Genetically Based Resistance of *Eucalyptus marginata* to *Phytophthora cinnamomi*

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ABSTRACT

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Sixteen half-sib families of jarrah (*Eucalyptus marginata*) seedlings were screened for resistance to *Phytophthora cinnamomi* using soil inoculation and stem inoculation in pot experiments, and soil inoculation in a *P. cinnamomi*-infested field site. Low mortality following soil inoculation and short lesion lengths following stem inoculation were used as indicators of *P. cinnamomi* resistance. Resistance levels varied continuously across families from high to low values in all experiments, but family rankings were consistent among experiments. The narrow-sense heritability of the resistance character was high at both family (0.74–0.85) and individual-tree (0.43) levels. The resistance of jarrah to *P. cinnamomi* is under strong genetic control. Selection of lines with high levels of resistance is feasible, and such lines can be used in rehabilitation plantings of jarrah forest sites. Selection of resistant parent trees in the forest based on a single assessment of crown health met with little success. Seedlings of five healthy parent trees in diseased forest exhibited a wide range of resistance levels and were only marginally more resistant than seedlings of trees with symptoms of root rot. Stem-inoculation of jarrah seedlings at least 9 mo old is recommended as the standard screening test to be used in selecting families and individuals resistant to *P. cinnamomi,* based on lesion size.

Jarrah (*Eucalyptus marginata* Donn ex Sm.) is a hardwood of major economic importance endemic in the southwest of Western Australia. A lethal disease of jarrah called "jarrah dieback," caused by the root-rotting fungus *Phytophthora cinnamomi* Rands (22), is responsible for extensive degradation in the jarrah forest. The fungus is believed to have been introduced to the jarrah forest ecosystem (22), probably soon after first European settlement; and in 1982, it was estimated that some 14% of the jarrah forest area in State Forests was affected (11). Occasional healthy jarrah trees persist in dieback-affected sites, but it has not been established whether these trees are resistant to the fungus or are disease escapes.

Resistance to *P. cinnamomi* has been demonstrated in individual plants in some tree species considered generally susceptible to the fungus, e.g., *Pinus echinata* Mill. (4), *Pinus radiata* D. Don (6), and *Persea americana* Miller (31). Resistance to *P. cinnamomi* has been reported in susceptible eastern Australian *Eucalyptus* species of the subgenus *Monocalyptus,* to which jarrah belongs. Seedlings of one of seven *E. muellerana* A. Howitt trees showing field tolerance to *P. cinnamomi* were tolerant of *P. cinnamomi* infection in a pot trial (20). Consistent phenotypic resistance to stem infection and mortality caused by *P. cinnamomi* was reported in seedlings of *E. regnans* F. Muell. growing on a site infested with *P. cinnamomi* (16).

A variety of responses to inoculation with *P. cinnamomi* have been reported in *E. marginata.* Podger (21,22) reported differences in the degree of root rot among nine jarrah provenances when seedlings were inoculated with *P. cinnamomi.* The rates of extension of root lesions varied widely among inoculated jarrah seedlings within one provenance under controlled conditions (13). Differences have been observed in levels of infection, growth reduction, and mortality among clonal lines of jarrah following inoculation with *P. cinnamomi,* in vitro and in pots (3). When jarrah coppice stems growing in the forest were inoculated under the bark with *P. cinnamomi,* the lengths of the resulting lesions differed among plants on the same site (25). Rockel (23) observed that smaller lesions were formed in inoculated roots of apparently healthy jarrah in diseased forest than in healthy trees in healthy forest. This paper reports a series of experiments designed to determine whether genetically based resistance to *P. cinnamomi* is present in *E. marginata.*

MATERIALS AND METHODS

Plant materials. Seed was collected from individual open-pollinated jarrah during September–December 1984. The parent trees were selected to cover a range of sites in the northern and central jarrah forests. Both healthy and diseased parent trees growing in several dieback-affected sites were included; these trees were classified as "apparently resistant" (R) to *P. cinnamomi* if their crowns were healthy (no. 1, 4, 8, 12, 14), or "apparently susceptible" (S) if their crowns showed obvious dieback symptoms (no. 2, 5, 9, 15, 16, 17). Healthy trees on apparently uninfested sites were included in a third category (U) whose resistance classes were unknown (no. 6, 7, 10, 11, 13). To improve the likelihood of outcrossing, seed was collected only from trees fruiting synchronously with neighboring trees in the stand (14).

Seeds were sown in December 1984 in peat pots containing a peat–sand (1:1) mixture sterilized by methyl bromide fumigation, and the seedlings were grown in a shadehouse. Seedlings derived from an individual parent tree were referred to as a family. Three experiments were conducted using separate sets of seedlings of 16 jarrah families.

Experiment 1: soil inoculation. Four seedlings, each of a different family selected at random, were removed from their peat pots and planted at an even spacing in an 8-L plastic pot containing peat–sand (1:1) potting medium. The design consisted of single-seedling family plots in 30 randomized complete blocks. A replicate block consisted of four pots.

