Breeding of eucalypt bud and flower lines

A report for the Rural Industries Research and Development Corporation

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Foreword

This project is the first in Australia or overseas to address the breeding of eucalypts for the cut stem sector of ornamental horticulture. All currently existing cultivars were developed for amenity horticulture, and are not suitable for the new and expanding trade in cut stems with buds, flowers or gumnuts. Experience with other crops has taught us that superior cultivars with the traits required by the consumer are essential to maintain a competitive advantage on international markets.

With the eucalypts, we, as Australians, are in the unique situation of having the full range of germplasm at our disposal. This offers an outstanding opportunity to the new fledgling eucalypt stem industry. The University of Adelaide’s approach of interspecific hybridisation combines superior characters from different species, often with the vigour associated with some hybrid combinations. Vegetative propagation methods are essential to capture the precise combination of superior characters, and are adapted to the selections of interest. All selections have a postharvest vase life sufficient to deliver to export markets.

The University of Adelaide study has developed twelve superior selections with outstanding characteristics of stunning buds and flowers. While it will be some years yet before this material can be proven under cultivated conditions, the resource secures the future of the cut stem industry in Australia, with the promise of superior adapted cultivars for the future.

This project was funded from RIRDC Core Funds which are provided by the Australian Government.

This report, a new addition to RIRDC’s diverse range of over 1000 research publications, forms part of our Wildflower and Native Plants R&D program, which aims to improve the productivity and sustainability of the Australian native plant industry.

Most of our publications are available for viewing, downloading or purchasing online through our website:

- purchases at www.rirdc.gov.au/eshop

Simon Hearn  
Managing Director  
Rural Industries Research and Development Corporation
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Abbreviations

RO water reverse osmosis water
CA citric acid
MoW Monarto Woodland, Monarto, SA
BAP 6-benzylaminopurine
IBA indole butyric acid
NAA naphthalene acetic acid
ppm parts per million
ANOVA analysis of variance
MS Murashige and Skoog
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Executive Summary

The total area of cut flowers in Australia exceeds 100,000 ha, with Australian exports of native fresh flowers valued at over $20 million. Australia has 8% of the Japanese market share. This project has the potential to increase production of Australian eucalypt bud and flower cut stems ten fold. At present, most native flower cultivation is confined to coastal areas with good soils. Eucalypts will grow in a wide range of soil types and water qualities, and the development and proving of novel lines, along with the propagation, production and postharvest information for optimum quality, will expand current production into new areas.

This project addressed the development of new eucalypt cultivars for cut stem production, a step that is essential for the future competitiveness of this Australian export industry. As relatively little work had been conducted in this area previously, the approach was wide-ranging. Research addressed the development of interspecific hybrids, evaluation of these hybrids for superior characteristics, investigation of their postharvest vase life, investigation of vegetative propagation, and investigation of pruning treatments.

An interspecific hybridisation approach was adopted as most current horticultural cultivars have complex pedigree. Parents with desirable characteristics were selected, and twelve superior selections have been produced for further evaluation. Postharvest vase life is important for any cut stem commodity, but particularly in product for export. The research has shown that clean water is the best postharvest treatment. Vegetative propagation of superior selections is essential to capture the advantages of genetic gain via breeding, as eucalypts are outcrossing so characters are not fixed via seed propagation. The research has shown that vegetative propagation is possible via cuttings, grafting and tissue culture. Cost-effective production depends upon pruning methods that stimulate the long stem length required on discerning export markets. The research has shown that pruning back to 1 m in spring is essential to stimulate required stem length.

The next stage of the project is registration of these selections for Plant Breeders Rights. Subsequent release to the industry will furnish Australian cut stem growers with a strong competitive advantage.
1. General introduction

1.1. Eucalypts for floriculture

The total area of cut flowers in Australia exceeds 101,140 ha, with Australian exports of native fresh flowers valued at over $20,000,000. Australia has 8% of the Japanese market share. This project has the potential to increase production of Australian eucalypt bud and flower cut stems ten fold. Eucalypt product has a small but significant place in the Australian floriculture industry with demand currently outstripping supply, on both local and export markets (Horsman & Delaporte 2002; Sedgley 1998). Current supply is erratic and seasonal, and has been severely affected by the recent drought. Other issues affecting production are product variability and range, and weight and form of product.

_Eucalyptus_ (family Myrtaceae) is a wide and diversified genus, with over 700 species (Brooker & Kleinig 2002) growing in all regions of Australia. There are many species suitable for floriculture; for foliage, bud and flower production. Bud and flower product can be divided into three types of products: filler, feature filler, and focal flowers. Filler lines, such as _E. leptophylla_ and _E. anceps_, are small bud and flower types and are used to provide background accent to arrangements. Larger bud and flower lines, such as _E. forrestiana_ and _E. pachyphylla_, are used as feature fillers, with numerous flowers providing a subtle focus. Focal buds and flowers are very large and are the focus of an arrangement, species such as _E. macrocarpa_ and _E. pyriformis_ are used in this way. The most popular species available currently is _E. tetragona_, a feature filler or focal line, which is sold as a stem with capsules.

At present, the bud and flower industry is based upon seed propagated seedling lines, which show the variability inherent in such material. This variability is important for selection programmes, as it means that outstanding individuals can be identified and become the focus for development: however in terms of product uniformity it is highly undesirable. Breeding, selection and clonal propagation are essential components of development. Previous breeding programmes (Delaporte et al. 2001a, 2001b, 2001c; Sedgley & Granger 1996; Ellis et al. 1991) have shown that interspecific hybridisation between close species can be highly successful; hybrids exhibit mainly intermediate characters in morphology and flowering time, many exhibit increased growth compared to parental species.

All selected hybrids must be clonally propagated to ensure genetic integrity. This may be difficult to achieve, but has been shown to be possible with some forestry species. Clonal propagation can be highly genotype dependant. Micropropagation, somatic embryogenesis, cuttings (micro, mini and macro) and grafting have been successful with forestry species and some ornamental varieties (Watt et al. 2003; Le Roux & van Staden 1991; Salmon 1990; Hartney 1980).

Bud and flower growers lack reliable information on the optimal training and pruning of trees for this industry. Some research has been conducted on postharvest vase life of eucalypt buds and flowers, which shows that each new line must be tested individually (Delaporte et al. 2000).

The process for development of ornamental eucalypts follows four steps: hybrids reach reproductive maturity; they are selected based on primary selection criteria (morphological characters); they are tested for secondary selection criteria (performance characters); and those that perform well in all aspects undergo final selection and evaluation, where they are tested in different environments and undergo PBR trails. Clonally propagated, tested superior plants can then be released to industry. This project covered all aspects of this development process. There is no other research in the area underway in Australia, and the project collaborated closely with industry to achieve its outcomes.
Schematic representation of the process for development of ornamental eucalypts

interspecific hybrid plants reach reproductive maturity

primary selection
based on morphological characters assessed in the field

select hybrids
those that score >75% move to next phase

final selection and evaluation
testing in different environments, PBR trials
release to industry

clonal propagation
grafting
cuttings
tissue culture

postharvest testing
sugar maturity
cold storage

production regimes
pruning
training
fertilizer
irrigation

marketing
industry
collaborator
florist
consumer
exporter
1.2.1 Project objectives

Objectives

1. Hybridisation of novel eucalypt lines for fresh bud and flower cut stems.
2. Field trial of the novel lines to develop training and pruning regimes for optimal production.
3. Investigation of clonal propagation of superior lines.
4. Development of postharvest treatments to allow transport of quality product to distant markets.
5. Investigation of marketability of lines.
2. Interspecific hybridisation

2.1. Interspecific hybridisation programme

2.1.1. Introduction

The eucalypt improvement and hybridisation programme has been underway at the University of Adelaide since the early 1990s, with the aim of producing interspecific hybrids with ornamental merit for the amenity horticulture and floriculture markets. Previous crossing programmes concentrated on species from Subgenus *Symphyomyrtus* section *Bisectae*, such as *Eucalyptus spathulata* and *E. platypus* (Sedgley & Granger 1996; Ellis et al. 1991), as well as *E. macrocarpa*, *E. gillii* and *E. stricklandii* (Delaporte et al. 2001a, 2001b, 2001c). From 2000 to 2004, the hybridisation programme focussed on a new range of species from Subgenus *Symphyomyrtus* section *Bisectae* (e.g. *E. caesia* ssp. *caesia*, *E. websteriana* ssp. *norsemanniana*), as well as species from Subgenus *Symphyomyrtus* section *Dumaria* (e.g. *E. lesouefii*, *E. forrestiana* ssp. *forrestiana*), and Subgenus *Corymbia* (e.g. *E. fycifolia*, *E. ptychocarpa*). These species were selected on the basis of their ornamental merit and their relatedness (Griffin et al. 1988), with an additional focus on summer and or red flowering types.

The pollination groups were named according to the taxonomic Series to which the female parent is assigned (according to Brooker 2000). Thus, there were eight groups: Preissianae, Curviptera, Cymbiformes, Erectae, Rufispermae, Maculatae, Heteropterae and Tetrapterae (Table 1).

Superior parental genotypes were selected from species that have desirable characters based on the A1 selection criteria (see chapter 3.1.).

Table 1: Pollination family according to female parent from the pollination programme 2000-2004. Families are named after the taxonomic Series to which the female parent belongs, species based on Brooker (2000).

<table>
<thead>
<tr>
<th>Pollination family</th>
<th>female parent</th>
<th>male parents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cymbiformes</td>
<td><em>E. calophylla</em></td>
<td><em>E. fycifolia</em>, <em>E. maculata</em>, <em>E. watsoniana</em></td>
</tr>
<tr>
<td>Disjunctae</td>
<td><em>E. fycifolia</em></td>
<td><em>E. fycifolia</em>, <em>E. maculata</em>, <em>E. ptychocarpa</em>,</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>E. citriodora</em>, <em>E. calophylla</em>, <em>E. watsoniana</em></td>
</tr>
<tr>
<td>Maculatae</td>
<td><em>E. citriodora</em></td>
<td><em>E. fycifolia</em>, <em>E. maculata</em>, <em>E. calophylla</em>,</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>E. watsoniana</em>, <em>E. ptychocarpa</em></td>
</tr>
<tr>
<td>Heteropterae</td>
<td><em>E. tetragona</em> (syn</td>
<td><em>E. tetragona</em> (syn <em>E. pleurocarpa</em>), *E.</td>
</tr>
<tr>
<td></td>
<td><em>E. pleurocarpa</em>),</td>
<td>conveniens*, <em>E. erythrocorys</em></td>
</tr>
<tr>
<td></td>
<td><em>E. conveniens</em>,</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>E. erythrocorys</em></td>
<td></td>
</tr>
<tr>
<td>Erectae</td>
<td><em>E. 9-5MuB</em>*, *E.</td>
<td><em>E. platypus</em>, <em>E. nutans</em>, <em>E. erythronema</em> var.</td>
</tr>
<tr>
<td></td>
<td><em>spathulata</em></td>
<td><em>marginata</em></td>
</tr>
<tr>
<td>Curviptera</td>
<td><em>E. orbifolia</em> (syn</td>
<td><em>E. kingsmillii</em>, <em>E. orbifolia</em> (syn <em>E. lata</em>),</td>
</tr>
<tr>
<td></td>
<td><em>E. lata</em>), *E.</td>
<td><em>E. rhodantha</em>, <em>E. synandra</em>, <em>websteriana</em> ssp.*</td>
</tr>
<tr>
<td></td>
<td><em>pachyphylla</em>,</td>
<td><em>norsemannica</em>, <em>E. pachyphylla</em>, <em>E. caesia</em></td>
</tr>
<tr>
<td></td>
<td><em>norsemannica</em></td>
<td><em>pyriformis</em>, <em>E. 144</em>, <em>E. 1926B</em>, *E.</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>youngiana</em>, <em>E. macrocarpa</em></td>
</tr>
<tr>
<td>Tetrapterae</td>
<td><em>E. forrestiana</em> ssp.</td>
<td><em>E. stotei</em>, <em>E. forrestiana</em> ssp. <em>forrestiana</em>,</td>
</tr>
<tr>
<td></td>
<td><em>dolichorhyncha</em>,</td>
<td><em>E. ceratocorys</em>, <em>E. corrugata</em>, <em>E. incerata</em>,</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>e. straiticalyx</em>, <em>E. lesouefii</em>, <em>E. torquata</em></td>
</tr>
<tr>
<td>Rufispermae</td>
<td><em>E. lesouefii</em></td>
<td><em>E. stotei</em>, <em>E. forrestiana</em> ssp. <em>forrestiana</em>,</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>E. forrestiana</em> ssp. <em>dolichorhyncha</em>, *E.</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>ceratocorys</em>, <em>E. corrugata</em>, <em>E. incerata</em>,</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>e. straiticalyx</em>, <em>E. torquata</em></td>
</tr>
<tr>
<td>Pressianae</td>
<td><em>E. preissiana</em></td>
<td><em>E. coronata</em></td>
</tr>
</tbody>
</table>

* Hybrid from previous breeding programme (Ellis et al. 1991).
2.1.2. Methodology

2.1.2.1. Pollination
Pollen was collected from desirable male species. Flowers at anthesis were collected and anthers removed and placed in a desiccator over silica gel for 48 hours to assist with anther dehiscence and pollen release. The pollen was stored at -20°C until required. Pollen was removed from the freezer several hours prior to pollination and kept on ice or at 4°C at all times, and discarded after five days.

The controlled pollination method followed that of Delaporte et al. (2001a) with modifications to suit specific species. Flowers on the selected female plant were emasculated at operculum lift by removing all stamens. The emasculated flowers were bagged and labelled. All open and immature flowers were removed from the branch prior to bagging. Pollen was applied to the stigma using a paint brush between 0 to 6 days later, depending on species. The flowers were re-bagged and left to develop. Bags were removed 4-8 weeks later and the number of developing capsules recorded.

2.1.2.2. Seed collection, storage and germination
Capsules were harvested between 9-12 months after pollination and dried at 37°C to promote opening. Seed released from the capsules was sorted, counted and stored at 4°C until planting. Seed was removed from storage and placed in wet vermiculite to stimulate germination. Germinated seeds were potted into 25 mm pots containing soil (sand and composted pine bark in a 1:2 ratio) and grown in a glasshouse, under conditions of natural daylength and temperatures of 18±3°C to 25±3°C. Plants were potted into 100 mm pots when required, and transferred to a shadehouse (natural environmental conditions) until planting.

2.1.3. Results
During the project, 84 different species combinations were attempted, with 13 repeated crosses. Of these, 15 crosses produced seedlings and 44 failed to produce viable seedlings. Table 2 summarises all pollinations conducted during the project and their success.

The Australian flower industry has identified a need for cut flower varieties that have red flowers and flower between the months of November and March. There has been an ongoing focus in this breeding programme towards summer flowering varieties and red colouration in the flowers and buds. There are a number of hybrids that have the potential to fit either category, due to the colour of the buds and flowers of the parent species and/or their time of flowering (Appendix A).

2.1.4. Discussion
The crossing programme endeavoured to combine as many horticulturally desirable species as possible, in combinations with a theoretically high chance of success. The data show that while crosses between closely related species were often successful, relatedness was not the only criterion influencing seed set. Other influences were female parent genotype and environmental conditions during pollination. Flower morphology may have played an important role as a barrier to hybridisation, as shown by the cross *E. tetragona* (small flower) x *E. erythrocorys* (large flower) producing seed, but the reciprocal cross producing none. This echoes the conclusions of Ellis et al. (1991) and Delaporte et al. (2001c) in regards to the influence of relatedness and flower morphology on hybridisation success.

The programme has enabled us to learn more about the inheritance of important characters, such as glaucousness and flower colour.

The scope for interspecific hybridisation as a tool to generate new and novel eucalypt bud and flower lines is significant, as this part of the project has shown. While a cross may produce few seedlings, each one is unique and may have very desirable characters that become evident as the plant matures. However, as it takes a minimum of five years from pollen collection to first assessment of a hybrid, implementation of hybridisation as a tool to generate new material must be not taken lightly, and provided with long term funding to adequately yield and assess results.
The breeding programme has produced a large valuable germplasm resource that should be maintained and protected for future work. Information generated through this programme has relevance to other areas such as eucalypt breeding for forestry and breeding with other preferentially outcrossing genera.
Table 2: Summary of results for pollinations conducted 2000-2004. Taxonomic classification according to Brooker (2000).

