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EARLY OBSERVATIONS IN THE IMPROVED SELECTION AND PROPAGATION OF *EUCALYPTUS LEUCOXYLON* FOR URBAN USE

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INTRODUCTION

In the past, the improved selection of Australian native trees by means of vegetative propagation has been limited to high value forestry trees, with little of this knowledge applied to important amenity trees. One tree worthy of such an approach is *Eucalyptus leucoxylon* (Yellow Gum, South Australian Blue Gum), which has been planted extensively throughout metropolitan Victoria and South-Australia. The use of *Eucalyptus leucoxylon* within urban areas has for the most part been of the variety 'Rosea'. Despite this, the natural origins of this cultivar are unknown. Consequently, as progeny have been derived from a tree in cultivation there is the distinct possibility there has been a loss of vigour due to inbreeding. This paper reports preliminary work which should lead to the identification of natural populations of *Eucalyptus leucoxylon* with qualities to equal those of 'Rosea'. The subsequent development of vegetative propagation methods will ensure that regardless of the extent of use, desirable characteristics will be retained with no loss of vigour.

PROVENANCE TRIAL

The variation within *Eucalyptus leucoxylon* is significant, with seven subspecies (Boland, 1979; Rule, 1989 -1992; Rule, 1998) described. One of these has been regarded as different enough to be elevated to species status, *Eucalyptus petiolaris* (Rule, 1989 - 1992). To understand the growth, development and variability within *Eucalyptus leucoxylon*, several of these subspecies were monitored for a period of nine months from germination.

Materials

Seed of *Eucalyptus leucoxylon* was sourced from eight locations (selected purely on the commercial availability of the seed) representing four subspecies (Boland, 1979), *Eucalyptus leucoxylon* ssp. *leucoxylon*, ssp. *megalocarpa*, ssp. *pruinosa* and ssp *petiolaris* (still used in this trial despite its current species status). For ease of labelling and identification, each population has been designated by a code letter, A-H (see Table 1). There is uncertainty as to the origins of the West Bendigo seed (D and E), but for the purposes of this paper they will be referred to as the West Bendigo provenance or population.

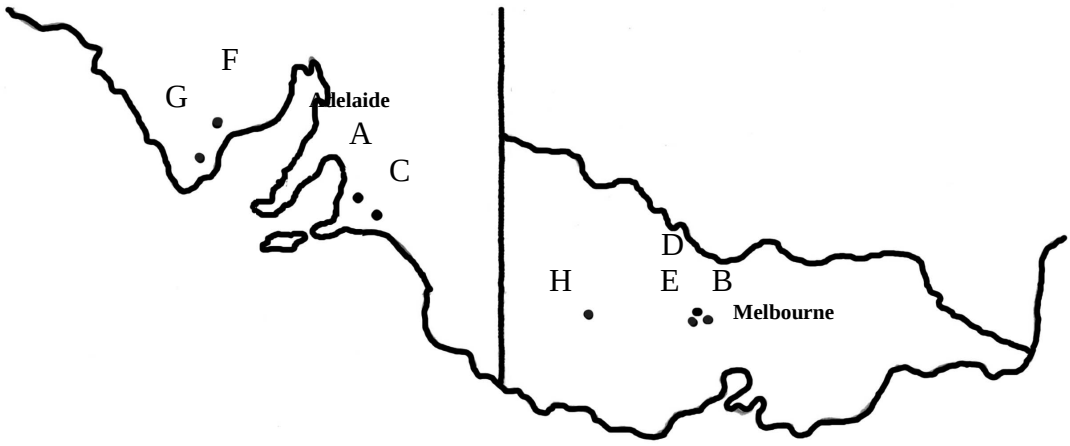
Table 1 Eight collection sites of *Eucalyptus leucoxylon* seed, including subspecies and identification code.