The seedlings were grown to develop extensive root systems prior to inoculation and were fertilized at 8-wk intervals with Aquasol, 125 mg per seedling (Hortico [Aust.] Pty. Ltd., 31

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Catalano Road, Canning Vale, W.A. 6155). When the plants were 11 mo old, pots were inoculated with *Pinus radiata* plugs (branch segments ~2.5 × 1.5 cm) which had been debarked and colonized by *P. cinnamomi* under aseptic conditions, as described by Butcher et al (6). Four local isolates of *P. cinnamomi* (type A2), which had been shown to be pathogenic to jarrah seedlings in a pilot trial, were used: 251N12 (isolated from *Pinus radiata*), Sc 381 (IMI 264385) (from *Allocasuarina fraseriana* (Miq.) L. Johnson), DCE 210 (from *E. marginata*), and 480R1 (from *Banksia* sp.).

Each pot was inoculated by burying eight pine plugs (two for each *P. cinnamomi* isolate) at equal distances from the seedlings and 5 cm deep. Each seedling was encircled by four plugs, each of which carried a different isolate of *P. cinnamomi.* Control pots received pine plugs inoculated with *P. cinnamomi* as described above and autoclaved (103.5 kPa [15 psi] for 20 min). Seedling heights were measured at the time of inoculation.

Immediately after inoculation, pots were watered to encourage *P. cinnamomi* sporulation and root infection. Free drainage was maintained. Subsequently, watering was done according to need, with an equal amount applied to each pot. During the full summer–autumn monitoring period, the pots were held in a glasshouse in which conditions were favorable to both seedling growth and fungal invasion. The maximum temperature did not exceed 28 C.

Seedling mortality was evaluated at approximately weekly intervals for 5 mo to determine the relative resistance of jarrah families to the fungus. Roots and root collars of a sample of freshly killed seedlings of each family were surface-sterilized and plated on *Phytophthora*-selective P_{10}VPH agar (29) to confirm *P. cinnamomi* infection. A sample of inoculum plugs was retrieved from pots at the conclusion of the trial and plated onto P_{10}VPH agar to check the survival of the fungus. The trial was repeated the following summer using 2-yr-old seedlings.

Experiment 2: stem inoculation. Jarrah seedlings were raised and potted as described for experiment 1, in 15 randomized complete blocks with single-seedling family plots. Seedlings with multiple leaders were pruned to leave a single stem. At 14 mo after sowing, in summer, the seedlings were inoculated with a single isolate of *P. cinnamomi* (type A2), Sc 72 (IMI 264384), using a modification of the method of Smith and Marks (26). Autoclaved polycarbonate membrane filter disks (pore size 8 μm) (Sartorius GMBH, Postfach 3243, Weender Landstrasse 94-108, D-3400 Gottingen, Germany) were placed on the surface of green pea agar (macerated frozen green peas at 200 g L^{-1}) in a petri dish ahead of the advancing margin of a young *P. cinnamomi* colony. When colonized, the filters were removed from the agar, cut into squares (2 mm) and floated on sterile distilled water in a petri dish to maintain high humidity. Microscopic examination of disks showed abundant *P. cinnamomi* hyphae, and *P. cinnamomi* readily grew from disks placed on an agar plate.

A shallow incision ~2.5 mm long was cut in the jarrah stem. One inoculum square was inserted, and the wound was immediately closed and sealed with petroleum jelly to prevent desiccation. Control stems received filter squares which had not been colonized by *P. cinnamomi.* A point at the center of the fourth internode from the apex was chosen where possible, to ensure that tissue of similar age on all seedlings was inoculated.

Seedling heights and stem diameters at the site of inoculation were measured. The lengths of lesions above and below the point of inoculation were measured approximately daily for up to 3 wk to determine the relative resistance of jarrah families to the fungus. No fertilizer was applied during this period. Glasshouse conditions were as described for experiment 1. Stems (<1%) which had not developed visible lesions 7 days after inoculation were reinoculated at a fresh site and measured at appropriate intervals. A random sample of stems of all families were plated on P_{10}VPH agar 2–4 wk after inoculation to compare the position of *P. cinnamomi* recoveries to the length of visible lesions. Sections 2 mm thick were cut and plated sequentially, starting in healthy tissue 10 mm ahead of the lesion front and continuing 10 mm inside the lesion. The trial was repeated using 9-mo-old seedlings in spring, 18-mo-old seedlings in winter, and 2-yr-old seedlings in summer.

Field experiment. A slightly sloping site of Havel type P (17) was selected in jarrah forest severely affected by dieback in the Jarrahdale District (lat. 32° 22′ S, long. 116° 09′ E). A disease-free site immediately upslope, of similar soil type, was used as a control. The vegetation was bulldozed, stacked, and burned. The soil was cultivated by ripping to a depth of 0.5 m at 4-m intervals on the contour. All machinery was washed down before entering the control site, and all operations were completed on this site before entering the diseased site, in order to avoid introducing *P. cinnamomi* to the control site.