<table>
<thead>
<tr>
<th>Female sp</th>
<th>Subgenus</th>
<th>Section</th>
<th>Series</th>
<th>Male sp</th>
<th>Subgenus</th>
<th>Section</th>
<th>Series</th>
<th># Flowers pollinated</th>
<th>% Capsules set/flowers pollinated at harvest</th>
<th># Seed produced</th>
<th>% Seed germinated</th>
<th># Plants alive</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. calophylla</em></td>
<td>Corymbia</td>
<td>Notiales</td>
<td>Cymbiformes</td>
<td><em>E. ficifolia</em></td>
<td>Corymbia</td>
<td>Notiales</td>
<td>Disjunctae</td>
<td>174</td>
<td>40</td>
<td>193</td>
<td>41</td>
<td>35</td>
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<td>Cymbiformes</td>
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<td>62</td>
<td>nh*</td>
<td>-</td>
<td>-</td>
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<tr>
<td><em>E. calophylla</em></td>
<td>Corymbia</td>
<td>Notiales</td>
<td>Cymbiformes</td>
<td><em>E. maculata</em></td>
<td>Corymbia</td>
<td>Septerionale</td>
<td>Maculatae</td>
<td>16</td>
<td>nh</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>E. calophylla</em></td>
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<td>Notiales</td>
<td>Cymbiformes</td>
<td><em>E. watsoniana</em></td>
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<td>Septerionale</td>
<td>Naviculares</td>
<td>12</td>
<td>nh</td>
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<tr>
<td><em>Pollination Family Disjunctae</em></td>
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<tr>
<td><em>E. ficifolia</em></td>
<td>Corymbia</td>
<td>Notiales</td>
<td>Disjunctae</td>
<td><em>E. citriodora</em></td>
<td>Corymbia</td>
<td>Septerionale</td>
<td>Maculatae</td>
<td>27</td>
<td>26</td>
<td>1</td>
<td>npy**</td>
<td>-</td>
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<tr>
<td><em>E. ficifolia</em></td>
<td>Corymbia</td>
<td>Notiales</td>
<td>Disjunctae</td>
<td><em>E. maculata</em></td>
<td>Corymbia</td>
<td>Septerionale</td>
<td>Maculatae</td>
<td>134</td>
<td>15</td>
<td>6</td>
<td>npy</td>
<td>-</td>
</tr>
<tr>
<td><em>E. ficifolia</em></td>
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<td>Notiales</td>
<td>Disjunctae</td>
<td><em>E. calophylla</em></td>
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<td>Notiales</td>
<td>Cymbiformes</td>
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<td>Disjunctae</td>
<td><em>E. watsoniana</em></td>
<td>Corymbia</td>
<td>Septerionale</td>
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<td>npy</td>
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<td><em>E. ficifolia</em></td>
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<td>Notiales</td>
<td>Disjunctae</td>
<td><em>E. ptychocarpa</em></td>
<td>Corymbia</td>
<td>Septerionale</td>
<td>Doriventerales</td>
<td>113</td>
<td>11</td>
<td>3</td>
<td>npy</td>
<td>-</td>
</tr>
<tr>
<td><em>E. ficifolia</em></td>
<td>Corymbia</td>
<td>Notiales</td>
<td>Disjunctae</td>
<td><em>E. ficifolia</em></td>
<td>Corymbia</td>
<td>Notiales</td>
<td>Disjunctae</td>
<td>531</td>
<td>32</td>
<td>493</td>
<td>npy</td>
<td>-</td>
</tr>
<tr>
<td><em>Pollination Family Maculatae</em></td>
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<td></td>
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Pollination family Tetrapterae

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<th>% Seed germinated</th>
<th># Plants alive</th>
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</tr>
<tr>
<td>E. forrestiana ssp. dolichorhyncha</td>
<td>Symphyomyrtus</td>
<td>Dumaria</td>
<td>Tetrapterae</td>
<td>E. forrestiana</td>
<td>Symphyomyrtus</td>
<td>Dumaria</td>
<td>Tetrapterae</td>
<td>20</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>E. forrestiana ssp. dolichorhyncha</td>
<td>Symphyomyrtus</td>
<td>Dumaria</td>
<td>Tetrapterae</td>
<td>E. torquata</td>
<td>Symphyomyrtus</td>
<td>Dumaria</td>
<td>Torquatae</td>
<td>21</td>
<td>10</td>
<td>14</td>
<td>npy</td>
<td>-</td>
</tr>
<tr>
<td>E. forrestiana ssp. dolichorhyncha</td>
<td>Symphyomyrtus</td>
<td>Dumaria</td>
<td>Tetrapterae</td>
<td>E. forrestiana ssp. forrestiana</td>
<td>Symphyomyrtus</td>
<td>Dumaria</td>
<td>Tetrapterae</td>
<td>20</td>
<td>30</td>
<td>159</td>
<td>npy</td>
<td>-</td>
</tr>
<tr>
<td>E. forrestiana ssp. dolichorhyncha</td>
<td>Symphyomyrtus</td>
<td>Dumaria</td>
<td>Tetrapterae</td>
<td>E. forrestiana ssp. dolichorhyncha</td>
<td>Symphyomyrtus</td>
<td>Dumaria</td>
<td>Tetrapterae</td>
<td>20</td>
<td>35</td>
<td>80</td>
<td>npy</td>
<td>-</td>
</tr>
<tr>
<td>Female sp</td>
<td>Subgenus</td>
<td>Section</td>
<td>Series</td>
<td>Male sp</td>
<td>Subgenus</td>
<td>Section</td>
<td>Series</td>
<td># Flowers pollinated</td>
<td>% Capsules set/flowers pollinated at harvest</td>
<td># Seed produced</td>
<td># Seed germinated</td>
<td># Plants alive</td>
</tr>
<tr>
<td>-----------</td>
<td>----------</td>
<td>---------</td>
<td>--------</td>
<td>---------</td>
<td>----------</td>
<td>---------</td>
<td>--------</td>
<td>---------------------</td>
<td>---------------------------------------------</td>
<td>----------------</td>
<td>------------------</td>
<td>--------------</td>
</tr>
<tr>
<td><em>E. lesouefii</em></td>
<td>Symphyomyrtus</td>
<td>Dumaria</td>
<td>Rufispermae</td>
<td><em>E. ceratocorys</em></td>
<td>Symphyomyrtus</td>
<td>Dumaria</td>
<td>Incrassatae</td>
<td>73</td>
<td>10</td>
<td>5</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><em>E. lesouefii</em></td>
<td>Symphyomyrtus</td>
<td>Dumaria</td>
<td>Rufispermae</td>
<td><em>E. corrugata</em></td>
<td>Symphyomyrtus</td>
<td>Dumaria</td>
<td>Corrugatae</td>
<td>74</td>
<td>62</td>
<td>186</td>
<td>154</td>
<td>43</td>
</tr>
<tr>
<td><em>E. lesouefii</em></td>
<td>Symphyomyrtus</td>
<td>Dumaria</td>
<td>Rufispermae</td>
<td><em>E. incerata</em></td>
<td>Symphyomyrtus</td>
<td>Dumaria</td>
<td>Corrugatae</td>
<td>34</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>E. lesouefii</em></td>
<td>Symphyomyrtus</td>
<td>Dumaria</td>
<td>Rufispermae</td>
<td><em>E. straticayx</em></td>
<td>Symphyomyrtus</td>
<td>Dumaria</td>
<td>Rufispermae</td>
<td>35</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>E. lesouefii</em></td>
<td>Symphyomyrtus</td>
<td>Dumaria</td>
<td>Rufispermae</td>
<td><em>E. concinna</em></td>
<td>Symphyomyrtus</td>
<td>Dumaria</td>
<td>Corrugatae</td>
<td>37</td>
<td>81</td>
<td>134</td>
<td>119</td>
<td>26</td>
</tr>
<tr>
<td><em>E. lesouefii</em></td>
<td>Symphyomyrtus</td>
<td>Dumaria</td>
<td>Rufispermae</td>
<td><em>E. stotei</em></td>
<td>Symphyomyrtus</td>
<td>Dumaria</td>
<td>Tetrapertae</td>
<td>82</td>
<td>33</td>
<td>34</td>
<td>25</td>
<td>12</td>
</tr>
<tr>
<td><em>E. lesouefii</em></td>
<td>Symphyomyrtus</td>
<td>Dumaria</td>
<td>Rufispermae</td>
<td><em>E. tetraptera</em></td>
<td>Symphyomyrtus</td>
<td>Dumaria</td>
<td>Tetrapertae</td>
<td>34</td>
<td>53</td>
<td>57</td>
<td>47</td>
<td>19</td>
</tr>
<tr>
<td><em>E. lesouefii</em></td>
<td>Symphyomyrtus</td>
<td>Dumaria</td>
<td>Rufispermae</td>
<td><em>E. torquata</em></td>
<td>Symphyomyrtus</td>
<td>Dumaria</td>
<td>Torquatet</td>
<td>91</td>
<td>25</td>
<td>84</td>
<td>63</td>
<td>18</td>
</tr>
<tr>
<td><em>E. lesouefii</em></td>
<td>Symphyomyrtus</td>
<td>Dumaria</td>
<td>Rufispermae</td>
<td><em>E. forrestiana ssp. dolicherophyha</em></td>
<td>Symphyomyrtus</td>
<td>Dumaria</td>
<td>Tetrapertae</td>
<td>51</td>
<td>11</td>
<td>18</td>
<td>7</td>
<td>0</td>
</tr>
</tbody>
</table>

Pollination family Preissianae

| *E. preissiana ssp. lobata* | Eucalyptus | Longistyli | Preissiana | *E. coronata* | Eucalyptus | Longistyli | Preissiana | 74 | 3 | 42 | npy | - |

*nh = not harvested yet
**npy = not planted yet
3. Evaluation of hybrids

3.1. Development of selection criteria
As large numbers of hybrids will reach reproductive maturity in any given time period, it is vital to have a quick method of selecting superior individuals. Thus, a two-phase selection protocol was developed, based on morphological and phenological characters (primary selection criteria) and performance characters (secondary selection criteria). As hybrids reach reproductive maturity, they can be quickly assessed using primary selection criteria for characters that make them superior for ornamental horticulture (e.g. floriferousness, bud and flower colour, leaf shape). Those selected can be further assessed using secondary selection criteria. These criteria are based on characters such as response to postharvest, clonal propagation, production and the overall marketability of the stems. Individuals that exhibit superior performance characters will be trialled extensively prior to commercial release. An additional benefit of the two-phase selection protocol is that it will ensure that assessment is standardised, reducing subjectivity and enabling uniform selection across different flowering seasons and at different locations.

The two-phase selection protocol enabled the rapid and efficient selection of individuals with characters suitable for ornamental horticulture (Delaporte & Sedgley 2004).

3.2. Selection and evaluation of hybrids

3.2.1. Introduction
Plants developed during the interspecific hybridisation programme were assessed at reproductive maturity for primary selection criteria.

3.2.2. Methodology
3.2.2.1. Primary selection criteria
Plants were individually assessed when one third of the flowers on the tree were open. Field measurements were made of each plant and digital images taken. A sample of buds, flowers stems and leaves were taken to the lab for further measurement. All data for each plant was recorded (refer Appendix B field score sheet) and data summarised for score calculation and comparison. Plants achieving a score greater than 75% were selected and a fresh sample sent to the industry partners for marketability assessment. The primary selection criteria score and the industry collaborator scores were collated and compared, and a final selection made of superior individuals to move into testing with the secondary selection criteria.

3.2.2.2. Secondary selection criteria
Plants are assessed for vase life (refer chapter 6.2), clonal propagation (refer chapter 5.2 and 5.3.) and marketability (refer chapter 7).

3.2.3. Results
3.2.3.1. Primary selection
Of the 577 hybrids available for assessment during the project, 574 have been assessed using the primary selection criteria. Of these, 36 were selected as superior, 363 required further observation and 175 were not suitable for selection or were dead. Fresh samples of the 36 selected plants were sent to the industry partners for comment, and a final selection of 12 individuals made. A summary of each of these can be found in Appendix C. A further 686 hybrids have been planted in the Laidlaw Plantation, but these had not reached reproductive maturity by project end.

The colours described in the assessment are adapted from the Royal Horticultural Society colour charts (1988) and are explained in Appendix D.
3.2.3.2. Secondary selection
As each of the superior individuals were selected for their primary criteria, they moved into the second phase of selection. The response to individuals tested so far are described in chapters 4, 6 and 7, with information for each selected hybrid listed in Appendix C.

3.2.4. Discussion
The development of a two phase selection protocol, encompassing selection for morphological and phenological characters first, then testing of selected individuals for performance characters, has enabled the speedy and effective assessment of hundreds of hybrids in a short period of time. The method has been refined with use, and now represents a system that can be used over different years by different people, at different locations. Data collected can be compared, allowing for a more uniform, repeatable and systematic assessment of hybrid individuals or superior genotypes, to ensure that only the very best progress to the secondary selection criteria, for more intensive assessment of performance characters.

The two-phase protocol was very useful during this project, and has meant that from over 500 possible candidates, only those with the highest rating and marketability tested for secondary characters.
4. Pruning and training

4.1. Trial at the University of Adelaide

4.1.1. Introduction
Eucalypts must be physiologically mature to produce buds, therefore coppicing, as used for foliage eucalypts, is not a suitable method of pruning to stimulate new shoots for bud production. Eucalypts produce new buds soon after, or during, flowering, so it is important to know when is the correct time to prune so as not to remove potentially harvestable stems. It is also important to know how long the stems take to produce buds, and whether this varies with time of pruning. Training to different shapes and pruning to different heights could be important in maximising stem number and length.

There is a need for proven information on optimal pruning and training regimes for the wide range of *Eucalyptus* species grown throughout Australia. Such trials were conducted initially in the Laidlaw Plantation. The Laidlaw plantation, est. 1994, contains a range of plants, with ten different species of eucalypt planted for cut bud and flower production. This growth trial became part of the pruning and training objective of project UA-52A.

The aim of the trial at the University of Adelaide was to determine, on a preliminary basis, the response of eucalypt species for cut bud production to different pruning treatments. Eucalypts generally change from juvenile to mature between node 20 and 30, or around 1-1.5 m from the lignotuber (Pryor 1976), so it is important to prune to a height that maintains maturity. Previous studies into pruning height for foliage production indicated that pruning between 60 cm and 1 m will produce the most number of stems compared to pruning at lower heights (Wirthensohn & Sedgley 1998). No information is currently available regarding pruning for cut bud and flower production, however it was determined from discussion with industry that pruning must occur to a minimum height of 100 cm.

4.1.2. Methodology

4.1.2.1. Plant material
The 10 species from the Laidlaw Plantation involved in the trial were *E. anceps*, *E. erythrocorys*, *E. forrestiana* subsp. *dolichorhyncha*, *E. leptophylla*, *E. lesouefii*, *E. stoatei*, *E. tetragona*, *E. uncinata*, *E. yalatensis* and *E. youngiana*. Trees were pruned straight after flowering, necessitating three pruning times: first pruning November 2000 (*E. anceps*, *E. youngiana*, *E. yalatensis*, *E. uncinata* and *E. leptophylla*); second pruning in January 2001 (*E. forrestiana*, *E. stoatei* and *E. tetragona*) and third pruning in late March 2001 (*E. erythrocorys* and *E. lesouefii*). Pruning after flowering reflects current practices, where plants are pruned whilst harvesting product.

