoculated with zoospores compared with the initial rankings based on stem inoculation (McComb *et al*., 1990; Stukely & Crane, 1994). In non-clonal jarrah saplings, Shearer *et al*. (1987a) reported comparable growth of *P. cinnamomi* in detached roots and in intact stems in the forest. Although Tippett *et al*. (1985) found that lesions induced by underbark inoculations of sapling stems and roots provided a similar resistance ranking for various *Eucalyptus* spp., the lesions on roots were smaller, by a factor of three in some cases. Also, they were unable to demonstrate a correlation between the results from detached roots and those from intact stems.

Recently, O'Gara *et al*. (1996); O'Gara *et al*. (1997) showed that intact stems of jarrah could be infected by zoospores. This supports observations where disease of jarrah collars or stems was often associated with ponded riplines in rehabilitated bauxite minepits (Hardy *et al*., 1996; O'Gara *et al*., 1996). To select *P. cinnamomi*-resistant genotypes of jarrah or other susceptible species, it is critical to assess the relative susceptibility of stems and roots before deciding which inoculation technique to use.

This study examined the relationship between length of lesion and length of colonization in lateral branches of resistant and susceptible clonal lines and seed-grown trees of jarrah after underbark inoculation with a number of isolates of *P. cinnamomi*. In addition, the relationship between lesion and colonization length in roots and branches was investigated and compared with previous resistance rankings of clonal lines.

Materials and methods

Experimental design

Seed-grown trees and clonal lines of 3·5–4·5-year-old jarrah growing on a rehabilitated jarrah forest mine site were underbark-inoculated on lateral branches (1995 trial), or simultaneously on lateral branches and lateral roots (1996 trial), with different isolates of *P. cinnamomi*. Both trials were completely randomized factorial designs. In autumn 1995, five branches on five replicate trees of each clonal line were inoculated per isolate, while in autumn 1996 three branches and three roots on 12 replicate trees of seed-grown and clonal lines were inoculated per isolate. There was one control inoculation of a branch or root per inoculated tree in each trial. All tree material was harvested 42 and 12 days after inoculation in 1995 and 1996, respectively. The 1996 trial was harvested earlier than in 1995 because examination of branches showed that lesion development progressed more rapidly in 1996.

Isolates and inoculum production

Five isolates of *P. cinnamomi* were selected based on results from inoculation of a 3-year-old RR1 clonal line (see below) growing on a rehabilitated forest mine site during summer 1994 (Hüberli, 1995). One (MP94-48) isolate that produced large lesions; one (MP116) producing medium lesions; and two (MP94-09 and MP112) producing small lesions were chosen for 1995 inoculations. Isolate MP94-48 was used again in 1996 together with MP94-17, which produced medium-sized lesions. All isolates were of A2 mating type and of the same clonal lineage (Hüberli *et al*., 2001).

Sterile Miracloth (Calbiochem Corporation, La Jolla, CA, USA) discs, 1 cm in diameter, were placed onto V-8 juice agar (Hüberli *et al*., 1997). Plates were inoculated individually with different isolates of *P. cinnamomi* that had been repassaged and incubated in the dark at 24°C for 10 days. Three weeks before inoculation, all isolates were repassaged through jarrah seedlings in a glasshouse as a precautionary measure against loss of pathogenicity (Erwin & Ribeiro, 1996).

Plant material, inoculation and growth conditions

Seed-grown trees and six clonal lines of 8-month-old jarrah were planted in July 1992 on a rehabilitated bauxite mine site in the jarrah forest at the Willowdale mine of Alcoa World Alumina Australia, located 110 km south-west of Perth, WA. Trees grown from seed were propagated from seeds collected from the northern jarrah forest of WA. Resistant (RR) and susceptible (SS) clonal lines of jarrah trees were propagated from individual seedlings that had been assessed for resistance to *P. cinnamomi* (McComb *et al*., 1990). In the 1995 inoculations, six clonal lines were used: 1J30 (RR1), 5J336 (RR2), 12J96 (RR3), 326J51 (RR4), 11J379 (SS1) and 11J402 (SS2). In 1996, seed-grown trees and all clonal lines used in 1995 were inoculated except 12J96 (RR3) and 11J402 (SS2). Both primary and secondary branches and roots were used for inoculations.

