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Progress in selection and production of jarrah (*Eucalyptus marginata*) resistant to *Phytophthora cinnamomi* for use in rehabilitation plantings

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Abstract. Resistance to *Phytophthora cinnamomi* in jarrah (*Eucalyptus marginata*) is under strong genetic control. It has high heritability, is probably polygenic, and is durable in field trials. Seedlings from healthy mother trees, either growing on long-term dieback sites or from a provenance collection made across the range of the species, were grown in the glasshouse and resistant seedlings selected from inoculation trials. These were micropropagated by tissue culture. The resulting clonal lines were planted in field validation trials on dieback-affected sites and soil at the base of the plants was also inoculated with *P.cinnamomi* to test survival and growth of resistant plants. In spite of some drought deaths, survival of most resistant lines has been high. Some 45 unrelated resistant lines have been selected. However, due to high costs of production and establishment problems in forest sites, it is not feasible to use the clonal jarrah directly in large-scale operational plantings. Clonal seed orchards are now being planted to supply seed of resistant jarrah for use in the rehabilitation of dieback-affected forest and plantings on cleared land.

Introduction

Phytophthora dieback (jarrah dieback), caused by *Phytophthora cinnamomi* Rands (Podger 1972), is the most serious and widespread disease of the Western Australian jarrah (*Eucalyptus marginata* Donn ex Sm.). In this paper we review the work that showed that resistance to *P. cinnamomi* in jarrah is under strong genetic control (Stukely and Crane 1994). We then describe the screening program undertaken to select a large number of genetically unrelated, but resistant, lines of jarrah, the field validation trials, and the establishment and use of seed orchards. The overall strategy followed in the program is outlined in Fig. 1. This project began in 1984 in parallel with a selection program for *Pinus radiata* D.Don with resistance to *P.cinnamomi* (Butcher *et al.* 1984).

Demonstration of resistance

Seed was collected from jarrah trees on a variety of sites, some of which were infested with *P.cinnamomi.* Seedling "families" (half sibs) derived from each of sixteen mother trees were grown and subjected to inoculation with *P.cinnamomi* in glasshouse and field trials (Stukely and Crane 1994).

Glasshouse trials

In the glasshouse, plants were either stem-inoculated or the soil was inoculated with plugs of infected wood.

Soil inoculation trials involved the introduction of four isolates of *P.cinnamomi* into the potting mix by way of infected plugs cut from branches of *Pinus radiata* (Butcher *et al.* 1984). Isolates were all of the A2 mating type and had been shown to be pathogenic to jarrah seedlings in a pilot trial: 251N12 (isolated from

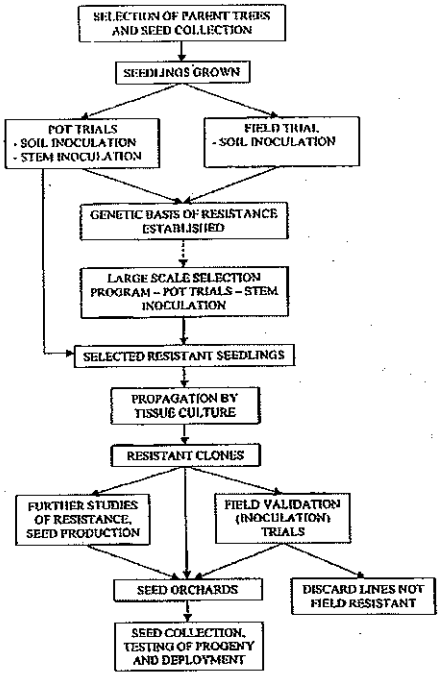

Fig. 1. Strategy followed in the selection, testing and deployment of dieback resistant jarrah.

from *Pinus radiata*), Sc 381 (*Allocasuarina fraseriana* (Miq.) L. Johnson), DCE 210 (*E.marginata*) and 480R1 (*Banksia* sp.). These trials were conducted over a full summer-autumn period, with low seedling mortality taken to indicate family resistance.

Stem inoculation trials involved the insertion of *P.cinnamomi* inoculum (isolate Sc 72, IMI 264384) into a surface incision on each seedling stem. The resulting lesions were measured over 14 days, with short lesions indicating seedling resistance.

Conditions in the glasshouse trials were highly favorable to the pathogen, and also to seedling growth. The trials were carried out in summer; soil was kept moist, and cooling on hotter days regulated the ambient temperature to a maximum of about 32C.

Field trial

A field trial was conducted on a cleared forest site already infested with *P.cinnamomi*, and four additional isolates of the fungus (as listed above) were introduced into the soil around each seedling by way of infected pine branch plugs. Low mortality was again taken to indicate family resistance.

There was a large degree of variation in response between the jarrah families in all trials, ranging from highly resistant to highly susceptible. Resistant plants were not immune to infection, but were able to restrict the growth of the pathogen sufficiently to survive and grow well. The performance of families, particularly those at the resistant and susceptible ends of the range, was consistent under the different treatment regimes and between the glasshouse and field trials.

