Mycorrhizal Inoculum for Propagation of *Epacris impressa*©

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Members of the Epacridaceae are traditionally difficult to propagate and are in decline in parts of Australia. Infection by *Phytophthora cinnamomi* has led to some species of Epacridaceae in Western Australia being listed as endangered. Propogation by seed is usually unsuccessful and cuttings often have a strike rate as low as 10%. Previous studies have demonstrated that introduction of soil collected from beneath adult plants improved the health and survival of cuttings of several epacrid species. In this study cuttings were grown in potting mix, potting mix containing mycorrhizal inoculum, or potting mix containing soil from beneath adult plants collected in the wild. Plants were grown under glasshouse conditions for 20 weeks and monitored for health and development before harvesting. Strike rate and mycorrhizal status were then determined. Statistical analysis of results indicated no significant difference between treatments and no mycorrhizas present in the roots of any cutting in any treatment.

INTRODUCTION

Epacris impressa Labill. (common heath) is a flowering woody shrub native to the states of Victoria (where it is the floral emblem), Tasmania, eastern South Australia and parts of southern New South Wales. Southern hemisphere Epacridaceae differ from the related northern hemisphere ericoids in that they grow in dry open sclerophyll woodland and grassy open forest, rather than in wet, subalpine conditions. Both ecological communities are known as "heathland" and exhibit poor edaphic conditions.

Members of Epacridaceae are generally difficult to propagate from seed and cuttings (Thompson, 1986) and have the potential to become endangered if not replaced in the wild. Several Western Australian epacrids have already been placed on the endangered plant list due to dieback resulting from *Phytophthora cinnamomi infection* (Environment Australia, 2002).

Discussions with revegetation groups, nurseries, and land reclamation consultants during this study indicate a market for *E. impressa* if it were more readily available. Potential commercial markets include councils, retail nurseries, cut flower production, and use in agricultural shelter-belt plantings.

Members of the Epacridaceae and the related northern hemisphere Ericaceae form a mycorrhizal relationship between their fine hair roots and symbiotic fungi.

This relationship is known to improve the uptake of nutrients such as nitrogen in ericaceous species with ericoid mycorrhizal relationships (Allen, 1991; Bajwa and Read, 1986; Stribley and Read, 1974). To date, it is not known whether ericoid mycorrhizal relationships in the epacrids also increase plant nitrogen uptake.

McLean et al. (1994) found that use of soil taken from around the plant's hair roots as an inoculum improved the health and survival of cuttings. These results indicate that mycorrhizal fungi present in the soil around the plant roots may enhance the strike rate of *E. impressa* cuttings. However, soil is not a practical inoculum for nursery use due to possible pathogens.

Direct application of fungal inoculum during sticking has increased the strike rate of epacridaceous and ericaceous cuttings (Lawrie et al, 2001; Scagel, 2001). This study will investigate the effect of a mycorrhizal inoculum on root initiation, shoot growth, and overall health of *E. impressa* cuttings.

MATERIALS AND METHODS

Cuttings of 30 to 50 cm in length were taken from Angahook-Lorne State Park (A) and two sites at the Royal Botanic Gardens, Cranbourne (C and CA). Appropriate permits were obtained from the Department of Natural Resources and Environment (DNRE) and Parks Victoria prior to tagging and collection. Fifty plants at each site were tagged for future reference and records of GPS position were taken. Flower colour was determined using the RHS Colour Guide (Royal Horticultural Society c.1995) and herbarium samples were retained.