4.1.2.2. Treatments
The four treatments were:

<table>
<thead>
<tr>
<th>Pruning</th>
<th>Detail</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Unpruned</td>
<td></td>
</tr>
<tr>
<td>Cube</td>
<td>All branches cut back to create a 1m cube</td>
<td></td>
</tr>
<tr>
<td>Flat</td>
<td>Branches removed to a height of 1m, but not into a cube</td>
<td></td>
</tr>
<tr>
<td>Thin</td>
<td>Approximately 1/3 of all branches removed</td>
<td></td>
</tr>
</tbody>
</table>

4.1.2.3. Statistical design
The plantation was set up as a randomised complete block design (RCBD) in which four different pruning treatments were tested on ten different *Eucalyptus* species at 3 time points. The trial consisted of five blocks with each *Eucalyptus* species represented in each. Comparisons between each species were not required, so each species was examined separately. Within each block were four trees of each species. One of the four pruning treatments was randomly applied to each tree.
4.1.2.4. Assessments

Initial measurements were on all 10 species at 4, 8 and 24 weeks post prune. Measurements were finalised on three species at reproductive maturity, when all stems were assessed. The remaining 7 species were empirically assessed.

The initial measurements were:

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>presence of nodes shooting at base</td>
<td>yes/no</td>
</tr>
<tr>
<td>presence of nodes shooting on cut wood</td>
<td>yes/no</td>
</tr>
<tr>
<td>presence of nodes shooting on existing or uncut wood</td>
<td>yes/no</td>
</tr>
<tr>
<td>actual maximum length of shoots on each tree</td>
<td>mm</td>
</tr>
<tr>
<td>number of shoots on each tree</td>
<td>0 = none, 1 = &lt;10 (few), 2 = &lt;20 (some), 3 = &lt;50 (lots), 4 = &gt;50 (many)</td>
</tr>
</tbody>
</table>

The final measurements were:

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>stem diameter</td>
<td>&lt;0.5cm, 0.5-1.0cm, 1.0-1.5cm, &gt;1.5cm</td>
</tr>
<tr>
<td>average number of umbels per stem</td>
<td>count</td>
</tr>
<tr>
<td>average number of stems</td>
<td>count</td>
</tr>
<tr>
<td>measured stem length</td>
<td>mm</td>
</tr>
<tr>
<td>length of stem to buds</td>
<td>&lt;30 cm, 30 - 49 cm, 50 – 68 cm, 70 – 99 cm, &gt;99 cm</td>
</tr>
<tr>
<td>new growth</td>
<td>yes/no</td>
</tr>
</tbody>
</table>

The number of months taken to reach reproductive maturity after pruning was also recorded for all species.

4.1.3. Results

4.1.3.1. Initial trial

Some species are mallee types, with more than one main stem, while others have one stem only. This impacted on the regrowth that occurred immediately after pruning. As the control treatment received no pruning, the stems were much longer than those measured for all other treatments. The position of shooting nodes was assessed, and it was determined that when pruned, nodes at the cut point or immediately below will re-shoot. In some cases the nodes on the lignotuber produced shoots, but this was not common. Growth continued from uncut stems regardless of treatment. There was little difference between treatments in number of new shoots or the rate of growth.

4.1.3.2. Final assessments

Three species, *E. yalatensis*, *E. tetragona* and *E. leptophylla*, were assessed at flowering for their regrowth after pruning. The remaining species were not assessed due to the erratic nature of their time to reproductive maturity. The data showed that the control treatment, while producing more stems than the pruning treatments, produced much shorter stems, with most being <30cm and therefore not saleable. No consistent trends became evident between the treatments for the three species due to the inherent variation within the population. Observation indicated that the pruning treatments of “cube” and “flat” produced many more stems than the other treatments, and these stems were much longer. The number of umbels on each stem was not affected by treatment.

4.1.3.3. Time to reproductive maturity

The 10 species could be divided into three flowering types:

- **Type 1**: macroscopic bud formation occurs on 1 year old growth, buds develop to maturity over a 12 month period while the stem continues to grow past the flower bud, new buds are set on this growth and the cycle continues;
- **Type 2**: macroscopic bud formation occurs on 1 year old growth, buds develop rapidly and flower 8 to 12 weeks later, as a result there is no over growth; and
- **Type 3**: macroscopic bud formation occurs on 1 year old growth, then buds continue to form and develop to flowering at regular intervals throughout the year, resulting in a stem that may have immature buds, mature buds, flowers and fruit on it at one time, there is usually a peak in flowering at a specific time of year.
E. yalatensis, E. youngiana, E. lesouefii, E. aniceps, E. uncinata and E. leptophylla were of Type 1. Of the six Type 1 species, E. yalatensis, E. youngiana and E. leptophylla produced buds on new growth approximately 12 months after pruning. Buds developed to maturity and flowered 12 months later. None of the E. uncinata plants had produced buds prior to pruning and did not produce any in the duration of the trial. E. aniceps was sporadic in bud production, with less than half of the plants producing buds during the trial. E. lesouefii produced buds within 30 months of pruning and flowered 6 months later. However, the response of this species to pruning was sporadic, with not all plants producing buds in the duration of the trial.

E. tetragona and E. erythrocorys are from Type 2. E. tetragona produced many buds on new stems 22 months after pruning, and flowered 2 months later. E. erythrocorys took 34 months to produce buds, flowering 2 to 6 months later, and not all plants had reached reproductive maturity at the end of the trial.

E. forrestiana and E. stoatei are from Type 3. Buds were produced on most plants from both species within 20 months of pruning, however new growth and buds were continually being produced, resulting in stems with immature buds, mature buds, flowers and fruit at the same time. Peak flowering for both species was in early summer.

The control plants for each species continued to grow and flower each year, resulting in very short stems.

4.1.4. Discussion
Training and pruning is a very important part of the production of eucalypts, however, due to the wide range of climates throughout Australia where eucalypts are grown commercially, it may be difficult to extrapolate information gathered from trials in Adelaide to plantations elsewhere. It is also difficult find significant effects, differences or interactions with this pruning trial, as the inherent variation within the seedling grown populations resulted in high variation between trees, confounding the results. However, observations suggested that for older plants of most species, a severe prune to 1 m in height is required to stimulate vigorous regrowth and to maximise productivity and length of new flowering stems. Severe pruning will result in a 24 month delay to next flowering, and the development of a rotational harvest system may be required to maximise the benefits of heavy pruning and the resulting biennial production.
4.2. Trials at Calperum Station, Bookmark Biosphere Reserve

4.2.1. Introduction

Based on the initial results from trials at University of Adelaide, two trials were implemented at Calperum Station, Bookmark Biosphere Reserve, Renmark, SA: one to test different pruning heights on four species and the other to assess the effect of pruning season on regrowth and time to flowering on a range of species. Previous studies have indicated that pruning in spring produces more regrowth than pruning in autumn (Wildy et al. 2003; Wirthensohn & Sedgley 1998), however the effect on flowering has not been documented.

4.2.2. Methodology

4.2.2.1. Trial 1: effect of pruning height on 4 species

The aim of this trial was to test three different pruning treatments on four Eucalyptus species located at the Hort Block, Calperum Station. Species used were E. tetragona (42 plants), E. stoatei (70 plants), E. pterocarpa (9 plants) and E. megacornuta (69 plants). The three pruning treatments tested were P1: remove central leader to a height of 1.0 m; P2: remove central leader to a height of 1.3 m; and P3: prune to simulate harvest (approx 1/3 flowering material removed).

4.2.2.2. Trial 2: effect of pruning season on regrowth

The second trial aimed to test one pruning treatment at two different seasons on a number of young Eucalyptus species. Thirteen species were incorporated into the trial, with numbers of each ranging from 9 to 96 individuals, and are listed below.

- E. erythronema (55)
- E. pyriformis (9)
- E. caesia (19)
- E. macrandra (35)
- E. macrocarpa (18)
- E. orbifolia (37)
- E. youngiana (96)
- E. forrestiana (43)
- E. tetraptera (26)
- E. websteriana (21)
- E. gillii (20)
- E. stricklandii (58)
- E. macrocarpa (18)

The species used in the first trial were not used in the second trial, as they were at the point where they were producing harvestable stems, and were left for commercial harvest.

The three treatments were

- Spring: cut all branches to a height of 1.0 m in late spring;
- Autumn: cut all branches to a height of 1.0 m in early autumn; and
- Control: where no pruning was applied, enabling a comparison of treatments.

Equal numbers of plants from each species were treated with each of the three treatments. Observations were made every four months until project end.

Assessments were made of the location of shooting nodes, the number of shoots at each node, the minimum and maximum lengths of shoots at the node. Observations were made of any damage to the trees from insects or environmental stresses, also for the onset of macroscopic bud production.

4.2.3. Results

4.2.3.1. Trial 1: effect of pruning height on four species

Observations were made at 6 and 12 weeks after pruning, however, no discernable or statistically significant differences between treatments were observed. The trees did not re-shoot at the cut points as expected. The conclusion was reached that eucalypts require a more severe prune to stimulate new vigorous growth.
4.2.3.2. Trial 2: effect of pruning season on regrowth

The data from this trial is summarised in Table 3. The data indicated that each species responded differently to the treatments, depending on the health and maturity of the individuals involved. Some general trends could be ascertained from the results:

- species pruned in spring produce more regrowth more quickly than those pruned in autumn,
- those pruned in spring had begun to show evidence of reproductive maturity,
- many plants not were big enough to be pruned, thus reducing the number of plants that could be assessed;
- there is a possible effect of environment on regrowth, as the weather conditions and soil type at Calperum Station are different to those in Adelaide.

4.2.4. Discussion

A recent study (Wildy et al. 2003) investigated the effect of coppicing on biomass of mallee eucalypts in the wheatbelt area of Western Australia. The study found that trees coppiced in spring grew much faster than those coppiced in late summer – early autumn. All trees showed fast growth rates into the 3rd year after coppicing, however, the root system of all plants did not show significant secondary root regrowth for over 2 years after cutting. The data from this study suggests that trees should be cut on rotation lengths of not less than 2-3 years to maximise above ground biomass and root regrowth, and that the vigour of the tree prior to pruning is more likely to effect the rate and volume of regrowth than the age. The conclusions drawn from this study may provide insights into the pruning regimes required to maximise production of cut stems from eucalypt species.

The Calperum trial involved a comprehensive list of species, of varying types from low growing mallees to tall single stemmed trees. All trees were 2-3 years old at the commencement of the trial, much younger than those used in the University of Adelaide trial. Original heights varied from 30 cm to 3 m, depending on species, tree health and age. The results from the trial suggest that pruning in spring produced a greater number of stems more quickly than those pruned in autumn. This may be due to the trees being in an active growth phase during the spring prune, while those pruned in autumn may have been entering a dormant phase.

The majority of the trees were not reproductively mature at the commencement of the trial, and by the end of the assessments only some trees had produced macroscopic buds. As a result, it is difficult to ascertain the effect of pruning on time to reproductive maturity, although in some species some control trees had begun to produce buds.

Understanding the physiology of each species and genotype would be beneficial, such as time to macroscopic bud appearance, age of wood required to produce buds, limiting new growth beyond the buds, enhancing bud production, time of pruning, biennial bearing regimes, tree versus mallee regrowth potential.
Table 3: Data from Calperum Station training trial: percentage of plants responding to different variables.

<table>
<thead>
<tr>
<th>Species</th>
<th>4 months post prune</th>
<th>8 months post prune</th>
<th>12 months post prune</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. caesia spp.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td># plants for each</td>
<td>Spring</td>
<td>Autumn</td>
<td>Control</td>
</tr>
<tr>
<td>in total</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>that were treated</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>that were not treated as &lt;1m</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>that responded with new shoots</td>
<td>83.33</td>
<td>33.33</td>
<td>100.00</td>
</tr>
<tr>
<td>that didn’t respond</td>
<td>16.67</td>
<td>66.67</td>
<td>0.00</td>
</tr>
<tr>
<td>with shoots growing from:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cut point to upper 50 cm</td>
<td>83.33</td>
<td>33.33</td>
<td>0.00</td>
</tr>
<tr>
<td>continued from uncut shoot</td>
<td>0.00</td>
<td>0.00</td>
<td>100.00</td>
</tr>
<tr>
<td>E. forrestiana spp.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td># plants for each</td>
<td>Spring</td>
<td>Autumn</td>
<td>Control</td>
</tr>
<tr>
<td>in total</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>that were treated</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>that were not treated</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>that responded with new shoots</td>
<td>80.00</td>
<td>81.82</td>
<td>100.00</td>
</tr>
<tr>
<td>that didn’t respond</td>
<td>20.00</td>
<td>18.18</td>
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</tr>
<tr>
<td>with shoots growing from:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cut point to upper 50 cm</td>
<td>80.00</td>
<td>81.82</td>
<td>0.00</td>
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<tr>
<td>continued from uncut shoot</td>
<td>0.00</td>
<td>0.00</td>
<td>100.00</td>
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<tr>
<td>E. macrandra</td>
<td></td>
<td></td>
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<tr>
<td># plants for each</td>
<td>Spring</td>
<td>Autumn</td>
<td>Control</td>
</tr>
<tr>
<td>in total</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>that were treated</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>that were not treated</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>that responded with new shoots</td>
<td>100.00</td>
<td>75.00</td>
<td>100.00</td>
</tr>
<tr>
<td>that didn’t respond</td>
<td>0.00</td>
<td>25.00</td>
<td>0.00</td>
</tr>
<tr>
<td>with shoots growing from:</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>cut point to upper 50 cm</td>
<td>100.00</td>
<td>75.00</td>
<td>0.00</td>
</tr>
<tr>
<td>continued from uncut shoot</td>
<td>0.00</td>
<td>0.00</td>
<td>100.00</td>
</tr>
<tr>
<td>E. orbifolia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td># plants for each</td>
<td>Spring</td>
<td>Autumn</td>
<td>Control</td>
</tr>
<tr>
<td>in total</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>that were treated</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>that were not treated</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>that responded with new shoots</td>
<td>0.00</td>
<td>100.00</td>
<td>0.00</td>
</tr>
<tr>
<td>that didn’t respond</td>
<td>0.00</td>
<td>100.00</td>
<td>0.00</td>
</tr>
<tr>
<td>with shoots growing from:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cut point to upper 50 cm</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>continued from uncut shoot</td>
<td>0.00</td>
<td>0.00</td>
<td>100.00</td>
</tr>
<tr>
<td># plants with presence of</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>new growth</td>
<td>100.00</td>
<td>75.00</td>
<td>100.00</td>
</tr>
<tr>
<td>flower buds</td>
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<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>open flowers</td>
<td>0.00</td>
<td>0.00</td>
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</tr>
<tr>
<td>fruit</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>
### E. sp. (E. transcontinentalis or E. socialis or unknown)

<table>
<thead>
<tr>
<th># plants for each</th>
<th>Spring</th>
<th>Autumn</th>
<th>Control</th>
<th>Spring</th>
<th>Autumn</th>
<th>Control</th>
<th>Spring</th>
<th>Autumn</th>
<th>Control</th>
<th>Spring</th>
<th>Autumn</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>in total</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>that were treated</td>
<td>58.06</td>
<td>96.67</td>
<td>100.00</td>
<td>58.06</td>
<td>96.67</td>
<td>100.00</td>
<td>58.06</td>
<td>96.67</td>
<td>100.00</td>
<td>58.06</td>
<td>96.67</td>
<td>100.00</td>
</tr>
<tr>
<td>that were not treated</td>
<td>41.94</td>
<td>3.33</td>
<td>0.00</td>
<td>41.94</td>
<td>3.33</td>
<td>0.00</td>
<td>41.94</td>
<td>3.33</td>
<td>0.00</td>
<td>41.94</td>
<td>3.33</td>
<td>0.00</td>
</tr>
<tr>
<td>that responded with new shoots</td>
<td>45.16</td>
<td>6.67</td>
<td>100.00</td>
<td>48.39</td>
<td>36.67</td>
<td>100.00</td>
<td>54.84</td>
<td>36.67</td>
<td>100.00</td>
<td>54.84</td>
<td>36.67</td>
<td>100.00</td>
</tr>
<tr>
<td>that didn’t respond</td>
<td>12.90</td>
<td>90.00</td>
<td>0.00</td>
<td>9.68</td>
<td>60.00</td>
<td>0.00</td>
<td>3.23</td>
<td>60.00</td>
<td>0.00</td>
<td>3.23</td>
<td>60.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