Five weeks prior to the 1996 inoculation, the soil from around six roots of each replicate tree per seed-grown and clonal line was carefully excavated and removed for later inoculation. The soil was replaced with sterile moist vermiculite around the roots to a depth of about 20 cm. Plastic sheeting was placed on top of the vermiculite and then covered with soil to prevent desiccation.

In both trials, a sterile razor blade was used to cut a bark flap (*c*. 1·5 cm long and 1 cm wide) through the cortex to the phloem on the adaxial position of the branch. Cuts on branches (1–2 years old) were always made towards the shoot apex, and on roots they were made

towards the root apex. A colonized Miracloth disc was inserted, mycelial side down, under the flap, the flap closed and the wound sealed with Parafilm (American National Can, Chicago, IL, USA) and silver duct tape (Norton Abrasives Pty Ltd, Lidcombe, NSW, Australia). Controls were underbark-inoculated with sterile Miracloth discs. The plant tissue in contact with the inoculum disc was referred to as the site of inoculation. The average branch diameter at the site of inoculation in both trials was 7 mm (range 5–11 mm). In the 1996 trial, root diameter at the site of inoculation averaged 10 mm (range 2–26 mm). After inoculation, roots were reburied as described earlier.

Rainfall data prior to and during both trials were obtained from the Willowdale mine site. The minimum and maximum temperatures during the trials were obtained from the nearest Bureau of Meteorology (Station No. 9642, Wokalup, WA), *c.* 25 km from the study site.

Harvest

Forty-two (1995 trial) and 12 (1996 trial) days after inoculation, the branches and/or roots were excised from the trees and transported to the laboratory for assessment of lesions and pathogen colonization. The outer bark was carefully scraped back with a sterile scalpel blade at the site of inoculation of branches and roots to expose the maximum extent of the lesion. The acropetal length of the lesion present in the phloem was measured. The acropetal and basipetal lesions were recorded separately in the 1996 trial.

In the 1995 trial, branches were cut into 0·5 cm sections from the mid-site of inoculation up the branch for eight sections. The sections were cut longitudinally to expose the bark and wood to the medium and plated sequentially onto NARPH, an agar selective for *Phytophthora* (Hüberli *et al*., 2000). In 1996, branches or roots were cut into 1 cm sections for 12 sections, as described earlier. Recovery of the pathogen from branch sections was assessed after 3 and 5 days' incubation at 24°C. The root or branch section furthest from the site of inoculation from which *P. cinnamomi* was recovered was used to determine the acropetal length of colonization. Colonization refers to the total amount of the branch or root tissue invaded by the pathogen, which in lesioned branches or roots included the lesion and the pathogen extension beyond the lesion (EBL). In branches or roots without lesions, colonization consisted only of the pathogen extension beyond the site of inoculation.

Statistical analysis

Following Tabachnick & Fidell (1996), data for parametric tests were screened for assumptions of homoscedasticity, presence of outliers, normality and noncorrelations of means and variances. All measurements of branches or roots on a tree were averaged and the means of the replicate trees were subjected to multivariate analysis of variance (MANOVA). The 1995 and 1996 trials were analysed separately. The dependent variables were lesion and colonization length, while the independent variables were plant genotype (seed-grown and clonal lines) and isolate. All significant main effects and interactions were compared using the least significant difference (LSD) test ($P = 0{\cdot}05$). Branch and root diameter at the site of inoculation were not used as covariates, as correlations with lesion data were low ($-0{\cdot}24 < r < 0{\cdot}36$, $P > 0{\cdot}05$). Also, there were no significant ($P > 0{\cdot}05$) differences in branch and root diameter among seed-grown and clonal lines.