Mycorrhizal inoculum was prepared 6 weeks prior to the sourcing of cuttings. The inoculum was grown in sterilized 100-ml jars as follows: sphagnum peat moss, composted pine bark (1 cm or less in size), and medium-grade vermiculite (7 : 2 : 1, by vol) (Starrett, pers. comm., 2000). The potting medium was moistened with 25 ml of 1% sterilized sucrose water and sterilized for 20 min at 120°C in an autoclave. The cooled mixture was then placed into 100-ml jars in a laminar-flow cabinet and a 5-mm cube of *E. impressa* mycorrhizal fungus E.1.1 (McLean et al., 1998) subcultured onto malt agar was placed in the centre of the media. A further 1 ml of sterilized 1% sucrose water was then added to rinse in the mycelia and speed the growth of the fungus. The jars were sealed with American National Can Parafilm® and stored in a dark area at room temperature. Hyphal growth was visible after 4 weeks.

Soil samples from each site were tested for *P. cinnamomi* prior to the transfer of soil from the sites to the nursery. Tests were negative for all sites.

During collection cuttings were tagged and placed in individual moistened plastic bags and stored at 4°C until used. All cuttings were stuck at the University of Melbourne, Burnley College nursery within 24 to 72 h after collection in the field. Cutting material retained turgidity and appeared to be in good condition.

Cutting material was washed in a 2.5% sodium hypochlorite solution for 30 sec. and rinsed in water. Diseased or insect infested material was removed and destroyed. A mixture of soft new season growth and woody second season growth was used for the cutting material. Uniform cutting material was not available due to plant development differences between sites.

Tip cuttings from 3 to 5 cm in length were taken from each larger field cutting. Major stem disturbance including stripping of leaves and cutting re-sticking has been found to cause death in epacrid cuttings (McLean et al., 1994). Based on these findings leaves were removed with scissors from the bottom centimetre of each cutting.

Table 1. Root scores for *Epacris impressa* cuttings at 20 weeks (root and shoot scoring percentages by Site (A, C, CA) and treatment (1, 2, 3, 4). Roots of cuttings were scored as follows: 0 - dead; 1 - alive but unrooted; 2 - rooted, roots 1 cm or less; 3 – rooted, less than 5 roots; 4 – rooted, 5 to 10 roots; 5 – rooted, more than 10 roots.

	Site and treatment											
Root score	A1 (%)	A2 (%)	A3 (%)	A4 (%)	C1 (%)	C2 (%)	C3 (%)	C4 (%)	CA1 (%)	CA2 (%)	CA3 (%)	CA4 (%)
0	30	43	37	28	50	52	50	50	32	23	35	18
1	10	34	28	25	15	22	30	27	18	20	32	20
2	37	5	10	15	20	3	7	3	10	3	5	5
3	5	0	5	5	3	5	3	5	20	10	3	5
4	15	0	10	17	5	3	0	5	5	22	0	15
5	3	18	10	10	7	15	10	10	15	22	25	37

Table 2. Shoot scores for *Epacris impressa* cuttings at 20 weeks (root and shoot scoring percentages by Site (A, C, CA) and treatment (1, 2, 3, 4). Shoots of cuttings were scored as follows: 0 - dead; 1 - extensive leaf loss or browning; 2 - 30% to 50% leaf loss or browning, little or no new growth; 3 - healthy, some leaf loss or browning, some new growth; 4 - very healthy, no leaf loss or browning, some new growth; 5 - very healthy, no leaf loss or browning, extensive shoot growth.

	Site and treatment											
Shoot score	A1 (%)	A2 (%)	A3 (%)	A4 (%)	C1 (%)	C2 (%)	C3 (%)	C4 (%)	CA1 (%)	CA2 (%)	CA3 (%)	CA4 (%)
0	30	43	38	28	50	53	50	50	30	23	35	18
1	8	13	8	10	10	8	5	5	5	18	15	15
2	15	13	0	15	18	18	18	10	13	13	5	10
3	18	13	28	0	15	18	28	30	15	23	18	38
4	30	18	28	48	8	5	0	5	33	15	28	20
5	0	3	0	0	0	0	0	0	5	10	0	0

The following four treatments were used on four groups of 40 cuttings: Treatment 1: Control group with nil stem treatment in pasteurised propagation medium;. Treatment 2: 6-sec quick dip in 1000 ppm IBA solution (Thompson, 1986) in pasteurised propagation medium; Treatment 3: 6-sec quick dip in 1000 ppm IBA solution, in pasteurised propagation medium with site soil (9 : 1, v/v) (McLean et al., 1994); Treatment 4: 6-sec quick dip in 1000 ppm IBA solution in pasteurised propagation medium with E.1.1 inoculum (3 :1, v/v) (Starrett, pers. comm., 2000; McLean et al., 1994).