Six-month-old jarrah seedlings were planted at 4 × 4 m spacing on the sides of the furrows in July 1985. The peat pots were removed from the root balls to facilitate rapid root growth. In addition to the 16 families, a bulked seed lot (containing seed from 20 parent trees not included in the experiments) from the routine seed collection was included for comparison. The experimental design consisted of 50 randomized complete blocks with single-seedling family plots. Twenty-five blocks were used on the control site.

A small number (3.5%) of seedlings died in the dieback site in the 2 mo prior to inoculation, and were replaced. The roots of the dead seedlings were excavated, surface sterilized, and plated onto P_{10}VPH agar. In September, the dieback site was inoculated with *P. cinnamomi* by surrounding each seedling with four buried pine plugs, each of which carried a different *P. cinnamomi* isolate (see experiment 1). The soil was moist at the time of inoculation, and rain fell on the following 2 days.

Seedling mortality was evaluated at regular intervals for 6 yr to determine the relative resistance of jarrah families to the fungus. During the first 2 yr, checks were done at 2- to 4-wk intervals; subsequently, the intervals were increased. In the first 4 yr, samples of roots and root-collar tissue from all dead seedlings were surface sterilized and plated onto P_{10}VPH agar to confirm *P. cinnamomi* infection. Inoculum plugs were retrieved from the soil below dead seedlings where possible in the inoculated site and plated onto P_{10}VPH agar to check the survival of the fungus. The heights of surviving seedlings in the two sites were measured after 6 yr.

Statistical analysis. Lesion length, seedling height, and stem diameter data were analyzed by analysis of variance (ANOVA) using the SPSS statistical package (27). Mortalities were compared by the chi-square test. Family rankings were compared among trials using the Spearman rank correlation coefficient. To permit the calculation of family heritability estimates, blocks were pooled to give family means for mortalities. Six pooled replicates of five noncontiguous family plots were used in experiment 1, while 10 pooled replicates of five noncontiguous family plots were used in the field experiment. Percent mortality data from pooled blocks were transformed by arcsine square root prior to ANOVA. Variance components were calculated according to Namkoong et al (19).

Estimates of the narrow-sense family heritabilities (h_F^2) of means for mortality in experiment 1 and the field experiment, and for lesion length in experiment 2, were calculated using the formula

$$h_F^2 = \sigma_f^2/(\sigma_f^2 + \sigma_e^2/b)$$

where σ_f^2 is the variance due to families, σ_e^2 is the residual variance, and b is the number of blocks.

The narrow-sense, individual-tree heritabilities (h_I^2) of lesion length, seedling height, and stem diameter in experiment 2 were estimated using the formula

$$h_I^2 = 2.5\sigma_f^2/(\sigma_f^2 + \sigma_e^2)$$

The coefficient of relationship (r) of 1/2.5 was used (15) rather than $r = 1/4$, which is applicable to true half-sibs, since jarrah, like other eucalypts, has a mixed mating system, and open-pollinated seed from natural stands will consist of a mixture of outcrossed and inbred seed. Under these circumstances, individual-tree h^2 estimates based on the assumption of $r = 1/4$

would be biased upwards (15). Standard errors for individual-tree heritability were calculated according to Swiger et al (28).

RESULTS

Experiment 1. Mortality was first observed 15 days after inoculation in families 11 and 13, each with four deaths, and families 1 and 2, each with one death. Dying seedlings exhibited symptoms of root rot, with sudden wilting of growing tips, followed rapidly by desiccation and yellowing of leaves. The numbers of dead seedlings increased sharply in the ensuing weeks, particularly in families 11 and 13, while mortality in other families such as 7 and 12 increased more slowly (Fig. 1). After 4 mo, mortality in family 13 had reached 100%; and families 2, 6, 11, 16, and 17 had 90% or more seedlings dead (Fig. 2). Other families exhibited a range of lower mortality levels, with the lowest mortality recorded in family 7 (43%). There were significant ($P < 0.05$) differences in mortality levels among families. In the fifth month, additional mortalities were recorded in some of the more susceptible families (Fig. 2); however, there were none in families 7, 1, 12, 8, or 5. Across all families, a total of 80% of inoculated seedlings had died after 5 mo. The family heritability of the resistance character was estimated at 0.78 (Table 1).

P. cinnamomi was recovered from the roots of all freshly killed seedlings sampled during the trial and from all pine plugs sampled at its conclusion. Mean heights of seedlings at inoculation differed among families ($P = 0.0006$), with a range of 287–389 mm. There was no correlation between seedling height and family mortality. Three seedlings died in the control pots. All of these were small, unthrifty individuals at inoculation; all died within the first 80 days. *P. cinnamomi* was not isolated from their roots.