Species	Subspecies	Code	Location	State
Eucalyptus leucoxylon	*leucoxylon*	A	Adelaide Hills	South Australia
		B	Bendigo	Vctoria
	megalocarpa	C	Monarto	South Australia
		D	West Bendigo (Red Flowered)	Victoria
		E	West Bendigo (Yellow flowered)	Victoria
	petiolaris	F	Ungarra-Cockaleechie*	South Australia
		G	Warunda-Koppio*	South Australia
	pruinosa	H	Horsham area	Victoria

* Eyre Peninsula

The distribution of the selected populations of *Eucalyptus leucoxylon* is shown in Figure 1.

Figure 1 *Eucalyptus leucoxylon* populations used in the trial from South-eastern Australia.



***Eucalyptus leucoxylon* ssp. *leucoxylon*, A; Adelaide Hills, B; Bendigo. *Eucalyptus leucoxylon* ssp. *megalocarpa*, C; Monarto, D&E; West Bendigo *Eucalyptus leucoxylon* ssp. *petiolaris*, F; Ungarra-Cockaleechie, G; Warunda-Koppio. *Eucalyptus leucoxylon* ssp. *pruinosa*, H; Horsham area.**

Experimental Design and Measurements

Seed Characteristics

All seed was separated from the chaff, to ensure there was no bias towards size. 30 replicates of 30 seeds were selected and weighed for each seed lot. From these weights the number of seeds per gram was derived (Figure 2).

Germination, Growth and Development

A glasshouse germination trial was established at Burnley College, in September 2001. Seedling trays were set up in a randomised block design with 12 blocks, each representing the eight populations. To satisfy the light requirement for germination (Turnbull & Doran, 1987), the seed was sown on top of Burnley seedling raising mix, and sprinkled with vermiculite. Initially there were three seeds per cell for a total of 720 seeds per provenance, however after recording daily germinants for six weeks these were randomly culled to only one plant per cell (240 per provenance). At four months of age these were transplanted to 14cm olive pots, and placed outside under 50% shade. Regular measurements were taken to assess the form and variability between and within all provenances (Table 2).

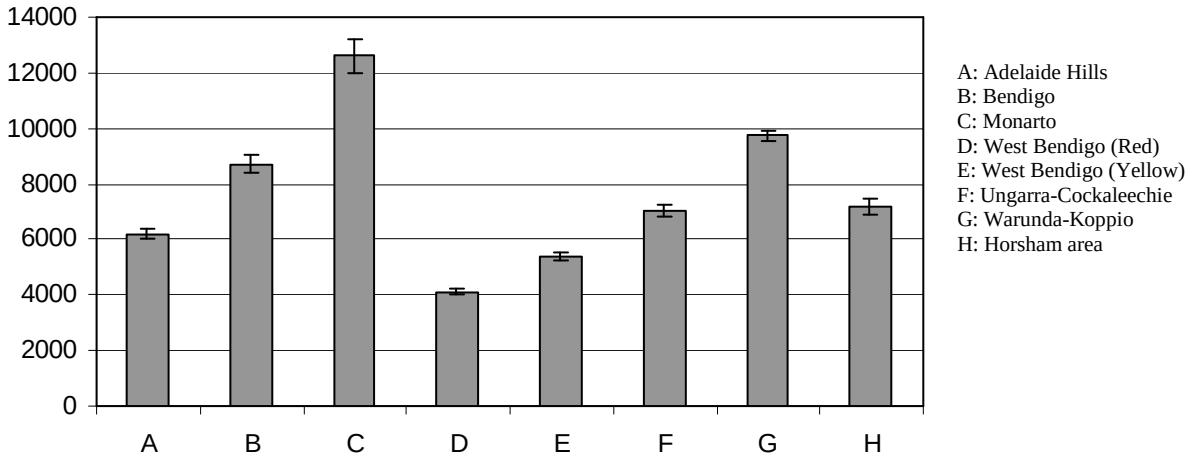
Table 2 Summary of the attributes recorded for *Eucalyptus leucoxylon* and the regularity of measurements taken.

