Eucalypts for Ornamental Horticulture: Selection, Interspecific Hybridisation and Postharvest Testing

Kate Louise Delaporte

Playford Memorial Trust Scholar in Ornamental Horticulture

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Department of Horticulture, Viticulture and Oenology

Waite Agricultural Research Institute

Adelaide University

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Frontspiece
Arrangement of *Eucalyptus* buds, flowers and leaves
(*E. kruseana*, *E. lesouefii*, *E. tetragona* and *E. youngiana*)
collected from the Laidlaw Plantation, October, 1999.

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Abstract

Of nine ornamental *Eucalyptus* species, *E. lesouefii* was the fastest growing, while *E. yalatensis* and *E. youngiana* were the slowest. Seventeen plants from three species showed superior characters, specifically bud size, shape and colour, precocity and floriferousness.

The parentage of the ornamental hybrid *Eucalyptus* 'Urrbrae Gem' was investigated using RAPD-PCR, adult morphology and seedling morphology. The results were analysed using hierarchical (UPGMA) and non-hierarchical distance methods (multidimensional ordination and minimum spanning tree analysis). The female parent was known to be *E. erythronema* var. *erythronema*, but previous opinion placed the male parent as either *E. stricklandii* or *E. gomphocephala*. All results indicated that *E. stricklandii* is the male parent of *E.* 'Urrbrae Gem'.

Crosses between *Eucalyptus macrocarpa*, *E. pyriformis* and *E. youngiana* were conducted, with all combinations producing fertile seed. The 166 seedlings, when measured for a range of leaf and stem characters at three different nodes, showed strong evidence of intermediacy between parents, with 1.2% grouped with the male parent, 94.6% clustered between the parent species, and the remaining 4.2% with the female parent.

Eucalyptus gillii and *E. socialis* were used as female parents in a controlled pollination program using pollen collected from sixteen species. The 425 seedlings produced were measured at three months for fifteen seedling characters. The UPGMA dendrograms showed 225 seedlings clustered with neither the male nor female parent seedlings, suggesting intermediacy. The remaining 200 seedlings clustered with the female parent. The multivariate analysis supported the UPGMA results, with the ordination point clusters

remaining consistent with the dendrogram groupings.

Continuous application of 0.5 to 5.0% sucrose in the vase solution reduced vase life of *E. tetragona* flowers by three days, while vase life of *E. youngiana* was not affected. Pulsing with 0.5 to 10% sucrose, in conjunction with cold dry storage at 3°C for one to two weeks, had no effect on vase life of *E. tetragona* flowers. Preliminary trials with *E. forrestiana* subsp. *forrestiana* and *E. stoatei* indicate that pulsing with sucrose at 2.0 to 5.0% may be beneficial to vase life. Sucrose did not increase flower opening after harvest of any species tested, nor did citric acid affect vase life. Significant differences in vase life were found between plants within a species.

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Dedication

To my father, who would be so proud,

To Mr Stuckey, for his inspiration, and

To Sir Thomas Playford, for his example.

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Glossary of Terms

acuminate: tapering gradually/suddenly to a protracted point, sides straight or convex, angle of convergence >90°

acute: with sides equally curved convexly to the base, whole included in 90 degree angle; sharp, terminating at once in a point not abruptly but not tapering; with 2 almost straight lines converging at an angle of <90°

Adnataria: one of the 8 informal sections of subgenera *Symphyomyrtus*

adult: one of the four recognised leaf phases of *Eucalyptus* plants, when the plant is physiologically mature and reproductive

alternate: leaves arranged in a spiral (1 at each node); placed singly and alternately one above the other on some common body, i.e. leaves on stem

anther: part of the stamen that bears pollen

apex: tip or point of leaf, opposite of base

apiculate: terminating abruptly in a little point

Arboretum: collection of cultivated trees

ascending: having a direction upward with an oblique base

attenuate: with convex curved sides narrowed gradually and concavely to the base

angular: having projected longitudinal angles

apiculate: ending abruptly in a short point

axillary: arising from the axil of ordinary foliage leaves

basal rim: rim at base of operculum

beaked: pointed

binary: character coded by two states, usually present (1) or absent (0)

Bisectaria: one of the 8 informal sections of subgenera *Symphyomyrtus*

bloom: white covering of wax; a flower or inflorescence

bud: immature flower, consisting of hypanthium and operculum, prior to operculum lift and anthesis

campanulate: bell shaped, inflated and gradually enlarged into a limb, base not being conical

calyx: the outermost of the floral envelopes

capsule: a dry, many seeded fruit, or 'gum nut', which opens at maturity at the valves

channelled: regularly ridged

citric acid: a six carbon tricarboxylic acid, from citrus plants (lemons)

clavate: club shaped

clonal propagation: the production of new plants by tissue culture

clone: a population of genetically identical cells or individuals

concave: rounded inward

concolorous: same colour throughout

conical: like a true cone

conspicuous: obvious, highly visible

continuous: character measured in specific units e.g. length mm

controlled pollination: placing the pollen on the stigma manually

flower: single structure, consisting of operculum, hypanthium, stamens, pistil and pedicel

fruit: alternative name for capsule

fusiform: spindle shaped i.e. narrower at both ends than at center, tapering to each end

genotype: the genetic constitution of an organism as opposed to its physical appearance.

genus: the smallest natural group containing distinct species

glandular: covered with hairs bearing glands at the tips

glaucous: with blue-ish green sheen or bloom

globular: spherical

Gower Metric: a range standardised Manhattan distance measure used to generated a matrix showing dissimilarity between individuals or operational taxonomic units (OTUs) see Appendix 1

habit: form or manner of growth

hemispherical: half a sphere, smoothly rounded

hierarchical classification: a system into which individuals are grouped into an ascending series of successively larger and broader categories, based on the affinities of the component units

hypanthium: flat or cup shaped receptacle, contains the ovary

inconspicuous: present but not readily visible

inflexed: suddenly bent inwards

inflorescence: arrangement of flowers on the floral axis, group of flowers

infrageneric: within a genus

inserted: inside the mouth of the capsule

internode: the portion of the stem between the level of insertion of 2 successive leaves or leaf pairs (or branches of an inflorescence)

inter: between

intermediate: one of the four recognised leaf phases of *Eucalyptus* plants, when the plant is between the juvenile leaves and the adult leaves, rarely seen, in some species may be physiologically mature and reproductive

intra: within

invariant: not variable or different

juvenile: one of the four recognised leaf phases of *Eucalyptus* plants, when the plant is between the seedling leaves and the intermediate leaves, in some species may be physiologically mature and reproductive

lamina: the blade of a leaf, thickness often measured

lanceolate: [L=4-6W] narrowly elliptic, tapering equally to each end

leaf margin: edge of leaf, may be smooth or jagged

leaf petiole angle: angle between the leaf petiole and the midrib

level: with respect to valves - neither exserted nor inserted, same height as the disc

linear: [3/6L:W] narrow, short, with the 2 opposite margins parallel

lobed: party divided with a determinate number of segments

mallee: a eucalypt with several stems arising from a lignotuber

manipulated hybridisation: controlled pollination using pollen from a different plant or species

midrib (midvein): the central, and usually most prominent, vein of a leaf or leaf-like organ

minimum spanning tree: a set of lines, representing the pair-wise associations from a group of objects, which interconnect all the objects.

mucronate: abruptly terminated with a hard short point; (*mucronulate*): diminutive

multidimensional scaling: ordination on more than one axis, usually three

multistate: character measured in more than two states, coded by numbers

multivariate analysis: analysis using a number of variates (characters)