Results

The initial MANOVA of 1995 data showed significant main effects for jarrah clonal line ($P < 0{\cdot}001$) and isolate ($P < 0{\cdot}001$), while the interaction was not significant ($P = 0{\cdot}29$). In 1996, the main effect of plant genotype (seed-grown and clonal lines) ($P < 0{\cdot}001$) was significant, while the isolate main effect ($P = 0{\cdot}60$) and the interaction ($P = 0{\cdot}99$) were not significant. In cases where the main effects were significant, both dependent variables of lesion and colonization length were highly significant (Table 1). The relationship between lesion and colonization length is shown in Figs 1 and 2.

Table 1 Results of univariate tests (following significant initial MANOVA) of acropetal lesion and colonization length of *Eucalyptus marginata* (jarrah) clonal lines and seed-grown trees growing in rehabilitated forest mine site that were underbark-inoculated in branches or roots with mycelial mats of *Phytophthora cinnamomi* isolates. Inoculations were conducted in late autumn 1995 and 1996

			Lesion length			Colonization length		
Year of trial	Tissue inoculated	Effect	df	*F*	*P*	df	*F*	*P*
1995	Branch	Host	5,92	17·93	<0·001[a]	5,92	13·45	<0·001[a]
		Isolate	3,92	14·35	<0·001[a]	3,92	12·46	<0·001[a]
1996	Branch	Host	4,106	7·56	<0·001[a]	4,106	6·11	<0·001[a]
		Isolate	–[b]	–	–	–	–	–
	Root	Host	4,106	5·23	<0·001[a]	4,106	3·16	0·02[a]
		Isolate	–	–	–	–	–	–

[a]Significant interaction at $\alpha = 0{\cdot}05$.

[b]Not determined as main effect of isolate was not significant ($P = 0{\cdot}60$).

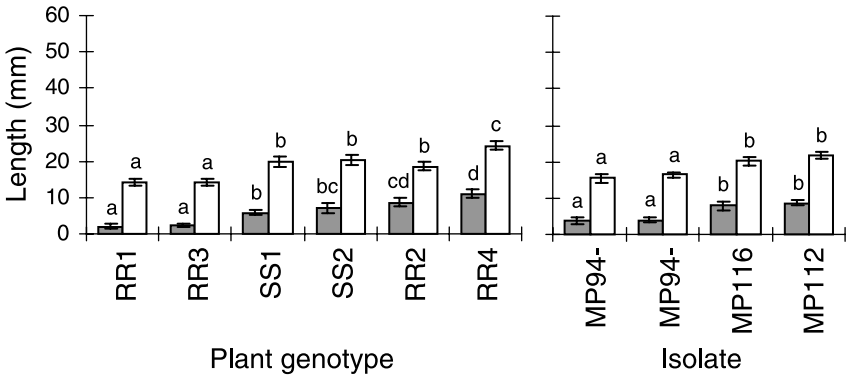

Figure 1 Mean length of acropetal lesions (closed bars) and acropetal colonization (open bars) in branches of six *Eucalyptus marginata* (jarrah) clonal lines after underbark inoculation with four *Phytophthora cinnamomi* isolates for 42 days in autumn 1995. Data for isolates are pooled within clonal lines; all clonal lines are pooled within isolates. Jarrah clonal lines with the prefix RR were classified as resistant, while those with the prefix SS were susceptible to *P. cinnamomi* (McComb *et al*., 1990). Error bars are standard errors of the mean; those with the same letter are not significantly different ($P = 0{\cdot}05$).