Cuttings were placed under fog on 24°C bottom-heated beds and hand watered daily. Dead cutting material was periodically removed. Control of the fungus-gnat larval pest (*Bradysia* species) was achieved by the introduction of a predator mite *Stratiolaelaps miles (Hypoaspis)*, to the top of the cutting mix (Biological Services, 2001).

RESULTS AND DISCUSSION

Cuttings were removed after 20 weeks and scored according to root initiation and shoot growth. A total of 37% of cuttings rooted and were potted on into individual 6-cm tubes. Root samples were taken for clearing and staining to determine the presence of mycorrhizal infection. The plants were then placed under mist for 5 days to harden off before transfer outdoors under shade cloth. Healthy unrooted cuttings were repotted in their original media in groups of seven. All cuttings that were re-stuck died.

Tables 1 and 2 illustrate root and shoot scoring percentages by Site (A, C, CA) and Treatment (1, 2, 3, 4).

A logistic regression analysis indicated no statistical difference between treatments ($P > 0.05$). The statistical difference in treatment results occurred between the individual plants from where the cuttings originated. Roots of the struck cuttings did not exhibit mycorrhizal infection at this time, consequently lack of statistical differences between treatments was not unexpected.

The negligible statistical differences in strike rates, shoot growth, and health may indicate that the inoculum is only effective following root initiation. Further studies will investigate whether or not this is the case.

Based on these preliminary findings future research within this project will include longer exposure of cuttings under fog, trial of site specific inoculums, and further study of the impact of stem disturbance on strike rates.

In conclusion, although there was no statistical difference between treatment results at this stage, statistical differences did occur between individual plant cuttings. In subsequent trials, cutting material will be taken from individual plants that displayed higher strike rates and better shoot growth, thus enabling more accurate measurement of treatment outcomes.

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Domestication and Improvement of *Kunzea pomifera*©

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The Australian native shrub species *Kunzea pomifera* F.Muell. (muntries) occurs in south-eastern South Australia and far western Victoria on sandy calcareous soils and is generally of prostrate habit. It produces edible berries of commercial potential, which are borne on the apical meristems of the plant in clusters of 3 to 9. The berries are succulent and range in size from 5 to 13 mm in diameter. They are mottled in colour from green, red to purple and possess a unique apple-like flavour.

***Kunzea pomifera* was sampled across the area of its natural distribution as cuttings, which were then grown in replication in the outdoor production area at Burnley College, University of Melbourne. Variation in plant habit, leaf and fruit traits, flowering time, and the nature of the breeding system were examined. Variation in plant and fruit traits are discussed in terms of the scope for breeding muntries for commercial production. Characters considered important in muntries improvement are, upright habit, wider soil-type tolerance, condensed flowering period, and consistent fruit qualities. These studies led to the proposal of an ideotype (ideal plant form) for the commercial production of muntries.**

INTRODUCTION

Detailed studies of variation within and between natural populations of *Kunzea pomifera* (muntries) are discussed in terms of its domestication for commercial production, culminating in the proposal of a plant form considered ideal for its commercial production (ideotype). Important preliminary research in the domestication of a wild plant species is that which aims to evaluate its variation for a range of plant characters considered best suited for its eventual commercial production. To maintain objectivity in this research, it is useful to conceptualise an ideal plant form (ideotype) that embodies desirable characteristics, both for the plant and its commercial product.