BREEDING OF *PINUS RADIATA* FOR RESISTANCE TO *PHYTOPHTHORA CINNAMOMI*

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ABSTRACT

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Glasshouse and field tests were used to screen one-year-old seedlings of elite Pinus radiata families for tolerance to Phytophthora cinnamomi. There is wide variation between families in disease tolerance. Very high and consistent family heritabilities were calculated, which suggests strong genetic control. It is feasible to develop a seed source of Pinus radiata that is resistant to the Phytophthora root rot disease.

Progeny testing of the Australasian breeding population of radiata pine has been intensified to search out additional resistant trees for breeding purposes. Stability of resistance to a range of Phytophthora inocula, and the persistence of resistance over a rotation of about 30 years are being investigated.

INTRODUCTION

The Forests Department of Western Australia has proposed the development of a 60000 ha pine re-afforestation programme in the Donnybrook Sunkland (Anon., 1975). The area, located at latitude 34°S and longitude 115°30'E, has a Mediterranean climate and averages 1150 mm of rainfall a year. Soils were described by McCutcheon (1978); generally they are pocrly drained lateritic sands characterized by an extremely poor natural fertility.

It is planned to convert a native hardwood forest of low productivity, predominantly Eucalyptus marginata (jarrah), into productive pine plantations. The jarrah dieback disease caused by Phytophthora cinnamomi (Podger, 1972) is prevalent in the area. In 1973, 16% of the total Sunkland area was recorded as diseased and because of the extreme susceptibility of the Sunkland forest it was seen as inevitable that some 60% of the forest would be affected by the extension of existing infections (Anon., 1975).

The preferred species for this programme is the highly productive Pinus radiata. However there have been reports in the literature citing P. radiata as susceptible to Phytophthora cinnamomi (eg. Newhook, 1959; Batini and Podger, 1968; Jehne, 1971). The main alternative species, Pinus pinaster, is tolerant to Phytophthora cinnamomi (Anon., 1971; Batini, 1978) although the pathogen has been associated with death of mature trees on an irrigated, riverine alluvium site (Batini and Podger, 1968).

Extensive planting of Pinus radiata in the Sunkland commenced in 1974. Chevis and Stukely (1982) were able to

consistently associate tree death with root infection by Phytophthora spp. and found a higher frequency of pine deaths in areas previously affected by jarrah dieback disease. However dead trees are apparently distributed randomly in these areas; this could be due to an irregular distribution of the pathogen in the surface soil, or there may be genetic variation in tolerance to the pathogen.

Improvement of genetic resistance as a means of controlling forest disease has received considerable attention (Bingham et al, 1960; Heimburger, 1962; Ivory and Patterson, 1970; Bazzigher, 1981), and Zobel et al. (1971) reported how such data can be used to help solve major forest management problems. The procedures are applicable in the Sunkland: the site is extensively manipulated and there is intensive silviculture of an introduced tree species in the presence of a fungal pathogen.

To our knowledge no natural association of Phytophthora cinnamomi with Pinus radiata at its original source has been reported. Therefore resistance cannot have naturally evolved. In an analagous situation, Bingham et al. (1971) reported that in the absence of selection, resistant genes to the introduced blister rust were dispersed through the North American white pine population. If there are resistant genes to Phytophthora cinnamomi dispersed through the Pinus radiata population, these genotypes can then be located and identified.

This paper reports screening tests of Pinus radiata orchard families which were conducted to locate genotypes resistant to the Phytophthora cinnamomi root rot disease. Our proposals to develop a disease resistant seed source and the implications for forest management are discussed.

METHODS

When starting an improvement programme for a relatively unknown genetic parameter such as disease resistance, two primary objectives for progeny testing have to be set. The first is to test whether all parents express the character equally, and the second is to estimate the heritability of the character.

Parent trees used in this study are part of the Western Australian radiata pine breeding population. This population consists of trees that have been selected from Australian and New Zealand plantations for their superior form and growth rate. Since there has been no report of Phytophthora cinnamomi in the areas where these trees were selected, it can be assumed that tolerance to this pathogen was not considered.

Cones were collected from a minimum of five widely separated ramets for each half-sib family from the Manjimup seed orchard in 1978. Open-pollinated seeds in the cone represent the maternal parent as crossed with an average cross section of the pollen produced in the orchard. This orchard was established over the period 1969 to 1972 using 91 grafted clones and is the major source of Pinus radiata seed for Western Australia.

Screening for resistance to a disease is best carried out at the affected site. Seedlings are tested for resistance under natural conditions and much larger populations of the host and pathogen can be dealt with. However, there is the disadvantage of non-uniform conditions, particularly the distribution of inoculum on the site. In glasshouse tests

each seedling can be exposed to a more uniform level of inoculum under controlled conditions, and the environment can be manipulated to favour infection.

The glasshouse test was chosen as the main screening method; here strategy was to provide an optimal environment for the growth of pine seedlings and a medium of sand and peat moss was preferred to the use of Sunkland soil. Complementary tests were also carried out in the field to assess the relevance of the glasshouse tests.

Glasshouse test No 1

In this first exploratory test, 18 families of Pinus radiata were screened for resistance to Phytophthora cinnamomi. Waterlogging had been described by Sutherland et al. (1959), Poutsma and Simpfendorfer (1963) and Batini and Podger (1968) as a site condition predisposing Pinus radiata to infection; this was included as a major treatment.

The experimental design was a four order factorial, with a split-split-plot layout in the glasshouse to prevent contamination of the controls and to facilitate the maintenance of watering treatments. The principal factors were Phytophthora cinnamomi inoculation (2 levels: inoculated or uninoculated), watering (2: saturated or periodic watering), Pinus radiata pedigree (18 families) and replication (4).

Equal parts of sand and peat moss were mixed, fumigated with methyl bromide and filled into 8 litre capacity plant pots. Pine seed was stratified by soaking in water for one day followed by 20 days cold treatment at approximately $3^{o}C$. Twenty seeds of a single family were sown into each pot in November 1978. The pots were randomized in a shadehouse,

mulched with pine litter containing the mycorrhizal fungi Rhizopogon luteolus and Thelephora terrestris, and the seedlings were grown with regular watering and periodic fertilizer application. Pots were thinned to seven seedlings in May 1979 and moved into a glasshouse where they were placed in individual water-holding containers and positioned according to the experimental design.

The water treatment was commenced in June 1979 (seedling age 7 months). One level maintained a saturated water layer to a height of about 8 cm with the remainder of the pot volume in the capillary fringe. For the other level, pots were watered to approximate field capacity and allowed to dry before more water was added. Pots were inoculated on the 16th October 1979, at a seedling age of 11 months; 56 seedlings of each family were subjected to Phytophthora cinnamomi inoculum. This test was terminated in March 1980, 160 days later.

Seedling death was usually scored weekly. Heights were measured at the commencement and completion of the study. During the experiment, dead seedlings and inoculum plugs were sampled and plated for the recovery of Phytophthora cinnamomi, and root systems of healthy seedlings were also examined. At the end of the study, all inoculum plugs were recovered from pots not containing dead seedlings, and plated to check the viability of the pathogen.

Glasshouse test No 2

Based on the result of glasshouse test No 1, the saturated watering treatment was eliminated. All pots were watered to approximate field capacity and allowed to dry before more water

was added. Thirty new families were screened in this test.

