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Susceptibility of *Eucalyptus marginata* and *Eucalyptus calophylla* Seedlings to Infection by *Phytophthora cinnamomi* in Nutrient Solution

by

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SUMMARY

Significant differences in the spread of infection between field susceptible and field resistant seedling eucalypt species were observed when these were inoculated with *Phytophthora cinnamomi* Rands in a nutrient solution.

These differences became less distinct as the trial progressed; at nine days severe root rot of both species was observed. The possibility of eliminating field resistant species in similar studies may be overcome by the inclusion of standard phytometers in each trial.

INTRODUCTION

Eucalyptus marginata Sm. (jarrah) and *Eucalyptus calophylla* Lindl. (marri) are the dominant tree species of the jarrah forest formation in Western Australia. The former is susceptible to root rot caused by *Phytophthora cinnamomi* Rands while the latter, though a host of *P. cinnamomi*, has demonstrated considerable resistance in pot trials and under field conditions (Podger 1968).

Screening trials in nutrient solutions are a standard procedure for comparing the resistance of host seedlings to disease caused by root pathogens such as *Phytophthora* (Oxenham and Winks 1963; Wong and Varghese 1966; Zak and Campbell 1958; Zentmyer and Mircetich 1965). In most instances the results of these trials correlate closely with the recorded host resistance in the field.

METHOD

Jarrah and marri seedlings were raised in steam-sterilized sand flats for four months. The roots were washed and the seedlings root-pruned and top-pruned to 76 mm. They were transferred to four battery cases, each containing seven litres of a modified Wong and Varghese (1966) nutrient solution (Table 1) adjusted to the pH range 5.3 to 5.6.

TABLE 1

Chemical Composition of the Nutrient Solution

Chemical	Concentration
$Ca(NO_3)_2$	0.25 millimoles per litre
$MgSO_4$	0.10 millimoles per litre
KH_2PO_4	0.10 millimoles per litre
H_3BO_3	0.025 parts per million B
$MnSO_4$	0.025 parts per million Mn
$ZnSO_4$	0.003 parts per million Zn
Iron Chelate	0.10 parts per million Fe

The seedlings were supported on floats of polystyrene foam and aerated with a continuous slow stream of air in an air-conditioned glass-house (temperature range 21 to 27°C). After an establishment period of three weeks, the seedlings were turgid, of good colour and had made considerable root and shoot growth.

76 mm lengths of lucerne stem were autoclaved in test tubes containing distilled water, inoculated with a local isolate of *P. cinnamomi* and incubated at 26°C. After one week the lucerne pieces were transferred to petri dishes, covered with a non-sterile soil extract to induce sporangial formation and re-incubated at 26°C for four days. One sealed and weighted nylon mesh bag containing ten lucerne stems bearing sporangia of *P. cinnamomi* was placed on the bottom of each of the three battery cases to be inoculated.

Three seedlings of each species (one from each of three battery cases) were removed 6, 30, 48, 72, 194, 218 and 360 hours after inoculation. The fourth battery case held three seedlings of each species and contained sterilised uninoculated lucerne stems.

Non-lesioned and lesioned root tips of the first order laterals were counted and the roots examined microscopically. Lesioned root pieces and non-lesioned portions 1 to 5 and 5 to 10 mm above a lesion were surface-sterilised for two minutes in calcium hypochlorite solution and then cultured on 3P agar (Eckert and Tsao 1962).

The lengths of lesion progression on the first order laterals were measured at 30, 54 and 78 hours after inoculation on seedlings finally harvested at 218 and 360 hours. At the completion of the trial, pieces from the seedling tap root, collar and stem were plated.

RESULTS

Six hours after inoculation, encysted and germinating zoospores were observed to be attached to the roots of both species. Only one root tip was lesioned and no recoveries of *P. cinnamomi* were obtained from plating. After thirty hours, all of the jarrah and a high percentage of the marri root tips were lesioned. *P. cinnamomi* was recovered from all seedlings at this and at subsequent platings.

The lesions were light to dark brown in colour and developed in the region just behind the root cap. Occasional lesions were also observed where the cortex of the first order lateral had been ruptured by an emerging root tip. Second order laterals of both species were also lesioned. In jarrah, the stele was darkened in advance of cortical lesioning.

After 72 hours, full, empty and proliferating sporangia were observed on the lesioned roots. At 194 hours, the steles of all jarrah seedlings were completely darkened, but cortical lesioning was restricted to the distal portion of these roots. The non-lesioned cortex was translucent and had a water-soaked appearance.

In marri, stelar and cortical lesioning occurred on the distal portion of the first order laterals. Short second order lateral roots were completely lesioned. The latter lesions penetrated the cortex but not the stele of the first order laterals.

