

LESION DEVELOPMENT IN SEEDLINGS OF JARRAH (*EUCALYPTUS MARGINATA*) FOLLOWING STEM INOCULATION WITH *PHYTOPHTHORA CINNAMOMI*, *P. CITRICOLA* AND *P. CRYPTOGEA*

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SUMMARY

Seedlings of ten half-sib jarrah (*Eucalyptus marginata*) families were stem-inoculated with either *Phytophthora cinnamomi* A₂, *P. citricola* or *P. cryptogea* A₂ to determine their reaction to these pathogens. Longitudinal and tangential spread of lesions and their appearance were recorded over a period of 42 days and compared between families and between *Phytophthora* species. There was a continuous range in lesion length across families for each *Phytophthora* species. Most plants inoculated with *P. citricola* and *P. cryptogea* had longer lesions and faster rates of lesion development than those inoculated with *P. cinnamomi* ($P < 0.05$). There were no significant differences in tangential spread of lesions between families or between *Phytophthora* species. Resistance to *P. cinnamomi* (expressed as short lesion length) did not necessarily indicate resistance to the other *Phytophthora* species. Further research is needed to determine whether assessment of resistance after stem inoculation of jarrah seedlings with *P. citricola* and *P. cryptogea* correlates with field resistance, as has been shown with *P. cinnamomi*.

RIASSUNTO

SVILUPPO DI LESIONI IN PLANTULE DI *EUCALYPTUS MARGINATA* A SEGUITO DI INOCULAZIONE ALLO STELO CON *PHYTOPHTHORA CINNAMOMI*, *P. CITRICOLA* E *P. CRYPTOGEA*. Plantule di *Eucalyptus marginata* ottenute da dieci famiglie di semi, ciascuna prodotta da un singolo albero soggetto ad impollinazione libera, sono state inoculate allo stelo sia con *Phytophthora cinnamomi* A₂ che con *P. citricola* o *P. cryptogea* A₂ al fine di determinare la loro reazione a questi patogeni. Sono stati eseguiti dei rilievi, durante un periodo di 42 giorni, sullo sviluppo longitudinale e tangenziale delle lesioni e sul loro aspetto comparando i dati sia tra le famiglie che tra le specie di *Phytophthora*. È stato osservato che esiste

un range continuo nella lunghezza delle lesioni tra le famiglie per ciascuna specie di *Phytophthora*. La maggior parte delle piante inoculate con *P. citricola* e *P. cryptogea* ha mostrato lesioni più lunghe ed una velocità di sviluppo delle lesioni superiore rispetto a quelle inoculate con *P. cinnamomi* ($P < 0,05$). Non sono state osservate differenze significative nello sviluppo tangenziale delle lesioni sia tra le famiglie che tra le specie di *Phytophthora*. La resistenza a *P. cinnamomi* (espressa come lunghezza ridotta delle lesioni) non indica necessariamente una resistenza nei confronti delle altre specie di *Phytophthora*. Saranno necessarie ulteriori ricerche al fine di determinare se la valutazione della resistenza, dopo inoculazione allo stelo di plantule di *Eucalyptus marginata* con *P. citricola* e *P. cryptogea*, è correlata con una resistenza di campo come è stato dimostrato per *P. cinnamomi*.

Key words: half-sib families, stem inoculations, resistance, field resistance.

INTRODUCTION

Jarrah (*Eucalyptus marginata* Donn. ex Smith) is a high value, hardwood timber species endemic to south-west Western Australia. The most important disease of jarrah, 'jarrah dieback', is caused by the soil-borne fungus *Phytophthora cinnamomi* Rands (Podger, 1972). Recently, variation has been found in the levels of resistance of jarrah to *P. cinnamomi*, and, based on soil and stem inoculation of seedlings, genetic resistance has been demonstrated in certain half-sib jarrah families (Stukely and Crane, 1994).

Stem inoculation has also proven to be useful in assessing resistance of apple rootstocks to *P. cactorum* (Lebert & Cohn) Schroet. (Browne and Mircetich, 1993), and *Banksia* spp. to *P. cinnamomi* (McCredie *et al.*, 1985). Stem inoculation permits repeated observation of a plants' reaction to a pathogen, and plant material from potentially resistant individuals can be rescued for propagation once infected tissue has been removed and assessment completed.

In addition to *P. cinnamomi*, *P. citricola* Sawada, *P.*

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cryptogea Pethybr. & Laff., *P. megasperma* Drechs. and *P. nicotianae* Breda de Haan have all been isolated in the jarrah forest, with *P. citricola* also occurring in disturbed areas such as logging roads and landings and bauxite pits (Shearer *et al.*, 1987). In the field, extension rates of lesions in sapling jarrah stem tissue inoculated with *P. citricola*, *P. cactorum*, *P. nicotianae* and *P. cryptogea* A₁ are not significantly different from that of *P. cinnamomi* (Shearer *et al.*, 1988). This investigation was conducted to examine, using stem inoculation, the reaction of jarrah seedlings to *P. cinnamomi*, *P. citricola* and *P. cryptogea*. A preliminary report of this work has been published (Bayliss *et al.*, 1996).