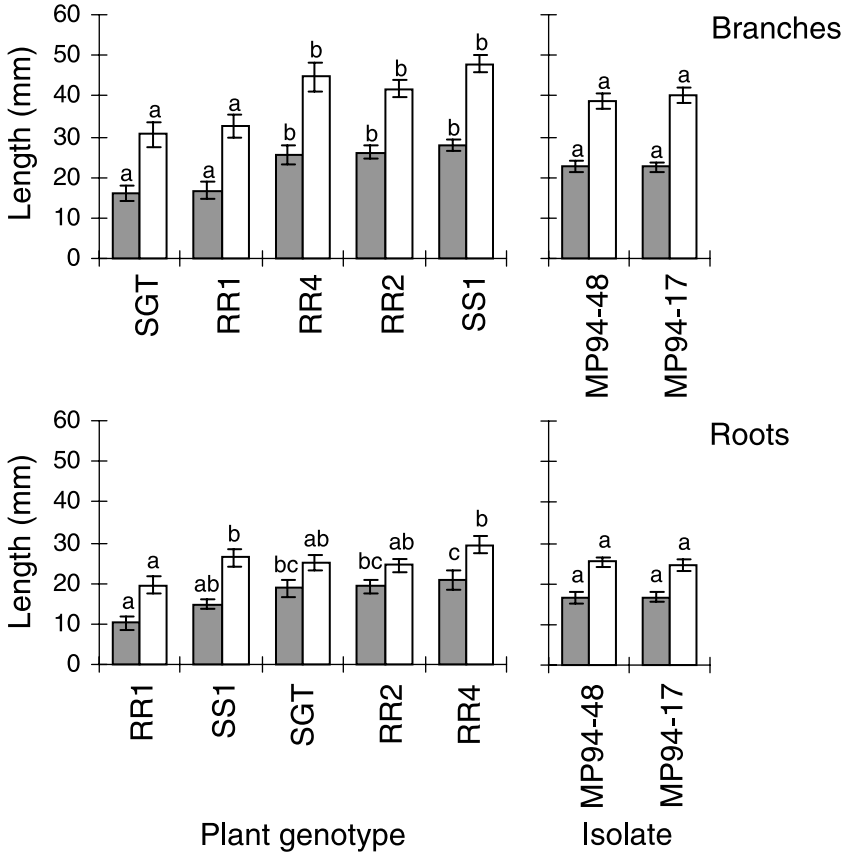

Figure 2 Mean length of acropetal lesions (closed bars) and acropetal colonization (open bars) in branches and roots of four clonal lines and seed-grown trees of *Eucalyptus marginata* (jarrah) after underbark inoculation with two *Phytophthora cinnamomi* isolates for 12 days in autumn 1996. Data for isolates are pooled within plant genotypes (seed-grown and clonal lines); all plant genotypes are pooled within isolates. Jarrah clonal lines with the prefix RR were classified as resistant, while those with the prefix SS were susceptible to *P. cinnamomi* (McComb *et al*., 1990). Error bars are standard errors of the mean; those with the same letter are not significantly different ($P = 0{\cdot}05$).

As there was no significant interaction between plant genotype (seed-grown and clonal lines) and isolates in lesion and colonization length of the 1995 ($P = 0{\cdot}29$) and 1996 ($P = 0{\cdot}98$) trial, the results are presented with isolates pooled within plant genotypes and plant genotypes pooled within isolates. The plant genotype main effect for lesion and colonization length was significant in 1995 ($P < 0{\cdot}001$) and 1996 ($P < 0{\cdot}02$), while the isolate effect was significant ($P < 0{\cdot}001$) only in 1995. Acropetal and basipetal lesions in both branches and roots were strongly correlated ($r > 0{\cdot}80$, $P < 0{\cdot}001$) in the 1996 trial. Therefore, for purposes of comparison with the 1995 trial, only acropetal lesion and colonization lengths were presented for the 1996 trial.

All control branches and roots were symptomless and *P. cinnamomi* was never isolated from these tissues.