ATTRIBUTE	TIME FRAME	DETAILS
Seed Weight	Initially	30 groups of 30 seed
Number of seeds with embryos	Initially	For each provenance, 100 seeds were checked for embryos (129 for *Eucalyptus leucoxylon* ssp. *leucoxylon* from Adelaide Hills)
Number of days to first germination		Emergence of radicle
Number of days to final germination		Last germinant from each population
Final Germination Percent	Day 42	(no germinates the preceding week)
Height	Weekly	Week 1 - 12
Height	Fortnightly	Week 16 -52
Leaf Size	6 Weekly	Largest Length Breadth Length from base to widest section. 6th leaf pair Length Width Length from base to widest section
Leaf Petioles	Fortnightly	Present / Absent Length Transition from sessile to petiolate
Leaf Arrangement	Fortnightly	Opposite / Alternate
Number of Leaf Pairs	Fortnightly	number
Leaves	Fortnightly	number
Lignotuber	Fortnightly	Present / Absent Number of nodes involved
Root Weight	6 Weekly	Fresh and Dry
Leaf Weight	6 Weekly	Fresh and Dry
Stem Weight	6 weekly	Fresh and Dry

Results

All of the seed groups had significantly different ($p<0.05$) weights, except for sample F (*E. leucoxylon* ssp. *petiolaris*) and H (ssp. *pruinosa*). In general (Figure 2) the seed of *Eucalyptus leucoxylon* ssp. *megalocarpa* was heavier than for the other subspecies, with the exception of the population from Monarto (C), which had the lightest seed, however the proportion of those with no embryo may have contributed to this result (Table 3).

Figure 2 **Number of seeds per gram for eight provenances. *Eucalyptus leucoxylon* ssp. *leucoxylon* (A & B), ssp. *megalocarpa* (C & D & E), ssp. *petiolaris*, (F & G), and ssp. *pruinosa* (H).**



The modified final germination percentage (Table 3) reflects the number that actually germinated as a percent of those that would be expected to be viable given that they have an embryo (although it is acknowledged that the presence of an embryo is not necessarily an accurate indication of viability). For all provenances, this is greater than 80%. The two provenances of *Eucalyptus leucoxylon* ssp. *leucoxylon* from the Adelaide Hills and Bendigo, and *E. leucoxylon* ssp *petiolaris* from Ungarra-Cockaleechie both had final germination percents, which were higher than those that were expected to be viable.

Table 3 **The percentage of seeds germinated, and the modified final germination percent, given the number of seeds without an embryo.**

Seed Source	Final (%)	N	Embryo (%) Present	Absent	N	Modified Final %
Eucalyptus leucoxylon ssp. *leucoxylon*						
Adelaide Hills	51.67	720	47.30	52.70	129	100.00
Bendigo	92.64	720	92.00	8.00	100	100.00
Eucalyptus leucoxylon ssp. *megalocarpa*						
Monarto	36.25	720	44.00	56.00	100	82.39
West Bendigo (Red)	85.83	720	88.00	12.00	100	97.53
West Bendigo (Yellow)	85.97	720	90.00	10.00	100	95.52
Eucalyptus leucoxylon ssp. *petiolaris*						
Ungarra-Cockaleechie	89.44	720	89.00	11.00	100	100.00
Warunda-Koppio	74.17	720	91.00	9.00	100	81.51
Eucalyptus leucoxylon ssp. *pruinosa*						
Horsham area	95.56	720	97.00	3.00	100	98.52

Table 4 outlines some of the characteristics that vary between the provenances. Many differences are evident, however the most prominent is probably the petiolate leaves in the two populations of ssp. *petiolaris* (*Eucalyptus petiolaris*) from the Eyre Peninsula. Generally these petioles are between 3 - 12mm in length, compared to the other subspecies which rarely

reach 5mm. This and the early development of alternate leaves - as early as the fifth leaf pair in this provenance - are among the main criteria for its elevation to species status (Rule, 1989-1992). For most of the other subspecies, alternating leaves occur rarely (although most plants still have less than 20 leaf pairs), and if so, it is generally not until at least the 11th leaf pair.

