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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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Abstract

Seed-grown trees and six clonal lines of 3.5 to 4.5 year-old *Eucalyptus marginata* (jarrah) growing in a rehabilitated bauxite minesite 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 colonisation lengths were measured. Overall, colonisation in roots and branches were more consistent as a measure of resistance than lesion length because colonisation 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 colonisation lengths in branches and roots. In contrast, the remaining two RR clonal lines used in both trials were no different to the SS line in their ability to contain lesions or colonisation. These latter two RR lines may therefore not be suitable for use in rehabilitation of *P. cinnamomi*-infested areas. Differences in lesion and colonisation lengths among *P. cinnamomi* isolates occurred only in the 1995 trial. Colonisation and lesion lengths in branches were up to eight times larger in 1996 than 1995, but the relative rankings of clonal lines were consistent between trials. Although colonisation lengths were always larger 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 resistance and susceptibility of young jarrah trees to *P. cinnamomi*.

Introduction

Phytophthora cinnamomi is a major soilborne phytopathogen and 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 the 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 minesites 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 colonisation. Studies with unselected trees have shown that stem and root inoculations are well correlated in terms of the relative resistance rankings of *Banksia* spp. (Dixon *et al.*, 1984), *Eucalyptus* spp. (Tippett *et al.*, 1985) and oak spp. (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 work directly compares between root and 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 to 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 correlate the results from detached roots with intact stems.

Recently, O'Gara *et al.* (1996, 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, assessing the relative susceptibility of stems and roots is critical before deciding which inoculation technique to use.

This study examined the *in planta* relationship of lesion and colonisation length in lateral branches of resistant and susceptible clonal lines and seed-grown trees (SGT) of jarrah after underbark inoculation with a number of *P. cinnamomi* isolates. We also investigated the relationship between lesion and colonisation length in roots and branches. We determined whether previous resistance rankings of the clonal lines are consistent for branch and root inoculations.

Materials and methods

Experimental design

SGT and clonal lines of 3.5 to 4.5 year-old jarrah growing on a rehabilitated jarrah forest minesite 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 randomised 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 twelve replicate trees of SGT 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 later) growing on a rehabilitated forest minesite during summer in 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 Mira cloth (Calbiochem Corporation, CA, USA) discs, 1 cm in diam., were placed onto vegetable-8 juice (Campbell's Soups Australia Pty. Ltd.) agar (Hüberli *et al.*, 1997). Plates were inoculated individually with the different *P. cinnamomi* isolates that had been repassaged and incubated in the dark at 24°C for 10 days. Three weeks before inoculation, all isolates were repassaged through potted jarrah seedlings in a glasshouse as a precautionary measure against loss of pathogenicity (Erwin & Ribeiro, 1996).

Plant material, inoculation and growth conditions

SGT and six clonal lines of 8-month-old jarrah were planted in July 1992 on a rehabilitated bauxite minesite in the jarrah forest at the Willowdale (32.55 south, 116.02 east) mine of Alcoa World Alumina Australia, located 110 km southwest of Perth, WA. SGT 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, SGT 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 SGT 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 (about 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 colonised Mira cloth disc was inserted, mycelial-side-down under the flap, the flap closed and the wound sealed with Parafilm (American National Can, Chicago, USA) and also silver duct tape (Norton Abrasives Pty Ltd, Lidcombe, NSW, Australia). Controls were underbark inoculated with sterile Mira cloth discs. The plant tissue in contact with the inoculum disc will be referred to as the site of inoculation (SOI). The average branch diam. at the SOI in both trials was 7 mm (range of 5 to 11 mm). In the 1996 trial, root diam. at the SOI averaged 10 mm (range of 2 to 26 mm). After inoculation, roots were reburied as described earlier.

Rainfall data prior to and during both trials were obtained from the Willowdale minesite. The minimum and maximum temperatures during the trials were obtained from the nearest Bureau of Meteorology (Station No. 9642, Wokalup, WA) approximately 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 colonisation. The outer bark was carefully scraped back with a sterile scalpel blade at the SOI 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-SOI 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 SOI from which *P. cinnamomi* was recovered was used to determine the acropetal length of colonisation. Colonisation 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, colonisation consisted only of the pathogen extension beyond the SOI.