At 360 hours, the root systems of both species were severely damaged, though one marri seedling was beginning to regenerate its root system. On plating, *P. cinnamomi* was recovered from the suberised roots and collars of both species. In jarrah it was also recovered from the stem, 13 mm above the collar region.

The time for lesioning of all root tips of the first order laterals was longer for marri than for jarrah. At 30 and 48 hours after inoculation these differences were most pronounced (significant at the one percent level).

Lesions progressed faster along the first order laterals of jarrah. The differences in lesion length between species were insignificant at 30, 54 and 78 hours after inoculation (Table 2).

TABLE 2

Rates of Progression of Lesions Along First Order Laterals of *E. marginata* and *E. calophylla* Seedlings Inoculated with *P. cinnamomi* in Nutrient Solution.

	No. of Roots Examined at			Mean Lesion Length (mm) ** at		
Species	30 hrs	54 hrs*	78 hrs*	30 hrs	54 hrs	78 hrs
E. marginata	27	24	12	13.7	33.0	53.0
E. calophylla	21	21	21	5.2	8.0	14.6

* Only well-defined lesions were measured.

** Differences between species are significant at the one percent level.

Percentage recoveries of *P. cinnamomi* by platings on 3P agar did not differ significantly between species for either the lesioned root tips or the non-lesioned portions 1 to 5 mm from a lesion. In the case of jarrah, the non-lesioned root pieces 5 to 10 mm from a lesion yielded significantly higher recoveries of the fungus (Table 3).

TABLE 3

Percentage Recovery of *P. cinnamomi* on 3P Plates From Root Pieces of *E. marginata* and *E. calophylla* Seedlings Inoculated in a Nutrient Solution.

Materials Plated	Recovery of *P. cinnamomi*	
	E. marginata	*E. calophylla*
All root pieces	51%	36%*
Lesioned root pieces	61%	55%
Non-lesioned root pieces 1 to 5 mm from a lesion	61%	47%
Non-lesioned root pieces 5 to 10 mm from a lesion	92%	29%*

* Differences between species significant at the one percent level.

Terminal root growth ceased and no new leaves were produced after inoculation. Three jarrah and one marri seedling had wilted after 72 hours; a further three jarrah wilted before the trial was terminated. The roots of seedlings in the uninoculated control remained non-lesioned and these plants continued to make good root and shoot growth.

DISCUSSION

Temperature, pH and aeration were in the optimum range for mycelial growth, sporangial production and infection by *P. cinnamomi* (Zentmyer and Mircetich 1965). The original inoculum was augmented by the formation of some sporangia on infected root tips, and the solution provided a suitable medium for zoospores movement. Under these conditions severe root rot of both species occurred.

P. cinnamomi was first recovered on 3P agar plates 30 hours after inoculation. Failure to recover the pathogen after six hours, an adequate period for zoospore release, encystment and germination, was possibly due to the surface sterilisation of root pieces for two minutes in calcium hypochlorite.

Several rating systems have been used when screening for resistance to *P. cinnamomi* in nutrient solution (op. cit.). The time of final assessment has ranged from 7 to 20 days. Time of assessment and the parameter used can markedly affect the results obtained, since the observed differences between jarrah and marri had largely disappeared 218 hours after inoculation.

Stelar invasion by *P. cinnamomi* may result from penetration of the suberised endodermal barrier through ruptures caused by developing secondary roots, as has been demonstrated with *Pythium debaryanum* Hesse (Hock and Klarman 1967), or by the upward spread of the pathogen from infection of the unsuberised root tips. When only the cortex tissues are colonised, regeneration of *Pinus radiata* D. Don roots has been observed (Newhook 1961); hence the degree of damage to a root system in this artificial environment depends on:

(1) The ease and rapidity of infection of the unsuberised root tips and of ruptures in the root's cortex.

(2) The rate of spread of the pathogen within the unsuberised roots.

(3) The rate of spread of the pathogen within the suberised roots, and the root tissues within which this spread occurs.

(4) The effects of environmental factors in favouring either root damage or root recovery.

The infection of unsuberised and non-mycorrhizal roots in this artificial environment is only one facet of disease resistance, and the extrapolation of these results to the field situation will be complicated by environmental and biological interactions. It is nevertheless an important one, since the pathogen gains entry in this way.

While nutrient culture may provide a rapid method of screening hosts for potential disease resistance, care needs to be taken both with the parameter used to assess the degree of damage and with the time of exposure. Severe root rot occurred in marri, a field resistant species, after 218 hours in this culture system. With this technique there exists the possibility of eliminating field resistant species, and the inclusion of standard phytometers in each trial is recommended.

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