Myrtaceae: family to which *Eucalyptus* genus belongs

nectary: nectar secreting organ

node: a point of the stem from which one or more leaves arise

numerical taxonomy: a method of classification based on the numerical analysis of the variation of a large number of characters in a group of organisms

ob-: prefix meaning inverted

oblong: [L=2-3W] elliptical, obtuse at each end, sides almost parallel

oblique: where the degree of inequality in the 2 sides is slight

obscure: not prominent

obtuse: terminating gradually in a blunt end, rounded enough to include a 90° angle

obovate: [L=11/2W] the reverse of ovate, broadest above the middle, narrow below

oil glands: a gland that secretes essential oil, usually on leaf surface

operculum: the budcap, or lid, formed by the fusion of the petals; an outer operculum may be formed by the calyx, or both calyx and corolla may be united

opposite: leaves arranged in 2 opposite rows (2 at each node)

orbicular: [L=W] perfectly circular

ordination: technique used to relate individuals or groups, with individuals ordered in the way that best reflects their similarities and differences

OTU: operational taxonomic unit, in this study an individual plant

ovary: ovule bearing unit of the pistil; simple if formed from a single carpel, compound if formed from more than one

ovate: [L=<2W] oblong or elliptical, egg shaped; broadest at the lower end

ovoid: ovate, egg shaped

PATN: computer program used for multivariate ordination

pedicel: attaches flower to umbel

peduncle: attaches flower or umbel to branch

pendulous: downward hanging, weeping

petal: individual organs that form the corolla

petiolate: having petioles

petiole: the stalk portion of a leaf

phenotype: the expression of characters of an organism determined by an interaction between the environment and the genotype, and between dominance and epistatic relationships within the genotype

pistil: collective term for ovary, style and stigma

pith glands: oil glands within the branchlets, twigs or stems

postharvest: occurring after harvest

precocious: forward in development

preharvest: occurring before harvest

prominent: highly visible

provenance: origin, source, place where found or produced, as a cultivar or selection of a taxon

pruinose: with deposits of powder or flakes of wax, often like flour and easily detached (farinose, mealy, scurfy)

pyramidal: like a cone with angular sides

quadrangular: four sided figure

ramet: any individual belonging to a known clone, preferred for studies of phenotypic plasticity in relation to environmental factors

RAPD: random amplified polymorphic DNA, method for generating DNA fingerprints

receptacle: the more or less enlarged or elongated end of the stem or floral axis on which some or all of the flower parts are borne

recurved: curved backwards

reflexed: bent abruptly backwards at more than 90°

retuse: terminating in a round end

ribbed: having ribs

rhomboid: [L=W] oval, a little angular in middle

rostrate: terminating gradually in a hard long straight point

seedling: young plant, from germination to 12 months; one of the four recognised leaf phases of *Eucalyptus* plants

sepals: the individual organs that form the corolla

senescence: the period between maturity and death of a plant or plant part

series: informal level of classification, below section, above species

sessile: flat, adjacent to surface, no attaching stalk (petiole or peduncle or pedicel)

shining: glossy surface, highly reflective, not dull or glaucous

SMC: simple matching coefficient,

smooth: smooth , not wrinkled, ridged or warty

spathulate: [L=4-6W] oblong with lower end much attenuated

species: the basic unit of classification; a population or system of populations that normally interbreed

square: four equal sides

stamen: collective term for anther and filament

stigma: the part of the pistil that receives the pollen; variable in shape and form according to the mode of pollination, with as many stigmatic surfaces provided as carpels unless fusion of the surfaces has taken place

stress: % degree of distortion of OTUs ordinates during multidimensional scaling, should be <20%

style: narrow constricted "neck" between the stigma and ovary, sometimes absent when the stigma is sessile; often with internal cavities (stylar canals) to facilitate penetration of pollen

tubes

sub: below

subspecies: informal level below species

taxon: general term applied to any taxonomical element, population or group irrespective of its classification level (taxa)

terete: slender cylindrical, but not so slender as filiform (threadlike)

truncate: as if cut straight across

turbinate: inversely conical with contraction towards the point

umbel: an inflorescence composed of a cluster of flowers whose pedicels arise from the same point

umbonate: round with projecting point in the centre

uncinate: hooked, curved back suddenly at the point

undulate: having an uneven alternating convex and concave margin

UPGMA: unweighted pair group method with arithmetic average

urceolate: same as campanulate but more contracted at orifice with an erect limb

valves: openings that dehisce to allow seed to be disseminated

vase life: period of time (days) a stem (including flowers and leaves) remains marketable after harvest

variate: character

variant: not all the same

variety: a "botanical variety' is a category below that of species, a 'cultivated variety' is a cultivar

vegetative propagation: the production of new plants without using seeds; cuttings or grafting

venation: collective vein pattern on leaves

ventral: upper surface

warty: small lumps on surface, not smooth

weeping: hanging vertically, pendulous, not erect

whorled: radial arrangement around a stem with 2 pairs of leaves at each node

winged: having wings on surface

Adapted from Brooker and Kleinig (1990), Tootill (1984), Soule and Sherman (1978)

Chapter One

Introduction

1.1. Introduction

Ornamentals are an expanding sector of the Australian horticulture industry. Within the ornamental sector is the area of floriculture, where plants are grown specifically to produce cut stems with flowers, buds and foliage. The *Eucalyptus* genus has received recent attention for use in floriculture; however, previous studies have focused on foliage rather than bud and flower production. The potential to expand exists; but before such potential can be realized, research is needed into relevant aspects, including selection of superior genotypes from suitable species, clonal propagation and post harvest physiology.

Selecting the most suitable specimen is the first step in plant improvement, and thus studies of growth habit are important prior to selection. *Eucalyptus* specimens suitable for floriculture are initially selected for their morphological appearance, such as the shape and colour of leaves and flowers. Attention should then be given to the postharvest physiology of the specimens, as this will influence their vase life. Aspects that require investigation relate to the rates of wilting, senescence and abscission of leaves, buds and flowers. The postharvest physiology of some *Eucalyptus* foliage species has been investigated, but the flowers and buds of a wider range of species require research.

The *Eucalyptus* forestry industry has investigated interspecific hybridisation and clonal propagation for improvement in timber production. The methodology involved could be applied to ornamental production, enabling the development and propagation of new hybrids. Molecular biology techniques can also be used to generate DNA profiles of plants, and to identify parentage or clarify hybrid status.

1.2. Native plants and ornamental horticulture

1.2.1. Introduction

The continent of Australia has been isolated for over 50 million years, and this has enabled the evolution of a diverse range of flora suited to a wide range of geographical and climatic conditions. This provides opportunities for development of new and unusual species for amenity horticulture.

1.2.2. Australian natives for cut flowers and foliage

Although sales of Australian native cut flowers worldwide are valued at $400m (wholesale), only around 10% is produced in Australia. In 1997-1998, total flower exports from Australia were valued at $27.4m, with 93% native flowers and exotic proteaceae (FECA, 1999). The top native flowers and exotic proteaceae exported, by volume and value terms in 1997-1998, are waxflower, kangaroo paw, *Thryptomene*, *Stirlingia*, *Protea*, *Banksia*, *Leucodendron*, Koala fern and *Scholtzia*, with unspecific eucalypt product tenth and general foliage rated fifteenth.

1.2.3. Development of ornamental *Eucalyptus* species

There are no superior varieties of eucalypt for cut flower production available in Australia (Sedgley, 1998), resulting in lack of uniformity and yield of product. A large number of *Eucalyptus* species show potential, however, development requires selection at both species and genotype level, for a number of criteria. Eucalypt selection programs are subject to long generation times and propagation difficulties and these must be overcome if ornamental eucalypts are to find their place on world markets.

