SELECTION AND PROPAGATION OF JARRAH FOR DIEBACK RESISTANCE: A PROGRESS REPORT

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INTRODUCTION

Jarrah (*Eucalyptus marginata* Donn ex Smith), a valuable hardwood restricted to Western Australia, provides two-thirds of the sawn timber from the State. Natural stands have been badly affected by dieback caused by the soil fungus *Phytophthora cinnamomi* Rands (5). It is suspected that a low, but significant number of jarrah plants may show resistance to the disease. If lines of jarrah known to be resistant were available, they could be used to replant "graveyard" areas where the disease has killed virtually all jarrah, and in reafforestation after bauxite mining.

Clones have been raised from mature trees which have survived in graveyard areas, using nodal explants from the crown of the trees. Some clonal lines showed a level of resistance to the disease (1), but the tissue culture process was very difficult. Branches from the crowns of the trees were hard to surface sterilize, shoot multiplication rate was low, shoots did not root until after a prolonged period (3), and the resultant plantlets showed the mature, rather than the juvenile leaf form (2) Consequently we have investigated the possibility of screening seedlings for resistance and cloning selected seedlings.

METHODS AND MATERIALS

Selection of "mother" trees. A number of jarrah trees were selected on the basis that they had survived in areas where dieback had killed neighbouring jarrah. Others were included because they showed dieback symptoms and were thought to be susceptible to the disease. Additional plants were selected in healthy forests to represent a particular jarrah forest site type. Open-pollinated seed was collected and seedlings from each mother tree are referred to as a "family."

Glasshouse screening of seedlings for resistance to *P. cinnamomi*. Seedlings 9 to 12 months old, grown 4 per 25 cm pot in an unheated glasshouse, were tested for resistance to

P. cinnamomi using an underbark inoculation method. An A2 strain of *P. cinnamomi* (IMI 264384), originally isolated from *Hibbertia subvaginata*, was cultured on plates of green pea agar (200g greenpeas l^{-1}) with sterile discs of polycarbonate membrane filters (pore size 8 μm) laid on the surface. When the fungal mycelium was growing thickly on the filters they were cut into squares (side approx 2mm) and a square was slipped under a flap cut in the bark of the stem. The flaps were sealed down with a smear of petroleum jelly. Care was taken to position the wounds midway between nodes, about 4 to 5 nodes from the stem apex on stems approximately 3mm in diameter. Control inoculations used filter membrane with no fungus. A brown to black lesion developed above and below the inoculation site and its extension was measured over a period of 13 days. Inoculations were carried out from January to March (summer) and repeated in July and August (winter) on a different set of plants.

Tissue Culture. Plants which were designated "susceptible" or "resistant" were pruned to remove inoculated stems and prevent further tissue invasion by the fungus, and to induce sprouting from basal regions of the plants. Shoots of 4 to 5 nodes were cut from the plants, the leaves removed, and the shoots sterilized using 2% benzylkonium chloride in 10% alcohol for 20 min. They were then rinsed three times in sterile water and nodal pieces cut and cultured in Murashige and Skoog (MS) (4) medium with 2% sucrose, 2.5 μM benzylamino-purine, 1.25 μM NAA to induce axillary sprouting (2). Sprouts were multiplied in the same medium and then rooted in a medium with ¼ strength MS macronutrients, ½ Fe, full strength micronutrients, 2% sucrose, and 10 μM IBA. Rooted shoots were potted into a 1:2.2 peat, sand, perlite mix and placed under a wet tent for 4 weeks before being hardened on a glasshouse bench.

Field planting of clones. Plants 4 to 5 months from the test tube were planted in June, 1988, on a former bauxite minesite at Dwellingup where dieback had been active prior to mining 2 years earlier. Ten plants of each of 17 clones were arranged in randomized blocks with plants spaced at 4m x 4m. In September 4 plugs of *Pinus radiata* wood inoculated with different *P. cinnamomi* A2 strains (480 R1, isolated from *Banksia*; DCE210 from *E. marginata*; SC381 from *Allocasuarina fraserana*; and 251N12 from *P. radiata*) were buried separately, around each plant. The field plantings were regularly surveyed for deaths, and the roots of all dead plants screened for *P. cinnamomi* infection by plating root pieces on selective agar (6). The pine inoculum plugs were also recovered from around dead plants and plated.

RESULTS

Glasshouse screening of seedlings. The extension of lesions differed widely among plants and between seasons. In summer, lesions grew very rapidly and often killed the shoot, yet some plants effectively contained the lesion under these conditions. In winter, lesion growth was very slow on all but the most susceptible plants. No lesions developed on the "control" plants. Best discrimination among plants was found after inoculation in January-March. "Families" were classified as "susceptible" or "resistant" according to the mean lesion lengths on the seedlings. The mother tree's appearance in the field was not always a reliable guide for predicting the performance of the seedlings, although more of the resistant "families" came from "resistant" mother trees than from other groups.

Tissue culture. Cultures were established for 40 lines Surface sterilization of material from the glasshouse was much easier than from trees in the forest. Fast-growing lines showed a multiplication rate of up to X4 over 4 weeks, and 80% rooting, but not all lines grew well. Some showed low multiplication (X1.5) and did not root. Internal contamination of many lines became apparent after 6 to 12 months in culture. This made the response in culture very variable and reduced survival on transfer to soil. Different lines showed 0 to 90% survival after transfer to soil. Plants have been produced for two field trials in 1988 and 1990.