Statistical analysis

Following Tabachnick & Fidell (1996), data for parametric tests were screened for assumptions of homoscedasticity, presence of outliers, normality and non-correlations 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 colonisation length, while the independent variables were plant genotype (SGT and clonal lines) and isolate. All significant main effects and interactions were compared using the Least Significant Difference (LSD) test ($P = 0.05$). Branch and root diam. at the SOI were not used as covariates, as correlations to lesion data were low ($-0.24 < r < 0.36$, $P > 0.05$). Also, there were no significant ($P > 0.05$) differences in branch and root diam. among SGT and clonal lines.

Results

The initial MANOVA of 1995 data showed significant main effects for jarrah clonal line ($P < 0.001$) and isolate ($P < 0.001$), while the interaction was not significant ($P = 0.29$). In 1996, the main effect of plant genotype (SGT and clonal lines) ($P < 0.001$) was significant, while the isolate main effect ($P = 0.60$) and the interaction ($P = 0.99$) were not significant. In cases where the main effects were significant, both dependent variables of lesion and colonisation length were highly significant (Table 1). The relationship between lesion and colonisation length is shown in Figs. 1 and 2.

As there was no significant interaction between plant genotype (SGT and clonal lines) and isolates in lesion and colonisation length of the 1995 ($P = 0.29$) and 1996 ($P = 0.98$) 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 colonisation length was significant in 1995 ($P < 0.001$) and 1996 ($P < 0.02$), while the isolate effect was significant ($P < 0.001$) only in 1995. Acropetal and basipetal lesions in both branches and roots were strongly correlated ($r > 0.80$, $P < 0.001$) in the 1996 trial. Therefore, for purposes of comparison with the 1995 trial, only acropetal lesion and colonisation 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 colonisation lengths were only up to 2.4-fold longer (Figs. 1 and 2). This is despite 1995 branches being harvested 30 days later than in the 1996 trial.

Lesion and colonisation lengths resulted in similar relative susceptibility rankings of clonal lines. RR3 (tested only in 1995), SGT (tested only in 1996) and RR1 (tested in both trials) had significantly ($P < 0.03$) smaller lesion and colonisation lengths than the remaining clonal lines (Figs. 1 and 2). In comparison to RR1, lesion and colonisation lengths tended to be relatively large for SS1, RR2 and RR4 in both trials.

Overall, isolates MP94-48 and MP94-09 had significantly ($P < 0.003$) smaller lesion and colonisation lengths than MP112 and MP116 in 1995 (Fig. 1). In 1996, no significant ($P = 0.60$) differences between isolates were found (Fig. 2).

Maximum and minimum temperatures during the 1996 trial were higher ($P < 0.003$) 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 rain received 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 mm and 64 mm of rain in 1995 and 1996, respectively.

Comparison of root and lateral branch inoculations

Correlations between lesion and colonisation lengths in jarrah branches and roots were calculated for individual clonal lines and the SGT. There was no correlation between jarrah branches and roots either for lesion ($-0.27 < r < 0.25$, $P > 0.22$) or colonisation ($-0.21 < r < 0.40$, $P > 0.05$) lengths in SGT and clonal lines. Mean lesion and colonisation lengths were approximately twice as long in branches than in roots for all clonal lines and *P. cinnamomi* isolates (Fig. 2). However, in SGT, lesion lengths were slightly (1.2-times) longer in roots than in branches, while for colonisation lengths it was *vice versa*.

The RR1 clonal line was consistently the most effective in containing *P. cinnamomi* lesion development and colonisation compared with all other clonal lines and SGT. The relative rankings of clonal lines were not consistent when lesion lengths were compared with colonisation for branch and root inoculations. SS1 was in the susceptible ranking for lesion and colonisation lengths on branches, but only for colonisation lengths on roots (Fig. 2). Root lesion lengths on SS1 were not significantly ($P = 0.10$) larger than those on RR1. The SGT, on the other hand, had a similar resistance to RR1 for branch inoculations (Fig. 2). In root inoculations, colonisation lengths on the SGT were not significantly ($P = 0.06$) different from those on RR1, while lesion lengths were larger ($P = 0.001$) than on RR1.