The experimental design was a factorial with split plots. Principal factors were Phytophthora cinnamomi inoculation (2 levels: inoculated or uninoculated), Pinus radiata pedigree (30 families) and replication (4). An extra four pots for each family were inoculated with Phytophthora cinnamomi and all pots were thinned to eight seedlings. Thus family tolerance assessment was based on 64 inoculated seedlings. Pots were inoculated on the 28th October 1980.

Uninoculated seedlings were top-harvested from each pot at the end of the main study in April 1981 (170 days) and foliar levels of N, P, K, Ca, Zn, Mn and Cu were analysed.

Inoculated pots were maintained for a further 160 days and the test terminated in August 1981 (330 days). During this time, seedlings were subjected to drought stress. Watering was infrequent and was only applied when plant wilting point was approached.

Field test

Stratified seed of each of 26 Pinus radiata families and 5 orchard seed sources was sown in open nursery beds at Wanneroo in September 1979. These orchard sources included Pinus radiata from our Manjimup orchard, Tallaganda (major seed source for New South Wales) and Korweinguboora (major seed source for Victoria), and the other major alternative species P. pinaster and P. taeda. Seedlings were lifted for transplanting to the field site in May 1980.

An area showing severe jarrah dieback symptoms in the native forest was chosen in the Sunkland for the field trial

site. It was windrow cleared, burnt, and mound ploughed prior to pegging of the field design. Plots were located in the windrow bays.

There was extra deliberation as to plot size and spacing. The area was considered to have a high natural infestation of Phytophthora cinnamomi but there was no knowledge on the distribution of inoculum on the site. Other standard progeny trials on jarrah dieback sites in the Sunkland (Australian trial numbers 6008, 6009, 6010, 6011) used a line plot of up to 10 seedlings per family, and a scattering of deaths in the more susceptible families there suggested a non-uniform distribution of inoculum. It was therefore necessary to use single tree plots, replicated many times to minimise the experimental error. Normal plantation spacing of 3.5 x 2.5m was used to enable study of tree age and susceptibility.

A factorial design was used with the principal factors as Phytophthora cinnamomi infection (2 levels: natural inoculum, or natural plus artificial inoculum) and a screening of 26 Pinus radiata families. Treatment number was increased to 35 by inclusion of seedlings from P. radiata, P. pinaster and P. taeda orchard sources. A split-plot field layout was used and there were 21 replications of treatment. For the analysis, a non-contiguous plot of 7 seedlings per family was used.

Seedlings were completely randomized in the principal factor blocks and hand planted by the ~~main~~ senior author in May 1980. After planting, each seedling received 100 g of an N-P fertilizer and was later treated with a foliar spray containing Zn, Cu and Mn. Phytophthora cinnamomi inoculum was applied on 26th September 1980 (17 weeks after transplanting). The trial

site was regularly inspected and all dead seedlings were removed and checked for Phytophthora infection.

Phytophthora cinnamomi inoculum

Pine branch-plug inoculum was prepared by the method of S.R. Shea, T.J. Boughton and B.L. Shearer (unpublished). Phytophthora cinnamomi, originally isolated from the roots of a dead Pinus radiata in the Sunkland, was incubated for 7 days in 10 ml lots of 10% V8 broth. Live P. radiata branches were cut into plugs 1 to 2 cm in diameter and approximately 2 cm long, after the bark was removed. The plugs were soaked overnight in distilled water, rinsed and placed in conical flasks (100 plugs per litre of flask capacity). Sufficient distilled water was added to cover the bottom of the flasks, which were plugged with non-absorbent cotton-wool and autoclaved for 30 minutes at 15 p.s.i., then cooled to room temperature. Phytophthora cinnamomi mycelial mats were aseptically lifted from the V8 broths and dropped onto the plugs (one mat per 100 plugs), which were shaken gently and then incubated at 25°C. After 2-3 days when the fungus had started to colonise the plugs, they were again shaken to disperse the inoculum. They were then incubated for a further 2-3 weeks.

In the glasshouse tests each treatment pot was inoculated with four infected plugs, equally spaced and buried 1 cm beneath the soil surface. The soil was saturated with water and maintained in this condition for one day. It was then allowed to drain freely and the appropriate watering regime was resumed. Control pots were treated identically except that the infected plugs were first autoclaved.

In the field trial, the artificial inoculum was applied by burying four infected plugs to a depth of 4 cm, equally spaced at a distance of 5 cm from each seedling.

In each study, inoculum was applied in spring when soil temperature and moisture are most favourable for the growth and proliferation of the pathogen (Shea, 1975; McKinnell, 1981). Pine seedlings were approximately one year old, healthy and growing vigorously at the time of inoculation.

Re-isolation of *Phytophthora*

At intervals during the experiments, dead seedlings were removed with sterile implements. Adhering soil was washed from roots and the root system surface-sterilised by immersion in 70% ethyl alcohol for 30 seconds followed by four rinses in distilled water. The lower stem and collar were cut into 8 mm segments and serially plated onto an agar medium selective for *Phytophthora* (Tsao and Guy, 1977). Randomly selected 1 cm root segments were also plated. After incubation for at least 48 hours at 25^{o}C the plates were examined and infection recorded. Inoculum plugs were also sampled and plated at various times to check for survival of the pathogen. These were split lengthwise and treated similarly to the plant material.

Heritability

Heritability was defined by Knight (1948) as the portion of the observed variance for which difference in heredity is responsible. It was viewed by Hanson (1963) as a valuable means of quantifying the concept of whether progress from selection for a plant character is relatively easy or difficult to make in a breeding programme.

Analyses of variance were performed on the arcsin square root transformation of the percentage of dead trees per plot for each of the open-pollinated progeny tests, and used for the calculation of expected mean squares (Falconer, 1960; Kung, 1972) as follows:

Source of variation	Degrees of freedom	Expected mean square
Between blocks (b)	b-1	$\sigma^2 + f\sigma^2_b$
Between families (f)	f-1	$\sigma^2 + b\sigma^2_f$
Within families	(b-1)(f-1)	σ^2

Assuming a random mating population, progenies are not inbred and a half sib relationship, family heritability (h^2) was estimated as:

$$h^2 = \frac{\sigma^2_f}{\sigma^2/b + \sigma^2_f}$$

RESULTS

Glasshouse test No 1

Before treatment, there was no difference in seedling height between the watering levels or the inoculation levels (Tables I and II). However there was a very significant difference between the heights of families ($p<0.001$). This was expected; some of these data are presented in Table III. It is interesting to note the performance of family 60017, a parent known for its superior vigour in Western Australian progeny trials. Other family rankings were in accord with their general field progeny performance.

(Tables I, II and III)

First deaths occurred 40 days after inoculation, in family 60017 (Fig. 1). After 50 days, mortality had increased to 16% in this family, while it was still negligible in other families. Seedling height was measured at this time (Table III), and the growth depression of inoculated compared with uninoculated seedlings of family 60017 was significant ($p<0.05$). At this stage there was no difference in the height of inoculated and uninoculated seedlings of any other family. There was a stepped progression of tree deaths with the most susceptible families 60017, 60001 and 12236 succumbing early, while the more tolerant families 60022 and 80007 survived over 100 days before the first loss (Fig. 1).

(Fig. 1, 2 and 3)

Seedling genotype had a significant ($p<0.001$) effect on growth and mortality at the end of the study (Tables II and III, Fig. 2). Susceptible family 60017 had the top ranking for height in the uninoculated series but bottom ranking after inoculation; this was because most of the inoculated seedlings died early. There was a significant difference in height growth between the inoculated and uninoculated seedlings of each family ($p<0.05$). This included the resistant family 80007 where all seedlings survived.