MATERIALS AND METHODS

Plant material. Seeds of ten half-sib jarrah families (each derived from a single, open-pollinated parent tree) were sown in late winter 1994 into jiffy pots containing a 1:3 peat-to-sand mix which had been fumigated with methyl bromide. Families were selected to represent a range of levels of resistance to *P. cinnamomi*, with seeds being collected from healthy trees on *Phytophthora*-infested dieback sites and non-infested sites. The seedlings were grown in a shadehouse and watered daily. In February 1995 they were repotted into 150 mm black plastic pots containing the same potting mix. The seedlings were watered daily and fertilised twice weekly with 1.6 g l⁻¹ of Aquasol (Hortico [Aust.] Pty. Ltd). All seedlings were periodically tip-pruned to maintain a single stem. Application of fertiliser ceased two weeks prior to the experiment. One week prior to the experiment, seedlings were moved into a glasshouse at ambient temperature.

Production of inoculum. Isolates of *Phytophthora cinnamomi* A₂ (IMI 264384, isolated from *Hibbertia subvaginata* (Steudel.) F. Muell.), *P. citricola* (DCE284, isolated from *E. marginata*) and *P. cryptogea* A₂ (227a-R, isolated from *Pinus radiata*) were obtained from the Department of Conservation and Land Management where they were maintained on slopes of cornmeal agar (Difco Laboratories, Detroit, MI) stored at 20°C. Inoculum of each species was prepared using colonised Mira cloth (Chicopee Mills, NY) as described by Davison *et al.* (1994).

Experimental procedure. Inoculation of seedlings was carried out in July 1995. A small tangential incision, approximately 2.5 mm long, was made in the stem of each jarrah seedling, in the centre of approximately the fourth internode from the shoot apex of each plant. One square (2 mm²) of Mira cloth colonised with one

species of *Phytophthora* was inserted into the incision which was then quickly closed and covered with petroleum jelly to prevent desiccation. Control plants were inoculated with sterile, uncolonised Mira cloth. All treatments were randomly allocated to plants, which were arranged in a completely randomised design in the glasshouse. There were five replicates per family.

Acropetal, basipetal and total extension of the lesions visible on the stem surface was measured every three days for 42 days. At 21 and 42 days, tangential spread of the lesion from the point of inoculation was assessed. Observations of lesion colour and appearance were recorded at 14-day intervals. Any plant without a visible lesion seven days after inoculation was re-inoculated and measured at the above intervals (approximately 2% of plants). At 42 days after inoculation, stems were harvested and the lesions and Mira cloth squares plated onto *Phytophthora*-selective agar (Tsao and Guy, 1977). The presence of each *Phytophthora* species in these stems was confirmed by comparing each with the 'mother' culture.

Statistical analysis. Mean lesion lengths 42 days after inoculation were compared between families infected with the same *Phytophthora* species, and within each family (infected with the three *Phytophthora* species) by analysis of variance. Tangential spread of lesions at 21 and 42 days after inoculation was compared both within and between families by analysis of variance. Correlation between family rankings was also calculated.

RESULTS AND DISCUSSION

Jarrah seedlings inoculated with each of the three *Phytophthora* species developed lesions extending above and below the point of inoculation (Fig. 1). The lesions varied in colour from light brown to black, but became woody in appearance between 14 and 28 days after inoculation (Fig. 2). Reddening of tissue above and below the lesion and development of callus around the lesion were also observed although this was most likely a general response to wounding of the stem as similar observations were made of stems inoculated with sterile Mira cloth. When lesions from plants inoculated with each of the three *Phytophthora* species were plated, the respective *Phytophthora* species was recovered from within the lesion, and often up to 10 mm beyond the lesion front in apparently symptomless tissue, similar to reports of reisolation of *P. cinnamomi* from field-grown jarrah after root and stem inoculation (Davison *et al.*, 1994). No lesions were observed in deeper tissues.



Fig.1. Typical lesion caused by *Phytophthora cryptogea* 14 days after stem inoculation of a jarrah seedling from a half-sib family susceptible to *P. cinnamomi*. Arrow indicates point of inoculation.



Fig.2. Typical woody appearance of a lesion 28 days after stem inoculation of a jarrah seedling with *Phytophthora cinnamomi*. Arrow indicates point of inoculation.