1.2.4. Native ornamental breeding and selection

Breeding and selection occur consecutively, following a series of logical steps (Figure 1.1)

(Sedgley, 1996). Selection initially occurs at the species level, where the gene pool from wild and cultivated populations is searched for species fitting the desired floral criteria. Selection proceeds to the genotype level, where plants of the same species are compared and assessed at primary and secondary levels. For this study, it was assumed that the phenotype expressed for any plant was fully indicative of the genotype of that plant in all criteria. Primary selection focuses on flowering, plant form and growth rates. Genotypes exhibiting superior characters at this level are moved on to secondary selection, where the response of the plant to cultivation, postharvest testing and clonal propagation is determined. Selected genotypes can be included in inter and intra specific breeding programs, for the development of hybrids combining superior characters from different plants. Once the selected genotype meets all criteria at all levels, it is registered as a cultivar and can be multiplied for commercial release.

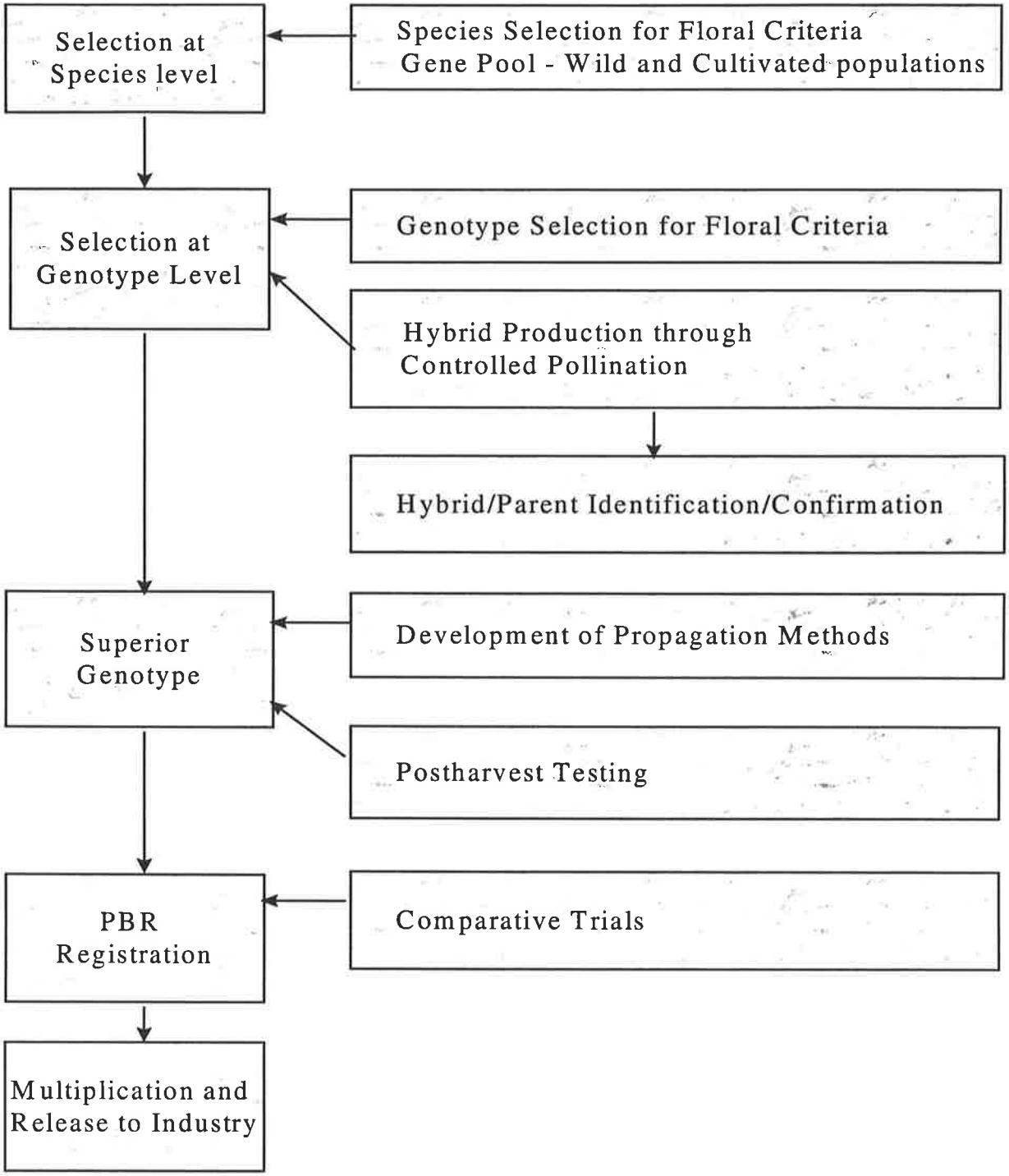


Figure 1.1. Ornamental eucalypt breeding flow chart.

1.3. Aims

The project aims to improve species from the *Eucalyptus* genus for ornamental horticulture by addressing three aspects of plant improvement:

1. Species and genotype selection: to grow a range of species suitable for the cut flower and bud industry under similar conditions, and to observe and record their growth and development over a three year period, with selection of genotypes for further development.

2. Interspecific hybridisation and hybrid identification: to investigate interspecific hybridisation, through controlled pollination, as a method to produce hybrids with increased ornamental merit, and to clarify hybrid status using DNA fingerprinting, and adult and seedling morphology comparisons.

3. Post harvest physiology and treatments: to perform postharvest trials on cut stems of *Eucalyptus*, with flowers and buds, to establish the optimal postharvest holding conditions for increased vase life and flower opening.

Chapter Two

Literature Review

2.1. The genus *Eucalyptus*

The genus *Eucalyptus* consists of over 700 species (Brooker and Klienig, 1999), almost all endemic to Australia. They are grown all over the world as ornamentals and as a valuable source of timber, fuel and shade (Brooker and Kleinig, 1990). Within Australia, eucalypts are widespread in all areas except the desert zone; their distribution depends mainly on climatic environment, with other factors, such as soil type, determining species placement and mix (Williams and Brooker, 1997).

Eucalyptus species are evergreen woody perennials, ranging from very large trees (e.g. *Eucalyptus regnans* up to 100 m) to small mallee types (e.g. *E. tetraptera* up to 3 m). Most can be described as forest (30 - 50 m) or woodland (10 - 25 m) species. Many are heteroblastic, that is the juvenile foliage is very different from that of the adult. Adult leaves have a distinctive appearance; they are petiolate, falcate-lanceolate, and mainly isobilateral. Juvenile foliage refers to that which occurs on seedlings up to 1 m tall (Pryor, 1976) and is particularly useful in species identification (Williams and Brooker, 1997). Juvenile characters are strongly inherited and can be identified a few months after germination. Within some species (e.g. *E. cinerea*), adult type leaves are not produced on some individuals, despite them being ontogenetically mature. Juvenile leaves are often round and glaucous, making them particularly attractive for floriculture.

Individual species are identified according to flower morphology and overall appearance. Stamens and anthers are important features, as are cotyledon shape, operculum shape, and ovule number and position. Overall appearance is determined by noting the main characters of

plant growth habit, bark, leaves, inflorescences, buds, fruit and seeds (Chippendale, 1988).

Pryor and Johnson (1971) developed a classification system for the eucalypts, which they used to identify the following subgroups: subgenus, section, series, subseries, species and subspecies. There are currently seven informal subgenera (*Blakella, Corymbia, Eudesmia, Guabaea, Idiogenes, Monocalyptus* and *Symphyomyrtus*) one of which, *Corymbia*, has recently been elevated to generic level (Hill and Johnson, 1995). *Symphyomyrtus* is by far the largest subgenus, containing eleven sections, and is the most important commercially. Revisions to the classification continue, but Pryor and Johnson's (1971) phylogeny will be followed in this research.