Mortality rate for each of the 18 families, 160 days after inoculation, is illustrated in Fig. 2. The range of mortality in these half-sib seedlots is quite remarkable, from a maximum of 91% for family 60017 to no death in family 80007 (Fig. 3). These families have been divided into four arbitrarily defined classes for the disease tolerance character (Table IV), in order to reference the population for orchard use, as well as to define those families requiring more intensive study.

Only the families categorized as tolerant will be considered as potential parents for a Phytophthora cinnamomi-tolerant source of Pinus radiata. Families listed as moderately tolerant require further evaluation.

(Tables IV and V)

Variance components for seedling mortality and for height at the completion of the study are shown in Table II. The dominating influence was inoculation with Phytophthora cinnamomi. This was highly significant ($p<0.001$) for both seedling mortality and height growth. The effects of inoculation and watering treatment on seedling mortality are summarized in Table V. The difference was not absolute as two seedlings in the uninoculated series died. These seedlings were plated and Phytophthora cinnamomi was not recovered. Survival in the saturated - inoculated pots was higher than in inoculated pots that were allowed to dry ($p<0.10$). The watering treatment had no effect on height growth of seedlings (Table 1).

Dead seedlings were sampled from 20 pots, 60 days after the inoculation treatment, and plated. Extensive Phytophthora cinnamomi infections were detected in the roots and collar of each plant, and from all but two of the seedling stems at a height of 2 cm above the soil level. The recovery rate of Phytophthora cinnamomi from 218 root segments plated was 70%. Sampling was repeated 20 days later. Again there was a positive Phytophthora cinnamomi recovery from the roots and collar of each of the 24 dead seedlings. Phytophthora cinnamomi was isolated from 75% of the 524 root segments plated. Similar high levels of infection were observed in the root systems of all families sampled.

Inoculum plugs from both the inoculated and uninoculated series were checked for survival of the Phytophthora cinnamomi, 130 days after application. This sample included plugs from inoculated pots containing all healthy seedlings and from pots with dead seedlings. Phytophthora cinnamomi was recovered from each of the plugs and surrounding soil of the inoculated series. There was no recovery from the uninoculated pots.

Glasshouse test No. 2

As with the first test series, there were highly significant differences ($p<0.001$) between the heights of families at the time of treatment (Tables VI and VII). The reader should note the contrasting height growth of the resistant families 60027 and 30043. There was no difference in seedling height between the inoculation levels.

(Tables VI and VII)

The first deaths occurred after 35 days, in family 20011. There was then a rapid proliferation of deaths through all inoculated pots of this susceptible family (Fig. 4). There were no deaths in the more tolerant families until about 90 days after inoculation. Family mortality from observation of 64 seedlings, 170 days after inoculation, is shown for each of the 30 test families in Fig. 5. Family 60038 is also shown on this figure, but it was not included in any of the analyses because observations were limited to 25 seedlings only. There was a quite remarkable segregation of tolerance levels according to the female parent of these open-pollinated families. Families 60027, 30043 and 30026 showed a high degree of tolerance whereas 20011, 60039 and 60023 were highly susceptible to the pathogen. As in the glasshouse test No. 1 the families have been

grouped into four classes based on their relative tolerance to Phytophthora cinnamomi infection (Table VIII).

(Fig. 4, 5 and Table VIII)

The variance analysis for height growth and mortality is summarized in Table VI. The dominant effect was again treatment with Phytophthora cinnamomi. Average mortality for inoculated pots was 44% and there was no loss in any of the uninoculated pots. The mean height of uninoculated seedlings 170 days after treatment was 65 cm, whereas that of living and dead inoculated plants was significantly lower at 52 cm ($p<0.001$). The genetic effect was also highly significant ($p<0.001$). Height growth in the inoculated pots for each family has been reduced (Table VII): this difference was not significant in tolerant families 60027, 30043, 30026, 12038 but was highly significant in susceptible families 20011, 60023, 60039 and 30004 ($p<0.001$).

There was no correlation between disease susceptibility and plant vigour. Uninoculated seedlings of tolerant families 60027, 12038 were ranked very high for height growth, while tolerant families 30043, 30026 were ranked low (Table VII).

Dead seedlings sampled during the test all gave a positive recovery of Phytophthora cinnamomi from the roots and collar. Inoculum plugs and soil from each of the 48 inoculated pots containing no dead seedlings at the end of the 170 day test period were plated, and the pathogen was readily re-isolated from both plugs and soil. Examination of these healthy plants showed that the root systems were infected with Phytophthora cinnamomi. A subsequent drought stress period of 160 days applied to these apparently healthy plants did not reduce their tolerance to infection (Fig. 4).

Foliar analysis of the uninoculated seedlings showed significant differences between families in the concentrations of elements N, P, K, Ca, Mn, Zn and Cu. All of the levels were reasonably high and there were no deficiencies. It was not possible to relate levels of P, N, Ca or any of the other elements with the disease susceptibility groupings. Differences in plant ability to utilize available nutrients do not provide a simple explanation for the observed variation in family mortality.

Field test

The seedlings were first inspected in August 1980, about 10 weeks after transplanting, and all appeared healthy. When the inoculum was applied 7 weeks later, most of the seedlings were healthy and growing vigorously, although some of the Pinus pinaster and P. taeda seedlings were still small. No immediate post-planting losses were observed.

The first seedling deaths occurred in late November, about 60 days after inoculation (Table IX). One occurred in the inoculated family 60017, a family found to be highly susceptible in the first glasshouse test (Table IV). Phytophthora cinnamomi infection was extensive in both the roots and collar of the 24 cm seedling, and the fungus was also recovered from the inoculum plugs and the soil. The other death involved family 60031 on an untreated site. No Phytophthora cultures developed from the plated root and collar segments or surrounding soil. The seedling top had been damaged and bent at right angles and this probably contributed to its death.

(Table IX)

Thirty days later, another 22 seedlings had died. Interestingly, similar numbers died on the inoculated and the untreated sites. There was a high recovery of Phytophthora cinnamomi from the inoculum plugs indicating survival of the inoculum. Phytophthora cinnamomi was isolated from the roots and collars of 14 dead seedlings. From two adjacent dead seedlings on an untreated block, only Phytophthora citricola was isolated. The pedigree of one of these seedlings was 60004, listed in Table IV as moderately susceptible to Phytophthora cinnamomi, and the other was a Pinus radiata checklot from the Manjimup orchard. The significance of this will be discussed. One seedling each of Pinus pinaster and P. taeda died but despite intensive sampling of roots and collar segments, no Phytophthora infections were detected.

By the end of January another 51 seedlings had died. Susceptible families 60017, 60024, 60004, (Table IV) and 60013, 60039, 60036 (Table VIII) accounted for 30 of these deaths. In each case, Phytophthora cinnamomi was recovered from root and/or collar segments of the dead seedlings.

After one year in the field, 120 seedlings had died. Phytophthora cinnamomi was the principal pathogen, associated with the death of 108 seedlings, while Phytophthora citricola was linked with 2 seedling deaths. Equal numbers of dead seedlings were found in the inoculated and untreated field plots. Eleven of the 126 Manjimup orchard seedlings died and this 9% mortality was the same as the general test mean. Mortality of Tallaganda and Korweinguboora orchard stock was rated as 7% and 5% respectively.