Mortality of the 2-yr-old seedlings was lower than that of the 1-yr-old group, with a range of 3% (family 12) to 67% (family

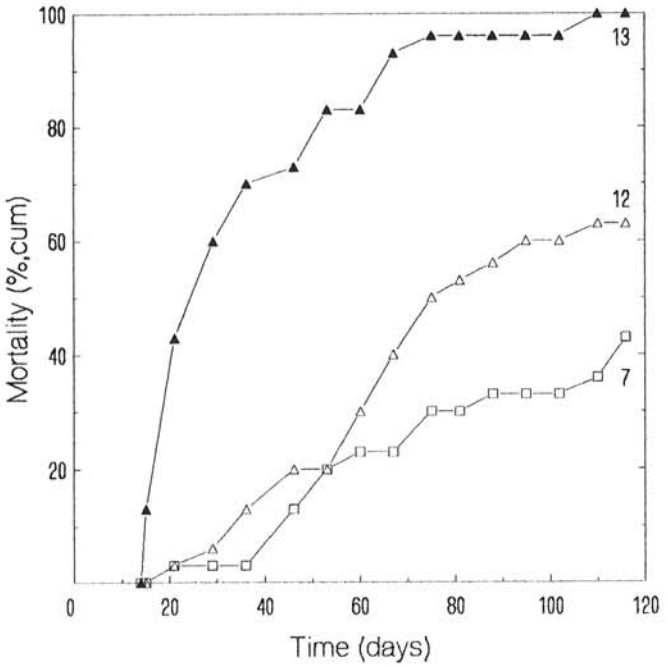

Fig. 1. Progressive mortality (%) of seedlings of half-sib *Eucalyptus marginata* families 7, 12, and 13 after inoculation of the potting medium with four isolates of *Phytophthora cinnamomi* in experiment 1. Thirty plants of each family were tested.

TABLE 1. Components of variance and estimates of the *Eucalyptus marginata* family heritability (h_F^2) and individual-tree heritability (h_I^2) of resistance to *Phytophthora cinnamomi*

Source of variation	Experiment 1[a]		Experiment 2[b]		Field experiment[c]	
	df	ms	df	ms	df	ms
Families	15	1,155	15	5,270	19	1,623
Blocks	5	72	14	3,859	9	379
Residual	75	251	204	1,309	171	248
	$h_F^2 = 0.78$		$h_F^2 = 0.74$ $h_I^2 = 0.43 \pm 0.18$		$h_F^2 = 0.85$	

[a] Data were arcsine square root transformations of percent mortality after 4 mo (pooled plots).
[b] Data were 13-day lesion lengths above the inoculation point.
[c] Data were arcsine square root transformations of percent mortality after 6 yr (pooled plots).

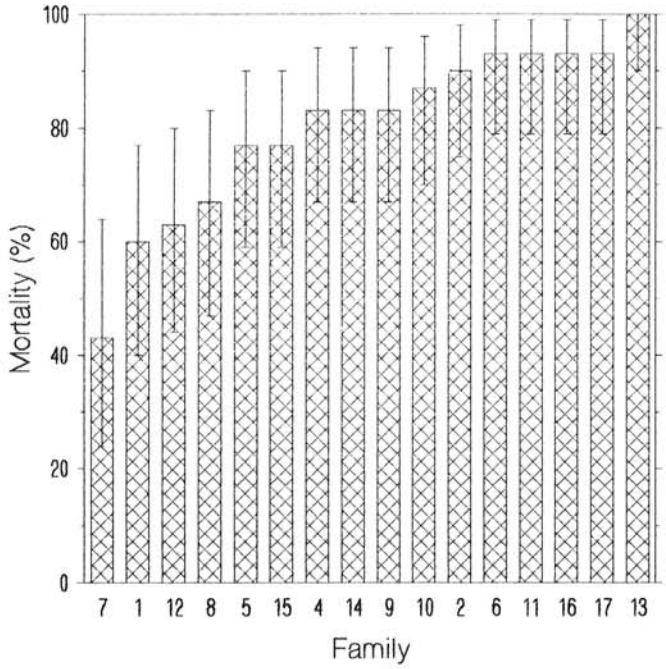

Fig. 2. Mortality (%) of seedlings of 16 half-sib *Eucalyptus marginata* families 4 mo after inoculation of the potting medium with four isolates of *Phytophthora cinnamomi* in experiment 1. Bars define 95% confidence intervals for the proportion (n = 30).