The resistance of jarrah to *P.cinnamomi* has high heritability (0.43 at individual seedling level; 0.74 – 0.85 at family level). The wide range of responses shown among families indicates that the resistance is probably polygenic. Furthermore, it is effective against a combination of *P.cinnamomi* isolates, and is durable in field trials (Stukely and Crane 1994).

Selection program

The stem inoculation of seedlings described above was adopted as the standard screening method to compare the resistance of seedling families derived from individual mother trees in the forest. A standard susceptible family (717) was included in the trials for comparison. Trials were carried out during the summer-autumn.

Seedlings were at least 250mm in height at inoculation. The method became less reliable on plants older than about one year, as reddish-brown surface pigmentation and bark then developed. At this stage the pathogen often preferentially invaded internal tissues so its progress was not visible as a continuous surface lesion (Stukely and Crane 1994).

Seed-lots from a jarrah provenance collection (R. Mazanec, Department of Conservation and Land Management, pers. com.), which covered forest areas affected by and also free of dieback, in both the Northern and Southern jarrah forest regions, were first tested. Between 17% and 56% of these families exhibited some resistance (Fig. 2). More recently, healthy mother trees growing on high-impact dieback sites, mostly located in the Northern jarrah forest and where the disease was known to have been present for over c.25 years, have been sought. Tests of seedling families derived from these trees have shown that they are indeed more likely to have higher levels of resistance (68-84% of families were resistant) (Fig. 2).

An additional level of stringency was applied to the test by maintaining the selected seedlings for at least two months after the fourteen-day measurement period, and discarding any that showed further lesion extension or girdling of the stem. The families with the highest resistance response (ie having the shortest mean lesions) were identified, and the outstanding seedlings from these families selected for clonal propagation.

Propagation of selected genotypes

The infected portion of the stem of each selected seedling was removed when measurements were completed, so that the seedlings could then be propagated and maintained free of the pathogen.

Micropropagation techniques for jarrah were developed by Bennett (McComb *et al.* 1996). These methods were applied to the seedlings showing the highest levels of resistance, and for comparative purposes, to some seedlings that were susceptible. Some 60 lines of jarrah (45 of which are unrelated) with resistance to *P.cinnamomi* have been selected and successfully propagated.

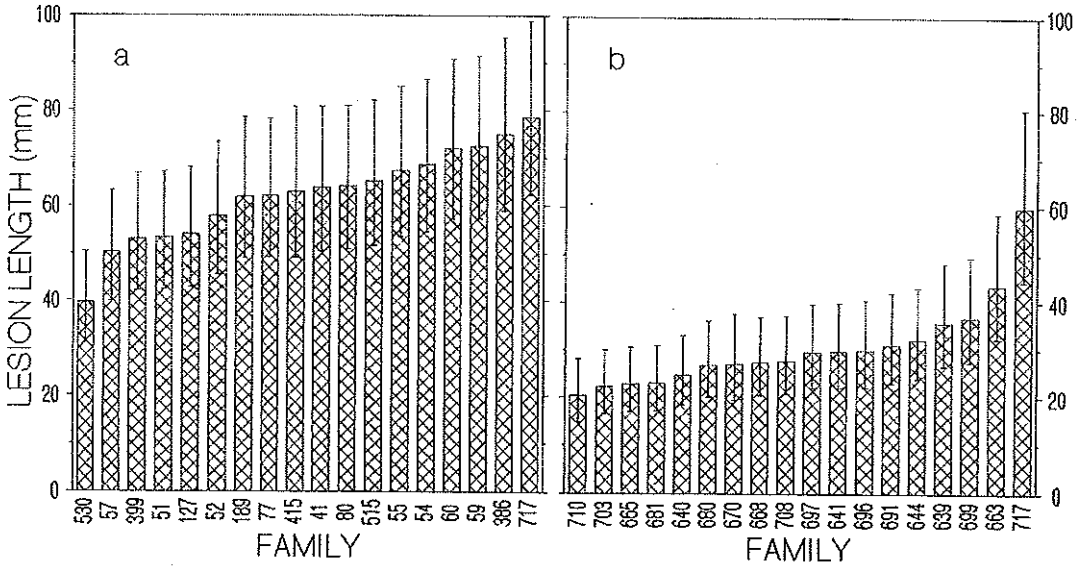

Fig. 2. Mean stem lesion lengths, 14 days after inoculation with *P.cinnamomi*, on seedlings of jarrah families grown under glasshouse conditions. Parent trees were (a) from a provenance collection; (b) survivors on old dieback sites. The standard susceptible family is 717. Bars indicate 95% confidence limits.