(Fig. 6, Tables X and XI)

Tree mortality attributable to Phytophthora was calculated for each non-contiguous seven tree family plot for the analysis

of variance (Table X). Inoculation of the field sites with the test isolate of Phytophthora cinnamomi did not increase mortality. There was an interesting significant effect of block, or site (Table X and XI). Similar numbers of dead seedlings were observed in the two blocks on type 4c soil (McCutcheon, 1978) and these were significantly higher than on the type 7 soil ($p<0.05$). The whole area was severely affected by jarrah dieback disease and a uniform distribution of the pathogen was expected over the sites. The heavier textured type 7 soil generally has a better soil moisture status and perhaps these seedlings were less stressed.

There was a highly significant family effect on tree mortality on the field sites (Table X, $p<0.001$). Individual family values are presented as a histogram in Fig. 6. No deaths occurred in the tolerant families 80007, 60027, 12038, 60022 and 60038, and most deaths were found in the susceptible families 60017, 60024, 60039 and 60036. An additional 25 seedlings each of the susceptible family 60017 and the tolerant family 60022 had been planted as a surround on the soil type 7 site. Mortality was assessed at 44% in family 60017 and was similar to the 43% value obtained for this family in the test. One seedling of 60022 died, but because of its small size this was considered to be a planting death.

Correlation of glasshouse and field test results

Results of the glasshouse and field trials were consistent for the most susceptible and the most tolerant families (Figs. 2, 5 and 6). This has been quantified by applying the analysis of variance to the seedling mortality data for common families and treating the field and glasshouse tests together as a split-

plot design. Mortality in the glasshouse tests (41%) was much higher than in the field (9%) ($p<0.001$). The difference between families was still significant ($p<0.001$), and there was no significant interaction of family with the test method, i.e. serial order of families was similar for both the glasshouse and field tests.

A non-parametric statistical test was also used to test the association of the tests. The Spearman rank correlation coefficient (Siegal, 1956) was applied to the family ranking for mortality, and was calculated as $r = 0.81$. This is highly significant ($p<0.001$), which suggests that families have the same ordinal scale for mortality in both the glasshouse and field tests.

The correlation of the glasshouse and field family performances from mortality data is illustrated in Fig. 7, using mean rank scores (this method is widely used in Australia for combining data across unbalanced progeny tests; mean rank scores are calculated by ranking the family means and then dividing the ranks by the number of families in the trial). Tolerance groupings (Tables IV and VIII) are shown in this figure.

(Fig. 7)

Heritability

Estimates of family heritability for disease tolerance were consistently high: 0.90 and 0.88 for the glasshouse tests and 0.86 for the field test (Table XII). It was pointed out by Falconer (1960) that heritability estimates are the property not only of the character but also of the genetic sample and the environment to which it has been subjected. In our tests,

genetic variance accounted for 85-90% of the total variance in resistance. Progeny test selection for this character should lead to a substantial improvement in the disease resistance of the population.

(Table XII)

DISCUSSION

The screening tests have given some remarkable results. Genetic variation in Pinus radiata for resistance to Phytophthora cinnamomi is quite large and the selection of a new resistant population has considerable promise. The development of this new population has commenced with the classification of 9 tolerant families from the 49 open-pollinated families studied.

The screening strategy employed for this study has been successful in that the glasshouse tests have reflected the field performance, albeit at a much higher order: average mortality in the glasshouse tests was 41% compared with 9% in the field. Glasshouse environment was near optimum for the development of the pathogen, and a uniform inoculation treatment was applied to the pots. This contrasted with the field where there was a more variable environment and a less uniform distribution of the pathogen. This suggests that an epidemic situation could occur in the field given high inoculum levels and warm, moist soil conditions. Improvement of genetic resistance is one way such an epidemic can be avoided.

The use of an artificial soil medium instead of the natural soil has not affected results. Healthy seedling growth was maintained throughout the experiments and segregation of families on their susceptibility to Phytophthora cinnamomi has

been achieved. T.J. Boughton (personal communication, 1981) confirmed these family rankings in a pot trial using a Sunkland soil medium.

It was not necessary to use any pre-disposing factor such as soil texture and nutrition (Campbell and Copeland, 1954), flooding or drought (Blaker and MacDonald, 1981) to achieve infection. Pre-disposing seedlings by waterlogging in the first glasshouse test did not influence survival, and extending the second test to subject root-infected seedlings to drought did not increase mortality. However, the drought was not applied until 160 days after inoculation and most of the susceptible families had already died.

Seedling death was early and rapid in the most susceptible families whereas death in the more tolerant families was not observed until much later in the summer period. This could reflect a greater buildup of inoculum in pots containing the more susceptible families, but there was a similar level of root infection in healthy seedlings of both tolerant and susceptible families. Future screening tests will use a multi-family pot to apply competition and to expose all families to similar inoculum levels.

The inoculation method for the glasshouse screening tests was successful. At the completion of the tests Phytophthora cinnamomi was recovered from inoculum plugs from all pots having no dead seedlings. Every dead seedling sampled had Phytophthora cinnamomi infections in both the roots and collar. Healthy seedlings had Phytophthora cinnamomi infections in the root system. Analysis of height growth showed a significant difference between inoculated and uninoculated seedlings of the same family, even in the case of family 80007 where no

inoculated seedlings died.

In the field it was quite different. There was no evidence to suggest that our artificial inoculation of the field site was successful as numbers of Phytophthora-induced deaths were the same on the untreated site. Phytophthora cinnamomi inoculum survived the summer and was recoverable from the plugs in late autumn, but it was apparently unable to move the few centimetres through the soil to colonize seedling roots. Roth and Kuhlman (1963) were unsuccessful when they tried to inoculate a Douglas-fir site although the fungus was able to persist in a greenhouse where soil moisture and temperature were adjusted favourably for it.

Each screening test used only the same single isolate of Phytophthora cinnamomi. This is a shortcoming of the study and will be remedied in future screening tests. Variation within the pathogen population is to be expected in the same way as there is variation in the host (Russell, 1978). Podger (1972) tested 49 isolates of Phytophthora cinnamomi and found 48 to be pathogenic to Eucalytpus marginata seedlings. Our field test has shown that the results are not specific to a single isolate of Phytophthora cinnamomi but effectively demonstrate a range of tolerance to a broader spectrum of the pathogen.

Phytophthora cinnamomi was the most commonly isolated Phytophthora sp. from the field site. The only other species isolated, Phytophthora citricola, was found on the roots and collar of a dead orchard stock seedling and one dead seedling of family 60004. More recently, an unidentified Phytophthora sp. was isolated from a dead seedling of family 60017 in our

second field trial. Both 60017 and 60004 have been classified as susceptible to Phytophthora cinnamomi, and their susceptibility to other Phytophthora spp. is most encouraging. Chevis and Stukely (1982) have reported that Phytophthora cinnamomi is the most prevalent Phytophthora sp. in pine plantations in the Sunkland area. They isolated P. cryptogea a number of times and P. citricola and P. megasperma var. sojae only rarely. Diseased young Pinus radiata trees growing on water gaining sites have been associated with Phytophthora drechsleri (Heather and Pratt, 1975) and Phytophthora cryptogea (Bumbieris, 1976). These reports indicate that there are a number of Phytophthora spp., with differing temperature and moisture requirements, which are pathogenic to Pinus radiata and must therefore be considered when developing a disease resistant seed source. "Resistant" and "susceptible" families of Pinus radiata are now being tested with a range of Phytophthora cinnamomi inocula and other species of Phytophthora.