When inoculated branches and roots were plated from both trials, *P. cinnamomi* was recovered from macroscopically symptomless tissue up to 6 cm ahead of the lesion margin. The pathogen was not always recovered from every sequential root or branch section plated. In some cases, *P. cinnamomi* was not recovered from symptomless tissue for up to 2 cm ahead of the lesion margin, but was recovered further up the root or branch (data not shown).

Comparison of 1995 and 1996 lateral branch inoculations

Lesion lengths in branches were up to 7·9-fold longer in 1996 than in 1995 for jarrah clonal lines and *P. cinnamomi* isolates, while colonization lengths were only up to 2·4-fold longer (Figs 1 and 2). This was despite 1995 branches being harvested 30 days later than in the 1996 trial.

Lesion and colonization lengths resulted in similar relative susceptibility rankings of clonal lines. RR3 (tested only in 1995), seed-grown trees (tested only in 1996) and RR1 (tested in both trials) had significantly ($P < 0{\cdot}03$) shorter lesion and colonization lengths than the remaining clonal lines (Figs 1 and 2). In comparison to RR1, lesion and colonization lengths tended to be relatively greater for SS1, RR2 and RR4 in both trials.

Overall, isolates MP94-48 and MP94-09 had significantly ($P < 0{\cdot}003$) smaller lesion and colonization lengths than MP112 and MP116 in 1995 (Fig. 1). In 1996, no significant ($P = 0{\cdot}60$) differences between isolates were found (Fig. 2).

Maximum and minimum temperatures during the 1996 trial were higher ($P < 0{\cdot}003$) than those during the 1995 trial. The average minimum and maximum temperatures during the 1995 trial were 8·9 and 17·8°C, respectively, and those during the 1996 trial were 11·5 and 23·0°C, respectively. The amount of rainfall in April (one month prior to inoculation) was 15 mm in 1995 and 40 mm in 1996, which is below the average of 61 mm for the region. During the trials, there was 208 and 64 mm of rain in 1995 and 1996, respectively.

Comparison of root and lateral branch inoculations

Correlation analysis between lesion and colonization lengths in jarrah branches and roots was carried out for individual clonal lines and seed-grown trees. There was no correlation between jarrah branches and roots either for lesion ($-0{\cdot}27 < r < 0{\cdot}25$, $P > 0{\cdot}22$) or colonization ($-0{\cdot}21 < r < 0{\cdot}40$, $P > 0{\cdot}05$) lengths in seed-grown trees and clonal lines. Mean lesion and colonization lengths were approximately twice as long in branches than in roots for all clonal lines and *P. cinnamomi* isolates (Fig. 2). However, in seed-grown trees, lesion lengths were slightly (1·2 times) longer in roots than in branches, and *vice versa* for colonization lengths.

The RR1 clonal line was consistently the most effective in restricting lesion development and colonization compared with all other clonal lines and seed-grown trees. The relative rankings of clonal lines were not consistent when lesion lengths were compared with colonization for branch and root inoculations. SS1 was in the susceptible ranking for lesion and colonization lengths on branches, but only for colonization lengths on roots (Fig. 2). Root lesion lengths on SS1 were not significantly longer than those on RR1 ($P = 0{\cdot}10$). The seed-grown trees, on the other hand, had a similar resistance to RR1 for branch inoculations (Fig. 2). In root inoculations, colonization lengths on seed-grown trees were not significantly ($P = 0{\cdot}06$) different from those on RR1, while lesion lengths were greater ($P = 0{\cdot}001$) than on RR1.