The shape (as described by Brooker & Kleinig (1999)) and size of the leaves vary considerably with most displaying ovate leaves for the first seven or eight leaf pairs. The Eyre Peninsula provenances have elliptic leaves, and the leaves from both populations of *E. leucoxylon* ssp. *megalocarpa* from West Bendigo are considerably larger (see Table 4) and generally cordate and amplexicaul. Also worth noting is the tendency to develop lateral stems. The Victorian *megalocarpa* subspecies very rarely did, however it is strongly pronounced in ssp. *megalocarpa* from Monarto, and *E. leucoxylon* ssp. *leucoxylon* from the Adelaide Hills. The leaves in these latter subspecies are becoming distinctly lanceolate.

The glaucous covering that is unique to *Eucalyptus leucoxylon* ssp. *pruinosa* (Boland, 1979) within this group is evident in only half of those from the Horsham area, and even these only have a very fine waxy covering, with usually only two or three leaf pairs affected.

Discussion

The differences evident within the selected subspecies of *Eucalyptus leucoxylon* have so far been limited to the juvenile characteristics. Differences have been observed in the growth rate, leaf size, shape, and arrangement; the presence or absence of petioles and lignotubers; seed weights, and the rates of germination. The effect that these differences will have on future growth and vigour is yet to be determined, however the close monitoring of these trees for a further 18 months should facilitate a greater understanding of how these traits relate to the development of mature trees.

Table 4 Growth characteristics of selected attributes in eight provenances of *Eucalyptus leucoxylon*.

Eucalyptus leucoxylon	**ssp. *leucoxylon***		**ssp. *megalocarpa***			**ssp. *petiolaris***		**ssp. *pruinosa***
	Adelaide Hills	**Bendigo**	**Monarto**	**West Bendigo (Red)**	**West Bendigo (Yellow)**	**Ungarra-Cockaleechie**	**Warunda-Koppio**	**Horsham**
Days to first germination	4	4	4	4	4	4	4	3
Days to 50% germination	10.92 ± 0.43	5.71 ± 0.11	6.75 ± 0.43	5.5 ± 0.15	6.17 ± 0.27	6.67 ± 0.14	9.33 ± 0.28	5.42 ± 0.46
Days to final germination	35	32	25	32	35	30	32	28
Lignotuber present	78.79% (n=33)	97.22% (n=36)	48.28% (n=29)	93.94% (n=33)	100.00% (n=34)	91.67% (n=36)	94.44% (n=36)	96.88% (n=32)
Leaf size (mm) – 6 months old (n = 36) 6th leaf pair								
Length	47.53±2.08	53.75±8.21	40.73±1.91	59.89±3.20	59.44±2.60	44.28±1.90	44.67±2.02	42.78±1.83
Breadth	18.67±0.95	24.19±1.17	17.91±0.97	27.53 ±1.69	35.17±2.02	28.83±1.37	25.56±1.11	25.69±1.27
Percent with petiolate leaves	80.55%	22.22%	88.25% (n=34)	61.76% (n=34)	16.67%	100.00%	100.00%	2.77%
Mean petiole length (mm)	1.55±0.21	1.88±0.64	2.73±0.30	1.33±0.20	1.17±0.17	5.75±0.46	4.00±0.29	1.67±0.67
Mean height increase per week (mm)								
Week 0 – 12	2.34	3.69	2.12	3.85	2.68	2.11	2.35	3.42
Week 12 – 24	26.31	22.84	16.96	24.35	22.05	19.33	23.05	20.65
Week 24 – 36	35.95	53.40	26.88	44.67	42.48	31.51	33.89	40.92
Current Height (44 weeks) (mm)	504.30± 84.64	610.20± 43.28	443.67± 110.33	662.22± 56.61	639.92± 32.14	600.87± 58.67	374.75 ±24.05	694.28 ± 41.78
Current number of leaf pairs (44 weeks)	16.20±1.17	16.60± 1.09	15.50± 2.18	16.67± 1.01	18.69± 0.86	18.50± 1.74	14.00 ± 1.08	17.42± 1.06

VEGETATIVE PROPAGATION

The inability to predict the final height and habit of *Eucalyptus* when growing in streetscapes is often the result of raising plants from seed. There is a need for the development of propagation systems to ensure offspring can be produced with desirable qualities, and a predictable mature height and form.