**with shoots growing from:**

<table>
<thead>
<tr>
<th></th>
<th>cut point to upper 50 cm</th>
<th>continued from uncut shoot</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>45.16</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>6.67</td>
<td>100.00</td>
</tr>
<tr>
<td></td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

### E. tetrapiera

<table>
<thead>
<tr>
<th># plants for each</th>
<th>4 months post prune</th>
<th>8 months post prune</th>
<th>12 months post prune</th>
</tr>
</thead>
<tbody>
<tr>
<td>in total</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>that were treated</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>that were not treated</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>that responded with new shoots</td>
<td>37.50</td>
<td>90.00</td>
<td>100.00</td>
</tr>
<tr>
<td>that didn’t respond</td>
<td>62.50</td>
<td>10.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

**with shoots growing from:**

<table>
<thead>
<tr>
<th></th>
<th>cut point to upper 50 cm</th>
<th>continued from uncut shoot</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>37.50</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>90.00</td>
<td>100.00</td>
</tr>
<tr>
<td></td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

### E. youngiana

<table>
<thead>
<tr>
<th># plants for each</th>
<th>4 months post prune</th>
<th>8 months post prune</th>
<th>12 months post prune</th>
</tr>
</thead>
<tbody>
<tr>
<td>in total</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>that were treated</td>
<td>87.13</td>
<td>75.76</td>
<td>100.00</td>
</tr>
<tr>
<td>that were not treated</td>
<td>21.88</td>
<td>24.24</td>
<td>0.00</td>
</tr>
<tr>
<td>that responded with new shoots</td>
<td>50.00</td>
<td>9.09</td>
<td>100.00</td>
</tr>
<tr>
<td>that didn’t respond</td>
<td>28.13</td>
<td>66.67</td>
<td>0.00</td>
</tr>
</tbody>
</table>

**with shoots growing from:**

<table>
<thead>
<tr>
<th></th>
<th>cut point to upper 50 cm</th>
<th>continued from uncut shoot</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>9.09</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

### E. thymifolia

<table>
<thead>
<tr>
<th># plants for each</th>
<th>4 months post prune</th>
<th>8 months post prune</th>
<th>12 months post prune</th>
</tr>
</thead>
<tbody>
<tr>
<td>in total</td>
<td>65.00</td>
<td>95.00</td>
<td>100.00</td>
</tr>
<tr>
<td>that were treated</td>
<td>35.00</td>
<td>5.00</td>
<td>0.00</td>
</tr>
<tr>
<td>that were not treated</td>
<td>50.00</td>
<td>40.00</td>
<td>100.00</td>
</tr>
<tr>
<td>that responded with new shoots</td>
<td>15.00</td>
<td>55.00</td>
<td>0.00</td>
</tr>
<tr>
<td>that didn’t respond</td>
<td>45.00</td>
<td>40.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

**with shoots growing from:**

<table>
<thead>
<tr>
<th></th>
<th>cut point to upper 50 cm</th>
<th>continued from uncut shoot</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>45.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>40.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

### E. crenulata

<table>
<thead>
<tr>
<th># plants for each</th>
<th>4 months post prune</th>
<th>8 months post prune</th>
<th>12 months post prune</th>
</tr>
</thead>
<tbody>
<tr>
<td>in total</td>
<td>50.00</td>
<td>40.00</td>
<td>100.00</td>
</tr>
<tr>
<td>that were treated</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>that were not treated</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>that responded with new shoots</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>that didn’t respond</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

**with shoots growing from:**

<table>
<thead>
<tr>
<th></th>
<th>cut point to upper 50 cm</th>
<th>continued from uncut shoot</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50.00</td>
<td>0.00</td>
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<tr>
<td></td>
<td>40.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

**with shoots growing from:**

<table>
<thead>
<tr>
<th></th>
<th>cut point to upper 50 cm</th>
<th>continued from uncut shoot</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.00</td>
<td>0.00</td>
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<tr>
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<tr>
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<tr>
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<td>0.00</td>
</tr>
<tr>
<td></td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>
### E. Gillii

<table>
<thead>
<tr>
<th></th>
<th>4 months post prune</th>
<th>6 months post prune</th>
<th>12 months post prune</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong># plants for each</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>in total</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>that were treated</td>
<td>57.14</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>that were not treated</td>
<td>42.86</td>
<td>0.00</td>
<td>100.00</td>
</tr>
<tr>
<td>that responded</td>
<td>57.14</td>
<td>33.33</td>
<td>100.00</td>
</tr>
<tr>
<td>that didn’t respond</td>
<td>0.00</td>
<td>66.67</td>
<td>0.00</td>
</tr>
</tbody>
</table>

**with shoots growing from:**

- cut point to upper 50 cm 57.14 33.33 0.00 57.14 83.33 0.00
- continued from uncut shoot 0.00 0.00 100.00 0.00 0.00 100.00

### E. macrocarpa

<table>
<thead>
<tr>
<th></th>
<th>4 months post prune</th>
<th>6 months post prune</th>
<th>12 months post prune</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong># plants for each</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>in total</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>that were treated</td>
<td>20.00</td>
<td>16.67</td>
<td>100.00</td>
</tr>
<tr>
<td>that were not treated</td>
<td>80.00</td>
<td>83.33</td>
<td>0.00</td>
</tr>
<tr>
<td>that responded</td>
<td>16.67</td>
<td>100.00</td>
<td>0.00</td>
</tr>
<tr>
<td>that didn’t respond</td>
<td>0.00</td>
<td>0.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>

**with shoots growing from:**

- cut point to upper 50 cm 20.00 0.00 0.00 20.00 0.00 0.00
- continued from uncut shoot 0.00 0.00 100.00 0.00 0.00 100.00

### E. pyriformis

<table>
<thead>
<tr>
<th></th>
<th>4 months post prune</th>
<th>6 months post prune</th>
<th>12 months post prune</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong># plants for each</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>in total</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>that were treated</td>
<td>0.00</td>
<td>0.00</td>
<td>100.00</td>
</tr>
<tr>
<td>that were not treated</td>
<td>0.00</td>
<td>0.00</td>
<td>100.00</td>
</tr>
<tr>
<td>that responded</td>
<td>0.00</td>
<td>0.00</td>
<td>100.00</td>
</tr>
<tr>
<td>that didn’t respond</td>
<td>0.00</td>
<td>0.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>

**with shoots growing from:**

- cut point to upper 50 cm 25.00 50.00 0.00 25.00 50.00 0.00
- continued from uncut shoot 0.00 0.00 100.00 0.00 0.00 100.00

### E. striklandii

<table>
<thead>
<tr>
<th></th>
<th>4 months post prune</th>
<th>6 months post prune</th>
<th>12 months post prune</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong># plants for each</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>in total</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>that were treated</td>
<td>0.00</td>
<td>0.00</td>
<td>100.00</td>
</tr>
<tr>
<td>that were not treated</td>
<td>82.35</td>
<td>52.94</td>
<td>100.00</td>
</tr>
<tr>
<td>that responded</td>
<td>17.65</td>
<td>47.06</td>
<td>0.00</td>
</tr>
<tr>
<td>that didn’t respond</td>
<td>0.00</td>
<td>0.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>

**with shoots growing from:**

- cut point to upper 50 cm 82.35 52.94 0.00 100.00 64.71 0.00
- continued from uncut shoot 0.00 0.00 100.00 0.00 0.00 100.00

### E. fruticosum

<table>
<thead>
<tr>
<th></th>
<th>4 months post prune</th>
<th>6 months post prune</th>
<th>12 months post prune</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong># plants for each</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>in total</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>that were treated</td>
<td>0.00</td>
<td>0.00</td>
<td>100.00</td>
</tr>
<tr>
<td>that were not treated</td>
<td>0.00</td>
<td>0.00</td>
<td>100.00</td>
</tr>
<tr>
<td>that responded</td>
<td>0.00</td>
<td>0.00</td>
<td>100.00</td>
</tr>
<tr>
<td>that didn’t respond</td>
<td>0.00</td>
<td>0.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>

**with shoots growing from:**

- cut point to upper 50 cm 25.00 50.00 0.00 25.00 50.00 0.00
- continued from uncut shoot 0.00 0.00 100.00 0.00 0.00 100.00

### E. macrocarpa

<table>
<thead>
<tr>
<th></th>
<th>4 months post prune</th>
<th>6 months post prune</th>
<th>12 months post prune</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong># plants for each</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>in total</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>that were treated</td>
<td>0.00</td>
<td>0.00</td>
<td>100.00</td>
</tr>
<tr>
<td>that were not treated</td>
<td>0.00</td>
<td>0.00</td>
<td>100.00</td>
</tr>
<tr>
<td>that responded</td>
<td>0.00</td>
<td>0.00</td>
<td>100.00</td>
</tr>
<tr>
<td>that didn’t respond</td>
<td>0.00</td>
<td>0.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>

**with shoots growing from:**

- cut point to upper 50 cm 82.35 52.94 0.00 82.35 52.94 0.00
- continued from uncut shoot 0.00 0.00 100.00 0.00 0.00 100.00
<table>
<thead>
<tr>
<th></th>
<th>4 months post prune</th>
<th>8 months post prune</th>
<th>12 months post prune</th>
</tr>
</thead>
<tbody>
<tr>
<td># plants for each</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>in total</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>that were treated</td>
<td>20.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>that were not treated as &lt;1m</td>
<td>80.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>that responded with new shoots</td>
<td>20.00</td>
<td>25.00</td>
<td>100.00</td>
</tr>
<tr>
<td>that didn’t respond</td>
<td>0.00</td>
<td>75.00</td>
<td>0.00</td>
</tr>
<tr>
<td>with shoots growing from:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cut point to upper 50 cm</td>
<td>20.00</td>
<td>25.00</td>
<td>0.00</td>
</tr>
<tr>
<td>continued from uncut shoot</td>
<td>0.00</td>
<td>0.00</td>
<td>100.00</td>
</tr>
<tr>
<td># plants with presence of</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>new growth</td>
<td>20.00</td>
<td>25.00</td>
<td>100.00</td>
</tr>
<tr>
<td>flower buds</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>open flowers</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>fruit</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>
5. Clonal Propagation

5.1. Introduction
Interspecific hybrids require vegetative propagation to ensure the propagules are identical to the original plant. Propagation techniques, such as cuttings and grafting, have been successful for a number of forestry species, as have standard methods of micropropagation. All of these methods were trialed here on various hybrid eucalypts. The development of a rapid, economic and reliable method of propagation for ornamental hybrid eucalypts is essential for their commercial development.

Vegetative propagation methods include grafting, cuttings, marcotting, budding and aerial layering and micropropagation in tissue culture. Cutting propagation requires juvenile material for successful propagation, this may not be a viable option for many ornamental trees. Grafting, marcotting, budding and aerial layering can be successful with adult material, and thus may be more useful in this project. The project investigated grafting, cuttings and micropropagation.

Previous research into clonal propagation of eucalypts for forestry lead to the development of a system for the propagation of ornamental eucalypts, where consideration for the maturity of the plant must be made. It was proposed that the systems involved 5 steps: 1) selection of superior hybrid; 2) graft superior hybrid onto suitable rootstock – referred to as the Mother Plant; 3) strike cuttings from the Mother Plant; 4) increase numbers of the selected hybrid by cuttings; and 5) investigate the selected hybrid in a PBR trial. The focus of the propagation objective became grafting of selected hybrids, and taking cuttings from these grafted plants in order to increase numbers. By developing a system such as this, plants not identified as superior until reproductively mature or of a significant size and maturity could be propagated without coppicing the tree.

Vegetative propagation by grafting can be achieved using the wedge graft, the splice graft and the whip and tongue graft. The age of the rootstock and its compatibility to the scion material are important factors to consider, as is the health of both rootstock and scion. Cutting propagation is achieved by taking pieces of juvenile stem material, wounding and treating with a root inducing plant hormone such as indole butyric acid (IBA), and placing in a humid, warm environment until roots appear.

Micropropagation involves the steps of plant collection and dis-infestation, initiation into culture, shoot growth/callus production, shoot multiplication/shoots from callus, rooting and hardening out to the environment. Somatic embryogenesis depends on callus production then embryo proliferation, development, maturation and germination. The different pathways of plant production are achieved mainly by varying the hormones used.

Clonal propagation was investigated on the superior hybrid E. ‘Urrbrae Gem’. This hybrid was selected for preliminary work as it is already recognised as a hybrid with potential for commercial development and material was readily available. Grafting, cuttings, micropropagation and somatic embryogenesis were investigated on this hybrid. The techniques developed for grafting and cuttings were tested on the hybrid families as selections of superior forms were made.

5.2. Cuttings and Grafting
5.2.1. Cuttings
Methodology developed in the forestry industry was investigated for use on the ornamental hybrid eucalypts. In most instances, juvenile material is required for successful propagation by cuttings. Genotype has a strong influence on cutting success, so selected hybrids must not only be superior morphologically, but they must also be able to be propagated by cuttings economically in order to be commercially viable.
Techniques most successful for clonal propagation of forestry species are the use of clonal hedges and mini-cuttings, although each region/nursery using the technology operationally has developed its own fine-tuning of the technique. Thus in order for this technology to be used at the University of Adelaide, it must be adapted to our conditions and facilities.

The techniques of micro-, mini- and macro- cuttings were investigated from a number of parental species and hybrids, to determine optimal conditions for cuttings from a range of species. Cutting material was taken from plants growing in Rocket Pots® (Trentcom APS Pty Ltd) in two greenhouses (at 18-25°C ± 3°C and 18°C ± 3°C), in a shadehouse (50% shade and external seasonal temperature range) and outside (external seasonal light and temperature range). Cuttings from coppiced field coppicing to 12 months after coppicing. Cutting material also ranged in number of nodes, number of leaf pairs, stem diameter and inter-nodal distance depending on the parental species. A range of concentrations of IBA and NAA were tested in a number of different freshly made and commercially available formulations. Environmental conditions were investigated by altering humidity, source of humidity, light levels, temperature, air movement, propagation media, and application of bottom heat. Seasonality was tested by taking cuttings at different times of the year.

Data from trials, information provided at the Clonal Forestry Workshop, Grafton 2004, and current forestry industry practice, indicated three elements crucial to cutting success: cutting age, cutting size and health status (nutritional and environmental) of the mother plant. Cuttings taken from coppice shoots less than 12 weeks old have a greater propensity to root than ones from older material. Small micro cuttings with healthy nodes are more likely to root than large, leggy, macro cuttings. Mother plants must have a high level of readily available nutrients to allow rapid regrowth of vigorous shoots after coppicing. The optimal environment under which the mother plant is grown varies according to species. As cutting age/cutting size/mother plant health interactions are crucial to propagation success, these aspects require ongoing investigation.

Optimal conditions for cutting propagation for the majority of plants are:
- cuttings taken between 4 and 12 weeks after coppicing, with between 2 and 4 healthy leaf pairs and a strong apical node
- cuttings treated with 3000 ppm IBA
- high humidity initially, gradually hardening off to low humidity conditions
- fogging or very fine mist is most successful
- there should be a minimum 2°C differential between the propagating medium and the surrounding air
- good hygiene is essential – fungal problems must be kept under control
- mother plants grown in rocket pots under conditions of high nutrient supply, as they improved plant health and number of shoots growing after coppicing
- species and genotype have a strong influence on rooting success

5.2.2. Grafting
Grafting has been successful for a number of ornamental eucalypt varieties, and the techniques used there were tested under Adelaide conditions and with the parental species of the families in the breeding program. Some time was needed to establish optimal methods due to the seasonality of conditions in Adelaide.