Another factor to be considered in developing a stable disease resistant population is the effect of tree age on resistance. The rotation in the Sunkland will cover a period of about 30 years and will involve a number of morphological and physiological changes. It will be necessary to demonstrate permanent resistance over this rotation period.

In the case of the Pinus echinata littleleaf disease caused by Phytophthora cinnamomi, Hepting and Cruikshank (1950) rarely found symptoms in trees younger than 20 years of age. Zak (1955) created an environment favouring infection by Phytophthora cinnamomi of one-year-old seedlings of apparently resistant and moderately diseased trees aged from 35 to 83 years.

He rated the root systems to measure relative resistance and found that this was heritable : the resistance of the seedlings reflected that of the parent trees.

For the Pinus radiata/Phytophthora cinnamomi association, Newhook (1959) reported that Monterey pine was highly susceptible as seedlings and in the juvenile stage to 4-5 years, and trees were then resistant until they became susceptible again at the age of 20-30 years. Batini and Podger (1968), Jehne (1971) and Chevis and Stukely (1982) confirmed this range of susceptibility in Pinus radiata.

Our screening tests are conducted when Pinus radiata is in its most susceptible growth phase. Under these conditions certain genotypes that are able to tolerate Phytophthora cinnamomi for most of the rotation may be eliminated, but this approach is favoured. To investigate the stability of the observed resistance, a study using girdled cuttings (Griffin et al., 1976) of tolerant families of physiological age 1 to 13 years and of mature ramets, has commenced. There is already some corroborative evidence from the Sunkland progeny trial number 6008, planted on a jarrah dieback site. Five-year-old trees of susceptible family 60001 died in the summer following an N-P fertilizer application, and Phytophthora cinnamomi was isolated from their roots and collars. No trees in tolerant families died.

The consistency of glasshouse and field data for particular parents indicates that resistance to Phytophthora cinnamomi in Pinus radiata is under strong genetic control and is transmissible. Our strategy is to locate additional resistant genotypes in the Australian and New Zealand breeding programmes using the glasshouse screening method, and later to

expand this work to include new Western Australian parents, selected for growth traits (Cotterill and Zed, 1980), and other Pinus radiata provenance sources (Cobb and Libby, 1968; Eldridge, 1978).

A seed production population based on parents with desirable bole, branching and vigour qualities is used for Pinus radiata afforestation in Western Australia. The additional character of disease tolerance can be added to create a seed source for the planting of jarrah dieback-susceptible sites. Stern (1959) suggests a minimum of 20 to 30 clones under random pollination in an orchard to avoid inbreeding effects. We are looking to establish a disease resistant orchard source of Pinus radiata using 50 clones.

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TABLE I

The effect of *Phytophthora cinnamomi* inoculation and watering treatment on the height of *Pinus radiata* seedlings.

Treatment	Height (cm) 22.8.79[1]	4.12.79[2]	26.3.80[3]
Inoculated + saturated	22.9	35.7	46.7
Inoculated + low water	23.0	36.2	45.9
Uninoculated + saturated	23.3	36.6	57.2
Uninoculated + low water	23.8	37.7	56.0
l.s.d. for p<0.05	1.9	2.6	3.4
Phytophthora cinnamomi factor			
Inoculated	22.9	35.9	46.3
Uninoculated	23.6	37.2	56.6
Watering factor			
Saturation	23.1	36.2	52.0
Low water	23.4	36.9	51.0
l.s.d. for p<0.05	1.4	1.8	2.4

[1] before inoculation; [2] 50 days after inoculation; [3] 160 days after inoculation.

TABLE II

Abbreviated analysis of variance table for height at the beginning and completion of glasshouse test No 1 and percent seedling mortality transformed to arcsin square root values.

Source of variation	df	Variance and significance		
		Height (cm)		Mortality
		22.8.79	26.3.80	26.3.80
Main plots				
Inoculation (I)	1	30	7603***	52758***
Block (B)	3	9	107	406
error	3	10	93	383
Sub plots				
Water (W)	1	4	70	882
I x W	1	6	4	601
error	6	22	68	366
Sub-sub plots				
Family (F)	17	25***	60**	1524***
I x F	17	5	78**	1428***
W x F	17	6	13	179
I x W x F	17	5	41*	191
error	204	6	23	148
Total	287			

* significant at p<0.05, ** p<0.01, *** p<0.001

TABLE III

The effect of inoculation with Phytophthora cinnamomi on the height of six Pinus radiata families in glasshouse test No 1.

22.8.79 - before inoculation			4.12.79 - 50 days after inoculation			26.3.80 - 160 days after inoculation		
Rank	Family	Height (cm)	Rank	Family	Height (cm)	Rank	Family	Height (cm)
1	60017	26.3	2	60017	40.1	1	60017	60.5
2	60015	25.2	10	60015	37.8	7	60015	57.8
3	†60017	25.1	11	60001	37.4	8	60022	57.0
4	†60015	25.0	12	60022	37.2	9	60001	56.8
4	60022	25.0	13	†60015	37.1	11	80007	56.2
8	†60022	24.4	14	60024	37.0	15	60024	54.1
14	60024	23.7	15	†60022	36.7	19	†80007	50.6
19	80007	23.0	16	†60001	36.6	20	†60022	49.9
26	60001	22.5	17	†60017	36.4	21	†60015	49.6
28	†60001	22.3	24	80007	35.7	33	†60001	43.5
29	†80007	22.1	25	†80007	35.5	35	†60024	42.5
33	†60024	21.4	33	†60024	34.0	36	†60017	38.7
lsd ($p<0.05$) = 2.5			lsd ($p<0.05$) = 3.4			lsd ($p<0.05$) = 4.7		

† inoculated with Phytophthora cinnamomi.

TABLE IV

Classification of *Pinus radiata* families on the character of tolerance to *Phytophthora cinnamomi* in glasshouse test No 1.

Classification	*Pinus radiata* family
Tolerant	80007, 60022, 60002, 30055.
Moderately tolerant	60015, 60003, 60007, 60008, 60009, 60010.
Moderately susceptible	60006, 60005, 60021, 60004.
Susceptible	12236, 60024, 60001, 60017.

TABLE V

The effect of inoculation with *Phytophthora cinnamomi* and watering treatment on seedling mortality of *Pinus radiata*.

Treatment	Seedling mortality[1]
Inoculated + saturated	24.2
Inoculated + low water	30.6
Uninoculated + saturated	0
Uninoculated + low water	0.6

l.s.d. for $p<0.05$ is 7.8 and for $p<0.001$ is 19.0.

[1] Mortality 160 days after inoculation, transformed into arcsin square root percentage values.

TABLE VI

Abbreviated analysis of variance table for seedling height before treatment, and height and mortality, transformed into arcsin square root percentage values, 170 days after *Phytophthora cinnamomi* was applied in glasshouse test No 2.

Source of variation	df	Variance and significance		
		Height (cm)		Mortality
		10.9.80	16.4.81	16.4.81
Main plots				
Inoculation (I)	1	13	10185***	96000***
Block (B)	3	9	12	104
error	3	6	102	104
Sub plots				
Family (F)	29	52***	205***	743***
I x F	29	11	178***	743***
error	87	7	55	190
Total	174			

*** significant at $p<0.001$

TABLE VII

The effect of inoculation with Phytophthora cinnamomi on the height of eight Pinus radiata families in glasshouse test No 2.