2.2. Taxonomy

2.2.1. Introduction

The term taxonomy, the study of the principles and practices of classification, is often used synonymously with systematics, which is the scientific study and description of the variation in living organisms and the relationships that exist between them. The field of taxonomy includes various sections and methods. Numerical taxonomy is a classification method based on the numerical analysis of a large number of characters in a group of organisms. To be highly predictive, a classification must be based on a large number of characters, each of equal weight (Toothill, 1984). A data matrix is compiled from measurements and observations of a range of characters for the individuals or operational taxonomic units (OTUs) used in the study. The data matrix can be subjected to a number of mathematical analyses, using different coefficients, to provide a measure of the similarity or dissimilarity between all OTUs, with the end product one or more dendrograms. Some analysis programs enable the generation of ordination plots for clearer resolution of the relationships between

OTUs, as well as providing information regarding the significance of particular characters.

2.2.2. Taxonomy and *Eucalyptus*

Eucalypts were first described by Charles L'Héritier in 1788, with the first classification of the whole genus based largely on staminal characters (Bentham, 1867). Later revisions were based on studies of other macroscopic characters, particularly cotyledons (Maiden, 1903-33), buds, fruits and anthers (Blake, 1953; Blakely, 1955). Other taxonomic investigations revealed a wider range of potentially diagnostic characters, including anatomical structures such as the seed coat (Gauba and Pryor, 1958, 1959, 1961), leaf waxes (Hallam and Chambers, 1970), trichomes (Ladiges, 1984), oil glands and ducts (Carr and Carr, 1969) and inflorescences or parts thereof (Carr and Carr, 1959, 1962; Boland and Sedgley, 1986). An informal classification suggesting an infrageneric hierarchy appeared in the early 70s (Pryor and Johnson, 1971) and has been widely adopted. The most recent classification of the genus was published by Chippendale (1988) as part of the *Flora of Australia* series, and presents a formal classification at the series level. Revisions to particular sections and series have been published since, including revisions of ser. *Dumosae* (Brooker, 1971), subgenus *Symphyomyrtus* sect. *Bisectaria* (Hill and Johnson, 1992), subgenus *Eudesmia* (Hill and Johnson, 1998), and particularly controversial, the revision of the bloodwoods and the separation of subgenus *Corymbia* as the genus *Corymbia* (Hill and Johnson, 1995). Other clarifications of relationships between species and series through studies of the morphology of both seedling and adult plants have been documented (Chappill and Ladiges, 1996; Ladiges *et al.*, 1984; Ladiges *et al.*, 1987). Morphological characters are used to study geographical variation within species/complexes (Boland, 1978; Jordan *et al.*, 1993; Potts, 1985) for intergrade classification (Doran and Burgess, 1993) and hybrid identification (Hopper *et al.*, 1978).

The advent of techniques enabling the study of plants at a molecular level has resulted in an influx of data regarding taxonomic relationships within the *Eucalyptus* genus. Genetic diversity within species has been investigated using techniques such as allozymes (Moran and Hopper, 1983; Sampson *et al.*, 1989, 1990; Kennington and James, 1997a, b, 1998), while linkage maps (Byrne *et al.*, 1995; Marques *et al.*, 1998) are used for studying genome structure and QTL studies. The combination of morphological and molecular techniques to determine relationships between individuals and species will enable further clarification of the hierarchy of *Eucalyptus*.

This investigation will use numerical taxonomy to determine similarities and dissimilarities between individuals and species. The resulting association matrices will enable the generation of a phenetic dendrogram and a multidimensional ordination clarifying the relationships of the individuals investigated.

2.3. Interspecific hybridisation

2.3.1 Introduction

Hybridisation, whether natural or artificial, is an important component in the development of superior lines of many horticultural plants. When two different species or strains are crossed, the resultant offspring may display high levels of variability, and may exhibit increased growth, improved vigour and adaptability, and higher resistance to diseases and pests than either or both of the parents. The offspring can be selected for further development based on superior characteristics, such as growth rate and habit, early flowering and unusual flowers or buds.

2.3.2. Interspecific Hybridisation in *Eucalyptus*

Interspecific hybridisation can occur naturally in *Eucalyptus*, often where two populations merge at the perimeters of their respective ecosystems. Natural hybrids have been studied and identified between *Eucalyptus preissiana* and *E. bruprestium* (Hopper *et al.*, 1978), *E.obliqua* and *E. pulchella* (Potts and Reid, 1983), *E. regnans* and *E. macrorhyncha* (Ashton and Sandiford, 1988), and between *E. tetraptera* and *E. stoatei* (Bennett, 1995). Most of these hybrids display morphological characters intermediate between the parent species. Natural hybridisation is rather rare across the genus, with only 15% of possible combinations expected on geographic/taxonomic grounds being recorded, and 37% of these are known from only a single herbarium record (Griffin *et al.*, 1988).

There are several breeding barriers to natural hybridisation in eucalypts: geographical isolation, ecological isolation and reproductive isolation. Geographical isolation is a major deterrent to natural hybridisation (Griffin *et al.*, 1988), the most striking example being the lack of natural hybridisation between species from the east and west of Australia, due to the presence of the Nullarbor Plain. The expanse of the plain means that pollen from either side cannot cross by natural means, and so natural cross pollination cannot occur. Ecological isolation is also important, as different habitat conditions (e.g. soil type) mean that some species will rarely or never grow in proximity to each other. Reproductive isolation occurs as different flowering periods, or by more complex post mating barriers and systematic affinity.

Post mating barriers within *Eucalyptus* are sometimes unilateral and relate to floral morphology. Large flowered species have longer styles than small flowered species; the barrier to hybridisation can occur when the larger flowered species is used as the female. The pollen tubes of the small flowered, short styled species are unable to penetrate the ovary of the large, long styled flower. This has been suggested as the simplest explanation for the

unilateral failure of hybridisation (Gore *et al.*, 1990). Boland and Sedgley (1986) determined that the anatomy of the stigma has implications with regard to the breeding systems of eucalypts. This may also affect the success of interspecific hybridisation.

Within *Eucalyptus*, the subgeneric groups are probably reproductively isolated. The frequency of natural hybridisation generally reflects the hierarchy of taxonomic affinities; although important exceptions have been noted in the subgenera *Monocalyptus* and *Corymbia* (Griffin *et al.*, 1988). There is considerable variation in rates of inter- and intra- sectional hybridisation within the *Symphyomyrtus* subgenus (Griffin *et al.*, 1988). The relationship between species, according to the particular systematic groups to which they belong, is an index of likely capacity to interbreed. With this in mind, manipulated interspecific hybridisation has been attempted, with most examples coming from the *Symphyomyrtus* subgenus (Griffin *et al.*, 1988). For example, Beardsell *et al.* (1979) performed crosses between *Eucalyptus caesia*, *E. sideroxylon* and *E. leucoxylon* to produce a small attractive tree suitable for street planting. *E. caesia* was found to be suitable as the male parent and *E. sideroxylon* and *E. leucoxylon* as females. Other work by Tibbits (1988) found that *E. nitens* (maternal parent) was capable of producing viable crosses with other closely related species from within the same series. The conclusion was reached that it is possible to artificially combine different species, or separate populations of the same species, that may be incapable of cross breeding naturally because of geographical/ecological isolation or differences in flowering times (Tibbits, 1988). However, there may be a higher frequency of viability problems with both seed and offspring with increasing taxonomic distance.

The offspring of interspecific hybridisation will most often exhibit morphological characters intermediate between the parents. Flower size and colour, leaf shape and growth habit are the most common morphological characters assessed to indicate hybrid status. Assessment can

also be carried out at an anatomical level, and these data examined statistically; and it may be supported by other characters such as the nature of the chemical constituents of the plant (e.g. Ashton and Sandiford, 1988).

This project will study interspecific hybridisation within the *Symphyomyrtus* subgenus, with the dual aim of understanding limits to compatibility and of producing new and unique hybrids with increased ornamental merit.

2.4. Molecular Biology

2.4.1. Introduction

The accurate, fast, reproducible and cost effective identification of plant populations and varieties is essential in agriculture as well as pure plant research (Morell *et al.*, 1995). Traditional methods of varietal identification involve assessing a range of morphological characters but these may have limitations, such as subjectivity in character analysis and delays in the expression of certain diagnostic characters (e.g. flower shape or fruit colour) particularly in long lived species. The environment in which the plant grows may also influence the morphological characters of the plant.