Discussion

This is the first study to investigate lesion and colonisation 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 inoculation (Hüberli *et al.*, 2002) provides further evidence that RR1 is resistant and has potential for the rehabilitation of *P. cinnamomi* infested forests and minesites. 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 SGT 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 colonisation lengths were 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 among Australian isolates in pathogenicity to jarrah clones (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 we reported for preliminary results (Hardy, 2000). On the basis of lesion lengths in root inoculations, SS1 would be considered as resistant as RR1, while SGT are more susceptible. This is in conflict with our data for root colonisation length and branch lesion and colonisation lengths. Lesion lengths were highly variable between trials, while colonisation lengths were more consistent for both trials. One possibility for variation in lesions is that the pathogen can consistently colonise 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 were considered a good measurement for assessing resistance to *P. cinnamomi* of jarrah plants aged less than 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 colonisation 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 SOI or from regions within 10 mm ahead of the lesion margin, but could be recovered at greater distances above the lesion. Therefore, we recommend that colonisation needs to be assessed in conjunction with lesions, particularly for older trees.

The length of both lesions and colonised tissue lesions in branches were shorter in 1995 than those in 1996 for all jarrah clonal lines. Other wound inoculation experiments with non-clonal 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, 1989). However, during the wetter months, lower temperatures rather than low bark moisture limits pathogen colonisation (Tippett & Hill, 1983). In our study, the maximum ambient temperature in the 1996 trial was very close to the optimum of 25 to 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 in differences between the 1995 and 1996 trials as trees received relatively similar amounts of rainfall prior to commencement of the experiment and in 1995 had over 3-fold more rainfall during this trial than in 1996. It has been shown for jarrah saplings that increased bark moisture predisposes the trees to more rapid pathogen colonisation and lesion development (Tippett & Hill, 1983; Tippett *et al.*, 1989). Furthermore, heavy summer rainfalls greater than 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 used in the comparison. Also, the data from branches showed larger differences among clonal lines than the data from roots. That branches were more susceptible to disease than roots confirms previous wound inoculation studies with jarrah saplings and poles (Tippett *et al.* 1983, 1985; Bunny *et al.*, 1995). In a year-long experiment at a jarrah forest site, Tippett *et al.* (1983) observed that large roots (3.2-10 cm diam.) 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 fluctuations than at the soil surface (Marks *et al.*, 1973; Shearer & Shea, 1987). While we have no data to make comparisons to 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 only reached 15°C for about 20 hours per week (Shea, 1975). These low temperatures in soils compared to the higher ambient temperatures in 1996 could account for the differences in lesion and colonisation observed in roots and branches.

The relative rankings of jarrah clonal lines for branch and root inoculations were consistent when colonisation 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 among isolates in pathogenicity in 1995 than in 1994. We 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). Hence, 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 to 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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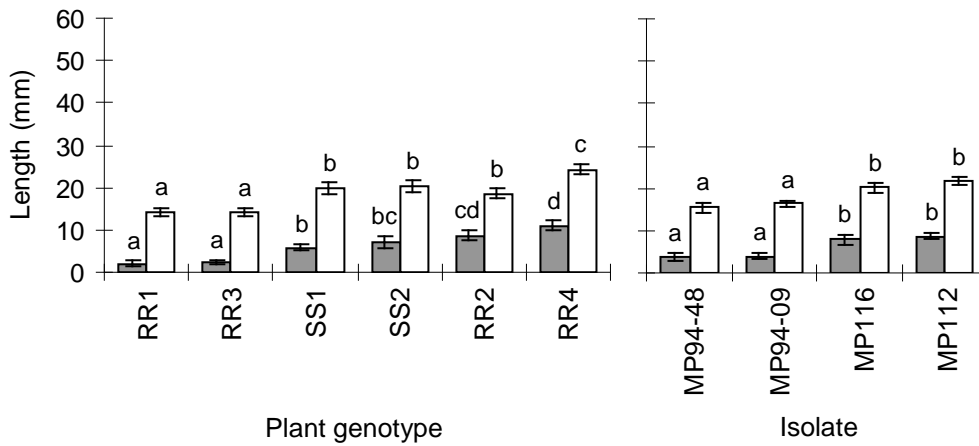


Figure 1 Mean length of acropetal lesions (■) and acropetal colonisation (□) in branches of six *Eucalyptus marginata* (jarrah) clonal lines after underbark inoculation with four *Phytophthora cinnamomi* isolates for 42 days in autumn 1995. Data for isolates are pooled within clonal lines and all clonal 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). Bars topped by the same letter do not differ significantly from each other among lesions or among colonisation lengths. Error bars are standard errors of the means.