10.9.80 before inoculation			16.4.81 170 days after inoculation		
Rank	Family	Height (cm)	Rank	Family	Height (cm)
3	†60027	28.2	3	60027	72.8
6	30004	26.7	5	12038	71.4
6	†60039	26.7	6	†60027	69.9
10	12038	26.0	7	30004	69.1
12	60027	25.8	9	20011	68.3
12	60039	25.8	11	60039	66.8
23	†12038	24.8	18	60023	64.6
24	†20011	24.7	19	†12038	64.4
28	†30004	24.1	26	30026	61.7
36	20011	23.4	40	30043	55.8
38	60023	23.1	43	†30043	54.4
46	†30043	21.6	46	†30026	52.8
55	†60023	19.5	53	†60039	45.5
57	30043	19.0	55	†30004	43.5
58	30026	18.9	58	†60023	41.0
59	†30026	17.8	60	†20011	33.2
lsd ($p<0.05$) = 3.7			lsd ($p<0.05$) = 10.3		

† inoculated with Phytophthora cinnamomi.

TABLE VIII

Classification of Pinus radiata families on the character of tolerance to Phytophthora cinnamomi in glasshouse test No 2.

Classification	Pinus radiata family
Tolerant	60027, 30043, 30026, 12038, 60038.
Moderately tolerant	60028, 50268, 60030, 60020, 60032, 60029, 20070, 50015, 60031, 20055.
Moderately susceptible	60026, 60033, 20088, 60025, 60034, 30010, 50012, 60036, 50009, 60035.
Susceptible	80055, 60013, 30004, 60023, 60039, 20011.

TABLE IX

Sampling of all dead seedlings in the field test for recovery of Phytophthora cinnamomi.

Date of Sample	Number of days inoculation	Number of dead seedlings	Recovery of Phytophthora cinnamomi from: collar	roots	soil	plug
25.11.80	60	†1	1	1	1	1
		1	0	0	0	
24.12.80	90	†9	6	6	7	8
		13	8(+2)††	9	9	
14.1.81	110	†18	17	17	12	4
		13	12	11	9	
27.1.81	123	†12	11	11	11	9
		8	7	7	8	
16.2.81	143	†12	8	12	12	5
		12	10	12	12	
23.4.81	208	†7	5	6	7	1
		10	9	10	10	
26.5.81	240	†1	1	1	1	1
		3	3	3	3	
Total		†60	49	54	51	29
		60	49(+2)	52	51	

† each seedling was inoculated with four Phytophthora cinnamomi inoculum plugs on 26.9.80; the second line of data for each date is for untreated seedlings.

†† Phytophthora citricola only was recovered from two dead seedlings.

TABLE X

Abbreviated analysis of variance table for percentage field mortality transformed into arcsin square root values, 240 days after the site was inoculated with *Phytophthora cinnamomi*.

Source of variation	df	Variance
Main plots		
Inoculation (I)	1	13
Block (B)	2	111*
error	2	4
Sub plots		
Family (F)	27	740***
I x F	27	89
error	108	105
Total	167	

* significant at $p<0.05$, *** $p<0.001$

TABLE XI

Effect of soil type on the field susceptibility of Pinus radiata to Phytophthora cinnamomi.

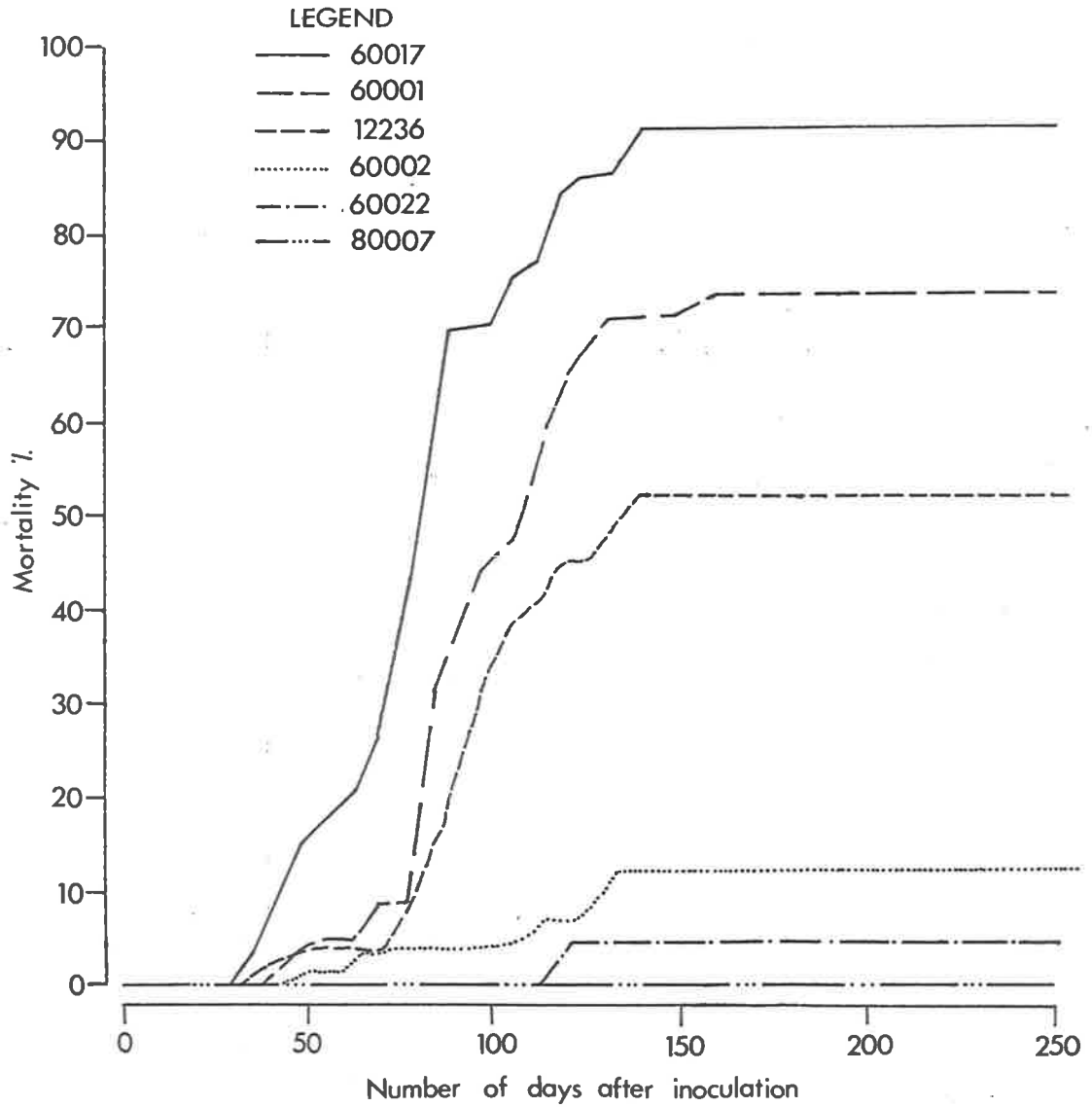
Block	Percent seedling mortality		
	Inoculum		
	Field	Field + artificial	mean
1 - soil type 4c	10	10	10^{a}
2 - soil type 4c	10	9	10^{a}
3 - soil type 7	7	7	7^{b}

Values with different superscripts are significantly different at $p<0.05$.