Varietal identification can use morphological characters in conjunction with molecular biology techniques, which have been reported in phylogenetic studies and varietal identification. Techniques include isozyme analysis (Eldridge, 1976; House and Bell, 1994), restriction fragment length polymorphisms (RFLP) (Steane *et al.*, 1991; Byrne *et al.*, 1994; Sale, 1995), simple sequence repeat (SSR) - anchored polymerase chain reaction amplification (Zietkiewicz *et al.*, 1994), microsatellite polymerase chain reaction (Wu *et al.*, 1994; Weising *et al.*, 1995), DNA amplified fingerprinting (Luro *et al.*, 1995); amplified

fragment length polymorphisms (AFLP) (Vos *et al.*, 1995), arbitrarily primed polymerase chain reaction (AP-PCR) (Welsh and McClelland, 1990), and random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) (Williams *et al.*, 1990).

The introduction of RAPD-PCR and AP-PCR has enabled a wider range of species to be studied genetically than previously, as no prior knowledge of the genome is needed (Morell *et al.*, 1995; Vos *et al.*, 1995). Both techniques utilize short primers of known sequence, arbitrarily chosen, to amplify specific regions of DNA. With appropriate primers, polymorphic DNA profiles can be developed within and between species. DNA fragments are separated on agarose gels and visualized under uv light by ethidium bromide staining to reveal distinct profiles (Rafalski and Tingey, 1993).

Since its inception, RAPD-PCR has been widely used for hybrid identification: in plants such as rice (Wang *et al.*, 1994); *Theobroma cacao* (N'Goran *et al.*, 1994); *Annona* sp. (Ronning and Schnell, 1995); grapevine (Moreno *et al.*, 1995); *Pinus sylvestris* (Lu *et al.*, 1995); Rabbiteye blueberry (Aruna *et al.*, 1995); and *Rhododendron* spp. (Iqbal *et al.*, 1995). Doubts have arisen concerning the accuracy and reproducibility of the RAPD-PCR technique (Heun and Helentjaris, 1993; Levi *et al.*, 1993; King, 1994); however problems can be limited by careful protocol establishment.

2.4.2. RAPD-PCR and *Eucalyptus*

Several studies have been conducted involving RAPD-PCR and *Eucalyptus*, looking into aspects including genetic linkage maps (Grattapaglia and Sederoff, 1994; Grattapaglia *et al.*, 1995), genotype discrimination and verification (Keil and Griffin, 1994), genetic variation (Nesbitt *et al.*, 1995) and hybrid verification (Sale, 1995). The DNA extraction procedure and the PCR protocol have varied slightly between studies, although the method described by

Doyle and Doyle (1990) remains the central link in terms of DNA extraction. PCR protocols generally follow that described by Williams *et al.* (1990) with some variations.

RAPD-PCR will enable identification of interspecific hybrids of *Eucalyptus* when used in conjunction with morphological characters. Seedling morphological characters are currently used as the initial step in hybrid identification. As the plant matures, adult morphological characters are used for further analysis. Identifying varieties and hybrids using RAPD-PCR will enable the separation of hybrid genotypes from self or parental genotypes in the offspring of breeding programs at an early stage. This will reduce the time and resources currently required to develop a new variety, as non-desirable genotypes can be culled quickly. New varieties qualify for Plant Breeders Rights registration; and molecular methods such as RAPD-PCR have the potential to supplement morphological examination and clarify hybrid status while the plant is still immature. RAPD-PCR may also provide a specific marker that identifies a variety (Sedgley, 1995), providing an easy method of varietal identification.

This project will use RAPD-PCR to develop DNA profiles of all parent plants used in the controlled interspecific hybridisation project, and all offspring produced. The profiles will be used in conjunction with morphological examination as a means to clarify hybrid status.

2.5. Postharvest physiology

2.5.1. Introduction

General post harvest physiology and its principles have been discussed at length for most horticultural produce, including the main lines of cut flowers. Halevy and Mayak (1979, 1981) reviewed aspects of cut flower physiology, determining the main principles of senescence in cut flowers. Flowers differ from most horticultural produce as they are often

picked at an immature stage and are extremely perishable. Flower and leaf tissues are highly susceptible to water loss and maintaining appropriate water relations is crucial to the maintenance of tissue quality and longevity (Halevy and Mayak, 1981). Other important components to be considered in the preservation of blooms are: the maturity of the bloom, nutrient supply, temperature, presence of ethylene gas, growth processes, physical injury and disease attack (Halevy and Mayak, 1979, 1981; Goszczynska and Rudnicki, 1988; Reid, 1991; Joyce, 1994; Williamson and Milburn, 1995). The maturity of the bloom can have an effect on the type and amount of nutrient required. Blooms picked at the bud stage require more carbohydrates, in order to continue their development, than those picked at maturity (Halevy and Mayak, 1979; 1981; Han, 1992; Doi and Reid, 1995). Blooms and foliage to be stored for long periods often require carbohydrates before and after, but rarely during, storage. Certain species are susceptible to the presence of endogenous (from the flower itself) and exogenous ethylene (from other ethylene producing products such as climacteric fruits or car exhaust fumes) as this increases the rate of senescence. Such blooms should be treated with an inhibitor of ethylene formation, such as STS (Halevy and Mayak, 1981; Tingley and Prince, 1990; van Doorn and Woltering, 1991) and kept well ventilated, away from ethylene producing products. Cool temperatures reduce metabolic processes and slow senescence, and must be maintained at all times (Goszczynska and Rudnicki, 1988). Most flowers can be kept at 0 to 2°C; others, such as tropical flowers, should be kept between 7 and 12°C to avoid chilling injury. Long term cold storage can be achieved, either wet or dry, if blooms are properly pre-treated before storage with carbohydrates and biocides (Jones, 1991; Rudnicki *et al.*, 1991; Jones and Hill, 1993). Blooms should be handled carefully to prevent physical injury; this may increase ethylene production or provide avenues for entry of disease organisms.

Once harvested, blooms and foliage are often transported long distances to market. Maintenance of cool temperatures and a supply of a vase solution (containing water, a biocide and carbohydrates) will enable the product to arrive at its final destination in a marketable condition with suitable vase life.

2.5.2. Post harvest treatment of *Eucalyptus*

Recent studies on the post harvest treatment of Australian native plants have focused on flowering species, such as *Banksia* spp. (Barth, 1992; Delaporte, 1995), *Telopea speciosissima* (Faragher, 1986a; 1986b; Jones & Faragher, 1991), *Anigozanthus* spp. cultivars (Teagle *et al.*, 1991), *Thryptomene* sp. (Jones and Faragher, 1991), *Verticordia* sp. (Jones and Faragher, 1991) and Geraldton Wax cultivars (Jones and Faragher, 1991). Foliage species of *Eucalyptus* and *Acacia* are now receiving more research attention in terms of post harvest treatment, especially long term cold storage and carbohydrate supply requirements.

Few *Eucalyptus* species have been fully investigated and most work has addressed foliage vase life. Trials into the cold storage and post harvest solution requirements of *E. crenulata* and *E. gunnii* foliage have been conducted (Jones *et al.*, 1993; Jones *et al.*, 1994); as well as trials to assess the effect of dry storage and floral preservatives on the vase life of *E. gunnii*, *E. delegatensis*, *E. rubida*, *E.* 'Silver Drop', *E. viminalis* and *E. globulus* foliage (Forrest, 1991). Wirthensohn *et al.* (1996) treated a range of foliage species with sucrose and dry storage. Current evidence suggests that species react differently to treatments, although most can be dry or wet stored for 4 weeks at 1 - 5°C without damage (Forrest, 1991; Jones *et al.*, 1993; Jones *et al.*, 1994; Wirthensohn *et al.*, 1996). Pulsing with inhibitors of ethylene formation has little effect, and the addition of biocide to the solution may enhance the vase life of some species but not others (Jones *et al.*, 1993; Jones *et al.*, 1994). Further trials with a wider range of species are required to facilitate the development of a standard protocol for

post harvest care of foliage, buds and flowers.