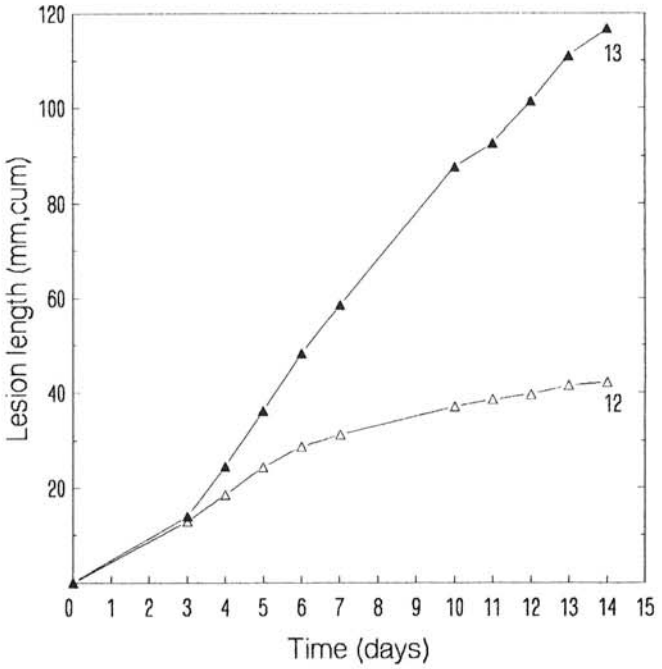

Fig. 3. Progressive stem lesion lengths (mm) on seedlings of half-sib *Eucalyptus marginata* families 12 and 13, following wound-inoculation with mycelium of *Phytophthora cinnamomi* isolate Sc72 (IMI 264384) in experiment 2. Each point is the mean of 15 replicates.

13) after 7 mo. However, family rankings of the 2-yr-old seedlings correlated well ($P < 0.01$) with those of the 1-yr-old seedlings.

Experiment 2. A distinctive brown-to-black lesion developed, extending above and below the point of inoculation, on many of the inoculated stems within 3 days. On some seedlings, however, the rate of lesion extension diminished after about 6 days, with the fungus being contained within a short distance of the inoculation point (resistant reaction) (Fig. 3). On others, lesions continued to extend at a rapid rate throughout the monitoring period (susceptible reaction). The lesions on some of these stems extended as much as 20 mm in 24 h. In some susceptible seedlings, the lesion had fully girdled the stem within 10 days. The distal portion of these plants wilted and died 6–9 days later, after which further measurements of the lesion were not possible. Numbers of replicates of some families were thus reduced after day 14. When inoculated stems were plated, *P. cinnamomi* was recovered from discolored tissue at the lesion front. It was sometimes also isolated from symptomless tissue up to 4 mm ahead of the lesion front.

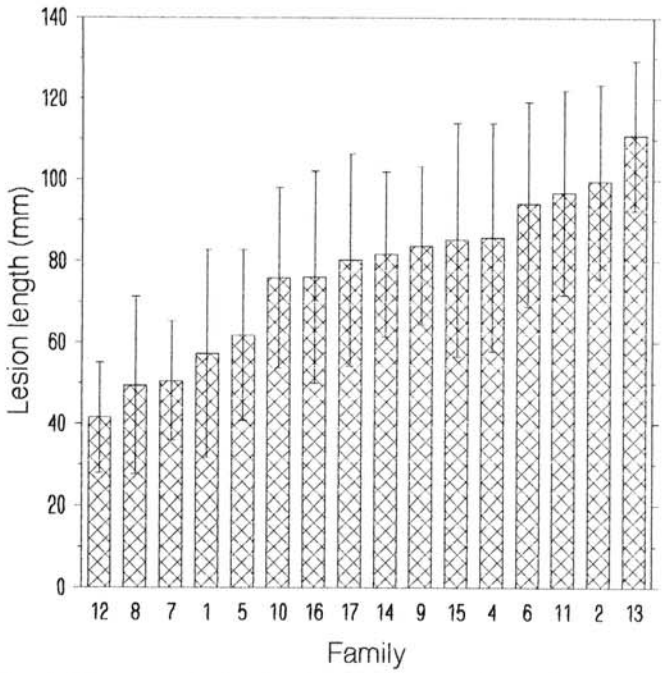

Fig. 4. Mean stem lesion lengths (mm) on seedlings of 16 half-sib *Eucalyptus marginata* families 13 days after wound-inoculation with mycelium of *Phytophthora cinnamomi* isolate Sc72 (IMI 264384) in experiment 2. Bars define 95% confidence intervals of the means (n = 15).

The mean lesion lengths above the inoculation point for the 16 families after 13 days are shown in Figure 4. Families exhibited a continuous range of lesion lengths, and there were significant differences ($P < 0.0001$) between those lying near the resistant (family 12) and susceptible (family 13) extremes. There was considerable variation in lesion length among seedlings within families. The range of lesion lengths at 13 days in resistant family 12 was 12–100 mm (mean 41.5 mm), while that of susceptible family 13 was 62–155 mm (mean 110.9 mm). Of the 240 inoculated seedlings in the trial, 10 showed a highly resistant reaction, with lesions contained at less than 20 mm each side of the inoculation point after 13 days. Eight of these individuals were from the five families (12, 8, 7, 1, and 5) with highest resistance. No lesions developed on the control stems. The family heritability of the resistance character was estimated at 0.74, the individual-tree heritability at 0.43 ± 0.18 (Table 1). The family rankings in experiments 1 and 2 were strongly correlated ($r = 0.74$, $P = 0.001$).