Budding and grafting of plants onto suitable rootstocks will ensure that subsequent scion growth will be representative of the material from which the plant was sourced. The success of this approach in *Eucalyptus leucoxylon* has not been well documented, so this preliminary trial was done to determine whether it is possible to graft six month old *Eucalyptus leucoxylon* ssp. *megalocarpa* plants onto other ssp. *megalocarpa* plants, of the same age and from the same seed source. It was also set up to determine whether a successful union could take place if a plant is grafted back to itself. Part of this trial will include the prevention of the cut surface from drying out, by keeping all surfaces wet during the grafting procedure.

Experimental Design

Eucalyptus leucoxylon ssp. *megalocarpa* plants were purchased from Mildura Native Nursery in October 2001. These were grown on until January 2002, 6 months of age. A total of 144 plants were used.

The plants were randomly allocated to groups and treatments, to ensure there was no bias as the budding and grafting techniques improved. Plants within the group were chip budded and grafted using the splice (whip) and whip and tongue graft (Hartmann *et al*, 2002).

To prevent the cut surface from drying out, half of the plants were fully submerged and the whole process was carried out under water, including the tying. For others, the time that the cut surfaces were exposed to air was kept to an absolute minimum, however in some cases this may have exceeded ten seconds. The four treatments for each graft type were as follows:

1.	The scion taken from a plant and grafted back to itself	EXPOSED
2.	Plants are paired and the scions swapped between them	EXPOSED
3.	The scion taken from a plant and grafted back to itself	SUBMERGED
4.	Plants are paired and the scions swapped between them	SUBMERGED

This trial was undertaken in January 2002 at Burnley College Nursery. It was carried out undercover, however it was still subjected to air drafts and temperature changes. Air temperature for this trial was between 16^{o}C and 22^{o}C (Bureau of Meteorology). Water temperature was between 20^{o}C and 22^{o}C, and the relative humidity was quite high (Bureau of Meteorology).

Grafted plants were placed in a fog house maintaining 90% humidity for two weeks. Following this they were placed in a misting house for a further three weeks until the tape was removed, and then taken outside.

Results

Overall results for this experiment were disappointing, with around a 10% success rate. This further fell to 4.17% in the following weeks, as the successful chip budded plants failed a week or so after tape removal.

Mortality rate was quite high with rootstock death occurring in 12 of 144 (8.33%) of the plants.

There was some sprouting of the scion buds on those plants that were whip and tongue, or splice grafted within the first week following the graft procedure. Some of these continued to develop; however in many trees the scion, and consequently the buds, died after a few days.

To date, the only successful grafts have been the whip and tongue, and the splice graft. These are still alive after seven months with even growth and a strong union.

Even though the numbers remaining are too small to get a clear indication as to the effect of submerging the cut surfaces, it can be seen that the plants are able to be grafted onto themselves, or others from the same seed stock, in either wet or dry conditions.

Discussion

Although not actually measured, it appeared that the successful grafts were generally on plants that had slightly thicker stems. Most of the failed grafts showed early necrosis beginning at the tip of the scion. The young age of these plants combined with the inexperience of the grafter may explain this low success rate. Further trials are planned for the future.

CONCLUSION

The preliminary results obtained, have emphasised the considerable variation that is present within *Eucalyptus leucoxylon*. The appearance and habit of the juvenile plants sourced from eight different sites throughout Victoria and South Australia have shown differences in both the pattern of development, and juvenile features. Whether these differences will be evident or significant in the mature plant, remains to be determined.

Despite the low success rate, the ability to vegetatively propagate *Eucalyptus leucoxylon* ssp. *megalocarpa* appears to be promising. The rate of failure could be attributed to the young age of the plants, and the small surface area over which the union was to occur. Further work has been planned which will involve the use of older plant material, with the effect of stock pre-treatment and season also investigated.

At the conclusion of this project - June 2004, it should be possible to recommend provenances of *Eucalyptus leucoxylon* that are suitable for planting in urban areas. A method of vegetative propagation, which will ensure that a sustainable number of plants can be produced, reliably exhibiting the various features the provenance was selected for, should also have been determined.

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