Tangential spread of lesions was observed in all plants, ranging from 90° to fully girdled (360°), but was not significantly different ($P > 0.05$) between families infected with the same *Phytophthora* species, at 21 or 42 days after inoculation. Within families, mean tangential spread of lesions caused by the three *Phytophthora* species was also not significantly different, although it was generally greatest in plants inoculated with *P. citricola*.

A continuous range in mean total lesion length was observed among jarrah families infected with the same *Phytophthora* species (Fig. 3). Families with the shortest lesion lengths were significantly different ($P < 0.05$) from those with the longest lesion lengths, for *P. cinnamomi*, *P. citricola* and *P. cryptogea*. There was little correlation ($r \leq 0.4$) in the ranking of families for their reaction to the three *Phytophthora* species based on lesion length (Fig. 3).

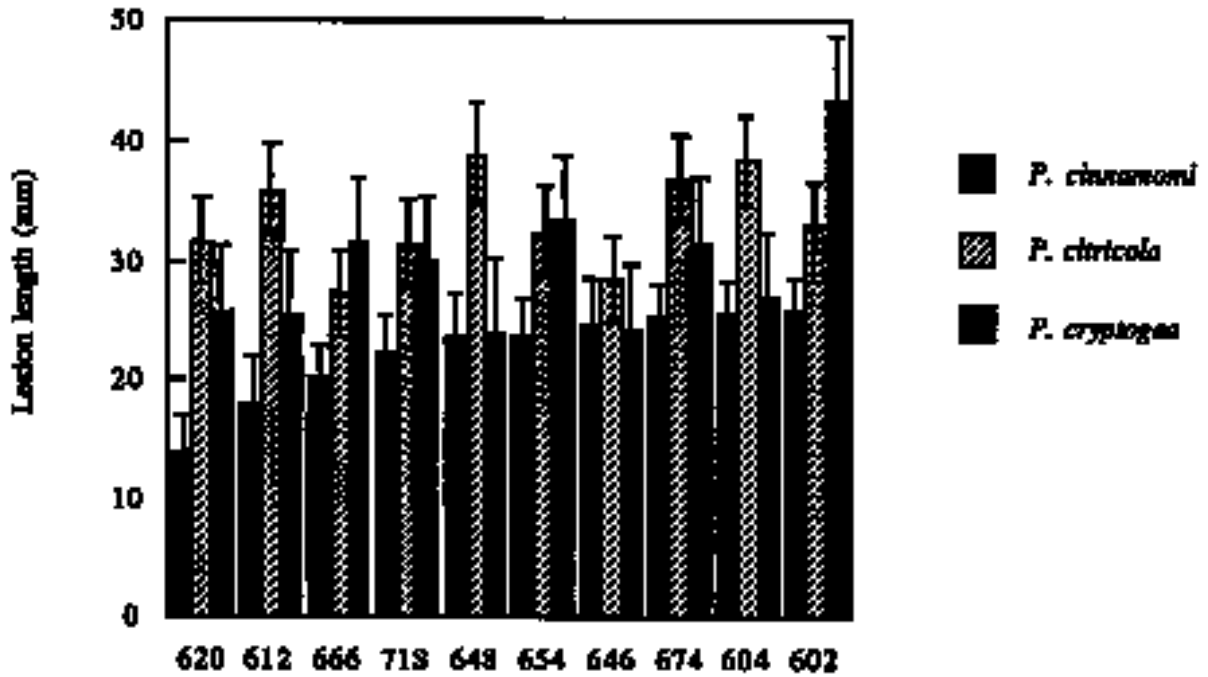


Fig.3. Mean lesion lengths in 10 half-sib jarrah families 42 days after stem inoculation with *Phytophthora cinnamomi*, *P. citricola* and *P. cryptogea*. Families are ranked for resistance to *P. cinnamomi*. Bars indicate standard errors ($n = 5$).