Mean seedling heights and stem diameters at the time of inoculation differed among families ($P = 0.0001$). Mean heights were 500–700 mm, while diameters at the inoculation point were 2.5–3.5 mm. The individual-tree heritabilities of seedling height and stem diameter were 0.24 ± 0.14 and 0.44 ± 0.19, respectively. There was no correlation between family mean lesion lengths and mean heights or diameters.

Inoculation of younger (9 mo old) stems in spring led to significant ($P < 0.05$) differences among families in lesion lengths. Mean lesion length after 8 days in resistant family 7 was 47.5 mm (susceptible family 13, 83.1 mm). However, the seedlings were considerably smaller (mean height 300–450 mm), and the inoculation incision caused significant physical damage to many of them. Most stems were girdled by the lesion in 6 days or less, and lesions could not be measured for more than 8 days on most plants. Smaller stems were therefore considered unsuitable for testing by this method. The family rankings correlated ($r = 0.51$, $P = 0.04$) with those of the 14-mo-old seedlings. Inoculation of 18-mo-old seedlings in winter resulted in much slower rates of lesion extension, probably due to the lower ambient temperatures (total hours above 20 C in 13 days in winter = 40, in summer = 185). Mean lesion length after 14 days in resistant family 12 was 2.3 mm (susceptible family 13, 15.0 mm). After 42 days, these means had increased to 6.9 mm and 28.5 mm, respectively. Differences among families were significant ($P = 0.0001$). The family rankings correlated well ($r = 0.58$, $P = 0.018$) with those of the 14-mo-old seedlings inoculated in summer.

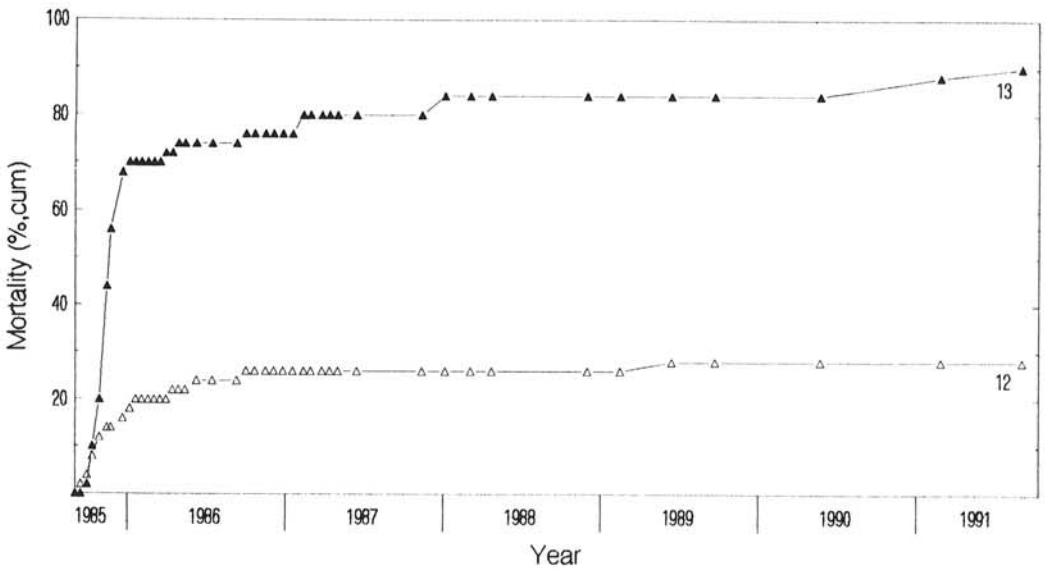

Fig. 5. Progressive mortality (%) of seedlings of half-sib *Eucalyptus marginata* families 12 and 13 after inoculation of the soil with four isolates of *Phytophthora cinnamomi* in the field experiment. Fifty plants of each family were tested.

Two-year-old seedlings, inoculated in summer, proved to be unsuitable. By this stage, they had often developed reddish brown surface pigmentation and/or considerable amounts of bark, and lesions were difficult to distinguish. In addition, on some stems the fungus preferentially invaded internal tissues, and its progress was not visible as a continuous surface lesion; subsequently, the surface lesion often broke out at a node. Visual measurements of lesions were thus unreliable on 2-yr-old plants.

Field experiment. *P. cinnamomi* was isolated from the root collars and/or roots of some of the seedlings which died in the dieback-affected site prior to the inoculation of the trial. This confirmed the presence of native *P. cinnamomi* inoculum in this site. Thirteen days after inoculation, mortalities were observed in several families. As an example of the extremes of response, progressive mortalities over 6 yr for families 12 and 13 are shown in Figure 5. A large number of seedlings died in the first spring–summer–autumn (1985–86): 74% in family 13 and 22% in family 12. Fewer deaths occurred in subsequent years. In family 12, the mortality level stabilized by the second summer, and 28% of the plants had died after 6 yr. In family 13, however, 80% of the seedlings had died by the second summer, and there was 90% mortality after 6 yr.