Discussion

This is the first study to investigate lesion and colonization development in branches and roots of clonal lines of jarrah trees. The inoculation trials on roots in 1996 and on branches in 1995 and 1996 confirmed the resistance status of RR1 to *P. cinnamomi* (McComb *et al*., 1990; Stukely & Crane, 1994). Previous and current work with 5–6-year-old RR1 trees in rehabilitated forest sites (McComb *et al*., 1994; Hüberli, 2001) and 1·5-year-old plants in temperature-controlled cabinets using zoospore inoculum (Hüberli *et al*., 2002) provides further evidence that RR1 is resistant and has potential for the rehabilitation of *P. cinnamomi*-infested forests and mine sites. In these trials, RR1 has been screened against a range of *P. cinnamomi* isolates varying in pathogenicity, and a number of different testing methods were used. RR3 was also highly resistant in the 1995 trial; however, root and branch inoculations need to be conducted in a second season to validate this finding. That the unselected seed-grown trees were as resistant as RR1 in branch and root inoculations indicates that the seed lot used was derived from more resistant genotypes of jarrah.

Clonal lines RR2 and RR4, previously selected as resistant from visible lesions in 14-month-old seedlings (McComb *et al*., 1990), were as susceptible as SS1 and SS2 in both trials, particularly when the length of colonization was considered. RR2 has been reported to be as resistant as RR1 in a field survival trial (McComb *et al*., 1994), and in a root inoculation study of 10-month-old seedlings (Cahill *et al*., 1993). There are several possible reasons for this disparity with our results, including age of host, environmental conditions, and pathogenicity of the *P. cinnamomi* isolates used. Two reports have demonstrated that there is large variation in pathogenicity to jarrah clones among Australian isolates (Dudzinski *et al*., 1993; Hüberli *et al*., 2001). Hüberli *et al*. (2001) found that 73 WA isolates ranged from killing all plants within 59 days to plants being symptomless 182 days after underbark inoculation of 1·5-year-old jarrah clonal line 77C40 (RR) in a glasshouse.

The relationship between lesion development in branches and roots of jarrah trees was not always as consistent as reported in our preliminary results (Hardy, 2000). On the basis of lesion lengths in root inoculations, SS1 would be considered as resistant as RR1, while seed-grown trees are more susceptible. This is in conflict with our data for root

colonization length and branch lesion and colonization lengths. Lesion lengths were highly variable between trials, while colonization lengths were more consistent for both trials. One possibility for variation in lesions is that the pathogen can consistently colonize beyond macroscopically visible lesions in tissue (O'Gara *et al*., 1997; Hüberli *et al*., 2000). Some reports indicate that *P. cinnamomi* has been isolated from symptomless tissue up to 5 cm in front of the lesion margin (Davison *et al*., 1994; Hardy *et al*., 1996; O'Gara *et al*., 1997; Hüberli *et al*., 2002), which is in agreement with the results in this paper.

The length of lesions was considered a good measurement for assessing resistance to *P. cinnamomi* of jarrah plants aged <2 years old (Stukely & Crane, 1994). In these plants, the pathogen was recovered only up to 4 mm ahead of the lesion margin. It should be noted, however, that only 10 mm of symptomless stem was plated. In older, 2-year-old plants, Stukely & Crane (1994) found that lesions were often unreliable as the pathogen progressed internally and its colonization was not visible as a continuous lesion. This study and others (O'Gara *et al*., 1997; O'Gara, 1998) showed that after inoculation of branches and roots of jarrah, *P. cinnamomi* might not be recovered from tissue adjacent to the site of inoculation or from regions within 10 mm ahead of the lesion margin, but could be recovered at greater distances above the lesion. Therefore it is recommended that the extent of colonization needs to be assessed in conjunction with lesions, particularly for older trees.