Due to limited selected superior hybrid material early in the project, preliminary trials aimed to find the best method for grafting the parent species of the hybrid families. This data could then be extrapolated to superior hybrids related to those species. The initial focus was on hybrid E. ‘Urrbrae Gem’ and the parents, E. erythronema var. erythronema and E. stricklandii. Once the majority of the artificial interspecific hybrids reached reproductive maturity and were assessed, superior selections could be moved into the programme.
Grafting trials involving selected hybrids have progressed, with each selected hybrid grafted onto parental rootstocks. Some combinations have been successful, however, others have proven difficult. Time of year is important for graft success, as is age and health of both rootstock and scion. Late spring to autumn has been identified as the optimal time to graft, when scion material is at its most vigorous. Grafting conditions of high humidity for a short period, then lower humidity for a longer period have proven most successful, but the duration at these conditions differs between families. The focus has shifted to producing many grafted plants of the selected hybrids. The grafted plants will become a clonal hedge for the collection of quantities of material suitable for cuttings.

Consultation with experts in the field has resulted in the inclusion of *E. camaldulensis* as a rootstock for all selected hybrids. Preliminary work has indicated that *E. camaldulensis* grows quickly and vigorously, resulting in the ability to graft with a higher success rate at a younger age, and thus increase throughput of grafted plants.

Optimal conditions for grafting for the majority of plants are:
- graft in late spring to autumn
- healthy vigorous rootstock less than 9 months old
- healthy scion material, firm growth with a strong apical bud
- wedge graft, maximum cambial contact
- high humidity initially, gradually hardening off to low humidity conditions

Optimal conditions for after-graft plant care were investigated. Rocket Pots® showed promise for their suitability for long term health of grafted stock plants. Four different environments were assessed for their suitability: Greenhouse 3 at 18-25°C ± 3°C, Greenhouse 13 at 18°C ± 3°C, shadehouse with 50% shadecloth and current external seasonal temperature range and Outside with current external seasonal light and temperature range. As plant response to environmental conditions varied with species, plant location may have a strong effect on cutting production from grafted plants, and this must also be considered.

5.2.3. Discussion
Successful propagation of hybrid eucalypts via both cuttings and grafting has been achieved. These techniques must now be refined for commercial production.

5.3. Tissue culture
5.3.1 Introduction
The objective was to investigate tissue culture techniques, such as somatic embryogenesis, as propagation methods for ornamental hybrid eucalypts (Arruda et al. 2000; Berney 2000; Watt et al. 2000, 2003). The research was conducted on the species *E. erythronema*, *E. stricklandii*, and hybrids between *E. erythronema* and *E. stricklandii*, such as hybrid *E. ‘Urrbrae Gem’* (Delaporte et al. 2001). Research needs to be focused towards finding optimum media, plant growth regulators and environmental conditions necessary for successful propagation, as the development of a rapid, economic and reliable method of propagation for ornamental hybrid eucalypts is essential for their commercial development. In addition, observation of tissue-cultured material at a microscopic level provided insights into organisation of callus and the development of normal and abnormal organogenesis.
5.3.2. Methodology
5.3.2.1. Plant Material
The plant material used was open pollinated seedlings of *E. erythronema*, *E. stricklandii* and *E. ‘Urrbrae Gem’*, located in the Waite Arboretum of the University of Adelaide, with limited work conducted on clonal material of *E. ‘Urrbrae Gem’* and of other *E. erythronema* x *E. stricklandii* hybrids from the breeding programme.

Three tissue culture pathways were investigated, somatic embryogenesis, organogenesis and micropropagation (Watt et al 2003).

5.3.2.2. Experiment 1: Effect of plant growth regulators on different genotypes and tissue types
Seeds of *E. erythronema*, *E. stricklandii* and *E. ‘Urrbrae Gem’* were sterilised and germinated in *vitro*. Hypocotyl, cotyledon and leaf explants were harvested from 2-3 week old seedlings and placed on MS medium. The auxins were NAA at 0, 5 and 10 µM, and 2,4-D at 0, 5 and 10 µM. The cytokinins were BAP at 0, 1, 5 µM and kinetin at 0 and 5 µM. The explants were placed on medium in 30 mL polycarbonate tubes with 10 mL medium per tube in a growth room at 24°C with 16 hours low light at 11 microeinsteins per m² per second, with 10 replicates per treatment. Data collected were callus growth, root number, shoot number and bud number and data were analysed by ANOVA.

5.3.2.3. Experiment 2: Effect of low concentration of BAP on bud and shoot regeneration from different genotypes and tissue types
Leaf and apex explants were taken from 2-3 week old seedlings of *E. erythronema*, *E. stricklandii* and *E. ‘Urrbrae Gem’*, and from *in vitro* grown shoots of *E. erythronema* x *E. stricklandii* hybrid 2.5. BAP levels were 0, 0.1, 0.25, 0.5 and 1 µM. Other conditions were as for Experiment 1.

5.3.2.4. Experiment 3: Effect of auxins on organogenesis from different genotypes and tissue types
This experiment tested a technique known to produce somatic embryos in *E. globulus* (Pinto et al 2002). Apices and 3 day old cotyledons were taken from seedlings of *E. erythronema*, *E. stricklandii* and *E. ‘Urrbrae Gem’*, and apices were taken from *in vitro* grown shoots of *E. erythronema* x *E. stricklandii* hybrid 2.5. NAA was used at 0, 4.5, 16, 26 and 80 µM for 1 - 4 weeks after which explants were subcultured to medium lacking plant growth regulators.

5.3.2.5. Experiment 4: Micropropagation
Nodal explants were taken from seedlings of *E. ‘Urrbrae Gem’* germinated in *vitro*. These were multiplied on MS medium and shoots were transferred to medium with IBA for root initiation.

5.3.3. Results
5.3.3.1. Experiment 1: Effect of plant growth regulators on different genotypes and tissue types
Organogenesis was achieved from leaf explants with the following combinations of plant growth regulators:

- **Callus**: 1 µM BAP and 5 µM NAA
- **Callus**: 5 µM BAP and 5 µM NAA
- **Buds and shoots**: 1 µM BAP alone
- **Callus**: 5 µM BAP and 5 µM NAA
- **Roots**: 5 µM NAA alone
- **Roots**: 10 µM NAA alone

Callus, roots, buds and shoots were produced, and microscopy showed that the shoots had normal meristematic and vascular organisation. No somatic embryos were produced.

Organogenesis from hypocotyls and cotyledons was varied. Low levels of BAP were effective, so the next experiment investigated even lower levels.
5.3.3.2. Experiment 2: Effect of low concentration of BAP on bud and shoot regeneration from different genotypes and tissue types
Apices were more responsive than leaves, with good shoot growth and less callus than in the previous experiment. No somatic embryos were observed.

5.3.3.3. Experiment 3: Effect of auxins on organogenesis from different genotypes and tissue types
Somatic embryos were produced, but did not develop beyond the globular stage. Shoots and roots developed producing plantlets after 1 week on 80 µM NAA.

5.3.3.4. Experiment 4. Micropropagation
*In vitro* shots were established from nodal explants. These were rooted with IBA.

5.3.4. Discussion
No previous research had been conducted on the ornamental species *E. erythronema*, *E. stricklandii*, and hybrids between *E. erythronema* and *E. stricklandii* (Watt et al. 2003). Hence, the first experiment was conducted to determine the best combination of plant growth regulators for these species. Leaf tissue was more responsive than hypocotyl, which was more responsive than cotyledon. Seedlings of *E. ‘Urrbrae Gem’* were more similar in response to the female parent *E. erythronema* than to the male parent *E. stricklandii*. They produced callus, buds, shoots and roots, whereas *E. stricklandii* produced fewer shoots. 2,4-D produced poor callus with no shoots and few roots. Kinetin was generally less effective than BAP. Because low levels of BAP were effective, it was decided to try lower levels, and to include apices in Experiment 2. Apices were more responsive than leaves, and better shoots with less callus were produced. These shoots were then rooted with IBA.

Experiment 3 followed a method shown to produce somatic embryos in *E. globulus* (Pinto et al. 2002), using high levels of NAA and 2,4-D. Cotyledons produced callus and roots and apices produced shoots and roots. Somatic embryos were produced but did not develop beyond the globular stage.

Leaves and apices were the most responsive in tissue culture. Callus from mature tissue produced only more callus and did not undergo organogenesis.

The conclusions that can be drawn from this work are that plants with shoots and roots were produced *in vitro* via organogenesis, plants with shoots and roots were produced *in vitro* via micropropagation, and somatic embryos were produced but did not develop beyond the globular stage.

Further research is required to harden off plants produced *in vitro*, and to apply the successful techniques to superior hybrids between *E. erythronema* and *E. stricklandii*. 

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6. Development of optimal postharvest solutions for eucalypt species & hybrids

6.1. Introduction
Hybrid eucalypts must have a postharvest vase life that is long enough to enable the product to reach distant markets. Treatments, such as the provision of sugar and germicides to the vase solution, can prolong vase life and in some cases enhance quality; cold storage can also prolong vase life.

Preliminary trials encompassing five treatments were trialled on stems from three species. Data from these and previous trials (Delaporte et al. 2000), was used to develop a protocol to determine vase life with minimal treatment for the testing of a large number of superior individuals. The protocol was developed after discussion with industry collaborators, Dr. Andreas Klieber and statisticians. The protocol enabled determination of vase life with water, a low sugar concentration supplied as a pulse or continuously, and a high sugar pulse. All plants that have passed primary selection are tested using this protocol.

Hybrid plants selected as superior using the primary selection criteria (Chapter 3.2) and Industry Collaborator rating systems (Chapter 7) are tested for vase life at reproductive maturity; those with a vase life of fewer than 10 days are culled from the programme, according to the secondary selection criteria.

6.2. Methodology
6.2.1. Plant material
Species testing requires many individuals from one species to be tested. A total of 8 plants for *E. tetragona* and *E. lesouefii*, and 6 for *E. yalatensis* were included in the trial. Thirty stems per plant of similar length, diameter, number of buds and number of leaves, were harvested and transported immediately to the laboratory. Here the stems were recut and six stems were randomly allocated to each treatment (Table 4). Each stem was placed in a separate bottle and assessed separately.

Fifteen to 30 stems from each selected hybrid were harvested and taken to the laboratory. A range of bud and flower maturities were present amongst the stem samples harvested. The stems were recut and five to ten stems were randomly allocated to each of three treatments (Table 5). All stems for each treatment were placed in a bucket containing the appropriate solution. The solution was changed every three days.

6.2.2. Treatments
Stems were assessed daily for the first five days, then once every two or three days. By replacing the water regularly the need for germicide within the solution was negated. The environmental conditions for the duration of all trials were 22°C room temperature and 12 hours day/night using standard fluorescent lights.
Table 4: Treatments used with E. tetragona, E. lesouefii and E. yalatensis. All species were treated with all solutions.

<table>
<thead>
<tr>
<th></th>
<th>Treatment Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Control RO water pH 3-4 CA</td>
</tr>
<tr>
<td>B</td>
<td>20% sucrose in RO water pH 3-4 CA pulse for 2 hours, then RO water pH 3-4 CA</td>
</tr>
<tr>
<td>C</td>
<td>5% sucrose in RO water pH 3-4 CA pulse for 24 hours, then RO water pH 3-4 CA</td>
</tr>
<tr>
<td>D</td>
<td>2% sucrose in RO water pH 3-4 CA continuous</td>
</tr>
<tr>
<td>E</td>
<td>dry storage at 3-4ºC for 24 hours, recut stem, then RO water pH 3-4 CA</td>
</tr>
</tbody>
</table>

Table 6: Postharvest solution treatments for Eucalyptus hybrids. All hybrids were tested with treatments A and B, and either C or D.

<table>
<thead>
<tr>
<th></th>
<th>Treatment Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Control RO water pH 3-4 CA</td>
</tr>
<tr>
<td>B</td>
<td>10% sucrose in RO water pH 3-4 CA pulse for 24 hours, then RO water pH 3-4 CA</td>
</tr>
<tr>
<td>C</td>
<td>2% sucrose in RO water pH 3-4 CA pulse for 24 hours, then RO water pH 3-4 CA</td>
</tr>
<tr>
<td>D</td>
<td>2% sucrose in RO water pH 3-4 CA continuously supplied</td>
</tr>
</tbody>
</table>

6.2.3. Measurements
At harvest stem weight, number of umbels, number of leaf pairs, colour of buds/umbels, stage of flower development (if appropriate), colour of leaves, colour of stem, and initial weight of stem were all recorded. Then, each day for 5 days, the weight of the stem and the condition of umbels and leaves were recorded, thereafter every second day until senescence.

Observations made during each measurement provided a picture of the overall rate of senescence of leaves, buds and flowers (Table 6). In some cases the leaves senesced more rapidly than the buds/flowers or vice versa, and it is important to identify the progress of degeneration of different tissues.

Table 6: Stages of bud, flower and leaf development and senescence after harvest.

<table>
<thead>
<tr>
<th>Stages of bud and flower development</th>
<th>Stages of senescence</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 = immature bud</td>
<td>6 = filaments desiccating/shriveling but not dropping</td>
</tr>
<tr>
<td>1 = mature bud</td>
<td>7 = bud browning/blackening</td>
</tr>
<tr>
<td>2 = operculum lift</td>
<td>8 = bud longitudinal or operculum split</td>
</tr>
<tr>
<td>3 = flower partially open (to ½)</td>
<td>9 = buds desiccating/softening</td>
</tr>
<tr>
<td>4 = flower fully open</td>
<td>10 = filaments wilting/collapsing but not desiccating</td>
</tr>
<tr>
<td>5 = flower (filaments) senescing and dropping to fully dropped</td>
<td>11 = filaments going brown</td>
</tr>
<tr>
<td></td>
<td>12 = nectaries going brown/black</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Leaf damage symptoms</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>wilting</td>
<td>browning or blackening</td>
</tr>
<tr>
<td>softening</td>
<td>dull and papery</td>
</tr>
<tr>
<td>spots</td>
<td>desiccating</td>
</tr>
</tbody>
</table>

6.2.4. Statistical design
For the purpose of analysis, the design of the species trial was assumed to be that of a randomised complete block design. Each species was considered for analysis separately, and analysed by analysis of variance (ANOVA) in order to test for differences between treatments. Where statistical significance between treatments was detected at the 5% level the Least Significant Difference (LSD) is used to make pair-wise comparisons between means. All analyses were performed in GenStat 6th Edition.

Vase life data from each hybrid were analysed using the data analysis component of Microsoft Excel to determine descriptive statistics such as the mean and standard error.
6.3. Results
Data indicated that a continual 2% sucrose solution is detrimental to vase life of all three species. Treating stems of *E. tetragona* with a 2 hour pulse in 20% sucrose produced a vase life of 29 days, while the control in water was 27 days. Treating stems of *E. lesouefii* with a 24 hours pulse in 5% sucrose produced a vase life of 17 days, with a control of 15 days. The vase life of *E. yalatensis* was around 13 days with all treatments.

Each individual reacts differently to the treatments tested. In over 50% of hybrids tested with control, low sugar and high sugar, the control produced the longest vase life, although the real difference between the treatments is not large. The high sugar pulse resulted in the appearance of spots and marks on the leaves of most hybrids within 24 hours, however this marking disappeared when the stems were moved into RO water. Many hybrids exhibited longitudinal bud splitting within 24 hours of harvest, and some exhibited splitting of the operculum above the join. The affected flowers continued to develop normally after the split. Any soft new growth present on the stems rapidly blackened and wilted after harvest, regardless of treatment, and did not recover.

6.4. Discussion
Eucalypt cut flowers have a poor postharvest reputation: they are perceived to be “messy” and have a “short” vase life. The hybrids tested with the 3 treatment protocol showed a range of vase life, from less than 8 days for some, to over 20 for others. The selected new varieties may therefore far outstrip perceptions of a short vase life and with further investigation of a wider range of postharvest treatments may consistently achieve a vase life over 15 days.

Stage of picking was very important to vase life and product display. Stems picked in bud rarely produced any open flowers and tended to last the longest, while those picked with some flowers already open often had all flowers open within 7 days. Others progressively opened flowers for up to a week after picking, and these flowers continued to develop normally. Understanding the stages of maturity will provide information on when to pick to maximise vase life and product marketability.

With the exception of encouraging flowers to open more quickly, sucrose in solution was more detrimental to leaves rather than flowers and buds. Continuous levels of sucrose, even at 2%, seemed to be detrimental to vase life with most individuals tested. High concentration of sucrose in short pulses initially caused damage to the leaves, however this damage was reversible and often this treatment resulted in longer a vase life when compared to the control.