TABLE XII

Components of variance (arcsin square root transformation of percentage mortality) and estimates of the family heritability of resistance to Phytophthora cinnamomi.

Source of Variation	Glasshouse test No 1		Glasshouse test No 2		Field test	
	df	ms	df	ms	df	ms
Blocks	7	850	7	491	5	48
Families	17	2944	29	2743	27	740
Within families	119	301	203	333	135	102
	$h^2 = 0.90$		$h^2 = 0.88$		$h^2 = 0.86$	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60001
12236
60002
60022
80007
Mortality %
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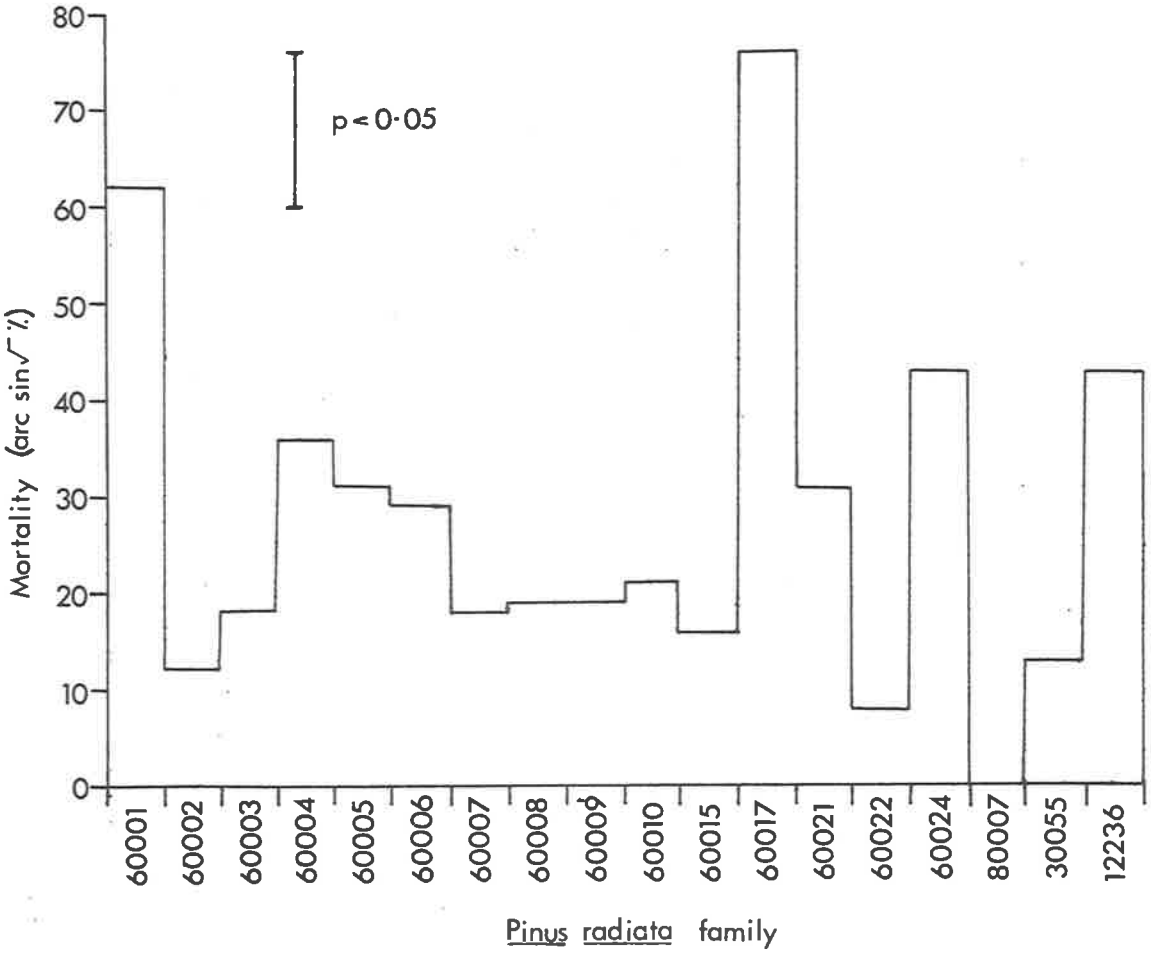
90
80
70
60
50
40
30
20
10
0
0
50
100
150
200
250
Number of days after inoculation


80
70
60
50
40
30
20
10
0
Mortality (arc sin √%)
p < 0·05
60001
60002
60003
60004
60005
60006
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60009
60010
60015
60017
60021
60022
60024
80007
30055
12236
Pinus radiata family