Eucalyptus species, suitable for foliage production, generally have numerous, small, roundish leaves and a 'blue' colouring which can be caused by the presence of leaf waxes. If leaf waxes are present, they may be damaged during post harvest handling (Jones & Sedgley, 1993) reducing the quality of the stem. Some species of *Eucalyptus* have attractive buds, flowers and fruit, however, their suitability for floriculture has not been investigated. The development of handling protocols and appropriate post harvest solutions for flowers and buds is required if the potential of this aspect of the *Eucalyptus* genus is to be realized.

There is an urgent need for further information into post harvest treatments of *Eucalyptus* species for foliage, flowers, bud and fruit production. This project will study the effect of carbohydrates, such as sucrose, and dry cold storage on the vase life of flowering *Eucalyptus* stems.

Chapter Three

Plant Material

3.1. Introduction

Of the 500 or so species of *Eucalyptus*, many exhibit varying levels of horticultural potential. This may take the form of showy or large buds, flowers or inflorescences, attractive leaves or capsules, or a neat compact tree form possibly with interesting bark or trunk shape. The major focus of this study is the cut bud and flower industry, although foliage and amenity potential is also acknowledged. Limited selection of species for floriculture has been undertaken, although several eucalypt species are already produced commercially for their foliage and many are grown as garden specimens. Selection at species level is required for floriculture, leading to selection of superior genotypes from suitable species.

3.2. Species used in the study and their natural distributions

For this study, thirty species, as named by Chippendale (1988), were assessed for their horticultural potential. Species for the hybridisation programs were selected on the basis of their floricultural characteristics as well as their taxonomic relatedness, according to the classification of Pryor and Johnson (1971) (Table 3.1). The morphological characteristics of all species studied are presented in Tables 3.2. (leaf), 3.3. (inflorescence) and 3.4. (capsule). General cultivation notes are presented in Table 3.5, and the use of each species in the project, their common name and natural distribution are listed in Table 3.6. The natural distributions of each species are illustrated with maps in Figure 3.1. Flowering times are illustrated in Figure 3.2. General notes on desirable horticultural characters are discussed. The sources of the plant material are described in section 3.4.

Table 3.1. Relatedness of *Eucalyptus* species investigated (Pryor & Johnson 1971).

Subgenus	Section	Series	Species	Subspecies
Eudesmia	Quadraria	Tetragonae	*tetragona*	
			erythrocorys	
Symphyomyrtus	Bisectaria	Cornutae	*gomphocephala*	
		Occidentales	*steedmanii*	
			eremophila	
		Erythronemae	*erythronema*	*erythronema*
				marginata
		Reduncae	*gardneri*	
		Grossae	*stricklandii*	
		Kruseanae	*kruseana*	
		Oleosae	*transcontinentalis*	
			socialis	
			gillii	
		Macrocarpae	*orbifolia*	
			websteriana	
			caesia	*caesia*
			macrocarpa	
			oldfieldii	
			pyriformis	
			youngiana	
		Foecundae	*leptophylla*	
	Dumaria	Dumosae	*anceps*	
			lesouefii	
		Incrassatae	*stoatei*	
			forrestiana	*forrestiana*
				dolichorrhyncha
	Adnataria	Pruinosae	*pruinosa*	
		Polyanthemae	*polyanthemos*	
		Melliodorae	*sideroxylon*	*sideroxylon*

E. yalatensis ser. *Subulatae* (Chippendale, 1988) was identified after the classification by Pryor and Johnson (1971), associated species include *E. gillii*, *E. socialis* and *E. transcontinentalis*.

Table 3.2. Leaf characters of all species investigated (adapted from Chippendale, 1973; 1988; Brooker and Kleinig, 1990).

Species	Adult (L x W cm)	Juvenile	Petiole (L x W mm)
anceps	lanceolate, thick, acuminate; shining, green; 5-12 x 1-2.5	elliptic to ovate; light green	terete or quadrangular; 10-19
caesia subsp. *caesia*	lanceolate, sometimes falcate, acute or acuminate; grey-green; 7-12 x 1.2-2.5	alternate; petiolate; orbicular or cordate; shining, green	glaucous; 10-40
eremophila	narrowly lanceolate to elliptical, acuminate or uncinate; green, glandular; 6-8 x 1-1.7	narrowly lanceolate	slightly flattened; 5-10
erythrocorys	opposite; narrow lanceolate, often falcate, acuminate or uncinate; bright green; 12-25 x 1-4	ovate; green; stellate hairy	quadrangular; 10-30
erythronema var. *erythronema*	narrowly lanceolate, uncinate; shining, green, glandular; 6-8 x 0.8-1.5	lanceolate	slightly flattened; 6-9
erythronema var. *marginata*	narrowly lanceolate, uncinate; shining, green, glandular; 6-8 x 0.8-1.5	lanceolate	slightly flattened; 6-9
forrestiana subsp. *forrestiana*	lanceolate, apiculate; shining, deep green, glandular; 6-9 x 1.2-2	ovate; green	flattened; 10-20
forrestiana subsp. *dolichorhyncha*	lanceolate, apiculate; shining, deep green, glandular; 6-9 x 1.2-2	ovate; green	flattened; 10-20
gardneri	lanceolate, uncinate; blue-green; 7-9 x 0.8-2	not seen	terete; 10-15
gillii	higher mature branches; lanceolate, acute; glaucous; 6-8 x 1.2-2	opposite; sessile; cordate or broadly ovate; glaucous	sessile
gomphocephala	lanceolate, acuminate; 9-16 x 1.6-2.5	alternate; petiolate; ovate, often cordate; discolorous	flattened or channelled; 15-
kruseana	opposite; orbicular, cordate; glaucous; 1.5-2 x 1.5-2	opposite; orbicular, persistent	sessile
leptophylla	narrowly lanceolate to lanceolate, moderately thick; shining, green; 4-8 x 0.4-	opposite; lanceolate; green	terete; 5-10
lesouefii	lanceolate, sometimes falcate, acuminate, thick; dull, green, glandular; 10-12 x 0.5-2.5	lanceolate; glaucous	terete; 15-20
macrocarpa	opposite; broadly ovate to elliptic-ovate, apiculate; glaucous; 8-12 x 5-8	opposite; broadly elliptic to suborbicular; grey-green and glaucous	sessile
oldfieldii	lanceolate to broadly lanceolate, acuminate; dull, grey-green; 7-10 x 1.2-2.5	alternate; petiolate; lanceolate-ovate; pale-green	terete; 15-20
orbifolia	alternate, sometimes opposite; suborbicular, retuse or emarginate; grey-green; 2.5-3.8 x 2.5-4.3	opposite; petiolate; suborbicular; grey-green	terete; 10-20; pruinose