Stems with very soft new growth at the tips should be avoided, as regardless of treatment or handling, the soft new growth desiccated and turned black within 24 hours of harvest. Understanding growth patterns and coordinating timing of harvest will reduce this effect.

Postharvest bud splitting, both longitudinally and around the operculum, was exhibited in a number of hybrids, regardless of treatment. The cause of this may be physiological and requires further investigation. Blackening of buds and leaves, principally observed on immature buds around oil glands, is another physiological problem requiring investigation.

The water status and general health of the plant may have an effect on vase life. Plants must be trialled in a number of different environments before release, to ensure that all aspects of cultivation are understood.
7. Marketability of selections

Assessment of marketability was assessed in three stages. Initially, due to the large numbers of hybrids that flowered over a short period of time, digital images of all flowering hybrids were sent to industry collaborators for comment, with their best selections recorded. The following year, samples of hybrids that rated well in the primary selection criteria phase (Chapter 3.2.) were sent to industry collaborators for comment, as part of the marketability assessment in the secondary selection criteria. The comments were used to make final selections. Refer to Appendix E for an example of the comment sheet. Samples of some superior hybrids were sent to Sally Sutton, AFEC, for market acceptability testing with a well-known Melbourne florist. Both industry collaborators visited the site at various times over the life of the project for a hands-on assessment of the hybrids.

Hybrids that survive the primary and secondary selection rounds are assessed for wider market acceptability. As soon as superior lines reach a point where sufficient material is available, market acceptability is assessed by sending product to the industry collaborators to disseminate to wider markets and report back.

Over the course of the project, it was found that personal, on-site observation and feedback was more useful than sending cut stems, which was more useful than sending photographs.
8. Plant Breeders Rights

8.1. Current test guidelines for eucalypts

The team is developing an understanding of the new Plant Breeders Rights requirements. The wide range of eucalypt species being utilized in this project will require a revised set of guidelines for testing. The current UPOV test guidelines are applicable to all vegetatively propagated varieties and interspecific hybrids of the species pertaining to the Sections Transversaria, Maidenaria and Exsertaria of the sub genus *Symphyomyrtus* of the genus *Eucalyptus* (Appendix F). A table of characteristics was developed for *E. gunnii* by the technical working party for ornamental plants and forest trees, Budapest, June 2000 (Appendix G). The taxonomic sections covered by both of these documents are not represented in the current breeding and development programme, and so the guidelines will have to be adapted to suit the interspecific hybrids under investigation. Additional examples can be found, such as the registered eucalypt varieties ‘Summer Beauty’ and ‘Summer Red’ (Appendix H). There may be some varieties developed from our programme in the future that can utilise these varieties as comparators. The team is in regular contact with the PBR Office and will remain in contact in the future as the hybrids are tested.
9. General Discussion

The major aim of this project is to provide the Australian wildflower industry with novel and improved varieties of *Eucalyptus* for the production of cut bud and flower stems. To achieve this aim, research was undertaken into hybridisation as a method to produce new and novel lines; pruning and training regimes for optimal production; clonal propagation methods, postharvest treatments and marketability of superior lines, and finally Plant Breeders Rights registration of new tested varieties prior to commercial release.

The eucalypt improvement and hybridisation programme has been underway at the University of Adelaide since the early 1990s, with the aim of producing interspecific hybrids with ornamental merit for the amenity horticulture and floriculture markets. Crossing programmes concentrated on species from Subgenus *Symphyomyrtus* section *Bisectae*, as well as species from Subgenus *Symphyomyrtus* section *Dumaria* and Subgenus *Corymbia*. The programme produced many hundreds of new and novel hybrids that require testing for their horticultural suitability. These hybrids were tested using a two-phase selection protocol devised to enable the rapid, repeatable testing of many hundreds of hybrids. The first phase required testing for primary selection criteria, based on morphological characters, and scored according to their number of ornamental characters. Those that score highly are assessed in the second phase, for the secondary selection criteria of vase life, clonal propagation, production requirements and marketability. Over 500 hybrids were assessed using the primary selection criteria, and 36 superior individuals were selected. Fresh material was sent to industry partners for comment, and a final 12 selected for investigation of secondary selection criteria.

Apart from providing hundreds of novel lines for selection, the hybridisation programme has produced a large valuable germplasm resource that should be maintained and protected for future work. Information generated through this programme has relevance to other areas such as eucalypt breeding for forestry and breeding with other preferentially outcrossing genera.

Training and pruning is a very important part of the production of eucalypts for floriculture, and there is a need for proven information on optimal pruning and training regimes. The trials suggest that for older plants of most species, a severe prune to 1 m height is required to stimulate vigorous regrowth and to maximise productivity and length of stems, but this will result in a 24 month delay to next flowering. The trials also showed that pruning in spring will result in more re-growth than pruning in autumn. Due to the wide range of climates throughout Australia where eucalypts are grown commercially, it may be difficult to extrapolate information gathered from these trials to plantations elsewhere. Also, the inherent variation within the seedling populations studied has resulted in empirical observations only. Understanding the physiology of each species and genotype would be beneficial.

Clonal propagation is vitally important to the production of hybrid eucalypts for commercial release, as the hybrids do not breed true from seed and thus must be propagated vegetatively. This was a major focus of the project. The techniques of grafting, cuttings and micropropagation were investigated intensively to develop optimal methods for superior hybrid genotypes. A procedure has been developed for clonal propagation of hybrids by grafting and cuttings, and will be used on the 12 superior selected hybrids. Micropropagation and organogenesis have been successful in producing plants *in vitro*, with further research required to harden off plants produced *in vitro*, and to apply the successful techniques to other superior hybrids. All mature horticultural industries are based on clonal material of superior cultivars, and this industry has a long way to go to achieve this level of sophistication.
One of the secondary selection criteria is a minimum postharvest vase life of 10 days. In order to test this, the selected hybrids were exposed to treatments of water, a high short sucrose pulse and a continuous treatment of low concentration. Most hybrids tested showed a vase life of over the minimum requirement, and some showed vase life of over 18 days, with continued floral development and maintenance of product form. The addition of sucrose to postharvest solution was found to be beneficial to some genotypes and detrimental to others. Plants so far tested are responding to treatments, with variability in response evident between both genotypes and species. Further testing is required on a wider range of treatments to determine optimal treatments for each hybrid. Studies were also conducted on material from three commercially acceptable species. The results from these studies shows that a continuous application of 2% sucrose is detrimental to vase life for most individuals. These studies highlighted the problems associated with testing a seedling derived highly variable population, where differences between genotypes were found to be more significant than those between treatments.

A number of postharvest issues were identified, including bud splitting, bud blackening and blackening of new growth. In-depth trials to assess the effect of treatments on these issues are required to provide a greater understanding of the postharvest physiology of the plants. Understanding growth patterns may also assist in reducing the effects of these issues.

Hybrids that survive the primary and secondary selection rounds are assessed for wider market acceptability. As soon as superior lines reached a point where sufficient material was available, market acceptability was assessed by sending product to Geoff Sullivan and Denis Tricks to disseminate to wider markets and report back. Personal observation and feedback regarding hybrids was found to be more useful than sending cut stems for comment, which in turn was more useful than sending photographs.

Hybrids/varieties that remain superior through all selection criteria will be trialled for distinctness, uniformity and stability, for eventual Plant Breeders Rights Registration. Current UPOV guidelines may require re-writing to suit the new hybrids.

Eucalypts will grow in a wide range of soil types and water qualities, and the development and proving of novel lines, along with propagation, production and postharvest information for optimum quality, will expand current production areas enormously into the inland irrigated areas across all mainland states. A full fertilizer, pruning and watering regime will see the hybrids produce at full capacity, but will also grow and produce with minimal attention. The benefits will flow to the Australian floriculture industry, to the depressed inland rural areas and to the economy of the country in improved export performance. Collaboration with industry leaders Longford Flowers and Ausbuds will ensure the success of the technology transfer.
10. Implications

The current demand for eucalypt bud and flower products has provided the stimulus for the recent investment in research into development of new varieties. The eucalypt sector of the floriculture industry stands poised to expand significantly, particularly in the area of export markets, with the new varieties arising from this project. The new varieties should be supported by best cultivation management practices and optimal postharvest treatments to ensure high-grade product reaches the destination markets.

Advancements in the area of clonal propagation of eucalypts will have benefits across the board, where recalcitrant woody plants require propagation. Information about compatibility between species will augment known information in this area.
11. Recommendations

The superior hybrid individuals identified during the current project must be investigated for their response to cultivation treatments and production systems before new cultivars are registered with the PBR office and recommendations made to the industry. Further investigation into cultural and production practices on a range of eucalypt species and hybrids at different sites should occur to support the information offered in this report.

Investigation into clonal propagation methods, primarily micropropagation, grafting and cuttings, should continue, to augment the findings in these areas from this study: dissemination of this information will assist forestry and ornamental nursery industries, as well as production of trees for land reclamation and biodiversity preservation, while benefiting the Australian wildflower industry by enabling the production of clonal plants for plantation production.

Project findings will be reported in various scientific and industry publications, as well as presentations at industry conferences.
Appendices

Appendix A: Interspecific eucalypt crosses that have, or may, produce red buds or flowers from December to March.

Appendix B: Primary selection criteria, single plant sheet (refer to Chapter 3.1.3).

Appendix C: Summary sheets of Top 12 hybrids.

Appendix D: RHS Colour Charts (Royal Horticultural Society, London, 1988) colours measured during hybrid assessments (refer to Ch 3.2.2 and 3.2.3.).

Appendix E: Example of comment sheet sent to industry collaborators for comment on superior selected hybrids.

Appendix F: Summary of information from UPOV working paper on test guidelines for Eucalyptus (sub genus Symphyomyrtus) (sections Transversaria, Maidenaria, Exsertaria).

Appendix G: Summary of Table of Characteristics for E. gunnii from the UPOV guidelines developed in 2000 by the UPOV Technical Working Party for Ornamental Plants and Forest Trees.

Appendix H: Summary of information provided with registered varieties in Australia.
Appendix A: Interspecific Eucalypt crosses that have, or may, produce red buds or flowers from December to March.

Crosses that have flowered in November 2003 – February 2004

<table>
<thead>
<tr>
<th>female parent colour</th>
<th>male parent colour</th>
<th>total number available</th>
<th>number that flowered in 03-04</th>
<th>dark pink-red</th>
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<tbody>
<tr>
<td><strong>E. erythronema var erythronema</strong></td>
<td>red</td>
<td>E. stricklandii green</td>
<td>50</td>
<td>29</td>
</tr>
<tr>
<td><strong>E. erythronema var erythronema</strong></td>
<td>red</td>
<td>E. gomphocephala cream</td>
<td>54</td>
<td>8</td>
</tr>
<tr>
<td><strong>E. youngiana</strong></td>
<td>yellow</td>
<td>E. macrocarpa red</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>peach</td>
<td>E. macrocarpa</td>
<td>15</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>red</td>
<td>E. macrocarpa</td>
<td>18</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

Crosses that have not yet flowered but one or other of the parent species flower between December and March, and one or other of the parents has red flowers or red buds

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<th>female parent colour</th>
<th>male parent colour</th>
<th>total number available</th>
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</thead>
<tbody>
<tr>
<td><strong>E. calophylla</strong></td>
<td>white</td>
<td>E. ficifolia dark pink red</td>
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<tr>
<td>white</td>
<td>4</td>
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<tr>
<td>orange</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>dusty pink</td>
<td>4</td>
<td></td>
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<tr>
<td><strong>E. 9-5MuB</strong></td>
<td>white</td>
<td>E. platypus red</td>
</tr>
<tr>
<td>E. nutans red</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td><strong>E. erythronema var marginata</strong></td>
<td>red</td>
<td>21</td>
</tr>
<tr>
<td><strong>E. tetragona</strong></td>
<td>white</td>
<td>E. erythrocorys red buds</td>
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Appendix B: Primary Selection Criteria, single plant sheet Ch 3.1.3.

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<td>&lt;25% stem b/f</td>
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<tr>
<td></td>
<td>average</td>
<td>25-50% stem b/f</td>
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<tr>
<td></td>
<td>good</td>
<td>50-75% stem b/f</td>
</tr>
<tr>
<td></td>
<td>excellent</td>
<td>&gt;75% stem b/f</td>
</tr>
<tr>
<td>Tree height</td>
<td>small</td>
<td>(&lt;4m)</td>
</tr>
<tr>
<td></td>
<td>medium</td>
<td>(4-8m)</td>
</tr>
<tr>
<td></td>
<td>large</td>
<td>(&gt;8m)</td>
</tr>
<tr>
<td>Tree form</td>
<td>single stemmed</td>
<td></td>
</tr>
<tr>
<td></td>
<td>multi stemmed</td>
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</tr>
<tr>
<td>Tree habit</td>
<td>erect</td>
<td></td>
</tr>
<tr>
<td></td>
<td>semi erect/pendulous</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pendulous</td>
<td></td>
</tr>
<tr>
<td>Tree shape</td>
<td>rectangular</td>
<td></td>
</tr>
<tr>
<td></td>
<td>square</td>
<td></td>
</tr>
<tr>
<td></td>
<td>circular</td>
<td></td>
</tr>
<tr>
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</tr>
<tr>
<td></td>
<td>triangle down</td>
<td></td>
</tr>
<tr>
<td></td>
<td>triangle up</td>
<td></td>
</tr>
<tr>
<td>Canopy density</td>
<td>sparse/open</td>
<td>&lt;10 leaf/30cm</td>
</tr>
<tr>
<td></td>
<td>dense</td>
<td>&gt;10 leaf/30cm</td>
</tr>
<tr>
<td>Canopy habit</td>
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</tr>
<tr>
<td></td>
<td>outer 25% only</td>
<td></td>
</tr>
<tr>
<td></td>
<td>continuous</td>
<td>through to trunk</td>
</tr>
<tr>
<td>Trunk</td>
<td>no colour contrast</td>
<td></td>
</tr>
<tr>
<td>Bark</td>
<td>persistent</td>
<td></td>
</tr>
<tr>
<td></td>
<td>shedding</td>
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</tr>
<tr>
<td>Vigour - unpruned</td>
<td>poor</td>
<td>&lt;10cm/year</td>
</tr>
<tr>
<td></td>
<td>vigorous</td>
<td>&gt;10cm/year</td>
</tr>
<tr>
<td>Flowering</td>
<td>Season Su Au Wi Sp</td>
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</tr>
<tr>
<td>General plant health</td>
<td>not healthy</td>
<td>(damage &gt;50%)</td>
</tr>
<tr>
<td></td>
<td>healthy</td>
<td>(damage &lt;50%)</td>
</tr>
<tr>
<td>Insects</td>
<td>caterpillars</td>
<td>damage % and area</td>
</tr>
<tr>
<td></td>
<td>leaf miners</td>
<td>damage % and area</td>
</tr>
<tr>
<td></td>
<td>sap suckers</td>
<td>damage % and area</td>
</tr>
<tr>
<td></td>
<td>scale</td>
<td>damage % and area</td>
</tr>
<tr>
<td></td>
<td>others</td>
<td>leaves yellow</td>
</tr>
<tr>
<td></td>
<td></td>
<td>leaves spotty/necrotic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>leaves distorted</td>
</tr>
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<td>Comments</td>
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**LEAF CHARACTERS**