The 6-yr mortality levels for the 16 families are shown in Figure 6, together with the corresponding data for the control site. There were clear differences among inoculated families in levels of mortality ($P < 0.001$), and the families showed a continuous range of mortality levels between the resistant (family 5) and susceptible (family 11) extremes. Family mortality levels in the inoculated site were clearly independent of those in the control site (Fig. 6). The total mortality across all families in the inoculated site after 6 yr was 54.5% (control site 11.7%). The 6-yr mortality level for the bulked seed lot planted in the inoculated site was also 54%. Differences among families in control mortality levels were not significant ($\chi^2 = 15.7$, $P = 0.4$). Family heritability of the resistance character was estimated at 0.85 (Table 1). Family resistance rankings in experiments 1 and 2 correlated well with those of the field experiment ($r = 0.56$, $P = 0.026$, and $r = 0.52$, $P = 0.038$, respectively).

Families within each of the three crown-health classes of parental trees (R, S, and U) exhibited a range of levels of mortality after 6 yr. Although overall seedling mortality levels in both the R and S groups were lower than those of the U group ($P < 0.001$), there was little difference between the R and S groups ($P = 0.08$).

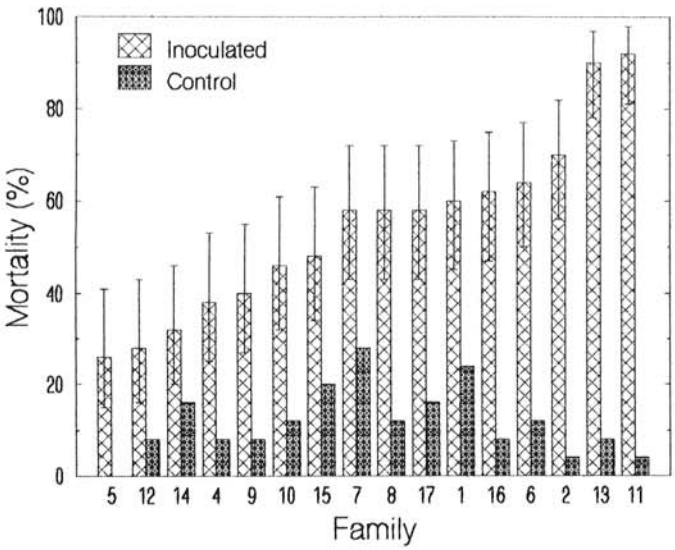

Fig. 6. Mortality (%) of seedlings of 16 half-sib *Eucalyptus marginata* families 6 yr after inoculation of the soil with four isolates of *Phytophthora cinnamomi* in the field experiment. Mortalities in the control site are shown for comparison. Bars define 95% confidence intervals for the proportion (inoculated site, n = 50; control site, n = 25).

P. cinnamomi was isolated from the root collars and/or roots of 71% of dead seedlings in the dieback site in the first 4 yr after inoculation, even though many of the younger dead plants had fully dried out before they could be sampled. The fungus was never isolated from dead seedlings in the control site. By December 1985, *P. cinnamomi* was still readily isolated from the inoculum plugs excavated with dead seedlings in the inoculated site. Recoveries from plugs declined rapidly as the soil dried during summer, and were rare after January 1986.

Growth rates varied widely among families ($P < 0.001$). After 6 yr, the mean height of surviving seedlings in the inoculated site was greatest for resistant family 5 (2.57 m) and smallest for susceptible family 11 (0.81 m). Most grew considerably taller in the control site than in the inoculated site. Depression of growth in the presence of *P. cinnamomi* was greatest in the susceptible families 11 and 13 (39 and 47% of control, respectively). Resistant families 5 and 12, however, attained 73 and 62% of control height, respectively. The degree of growth depression varied considerably among the intermediate families (59–97% of control). While family mean heights reached a maximum of only 2.57 m in 6 yr in the inoculated site (3.52 m, control site), some individual inoculated plants attained heights of 4–5 m.

DISCUSSION

The 16 jarrah families exhibited a wide and continuous range of levels of resistance or susceptibility to *P. cinnamomi.* Their rankings were consistent for different inoculation methods and test environments (glasshouse or field). No family was immune to *P. cinnamomi,* as some mortalities occurred in resistant families, and the growth of survivors was depressed. The resistance of families was independent of seedling size at inoculation. The narrow-sense heritability of resistance was remarkably high and consistent, at both family ($h^2 = 0.74 - 0.85$) and individual-tree ($h^2 = 0.43 \pm 0.18$) level, even though the seedlings were derived from open-pollinated parent trees. For disease resistance traits in forest trees, individual-tree (narrow-sense) heritability is seldom higher than $h^2 = 0.3$ (10). We therefore conclude that the observed resistance of jarrah to *P. cinnamomi* is under strong genetic control. There is potential for selection of jarrah families with useful levels of *P. cinnamomi* resistance; and within these families, individuals with outstanding levels of resistance can be selected. The high heritabilities indicate that this will lead to substantial gains in *P. cinnamomi* resistance in the resulting population. Resistant lines can then be propagated for inclusion in forest rehabilitation plantings.