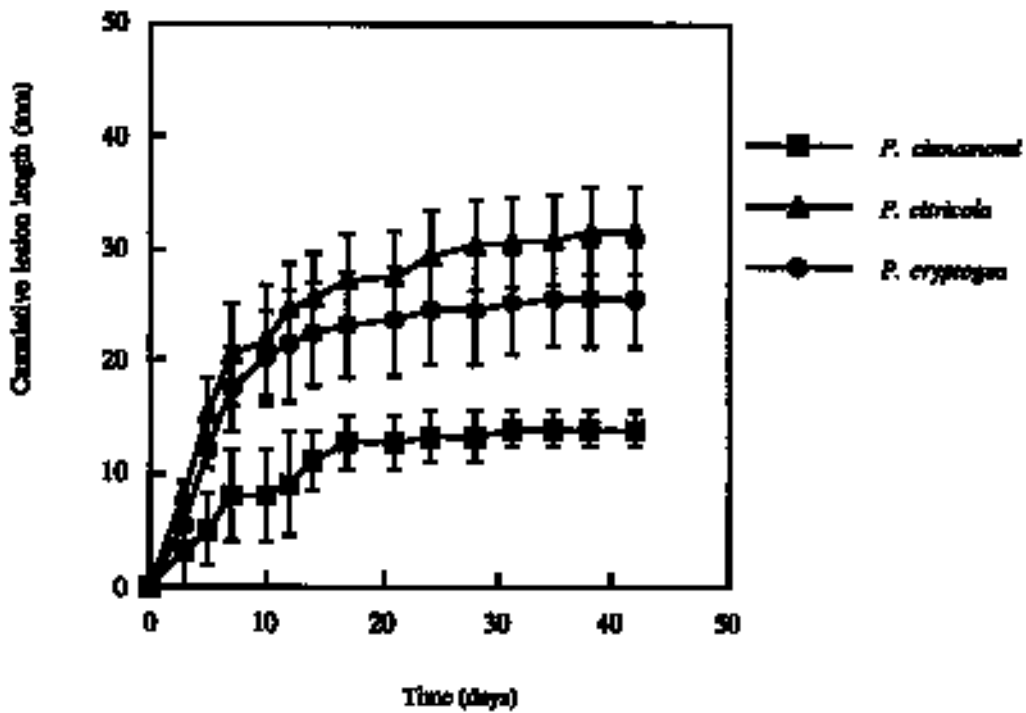


Fig.4. Lesion development in a *Phytophthora cinnamomi*-resistant family (620) after stem inoculation with *P. cinnamomi*, *P. citricola* and *P. cryptogea*. Bars indicate standard errors ($n = 5$).

Within families, lesions caused by *P. citricola* and *P. cryptogea* were, in most cases, longer ($P < 0.05$) and had a faster rate of development than those caused by *P. cinnamomi* (Fig. 3 and 4). The lengths of lesions caused by *P. cinnamomi* were similar to those reported by Stukely and Crane (1994) for winter inoculations of jarrah seedlings.

Based on glasshouse stem inoculation, jarrah families showing a resistant reaction (expressed as short lesion length) to *P. cinnamomi* did not exhibit comparable resistance to the other *Phytophthora* species. However, whilst stem lesion length is known to be correlated with field resistance to *P. cinnamomi* (Stukely and Crane, 1994) there is a need for further research including field testing, to determine whether stem inoculation of jarrah is a valid method for assessing its resistance to *Phytophthora* species other than *P. cinnamomi*. Further work using a larger number of isolates may also be required to confirm the results of this study, although Shearer *et al.* (1988) found no significant difference in lesion extension rates between isolates of *P. cinnamomi* and four out of five isolates of *P. citricola* and *P. cryptogea* A₂ inoculated on jarrah saplings in the field. Stukely and Crane (1994) also found that jarrah family resistance rankings remained constant when they were inoculated with four isolates of *P. cinnamomi* and Bunny (1996) found that aggressive isolates of *P. citricola* consistently caused longer lesions than *P. cinnamomi*, in resistant and susceptible clones of jarrah seedlings. The latter finding also indicates that the *P. citricola* isolate used in our study was an aggressive isolate, while the virulence of our *P. cinnamomi* isolate has been extensively confirmed on jarrah, causing stem lesions to increase by up to 20 mm within 24 hours on some susceptible jarrah seedlings (Stukely and Crane, 1994).

That *P. citricola* can cause lesions in jarrah stem tissue that are equal to, or greater in length than those caused by *P. cinnamomi* is in agreement with previous work by Shearer *et al.* (1988) and Bunny (1996). However, in contrast to our observation, Shearer *et al.* (1988) reported the lesion extension rate of *P. cryptogea* A₂ to be slower than *P. cinnamomi*. Since the same isolates were used in both studies, it is likely that the hosts and/or environmental conditions were the main factors contributing to the observed differences, particularly as Shearer *et al.* used saplings in the field, while we used seedlings inoculated in the glasshouse.

P. citricola and *P. cryptogea* are both present in the jarrah forest and jarrah stem tissue can be rapidly invaded by each. This suggests that both have the potential to cause disease. However, neither has been reported as causing significant disease of jarrah in forests where they are present, although *P. citricola* has been

isolated from young dying jarrah trees on replanted minesites (F. Bunny, personal communication). Thus, while conditions conducive to the invasion of jarrah by these fungi may rarely occur in the forest, it is possible that they exist in disturbed areas such as minesites. Hardy *et al.* (1996) demonstrated that the ponding that occurs in riplines on bauxite mine sites is consistently associated with the direct invasion of jarrah collars and stems by *P. cinnamomi*. It is likely that other *Phytophthora* species may also take advantage of such conditions. This indicates the importance of continued surveillance of *Phytophthora* species across the jarrah forest and monitoring of any infection of jarrah by these species.

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