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<thead>
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<tbody>
<tr>
<td>Leaf shape (sketch)</td>
<td>linear</td>
<td></td>
</tr>
<tr>
<td></td>
<td>lanceolate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ovate</td>
<td></td>
</tr>
<tr>
<td>Leaf colour</td>
<td>yellow</td>
<td></td>
</tr>
<tr>
<td></td>
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</tr>
<tr>
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<td>grey</td>
<td></td>
</tr>
<tr>
<td></td>
<td>blue</td>
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</tr>
<tr>
<td>Leaf colour</td>
<td>RHS chart</td>
<td>upper surface</td>
</tr>
<tr>
<td></td>
<td>RHS chart</td>
<td>lower surface</td>
</tr>
<tr>
<td>Leaf size (LxW)</td>
<td>actual length</td>
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</tr>
<tr>
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<td>width</td>
<td></td>
</tr>
<tr>
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<td></td>
</tr>
<tr>
<td></td>
<td>glaucous - low</td>
<td></td>
</tr>
<tr>
<td></td>
<td>glaucous - medium</td>
<td></td>
</tr>
<tr>
<td></td>
<td>glaucous - high</td>
<td></td>
</tr>
<tr>
<td>Leaf wax</td>
<td>dull</td>
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</tr>
<tr>
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<tr>
<td>Leaf margin</td>
<td>RHS colour chart</td>
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<td>Petiole colour</td>
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<td>full</td>
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</tr>
<tr>
<td>Stem colour</td>
<td>RHS colour chart</td>
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<tr>
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<td>RHS colour chart</td>
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</tr>
<tr>
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<td>glaucous – low</td>
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</tr>
<tr>
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<td>glaucous - medium</td>
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</tr>
<tr>
<td></td>
<td>glaucous - high</td>
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</table>

**DESCRIBE EACH FLOWERING STEM:**

<p>| # stems umbels | # umbels/m stem | stem length | stem diameter | length overgrow |
|----------------|-----------------|-------------|---------------|----------------|----------------|
|                |                 |             |               |                |                |
|                |                 |             |               |                |                |
|                |                 |             |               |                |                |
|                |                 |             |               |                |                |
|                |                 |             |               |                |                |
|                |                 |             |               |                |                |
|                |                 |             |               |                |                |
|                |                 |             |               |                |                |
|                |                 |             |               |                |                |
|                |                 |             |               |                |                |
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<tr>
<td>BUD &amp; FLOWER CHARACTERS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bud position</td>
<td>terminal</td>
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</tr>
<tr>
<td></td>
<td>not terminal</td>
<td></td>
</tr>
<tr>
<td>Bud colour</td>
<td>RHS - operculum</td>
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</tr>
<tr>
<td></td>
<td>RHS - hypanthium</td>
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<tr>
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<td>RHS - blush</td>
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<tr>
<td>Bud colour</td>
<td>similar to leaf/stem</td>
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<tr>
<td></td>
<td>white/cream</td>
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<td>green (contrast to leaf/stem)</td>
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<td>red</td>
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<td>purple</td>
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</tr>
<tr>
<td>Bud shape</td>
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<td></td>
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<td>Diagram of bud shape</td>
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<th>state</th>
<th>yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peduncle</td>
<td>sessile</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(sketch)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>erect</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pendulous</td>
<td></td>
</tr>
<tr>
<td>Peduncle length (cm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pedicel length</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flower colour intensity</td>
<td>dull</td>
<td></td>
</tr>
<tr>
<td>Flower colour similar to leaf</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flower colour</td>
<td>white/cream</td>
<td></td>
</tr>
<tr>
<td></td>
<td>green (contrast to leaf/stem)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>yellow</td>
<td></td>
</tr>
<tr>
<td></td>
<td>orange</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pink</td>
<td></td>
</tr>
<tr>
<td></td>
<td>red</td>
<td></td>
</tr>
<tr>
<td></td>
<td>purple</td>
<td></td>
</tr>
<tr>
<td>Flower colour RHS chart</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stamen density</td>
<td>sparse</td>
<td></td>
</tr>
<tr>
<td></td>
<td>medium</td>
<td></td>
</tr>
<tr>
<td></td>
<td>full</td>
<td></td>
</tr>
<tr>
<td>Anthers</td>
<td>not prominent</td>
<td></td>
</tr>
<tr>
<td></td>
<td>prominent (contrast or size)</td>
<td></td>
</tr>
<tr>
<td>bud arrangement</td>
<td>close line</td>
<td></td>
</tr>
<tr>
<td></td>
<td>medium line</td>
<td></td>
</tr>
<tr>
<td></td>
<td>broad line</td>
<td></td>
</tr>
<tr>
<td></td>
<td>close triangle</td>
<td></td>
</tr>
<tr>
<td></td>
<td>medium triangle</td>
<td></td>
</tr>
<tr>
<td></td>
<td>broad triangle</td>
<td></td>
</tr>
<tr>
<td>Floral display diameter</td>
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<td></td>
</tr>
<tr>
<td>Filaments</td>
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</tr>
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<td></td>
</tr>
<tr>
<td></td>
<td>medium</td>
<td></td>
</tr>
<tr>
<td></td>
<td>full</td>
<td></td>
</tr>
<tr>
<td>Anthers</td>
<td>not prominent</td>
<td></td>
</tr>
<tr>
<td></td>
<td>prominent (contrast or size)</td>
<td></td>
</tr>
<tr>
<td>bud arrangement</td>
<td>close line</td>
<td></td>
</tr>
<tr>
<td></td>
<td>medium line</td>
<td></td>
</tr>
<tr>
<td></td>
<td>broad line</td>
<td></td>
</tr>
<tr>
<td></td>
<td>close triangle</td>
<td></td>
</tr>
<tr>
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<td>medium triangle</td>
<td></td>
</tr>
<tr>
<td></td>
<td>broad triangle</td>
<td></td>
</tr>
<tr>
<td># generations of buds</td>
<td></td>
<td></td>
</tr>
<tr>
<td># stems umbels</td>
<td></td>
<td></td>
</tr>
<tr>
<td># umbels/m stem</td>
<td></td>
<td></td>
</tr>
<tr>
<td>stem length</td>
<td></td>
<td></td>
</tr>
<tr>
<td>stem diameter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>stem length overgrowth</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DESCRIBE EACH FLOWERING STEM:

# stems umbels | # umbels/m stem | stem length | stem diameter | stem length overgrowth |
Appendix C: Summary sheets for top 12 individuals

EcEg001
EcEs001
EcEs002
EcEs003
EmEy001
EyEm001
EyEp001
EpEm001
EpEm002
EpEm003
EpEm004
EpEm005
<table>
<thead>
<tr>
<th>NUMBER:</th>
<th>EeEg001</th>
<th>DATE OF GERMINATION:</th>
<th>Oct 1998</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOCATION:</td>
<td>LaPS3</td>
<td>DATE OF PLANTING:</td>
<td>May 2000</td>
</tr>
<tr>
<td>PRIMARY SELECTION SCORE 2002:</td>
<td>na</td>
<td>DT SCORE:</td>
<td>na</td>
</tr>
<tr>
<td>PRIMARY SELECTION SCORE 2003:</td>
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<td>GS SCORE:</td>
<td>na</td>
</tr>
<tr>
<td>DT SCORE:</td>
<td>2</td>
<td>GS SCORE:</td>
<td>2</td>
</tr>
</tbody>
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**DESCRIPTION:** Medium (A) green yellow cream - (A) dark apple green buds with (A) dark purple pink / (A) dark bright red blush; (A-C) mid purple pink flower; (A) dark brown stems with no glaucousness; green leaves.

**POSTHARVEST DATA:**
Basic vase life 10.2 days in H2O.

**CURRENT TREE STATUS:**
Tree height <4m, trunk single at base; new growth; fruit & new buds.

**COMMENTS:**
Bright pink flower and bright red buds contrasting with stems and green leaves, medium to large flowers, good vase life, flowers mid summer.
NUMBER: EeEs001
LOCATION: LaPS3
DATE OF GERMINATION: Oct 1998
DATE OF PLANTING: May 2000

<table>
<thead>
<tr>
<th>PRIMARY SELECTION SCORE 2002: na</th>
<th>PRIMARY SELECTION SCORE 2003: 82%</th>
</tr>
</thead>
<tbody>
<tr>
<td>DT score: na</td>
<td>GS score: na</td>
</tr>
<tr>
<td>DT score: 1</td>
<td>GS score: 1</td>
</tr>
</tbody>
</table>

DESCRIPTION: Medium (A) light yellow green buds with (A) dark red blush; (A) dark purple pink flower; (A) dark purple stems with medium glaucousness; green leaves.

POSTHARVEST DATA:
Basic vase life 12.2 days in H₂O.

CURRENT TREE STATUS:
Tree height <4m, trunk single at base; new growth; fruit & buds.

COMMENTS:
Strong red flower colour contrasting with glaucous stems and green leaves, medium flowers, summer flowering time, highly floriferous, good vase life.
<table>
<thead>
<tr>
<th>NUMBER: EeEs002</th>
<th>DATE OF GERMINATION: Oct 1998</th>
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</thead>
<tbody>
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<td>LOCATION: LaPS3</td>
<td>DATE OF PLANTING: May 2000</td>
</tr>
<tr>
<td>PRIMARY SELECTION SCORE 2002: na</td>
<td>DT SCORE: na</td>
</tr>
<tr>
<td>PRIMARY SELECTION SCORE 2003: 70%</td>
<td>GS SCORE: na</td>
</tr>
<tr>
<td>DT SCORE: 1</td>
<td>GS SCORE: 1</td>
</tr>
</tbody>
</table>

**DESCRIPTION:** Medium (A) light yellow green - (A) dark apple green buds with (A) rust brown blush; (A) bright red purple flower; (A) bright red purple stems with medium glaucousness; green leaves.

**POSTHARVEST DATA:**
Basic vase life 11.0 days in H₂O.

**CURRENT TREE STATUS:**
Tree height <4m, trunk single at base; new growth; fruit & buds.

**COMMENTS:**
Strong red flower colour contrasting with glaucous stems and green leaves, medium flowers, summer flowering time, highly floriferous, good vase life.
<table>
<thead>
<tr>
<th>NUMBER:</th>
<th>EeEs003</th>
<th>DATE OF GERMINATION:</th>
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<td>LOCATION:</td>
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<td>DATE OF PLANTING:</td>
<td>May 2000</td>
</tr>
<tr>
<td>PRIMARY SELECTION SCORE 2002:</td>
<td>na</td>
<td>DT SCORE:</td>
<td>na</td>
</tr>
<tr>
<td>PRIMARY SELECTION SCORE 2003:</td>
<td>70%</td>
<td>GS SCORE:</td>
<td>na</td>
</tr>
<tr>
<td>DT SCORE:</td>
<td>na</td>
<td>GS SCORE:</td>
<td>na</td>
</tr>
</tbody>
</table>

**DESCRIPTION:** Medium (A) light yellow green/(D) - (A) mid yellow green/dark mustard buds with no blush; (C) dark purple pink / (B) bright red purple flower; (A) bright red purple stems with low glaucousness; green leaves.

**POSTHARVEST DATA:**
Basic vase life 13.0 days in H₂O.

**CURRENT TREE STATUS:**
Tree height <2m, trunk multi-stemmed at base; new growth; fruit & buds.

**COMMENTS:**
Strong pink flower colour contrasting with glaucous stems and green leaves, medium flowers, highly floriferous, summer flowering time, good vase life.
NUMBER: EmEy001
LOCATION: LaPS3
DATE OF GERMINATION: Dec 1998
DATE OF PLANTING: May 2000

DATE OF PLANTING: May 2000

PRIMARY SELECTION SCORE 2002: 79
PRIMARY SELECTION SCORE 2003: 70
DT SCORE: na  GS SCORE: na
dt score: na  gs score: na

DESCRIPTION: Extra large (A) mid yellow green bud with (A) dull dark pinky red blush; (D) pale light green / (D) greenish yellow or (B) pale pink flower; (A) rust brown stem; medium glaucousness; glaucous leaves.

POSTHARVEST DATA:
ot assessed

CURRENT TREE STATUS:
Tree height <4m, trunk multi-stemmed at base; new growth; no fruit, buds.

COMMENTS:
Yellow/pink flower contrasting with green buds, all glaucous, extra large flowers, spring flowering time.
**NUMBER:** EyEm001  
**DATE OF GERMINATION:** Dec 1998  
**LOCATION:** LaPS3  
**DATE OF PLANTING:** May 2000

<table>
<thead>
<tr>
<th>PRIMARY SELECTION SCORE 2002:</th>
<th>86</th>
</tr>
</thead>
<tbody>
<tr>
<td>DT SCORE:</td>
<td>1</td>
</tr>
<tr>
<td>GS SCORE:</td>
<td>1</td>
</tr>
</tbody>
</table>

**DESCRIPTION:** Extra, extra large (A) light yellow green bud with (A) dark red / (A) dull dark orange / (A) bright red purple blush; (C) dark purple pink / (B) mid red purple flower; (A) bright red purple stem, medium to high glaucousness; glaucous leaves.

**POSTHARVEST DATA:** not assessed

**CURRENT TREE STATUS:** Tree height <4m, trunk multi-stemmed at base; new growth; fruit & buds.

**COMMENTS:** Excellent colour and form, dark red flower contrasting with buds, all glaucous, extra, extra large flowers, late spring early summer flowering time.
NUMBER: EyEp001  
LOCATION: LaPS3  
DATE OF GERMINATION: Dec 1998  
DATE OF PLANTING: May 2000  

**Primary selection score 2002:** 79%  
**Primary selection score 2003:** 76%  
**DT score:** na  
**GS score:** na  
**DT score:** 2.5  
**GS score:** 3

**Description:** Extra large (A) mid yellow green bud with (A) hot bright red / (A) dull dark orange blush; (B) dark peach pink flower; (A) dark purple stem, no glaucousness; green leaves.

**Current tree status:**  
Tree height <4m, trunk multi-stemmed at base; new growth; fruit & buds.

**Comments:**  
Bright red flower contrasting with green buds, red stems and green leaves, extra large flowers, spring flowering time.

---

**Postharvest data:**  
not assessed

---

**Buds**

**Flowers**

**Tree**
**NUMBER:** EpEm001  
**DATE OF GERMINATION:** Dec 1998  
**LOCATION:** LaPS3  
**DATE OF PLANTING:** May 2000  
**PRIMARY SELECTION SCORE 2002:** 88%  
**DT SCORE:** 1  
**GS SCORE:** 1  
**PRIMARY SELECTION SCORE 2003:** 86%  
**DT SCORE:** 1  
**GS SCORE:** 1

**DESCRIPTION:** Extra large (A) light mid yellow green bud with no blush; (C-D) dark purple pink flower; (A) dark mustard stem, low glaucousness; grey green leaves.

**POSTHARVEST DATA:**  
H₂O = 16.6; 10% sucrose 24 hour pulse = 13.4; 2% sucrose continuous treatment = 13.4 days.

**CURRENT TREE STATUS:**  
Tree height <3m, trunk multi-stemmed at base; new growth; no fruit, buds.

**COMMENTS:**  
Red flower contrasting with green buds, red stems and green leaves, all glaucous, extra large flowers, spring flowering time.
NUMBER: EpEm002
LOCATION: LaPS3
DATE OF GERMINATION: Dec 1998
DATE OF PLANTING: May 2000
PRIMARY SELECTION SCORE 2002: 77%
PRIMARY SELECTION SCORE 2003: 92%
DT score: 1
GS score: 1

DESCRIPTION: Extra large (A) light yellow green – (A) mid yellow green bud with no blush; (B) dark purple pink flower; (A) dark mustard stem, low glaucousness; grey green leaves.

POSTHARVEST DATA:
not assessed

CURRENT TREE STATUS:
Tree height <2m, trunk multi-stemmed at base; new growth; no fruit, buds.