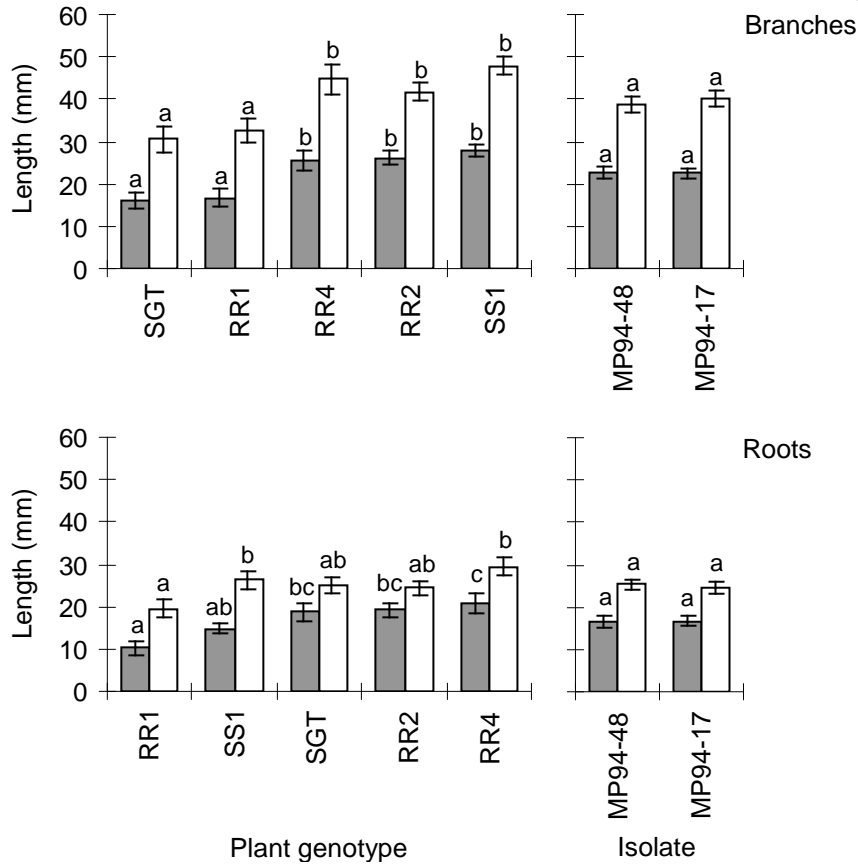


Figure 2 Mean length of acropetal lesions (■) and acropetal colonisation (□) in branches and roots of four clonal lines and seed-grown trees (SGT) of *Eucalyptus marginata* (jarrah) after underbark inoculation with two *Phytophthora cinnamomi* isolates for 12 days in autumn 1996. Data for isolates are pooled within plant genotypes (SGT and clonal lines) and 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). Bars topped by the same letter do not differ significantly from each other among lesions or among colonisation lengths. Error bars are standard errors of the means.

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

Year of trial	Tissue inoculated	Effect	Lesion length			Colonisation length		
			df	F	P-value	df	F	P-value
1995	Branch	Clonal line	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	Clonal line	4, 106	7.56	<0.001 ^A	4, 106	6.11	<0.001 ^A
		Isolate	— ^B	—	—	—	—	—
	Root	Clonal line	4, 106	5.23	<0.001 ^A	4, 106	3.16	0.02 ^A
		Isolate	—	—	—	—	—	—

^ASignificant interaction at $\alpha = 0.05$.

^BNot determined since main effect of isolate was not significant ($P = 0.60$).