The length of both lesions and colonized tissue lesions in branches were shorter in 1995 than those in 1996 for all jarrah clonal lines. Other wound inoculation experiments with nonclonal jarrah saplings have shown that both ambient temperature and water status of jarrah trees during invasion affect lesion development by *P. cinnamomi* (Shearer *et al*., 1987b; Tippett *et al*., 1987; Tippett *et al*., 1989). However, during the wetter months, lower temperatures rather than low bark moisture limits pathogen colonization (Tippett & Hill, 1983). In our study, the maximum ambient temperature in the 1996 trial was very close to the optimum of 25–30°C for lesion development of WA isolates (Shearer *et al*., 1987b; Hüberli *et al*., 2002). It is unlikely that rainfall was a contributing factor to differences between the 1995 and 1996 trials, as trees received relatively similar amounts of rainfall prior to commencement of the experiment, although in 1995 there was three times more rainfall during the trial than in 1996. It has been shown for jarrah saplings that increased bark moisture predisposes trees to more rapid pathogen colonization and lesion development (Tippett & Hill, 1983; Tippett *et al*., 1989). Furthermore, heavy summer rainfalls >200 mm in a month have been associated with *P. cinnamomi* disease epidemics (Marks *et al*., 1972; Tippett & Hill, 1983).

Branches were generally more susceptible than roots to the two *P. cinnamomi* isolates, confirming previous wound inoculation studies with jarrah saplings and poles (Tippett *et al*., 1983; Bunny *et al*., 1995; Tippett *et al*., 1985). Also, the data from branches showed larger differences among clonal lines than the data from roots. In a year-long experiment at a jarrah forest site, Tippett *et al*. (1983) observed that large roots (3·2–10 cm diameter) resisted circumferential spread of the pathogen more effectively than coppice stems. The factors responsible for this lower susceptibility in roots are not known, although soil temperatures have been reported to be lower, with less fluctuation than at the soil surface (Marks *et al*., 1973; Shearer & Shea, 1987). While this study provides no data to make comparisons with ambient temperatures, another study in the WA jarrah forest has shown that soil temperatures at a depth of 7·5 cm during late autumn were always less than 18°C, and reached 15°C for only about 20 h per week (Shea, 1975). These low temperatures in soils, compared with the higher ambient temperatures in 1996, could account for the differences in lesion and colonization lengths observed in roots and branches.

The relative rankings of jarrah clonal lines for branch and root inoculations were consistent when colonization lengths were measured. Branch inoculations provide a convenient, rapid, low-cost and easily repeatable method for initial resistance–susceptibility screening of large numbers of jarrah genotypes.

The relative pathogenicity rating of isolates used in the 1995 inoculations of RR1 was not consistent with that of earlier underbark inoculations of 3-year-old plants of this clonal line conducted in summer 1994 (Hüberli, 1995). Additionally, there was less differentiation in pathogenicity among isolates in 1995 than in 1994. It was found that some isolates switched from producing large lesions to small lesions (MP94-48) in RR1, and *vice versa* (MP112). This inconsistency between these two trials may be explained by seasonal factors. The variation in individual isolate pathogenicity rankings indicates that their capacity to produce disease is variable, and may be influenced to different extents by environmental and plant physiological factors. Recently, we showed that all five isolates used in the current study were ranked as intermediate pathogenic phenotypes on the basis of their capacity to kill jarrah line 77C40 (RR) (Hüberli *et al*., 2001). Further work is needed to investigate the variability in pathogenicity and its interaction with plant age.

Given that jarrah is a long-lived species with a life cycle of 500–1000 years (Abbott *et al*., 1989), the durability of resistance of the clonal lines in the field remains uncertain. However, it is encouraging that resistance of RR1 has been shown in a number of inoculation tests using plants up to 6 years old (McComb *et al*., 1994; Stukely & Crane, 1994; Hüberli, 2001; Hüberli *et al*., 2002). Also, clonal lines have been exposed to a range of aggressive *P. cinnamomi* isolates.

This study has important implications for breeding and selection programmes for resistance to *P. cinnamomi*, and provides a basis for improving resistance-screening procedures. Further work is in progress to assess the ability of 3–6-year-old clonal lines growing in rehabilitated forest sites to survive underbark inoculations with the pathogen (Hüberli, 2001). Currently, RR1 is showing promising results in these trials which commenced in November 1997.

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