COMMENTS:
Red flower contrasting with green buds, red stems and green leaves, all glaucous, extra large flowers, highly floriferous, spring flowering time.
**NUMBER:** EpEm003  
**LOCATION:** LaPS3  
**DATE OF GERMINATION:** Dec 1998  
**DATE OF PLANTING:** May 2000  
**PRIMARY SELECTION SCORE 2002:** 80%  
**PRIMARY SELECTION SCORE 2003:** 82%  
**DT SCORE:** 1  
**GS SCORE:** 1  

**DESCRIPTION:** Extra large (A) mid yellow green bud with (A) dull rust brown blush; (D) pale light green flower; (A) dull rust brown stem, medium glaucousness and grey green glaucous leaves

**POSTHARVEST DATA:**  
H$_2$O = 19.6; 10% sucrose 24 hour pulse = 17.0; 2% sucrose continuous treatment = 15.2 days.

**CURRENT TREE STATUS:**  
Tree height <2m, trunk multi-stemmed at base; new growth; fruit & buds.

**COMMENTS:**  
Light green flower contrasting with green buds, red stems and green leaves, all glaucous, extra large flowers, spring flowering time.
NUMBER: EpEm004  
LOCATION: LaPS3

<table>
<thead>
<tr>
<th>DATE OF GERMINATION: Dec 1998</th>
<th>DATE OF PLANTING: May 2000</th>
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</thead>
<tbody>
<tr>
<td>PRIMARY SELECTION SCORE 2002: 90%</td>
<td>PRIMARY SELECTION SCORE 2003: 86%</td>
</tr>
<tr>
<td>PRIMARY SELECTION SCORE 2002: 90%</td>
<td>PRIMARY SELECTION SCORE 2003: 86%</td>
</tr>
<tr>
<td>DT score: 1</td>
<td>GS score: 1</td>
</tr>
<tr>
<td>DT score: 1</td>
<td>GS score: 1</td>
</tr>
</tbody>
</table>

**DESCRIPTION:** Extra large (A) mid yellow green bud with no blush; (C) dark purple pink flower; (A) dark mustard stem, medium glaucousness; grey green glaucous leaves.

**POSTHARVEST DATA:**
H₂O = 19.0; 10% sucrose 24 hour pulse = 19.6; 2% sucrose continuous treatment = 21.2 days.

**CURRENT TREE STATUS:**
Tree height <2m, trunk multi-stemmed at base; new growth; fruit & buds.

**COMMENTS:**
Red flower contrasting with green buds, red stems and green leaves, all glaucous, extra large flowers, spring flowering time.
| NUMBER: EpEm005 | DATE OF GERMINATION: Dec 1998 |
| LOCATION: LaPS3 | DATE OF PLANTING: May 2000 |
| PRIMARY SELECTION SCORE 2002: 79% | DT SCORE: na |
| PRIMARY SELECTION SCORE 2003: 78% | GS SCORE: na |

| DT SCORE: 2 | GS SCORE: 2 |

**DESCRIPTION:** Extra large (A) mid yellow green bud with (A) mid khaki green blush; (B) hot mid pink flower; (A) dark mustard stem, medium glaucousness; glaucous leaves.

**POSTHARVEST DATA:**
not assessed

**CURRENT TREE STATUS:**
Tree height <2m, trunk multi-stemmed at base; new growth; some fruit & buds.

**COMMENTS:**
Red flower contrasting with green buds, red stems and green leaves, all glaucous, extra large flowers, spring flowering time.
Appendix D: RHS Colour Charts (Royal Horticultural Society, London, 1988) colours measured during hybrid assessments

<table>
<thead>
<tr>
<th>colour group</th>
<th>name group</th>
<th>colour listed</th>
<th>colour description</th>
<th>brightness</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>A -</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>B -</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C -</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>D - pale</td>
</tr>
<tr>
<td>1</td>
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<td>1</td>
<td>greenish yellow</td>
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<td>8</td>
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<td>8</td>
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<td>10</td>
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<td>hot yellow orange</td>
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<tr>
<td>16</td>
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<td>orange</td>
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<td>red</td>
<td>51</td>
<td>light mid pink</td>
<td>✗ ✗ ✗ ✗</td>
</tr>
<tr>
<td>52</td>
<td>red</td>
<td>52</td>
<td>pink</td>
<td>✗</td>
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<tr>
<td>53</td>
<td>red</td>
<td>53</td>
<td>dark purple pink</td>
<td>✗ ✗ ✗ ✗</td>
</tr>
<tr>
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<td>red</td>
<td>54</td>
<td>mid purple pink</td>
<td>✗ ✗ ✗ ✗</td>
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<tr>
<td>59</td>
<td>red purple</td>
<td>59</td>
<td>dark red purple</td>
<td>✗ ✗ ✗</td>
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<tr>
<td>60</td>
<td>red purple</td>
<td>60</td>
<td>mid red purple</td>
<td>✗</td>
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<table>
<thead>
<tr>
<th>Colour group</th>
<th>Name group</th>
<th>Colour listed</th>
<th>Colour description</th>
<th>Brightness</th>
</tr>
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<tbody>
<tr>
<td>A - bright</td>
<td>B - mid</td>
<td>C - light</td>
<td>D - pale</td>
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<tr>
<td>137 green</td>
<td>137</td>
<td>dark olive green</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>138 green</td>
<td>138</td>
<td>pale olive green</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>139 green</td>
<td>139</td>
<td>dark tree green</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>143 green</td>
<td>143</td>
<td>dark apple green</td>
<td>x   x</td>
<td></td>
</tr>
<tr>
<td>144 yellow green</td>
<td>144</td>
<td>mid yellow green</td>
<td>x   x   x</td>
<td></td>
</tr>
<tr>
<td>146 yellow green</td>
<td>146</td>
<td>mid dark yellow green</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>147 yellow green</td>
<td>147</td>
<td>dark yellow green</td>
<td>x   x</td>
<td></td>
</tr>
<tr>
<td>149 yellow green</td>
<td>149</td>
<td>bright light green</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>150 yellow green</td>
<td>150</td>
<td>light green</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>151 yellow green</td>
<td>151</td>
<td>light khaki green</td>
<td>x   x   x</td>
<td></td>
</tr>
<tr>
<td>152 yellow green</td>
<td>152</td>
<td>dark khaki green</td>
<td>x</td>
<td></td>
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<td>153</td>
<td>mid khaki green</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>154 yellow green</td>
<td>154</td>
<td>pale light green</td>
<td>x   x   x</td>
<td></td>
</tr>
<tr>
<td>158 yellow white</td>
<td>158</td>
<td>beige cream</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>160 greyed yellow</td>
<td>160</td>
<td>green yellow cream</td>
<td>x   x</td>
<td></td>
</tr>
<tr>
<td>161 greyed yellow</td>
<td>161</td>
<td>mustard cream</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>163 greyed orange</td>
<td>163</td>
<td>dark mustard</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>168 greyed orange</td>
<td>168</td>
<td>mid orange brown</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>169 greyed orange</td>
<td>169</td>
<td>bright orange brown</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>170 greyed orange</td>
<td>170</td>
<td>light orange brown</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>171 greyed orange</td>
<td>171</td>
<td>dark orange brown</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>172 greyed orange</td>
<td>172</td>
<td>rust brown</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>173 greyed orange</td>
<td>173</td>
<td>dull rust brown</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>175 greyed orange</td>
<td>175</td>
<td>dark brown</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>177 greyed orange</td>
<td>177</td>
<td>dull dull red brown</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>179 greyed red</td>
<td>179</td>
<td>dull red</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>181 greyed red</td>
<td>181</td>
<td>dull dark pinky red</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>182 greyed red</td>
<td>182</td>
<td>dull pinky red</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>183 greyed purple</td>
<td>183</td>
<td>bright purple brown</td>
<td>x   x</td>
<td></td>
</tr>
<tr>
<td>184 greyed purple</td>
<td>184</td>
<td>dull red purple</td>
<td>x   x</td>
<td></td>
</tr>
<tr>
<td>185 greyed purple</td>
<td>185</td>
<td>bright red purple</td>
<td>x   x   x   x</td>
<td></td>
</tr>
<tr>
<td>187 greyed purple</td>
<td>187</td>
<td>dark purple</td>
<td>x   x   x   x</td>
<td></td>
</tr>
<tr>
<td>188 greyed green</td>
<td>188</td>
<td>light grey</td>
<td>x   x</td>
<td></td>
</tr>
<tr>
<td>189 greyed green</td>
<td>189</td>
<td>dark grey strong green tinge</td>
<td>x   x</td>
<td></td>
</tr>
<tr>
<td>191 greyed green</td>
<td>191</td>
<td>mid grey with green hints</td>
<td>x</td>
<td></td>
</tr>
</tbody>
</table>

Bud size descriptions
S: small buds <7mm diameter
M: medium buds, 7-20mm diameter
L: large buds, 20-40mm diameter
XL: extra large buds, 40-60mm diameter
XXL: extra, extra large buds, >60mm diameter
Appendix E: Example of comment sheet sent to industry collaborators for comment on superior selected hybrids

20th January 2004

Name: Geoff Sullivan rating 1 = very best, through to worst = 9

<table>
<thead>
<tr>
<th>Sample #</th>
<th>Comment</th>
<th>Rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>22B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22E</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23G</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23K</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23U</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21G</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

additional comments:
Appendix F: Summary of information from UPOV working paper on test guidelines for Eucalyptus (sub genus Symphyomyrtus) (sections Transversaria, Maidenaria, Exsertaria).

I. Subjects of these guidelines
1. apply to all vegetatively propagated varieties and interspecific hybrids of the species pertaining to the Sections Transversaria, Maidenaria and Exsertaria of the subgenus Symphyomyrtus of the genus Eucalyptus.

II. Material required
1. minimum 10 young plants (six months old at least)
2. visibly healthy, no impairment of disease or insect pest
3. free form any treatment (e.g. hormone) unless thoroughly stated
4. if distinguishing characters come from adult trees, a population of adult trees must be indicated.

III. Conduct of Tests
1. conducted over three growing periods after the year of establishment until distinctness/uniformity is sufficiently established.
2. must be done at the same place.
3. tests carried out under conditions of normal growth, pieces removed for observational purposes must not prejudice future observations, separate plots for observation and measuring have to be subject to similar environmental conditions.
4. additional tests for special purposes may be established.

IV. Methods and Observations
1. unless otherwise stated, all observations and measurements should be made on 10 typical organs of at least 10 plants at the time of full flowering.
2. for full assessment of uniformity a population standard of 1% and an acceptance probability of at least 97% should be applied, in the case of a sample size of 10 plants, the max number of off types allowed is 1.
3. Bark observations on new bark after peeling, main stem observations summer only, rhytidome observations on dead bark once peeling is significant, leaf observations on terminal shoots in vegetative growth.
4. Colour determinations made against a colour chart in artificial daylight or in the middle of a room with no direct daylight (specifications for artificial daylight in manual) against a white background.

V. Grouping of Varieties
1. Collection divided into groups to facilitate assessment of distinctness, based on characteristics known from experience not to vary within a variety.
2. Recommended characters for grouping varieties:
   a) Plant: shape (characteristic 2)
   b) Main stem: colour of non-dead bark at the base before peeling (characteristic 6)
   c) Main stem: glaucescence (characteristic 8)
   d) Bark; anthocyanin (characteristic 13)

VI. Characteristics and Symbols
1. To assess distinctness, homogeneity and stability, the characters and their states as given in the three UPOV languages should be used.
2. Notes are provided
3. Legend
## Appendix G: Summary of Table of Characteristics for *E. gunnii* from the UPOV guidelines developed in 2000 by the UPOV Technical Working Party for Ornamental Plants and Forest Trees

<table>
<thead>
<tr>
<th>Number</th>
<th>Characteristic</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Plant: growth (height at 4 years)</td>
</tr>
<tr>
<td>2</td>
<td>Plant: shape (outline at 3 years)</td>
</tr>
<tr>
<td>3</td>
<td>Leaves: density</td>
</tr>
<tr>
<td>4</td>
<td>Main stem: straightness</td>
</tr>
<tr>
<td>5</td>
<td>Main stem: straightness of the apex when flushing</td>
</tr>
<tr>
<td>6</td>
<td>Main stem: colour of non dead bark at the base before peeling (at one year)</td>
</tr>
<tr>
<td>7</td>
<td>Main stem: colour of the non dead bark of the upper part before peeling (at one year)</td>
</tr>
<tr>
<td>8</td>
<td>Main stem: glaucescence (on tree from 1 to 3 years)</td>
</tr>
<tr>
<td>9</td>
<td>Main stem: intensity of glaucescence (on tree from 1 to 3 years)</td>
</tr>
<tr>
<td>10</td>
<td>Main stem: colour of the base of the non dead bark (as from 3 years)</td>
</tr>
<tr>
<td>11</td>
<td>Main stem: colour of the upper part of the non dead bark (as from 3 years)</td>
</tr>
<tr>
<td>12</td>
<td>Main stem: age of the tree on disappearance of glaucescence from the main stem</td>
</tr>
<tr>
<td>13</td>
<td>Bark: anthocyanin</td>
</tr>
<tr>
<td>14</td>
<td>Bark: intensity of anthocyanin</td>
</tr>
<tr>
<td>15</td>
<td>Rhytidome (dead bark): appearance of the surface (tuberoze cells) on tree between 1 and 5 years</td>
</tr>
<tr>
<td>16</td>
<td>Primary branches: density of branching between 2 and 5 years (number of branches per linear meter) before natural pruning before 4 to 5 years</td>
</tr>
<tr>
<td>17</td>
<td>Primary branches: angle in degrees of the insertion compared to main stem (on one year old branch on 2 to 3 year old tree)</td>
</tr>
<tr>
<td>18</td>
<td>Primary branch: curve of the terminal</td>
</tr>
<tr>
<td>19</td>
<td>Primary branch: colour</td>
</tr>
<tr>
<td>20</td>
<td>Stem: shape of the primary branch insertion excrescence on the main stem</td>
</tr>
<tr>
<td>21</td>
<td>Leaf: early appearance of the adult form (petiolate and lanceolate)</td>
</tr>
<tr>
<td>22</td>
<td>Leaf: glaucescence (on 6 month seedling at most)</td>
</tr>
<tr>
<td>23</td>
<td>Leaf: glaucescence (on 2 year seedling at least)</td>
</tr>
<tr>
<td>24</td>
<td>Petiolate and lanceolate adult leaf: length without petiole (on 2 year seedling at least)</td>
</tr>
<tr>
<td>25</td>
<td>Petiolate and lanceolate adult leaf: width (on 2 year seedling at least)</td>
</tr>
<tr>
<td>26</td>
<td>Flower bud: length</td>
</tr>
<tr>
<td>27</td>
<td>Flower vessel: glaucescence</td>
</tr>
<tr>
<td>28</td>
<td>Operculum: mucro</td>
</tr>
<tr>
<td>29</td>
<td>Operculum: colour</td>
</tr>
<tr>
<td>30</td>
<td>Flower vessel: glaucescence (repeat of 27)</td>
</tr>
<tr>
<td>31</td>
<td>Fruit: shape</td>
</tr>
<tr>
<td>32</td>
<td>Age of tree at first flowering</td>
</tr>
</tbody>
</table>
Appendix H: Summary of information provided with registered varieties in Australia

“Summer Beauty” and “Summer Red”

Description: as for the Flora, with any special features noted, and flower/bud/leaf colour noted with RHS values. Flowering period also noted.

Origin and breeding: where it came from (controlled pollination), year, parental selection with flower colours with flower colours (RHS codes). Breeder noted, and reason for selection. Method of propagation.

Comparative trials: Comparator female parent, over 5 years. Samples taken from 20 leaves/flowers from seven specimens of the variety and six specimens of the comparator. Plants were raised in the field, observations made of grafted clones and the original seedling clone. Detailed description in Flora of Australia, vol 19. In the case of the second variety, the comparator was the first PBR variety applicant.

Prior and sales application:

Table nn Eucalyptus varieties

Flowering time
Leaf length (cm): mean, std dev., LSD/sig.
Leaf width (cm): mean, std dev., LSD/sig.
Petiole length (cm): mean, std dev., LSD/sig.
Operculum length (mm): mean, std dev., LSD/sig.
Operculum width (mm): mean, std dev., LSD/sig.
Hypanthium length (mm): mean, std dev., LSD/sig.
Hypanthium width (mm): mean, std dev., LSD/sig.
Peduncle length (mm): mean, std dev., LSD/sig.
Peduncle width (mm): mean, std dev., LSD/sig.
Flower bud glaucosity (yes, no)
Stamen colour (RHS colour chart)
Fruit shape, ribbing, number of valves
References


Pryor, LD 1976, *The Biology of Eucalypts*, The Institute of Biology’s Studies In Biology # 61, Edward Arnold.


Wildy, D, Pate, J & Bartle, J 2003, Silviculture and water use of short-rotation mallee eucalypts’, RIRDC Publication no. 03/033, Rural Industries Research and Development Corporation, Canberra.