The two seedling inoculation methods used in our pot experiments gave consistent rankings of families in their response to *P. cinnamomi.* Stem inoculation is preferred for use as the standard screening test, since under optimum conditions (seedlings ~14-mo-old, inoculated in summer), it gives the best separation among families and among individuals. Individual-tree heritabilities can also be estimated using this method. The soil inoculation was a particularly severe test, where seedlings were exposed to live *P. cinnamomi* inoculum continuously for 5 mo, and a total of 80% of seedlings died across all families.

Our selection of parent trees in the forest for resistance to *P. cinnamomi* on the basis of a single assessment of crown health met with little success. Inoculation of seedlings of five apparently resistant parents showed that their resistance was only marginally higher than that of seedlings from six trees selected as apparently susceptible. Some trees with healthy crowns surviving in dieback sites may have been disease escapes or in the early stages of infection (prior to symptom development) at the time of assessment. The field mortality levels of families 8 and 1 were high in comparison with other apparently resistant families. Family 5, which gave a resistant response in the trials, had been classified apparently susceptible because the parent tree exhibited severe crown dieback symptoms (and in fact died soon after seed was collected). However, this was a large, veteran tree which could well have been senescent, irrespective of the activity of *P. cinnamomi.* Highly susceptible trees would likely have died out rapidly

in *P. cinnamomi*-infested sites, but could still be present in sites unaffected by the disease. It is therefore not surprising that the two most susceptible families (11 and 13) both lie in the unknown group, whose parent trees were growing in apparently uninfested sites.

Symptom development is a dynamic process, linked closely to climate and environment, which in jarrah may stretch over several years after the initial infection of the plant (25). A single visual assessment of crown symptoms is thus unlikely to be a reliable basis on which to classify potentially *P. cinnamomi*-resistant parent trees, especially if the site has been infested with *P. cinnamomi* for a relatively short time. Seedling progeny of a larger sample of surviving trees on older dieback sites should be screened to determine whether such trees are more likely to carry the resistance trait. It is not yet possible to apply a test for *P. cinnamomi* resistance to potential parent trees prior to collection of seed for use in forest rehabilitation programs.

The genetic and physiological bases of the resistance to *P. cinnamomi* in jarrah need to be examined further. The continuous range of resistance–susceptibility levels among families which was shown in all our experiments suggests that the character involves multiple genes rather than a single gene. Polygenic inheritance is synonymous with continuous variation (30): resistance varies from high to low values without breaks in distribution, and intermediate values are the most common. There are similarities between the expression of the *P. cinnamomi* resistance in jarrah reported here and that reported in *Pinus radiata,* which is believed to be controlled by multiple genes (5) and also has very high and consistent heritabilities (5,6).

Recent work by Cahill et al (7) on clonal, micropropagated jarrah developed from seedlings selected in our stem inoculation experiments has demonstrated that the roots of a resistant line (but not of susceptible seedlings) were able to restrict and confine colonization by *P. cinnamomi.* Increased activity of phenylalanine ammonia lyase and increases in lignin and phenolic synthesis were reported in roots of resistant (but not of susceptible) lines following inoculation (8). Similar changes were observed following the inoculation of roots of a field resistant species, *Eucalyptus calophylla* R.Br., with *P. cinnamomi* (9).

The durability of the resistance in the field through the life of a jarrah tree obviously cannot yet be predicted with certainty. However, the fact that the resistance operates in the field during the very vulnerable seedling stage, and in plants up to 6 yr old, is most encouraging. Furthermore, the resistance is effective against not only the four *P. cinnamomi* isolates introduced at inoculation, but also the *Phytophthora* population already present in the test site.

A variety of sites in the jarrah forest are in need of rehabilitation planting. These include areas severely degraded by dieback disease, mine sites, river catchments, cleared land, amenity areas, and road verges. In the 1970s and early 1980s, *Eucalyptus* spp. from eastern Australia and other exotics such as *Pinus* spp. were used in the replanting of dieback sites (2). Jarrah was excluded from early forest rehabilitation plantings, and also from most early mine site rehabilitation plantings, due to its presumed general susceptibility to *P. cinnamomi* (24). The emphasis has recently shifted back to the use of indigenous tree species in these sites (1,12), and jarrah is now a major component of the species mix planted in bauxite mine pits (1). It is important that jarrah plantings in these sites utilize the highest quality stock in terms of *P. cinnamomi* resistance and other desirable growth characters. For this to be possible, reliable and plentiful supplies of dieback-resistant planting stock must be available. Information on the suitability of individual jarrah lines for planting on particular site types is also required. Currently, we are screening a large number of open-pollinated jarrah families for *P. cinnamomi* resistance by stem inoculation. These families have been planted recently in extensive provenance trials on several sites, from which information on their growth and form will be available. Resistant seedlings are being propagated by tissue culture (18), and their clones are to be established in seed orchards which will supply seed for use in future rehabilitation plantings.

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