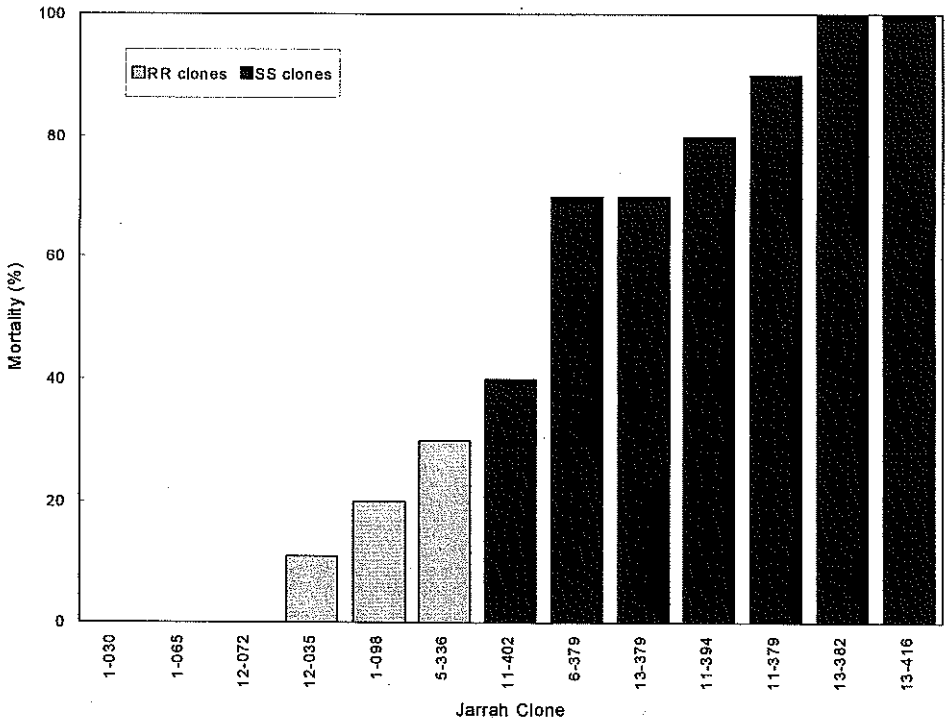

Fig. 3. Mortality of 13-year-old jarrah clones, propagated from seedlings selected as resistant (RR) or susceptible (SS) to *P.cinnamomi*, in a field validation trial.

Field testing of selected genotypes

Tissue cultured clones of resistant seedlings were planted in field validation trials on dieback-affected sites, and soil at the base of the plants was also inoculated with four isolates of *P.cinnamomi* (listed above) to test survival and growth. Susceptible clones were included for comparison. Seventy-four clones were grown in 10 trials. Data for the oldest validation trial are given in Fig. 3.

Survival of most resistant lines has been high (Fig. 3) and they have shown excellent growth, while susceptible lines have had high mortality. Drought deaths in the first summer have been a problem in some trials. These

validation trials are an essential component of the selection process, complementing the glasshouse selections and giving a further opportunity for the removal of any inferior lines from the program.

Deployment of dieback resistant jarrah

There is wide variation in the rooting ability of jarrah clones in tissue culture, with successful root production ranging from 0% to c.80% of shoots placed in culture. The performance of individual clones is consistent, and those with poor root production *in vitro* show poor survival on transfer to soil. Such characteristics add significantly to the cost of production of these clones, which are also likely to suffer high mortality after transplanting to the field due to restricted root development.

Due to generally high costs of production of the clones, and establishment problems in some forest sites, it is not feasible to use the clonal jarrah directly in large-scale operational plantings. However, some small-scale plantings have been established in operational forest rehabilitation areas.

Clonal seed orchards are now being planted to supply seed of resistant jarrah for rehabilitation plantings in dieback-affected forest and on cleared land. To maintain genetic diversity, at least 30 unrelated jarrah clones will be included in each production orchard. The orchards will be culled and upgraded as necessary to ensure that only the most resistant available jarrah lines are used. It is expected that due to the high heritability of the resistance to *P.cinnamomi*, the resulting seed crops will carry high levels of resistance and this will be tested in ongoing trials. Methods of maximising seed production in jarrah seed orchards are currently being investigated (M. Wheeler, Murdoch University, pers. com.).

In the forest, small plots of resistant jarrah trees will be established in dieback areas, or those under potential threat; it is hoped that these will cross-pollinate with surviving trees to produce a new generation of resistant seedlings. A forest rehabilitation program using dieback resistant jarrah will enable jarrah to be re-established on sites severely impacted by *Phytophthora* dieback. Dieback resistant jarrah will become an important element in the integrated management of the dieback problem in the jarrah forest. In addition, jarrah is now being included in trials on groundwater recharge sites on agricultural land where increasing soil salinity is a long-term problem. There is some potential for the use of jarrah to lower water tables on appropriate sites in the western part of the Western Australian wheatbelt.

Acknowledgments

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