Field trial of clones. Clones planted in the field in June, 1988, had a survival rate of 98% at inoculation in September. There were 44 deaths over the summer of 1988/89 and a further 26 in 1989/90. *P. cinnamomi* was isolated from the roots of 94% of dead plants. Clones of "susceptible" plants from susceptible "families" showed highest numbers of deaths (40 to 100%, Figure 1); clones of "resistant" plants from susceptible "families" also showed significant numbers of dead plants (30 to 70%). Two plots of clone 11.93 showed 60% and 70% deaths, respectively. Amongst clones of "resistant" plants from resistant "families" there were very few deaths; in four lines there were no deaths, and one plant died in each of the other two lines.

P. cinnamomi was reisolated from almost all pine inoculation plugs to late December, 1988, and although reisolation has become increasingly difficult, *P. cinnamomi* has been reisolated from some plugs to March, 1990, indicating that viable inoculum is still present.

DISCUSSION

Micropropagation techniques allow cloning of jarrah from mature trees of 1-year-old seedlings, but the technique is very much easier to apply to seedlings than to the mature plants. Even so, before large scale propagation can be undertaken, problems of internal contamination, low percentages of rooting, and low survival on transfer to soil remain to be solved.

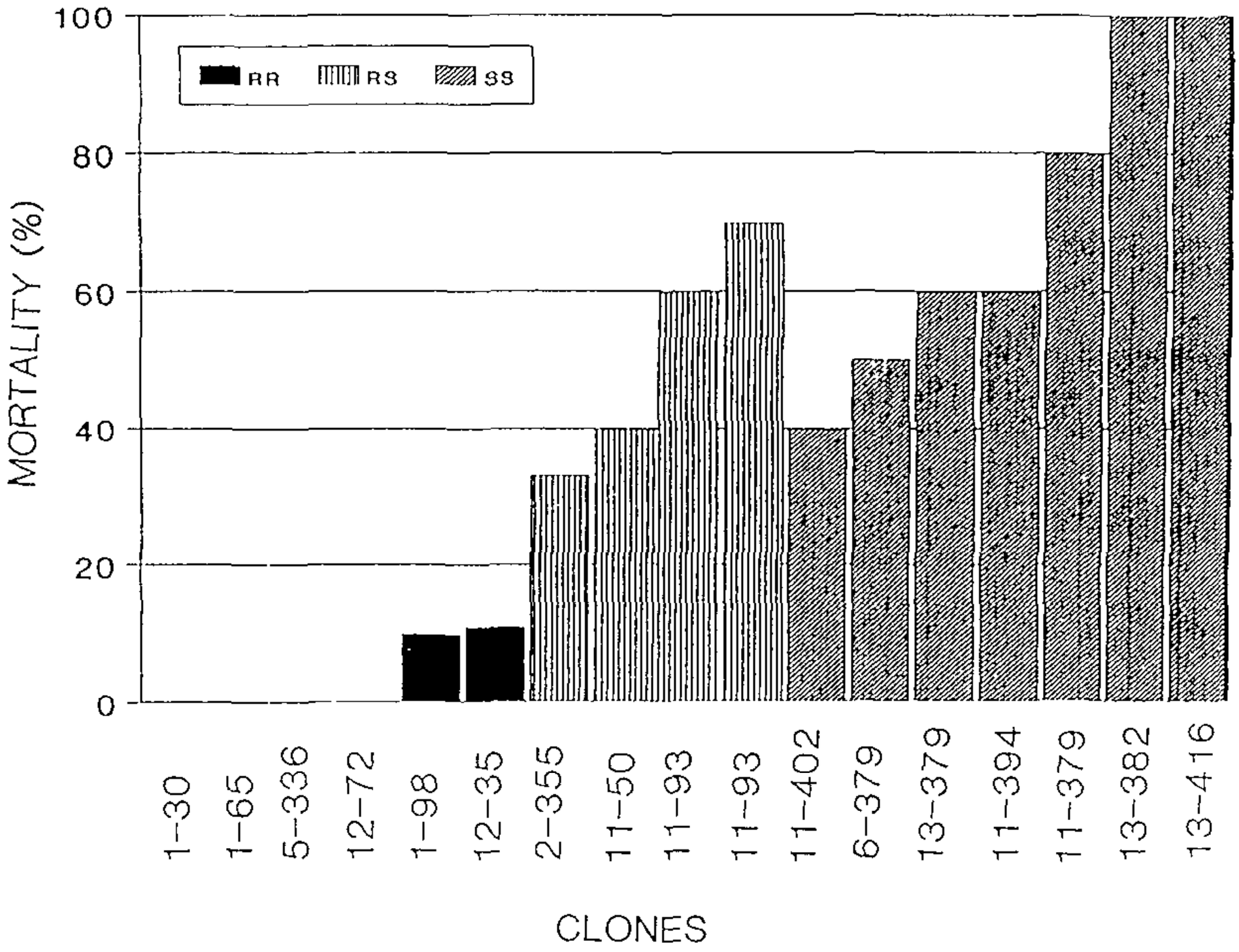

Figure 1. Deaths in clonal lines of jarrah in the field from September, 1988, to May, 1990 Clonal numbers show the family number followed by the plant number within the family RR—clones of "resistant" plants from "resistant" "families", RS—clones of "resistant" plants from "susceptible" "families", SS—clones of "susceptible" plants from "susceptible" "families"

Clones of seedlings selected for resistance to *P. cinnamomi* in glasshouse screening trials have survived in the field while exposed to *P. cinnamomi*. However, not all seedlings that appear resistant in the glasshouse maintain their resistance as clones in the field; this is particularly the case for the rare "resistant" individuals that appear in otherwise susceptible "families". It is likely that the "resistant" rating of such individuals is largely the result of experimental error. On the other hand, clones of "resistant" seedlings from "resistant" "families" all maintain high levels of resistance in the field.

Monitoring of deaths from the 1988 field planting over the summer of 1990/91 and the planting of additional clones in the winter of 1990 will be undertaken to further assess the validity of our selection of resistant jarrah.

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