Continued

Table 3.2. Continued

Species	Adult (L x W cm)	Juvenile	Petiole (L x W mm)
polyanthemos	ovate to broadly lanceolate, apiculate; grey or glaucous; 5.5-9 x 1.5-3.5	alternate; petiolate; orbicular, emarginate; dull, grey-green, concolorous	terete or flattened; 15-25
pruinosa	broadly ovate to elliptic-ovate, cordate, apiculate; grey-green to pruinose; 5-13 x 2.5-9	opposite; ovate or broadly lanceolate; glaucous, concolorous	absent
pyriformis	lanceolate to ovate-lanceolate, acuminate; light green; 6-8 x 1.3-2.5	alternate; petiolate; ovate to lanceolate, sometimes orbicular; green	flattened; 12-25
sideroxylon subsp. *sideroxylon*	lanceolate to narrowly lanceolate, acuminate or uncinate; green, grey-green or blue-green; 7-14 x 1.2-1.8	alternate; petiolate; lanceolate to linear; grey or grey-green	terete; 10-20
socialis	lanceolate, uncinate; dull, grey-green; 6-9 x 1.2-2	alternate; sessile to shortly petiolate; elliptic to ovate; dull, green	terete; 10-20
steedmanii	narrowly elliptic or lanceolate; 4.5-6.5 x 0.8-1.3	alternate; petiolate; lanceolate or ovate; green	flattened; 2-6
stoatei	oblong or elliptic to ovate or broadly lanceolate, apiculate; shining, dark green; 6-8 x 2-3	alternate; petiolate; ovate; green	quadrangular; 13-16
stricklandii	lanceolate; thick; shining, green; 10-13 x 2-3	alternate; petiolate; elliptic to ovate	terete; 20-35
tetragona	opposite; broadly elliptic, apiculate, thick; glaucous or green; 7-15 x 2.5-7	similar to adult	edges continuous into stems; 10-20
transcontinentalis	lanceolate to narrowly lanceolate, acuminate; blue-grey or grey-green, dull, glandular; 7-15 x 1-2.2	alternate; petiolate or opposite; sessile; decurrent, ovate; blue-green	terete; 15-25
websteriana	alternate, sometimes opposite; obovate, retuse; dull, grey-green; 2-4 x 1.5-2.5	opposite to alternate; petiolate; orbicular; subglaucous	terete; 6-15
yalatensis	lanceolate, uncinate; grey-green, dull, glandular; 6-11 x 1-2	subopposite or alternate; shortly petiolate, ovate or broadly lanceolate; green	terete or slightly flattened; 8-12
youngiana	lanceolate, to falcate, acuminate; pale green; 10-15 x 2-3.5	not seen	terete; 20-30

Table 3.3. Inflorescence characters of all species investigated (adapted from Chippendale, 1973; 1988; Brooker and Kleinig, 1990).

Species	# buds/ umbel	Bud shape; pedicel (L mm)	Operculum (L x W mm)	Hypanthium (L x W mm)	Peduncle (L mm)
anceps	7	cylindrical; to 2mm or obscure	conical, faintly striate; 3-4 x 4-5	cylindrical or hemispherical, faintly striate, 4-5 x 4-5	slightly flattened; 5 - 12
caesia subsp. *caesia*	3	clavate; glaucous; 10-30;	conical; 8-10 x 10-12	obconical or campanulate; 8-12 x 10-12	terete; 20-30
eremophila	7	horn-shaped; 4-10	horn-shaped; 10-25 x 3-5	hemispherical; 5-8 x 4-6	slightly flattened; 15 - 25
erythrocorys	3	broadly turbinate	rugose, depressed hemispherical, cross shaped; 10 x 10; red	quadrangular, ribbed; 15 x 15; green	quadrangular; 10-30
erythronema var. *erythronema*	7	conical or ± fusiform; often quadrangular, smooth; 10-18	conical; 10-15 x 7-10	obconical; 6-7 x 8-9	terete or angular, recurved; 15-20
erythronema var. *marginata*	3	conical or ± fusiform; often quadrangular, faintly ribbed; 10-18	conical; expanded basal rim; 10-15 x 7-10	obconical; 6-7 x 8-9	terete or angular, recurved; 15-20
forrestiana subsp. *forrestiana*	1, rarely 3	pyriform, quadrangular, 4-winged; absent	pyramidal to hemispherical or flattened; 5-10 x 12-15	obpyramidal or obovate, quadrangular, 4-winged; 30-40 x 12-15	flattened at top, recurved; 30-50
forrestiana subsp. *dolichorhyncha*	1, rarely 3	pyriform, quadrangular, 4-winged; absent	narrowly rostrate; 12-22 x 7-14	obpyramidal or obovate, quadrangular, 4-winged; 13-15 x 7-17	flattened at top, recurved; 30-50
gardneri	7-11	narrowly horn-shaped; 2-3	incinate; 10-15 x 3-4	obconical; 4-5 x 3-4	flattened; 10-15
gillii	7-11	glaucous; 1-4	rostrate; 5-8 x 4-5	cylindrical; 3-4 x 4-5	terete; 6-15
gomphocephala	7	clavate; absent or to 4	hemispherical; 8-10 x 9-13	obconical or campanulate, often faintly ribbed; 13-22 x 13-17	flattened; 13-27
kruseana	7	ovoid to conical; glaucous; 3-6	conical; 4-6 x 4-5	obconical; 3-4 x 4-5	terete or flattened; 3-10
leptophylla	7-13	fusiform; terete; 2-4	conical; 3-4 x 3-4	obconical; 3-4 x 3-4	quadrangular; 6-8
lesouefii	7	broadly fusiform; 2-6; glaucous	conical, ribbed; 10-15 x 10-12	obconical, ribbed; 6-7 - 6-9	slightly flattened or angular
macrocarpa	1	ovoid; pedicel absent	conical-hemispherical; 25-35 x 25-40	hemispherical or obconical; 15-25 x 25-40	terete, thick; glaucous;
oldfieldii	3	globular to ovoid; pedicel absent	hemispherical, shortly rostrate; 6-10 x 9-12	hemispherical; 5-8 x 9-12	terete, thick; 6-12

Continued

Table 3.3. Continued

Species	# buds/ umbel	Bud shape; pedicel (L mm)	Operculum (L x W mm)	Hypanthium (L x W mm)	Peduncle (L mm)
orbifolia	7,	globular to ovoid, sometimes shortly umbonate; 4-7	conical or hemispherical-conical; 7-13 x 6-8	hemispherical; 3-5 x 6-8	terete; 10-25
polyanthemos	7	paniculate; clavate to fusiform, glaucous; 2-5	conical; 1-2 x to 2	obovoid to obconical; 3 x 3	terete; 5-10
pruinosa	7	fusiform to pyriform, pruinose; 5-10	conical, sometimes slightly rostrate; 4-6 x 3-6	obconical; 4-6 x 3-6	slightly flattened to terete; 12-25
pyriformis	3	pyriform; thick 8-35	hemispherical, umbonate, ridged; 20-30 x 25-38	obconical or obpyramidal, ridged; 20-30 x 25-38	terete, thick, pendulous; 20-65
sideroxylon subsp. *sideroxylon*	7	ovoid, rostrate; 2-15	conical to rostrate; 3-5 x 4-5	ovoid to hemispherical; 4-6 x 4-5	7-20
socialis	7 - 13	fusiform; 2-8	conical, rostrate; 5-8 x 4-5	hemispherical; 4-5 x 4-5	slightly flattened; 8-20
steedmanii	3-7	ovoid or pyramidal, 4-winged;	pyramidal; 10-15 x 5-8	turbinate; 10-18 x 8-12	down curved, flattened; 15-30
stoatei	1	pyriform or turbinate; 2-10	conical, smooth or shallowly ribbed; 5-10 x 8-19	pyriform, ribbed (6-10 ribs); 20-30 x 15-20	flattened, dilated, recurved; 20-30
stricklandii	7	cylindrical; absent	conical; glaucous; 10-15 x 7-10	campanulate; glaucous; 8-10 x 7-10	glaucous, thick, flattened; 10-20
tetragona	3	clavate, quadrangular, glaucous; angular 4-10	hemispherical, striate, cross; 3-4 x 6	4-angled, toothed at corners; 5-6 x 5-6	flattened or angular; 5-15
transcontinentalis	7	glaucous; 4-7	hemispherical at base, narrowly rostrate; 8-13 x 5-6	± cylindrical or suburceolate; 4-5 x 5-6	terete or angled; 7-13
websteriana	7	globular or broadly ovoid; glaucous; angular, 6-8	hemispherical-conical; 3-4 x 4-6	shallow hemispherical; 2-3 x 3-5	terete; 10-15
yalatensis	7-11	fusiform; 2-3	conical or rostrate; 4-6 x 3-4	obconical or hemispherical; 2-3 x 3-4	terete or quadrangular; 4-8
youngiana	3	subglobular; pedicels absent or thick; to 8	hemispherical or sometimes conical, strongly ribbed; 20-25 x 20-35	hemispherical to turbinate, strongly ribbed; 20-25 x 25-25	terete, thick, recurved; 10-20

Table 3.4. Capsule characters of all species investigated (adapted from Chippendale, 1973; 1988; Brooker and Kleinig, 1990).