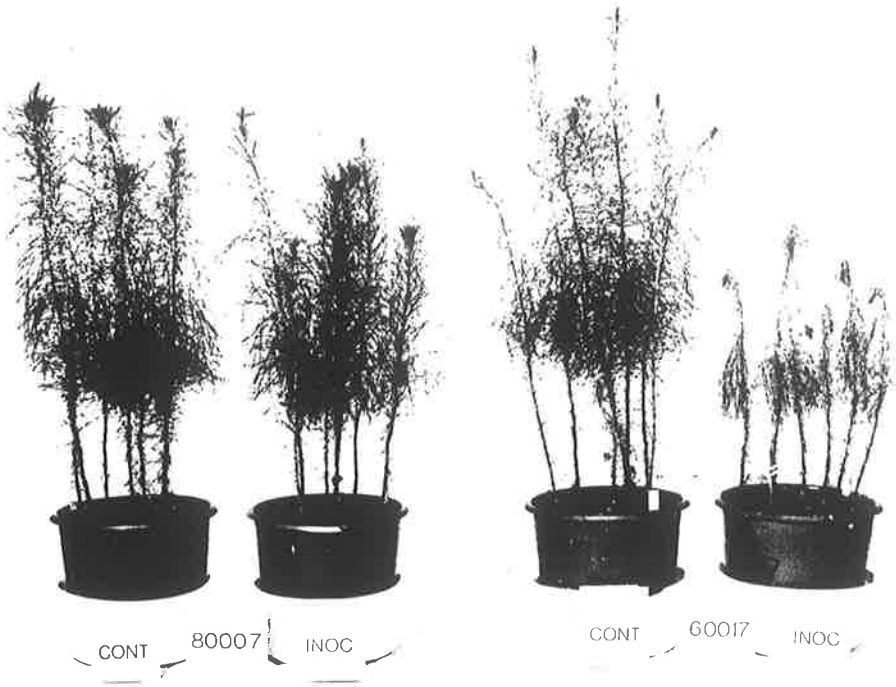
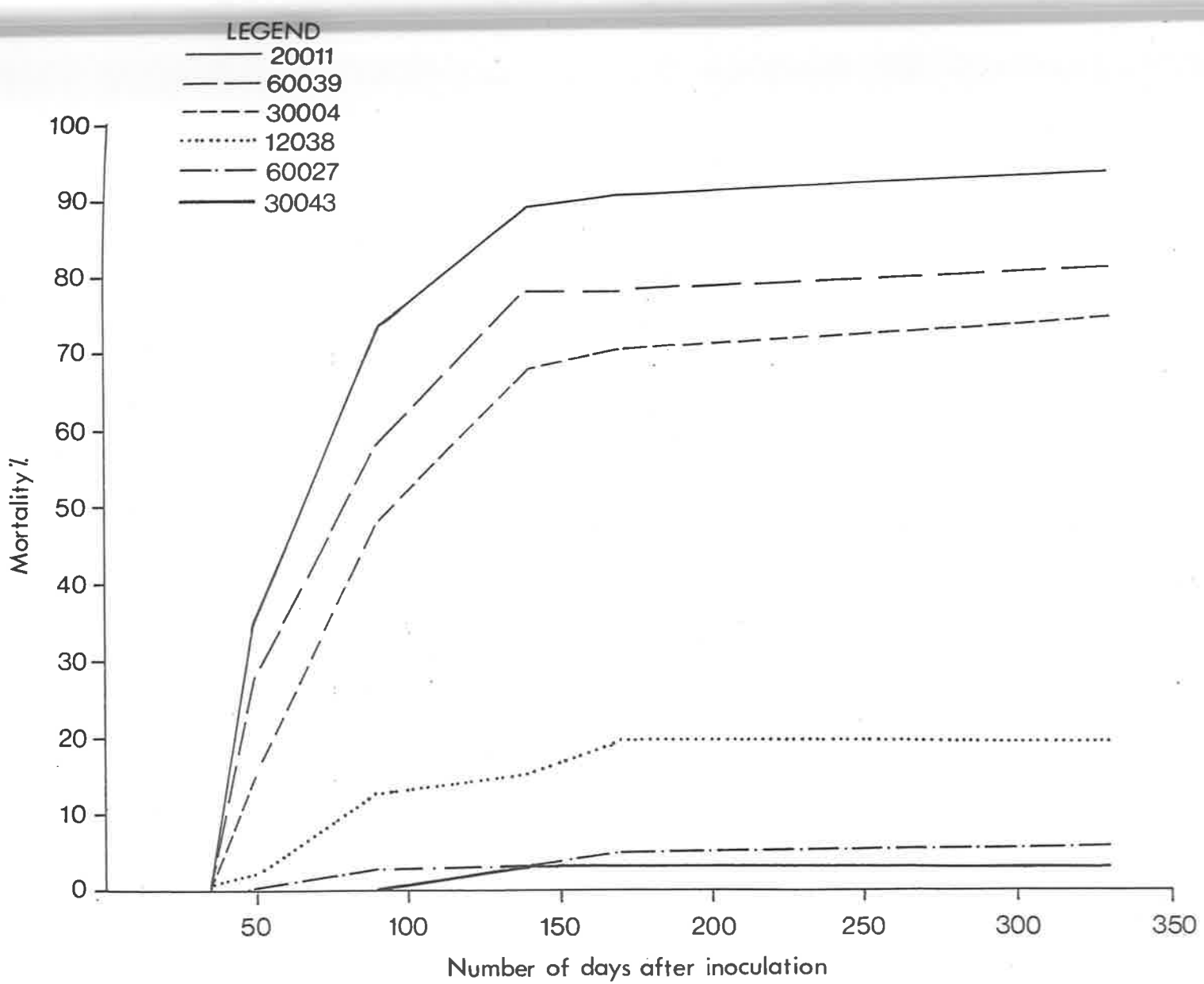
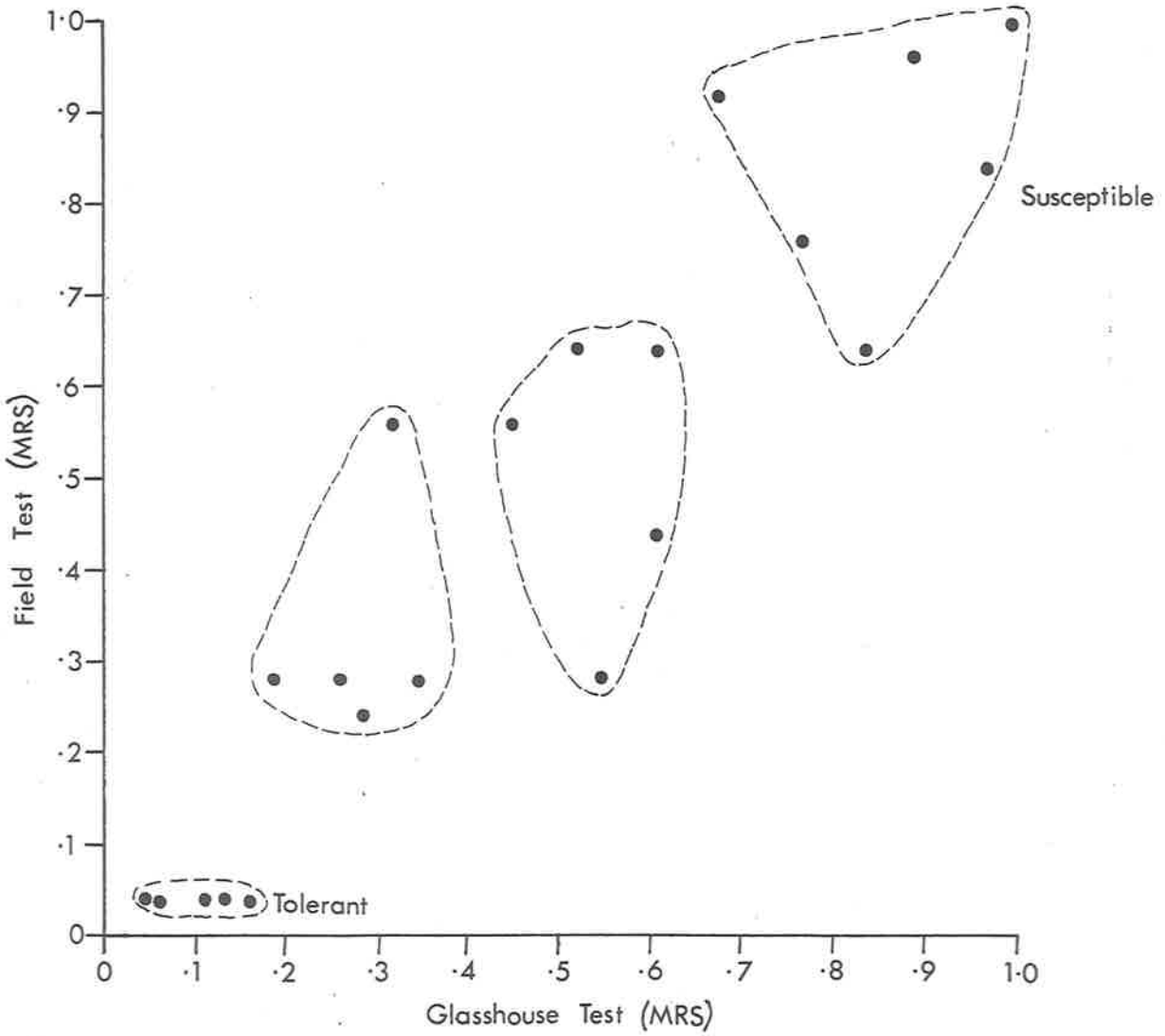


Figure 3.


LEGEND
20011
60039
30004
12038
60027
30043
100
90
80
70
60
50
40
30
20
10
0
Mortality %
50
100
150
200
250
300
350
Number of days after inoculation

Pinus radiata family

Pinus radiata family


1·0
·9
·8
·7
·6
·5
·4
·3
·2
·1
0
Field Test (MRS)
Susceptible
Tolerant
0
·1
·2
·3
·4
·5
·6
·7
·8
·9
1·0
Glasshouse Test (MRS)