Species	Fruit (L x W mm)	Valves	Disc	Seed
anceps	cylindrical, 5-10 x 5-7	level; 3 or 4	descending	D shaped; red brown
caesia subsp. *caesia*	urceolate; glaucous; 20-30 x 18-25	included; 5-6	descending	irregular crescent; grey-black
eremophila	subpyriform or cylindrical; 8-10 x 7-9	exserted; 3-4	obscure	crescent; brown
erythronema var. *erythronema*	obconical or turbinate, striate; 7-12 x 10-15	just exserted; 4-5	broad, level or obliquely descending	crescent; brown
erythronema var. *marginata*	obconical or turbinate, striate; winged by expanded horizontal rim; 7-12 x 10-15	just exserted; 4-5	broad, level or obliquely descending	crescent; brown
erythrocorys	broadly campanulate, 4-toothed; 25-40 x 30-50	enclosed; 4	broad, prominently domed	pyramid; dark brown
forrestiana subsp. *forrestiana*	pyriform, quadrangular, 4-winged; 30-50 x 15-35	; 3 or 4		orbicular, pyramid, winged
forrestiana subsp. *dolichorhyncha*	pyriform, quadrangular, 4-winged; 30-50 x 15-35	; 3 or 4		orbicular, pyramid, winged
gardneri	pyrimidal or cylindrical; 7-8 x 5-6	± level; 3-4	descending	round, oval; light brown
gillii	globular; glaucous; 5-7 x 5-7	exserted; 3-4	descending	
gomphocephala	campanulate or cylindrical, often faintly ribbed; 7-9 x 7-8	level or slightly exserted; 4	broad, level, convex or ascending	round - oval; black
kruseana	cylindrical to obconical; 6-7 x 6-7	included or exserted; 3-4	narrow; descending	crescent; brown
leptophylla	hemispherical or subglobular; 3-6 x 3-6	exserted; 3-4	descending	elliptic; brown
lesouefii	hemispherical, ribbed; glaucous; 8-10 x 8-10	exserted; 3 or 4		D shaped, teardrop; dark red brown
macrocarpa	shallowly hemispherical or tubinate; 30-50 x 50-90	exserted; 4-7	ascending	orbicular or irregular pyramidal, ribbed on ventral surface, narrowly winged; brown
oldfieldii	subglobular, obconical or hemispherical; 10-12 x 12-18; usually sessile	exserted; 4	ascending	irregular crescent or orbicular; brown
orbifolia	hemispherical or campanulate; pruinose; 6-11 x 12-18	exserted; 3-4	ascending	irregular crescent; grey-brown
polyanthemos	obconical to pyriformis, often glaucous; 4-7 x 3-6		broad	
pruinosa	cylindrical to almost obconical; glaucous when young; 7-10 x 6-8	just exserted; 3-5	narrow, descending	

Continued

Table 3.4. Continued

Species	Fruit (L x W mm)	Valves	Disc	Seed
pyriformis	broadly turbinate, strongly ribbed; 25-38 x 30-65	level or exserted; 4-6	steeply ascending	irregular pyramidal, narrowly winged, ridged on ventral surface; dark brown
sideroxylon subsp. *sideroxylon*	ovoid, subglobular or urceolate; 5-11 x 6-10	included; 5		
socialis	globular; 5-8 x 5-8	exserted; 3-4	descending	compressed ovoid; grey - dark brown
steedmanii	turbinate, 4-winged; 10-18 x 10-15	exserted; 4	narrow, descending	crescent; brown
stoatei	pyriform or turbinate, strongly ribbed; 25-35 x 25-30	; 3		pyramid, winged; black
stricklandii	campanulate, faintly several ribbed; 13-15 x 10-13	exserted; 3-4	broad, descending	crescent; red brown
tetragona	subglobular; glaucous; 12-18 x 11-18	included; 4	broad, descending obliquely	pyramid; dark brown
transcontinentalis	globular; 5-8 x 5-8	exserted; 3-4	broad, descended	D shaped, elliptic; dark brown
websteriana	shallow hemispherical; glaucous; 5-8 x 8-12	exserted; 4	ascending, convex	irregular crescent; red brown
yalatensis	hemispherical or obconical; 4-5 x 5-6	exserted; 3-4	broad, descended	
youngiana	turbinate or hemispherical, strongly ribbed; 25-38 x 35-70	exserted; 4-6	concave, ascending	irregular crescent, narrowly winged, ridged on ventral surface; brown-dark brown

Table 3.5. Cultivation characters of species investigated (adapted from Chippendale, 1973; 1988; Brooker and Kleinig, 1990).

Species	Habit	Rainfall (mm)	Months of flowering; flower colour	Soil type	Tolerance
anceps	Mallee to 6m	300-500	2-4; cream	sandy loam - clayey flats	drought, frost, coastal, wind
caesia subsp. *caesia*	Mallee to 10m	250-500	6-9; pink	shallow sandy-loam	drought, frost, clay
eremophila	Tree to 4.5m	200-500	6-10; yellow, pink		drought, frost, coastal, wind
erythrocorys	Tree to 8m	470	3-5; yellow	sandy	drought, frost, coastal
erythronema var. *erythronema*	Mallee or tree to 6m	300-380	9-11; red	sandy	drought, frost, wind
erythronema var. *marginata*	Mallee of tree to 6m	300-380	10-1; red, yellow	sandy	drought, frost, wind
forrestiana subsp. *forrestiana*	Tree to 5m	330-400	12-3; yellow		drought, frost, wind, saline
forrestiana subsp.*dolichorhyncha*	Tree to 5m	330-400	12-3; yellow		drought, frost, wind, saline
gardneri	Tree to 9m	330-500	5-6; yellow-green	sandy-loam, gravelly	drought, frost, wind, timber
gillii	Mallee or tree to 7.5m	330-400	5-10; creamy		drought, frost, wind
gomphocephala	Tree to 40m	750-1000	2-3; creamy	limestone, sandy	drought, coastal, saline
kruseana	Mallee to 2.5m	200-250	4-7; yellow-green		drought, frost
leptophylla	Mallee to 5m, tree to 8m	200	3-8; creamy		drought, frost, saline
lesouefii	Tree to 18m	230-300	10-12; cream		drought, frost, saline
macrocarpa	Mallee to 5m	380-500	9-12; red		drought, frost
oldfieldii	Mallee or tree to 6m		7-9; cream	sandy	drought, frost, wind
orbifolia	Mallee or tree to 6m	230-250	5-8; cream	sandy	drought, frost, shallow soil

Continued

Table 3.5. Continued

Species	Habit	Rainfall (mm)	Months of flowering; flower colour	Soil type	Tolerance
polyanthemos	Tree to 25m		9-12; white		
pruinosa	Tree to 10m		4-6; cream yellow		
pyriformis	Mallee to 4.5m	280-400	7-10; red, yellow, peach		drought, frost
sideroxylon subsp. *sideroxylon*	Tree to 35m	variable	5-10; white, pink, red or pale yellow		
socialis	Mallee to 9m or tree to 12m		7-10; creamy white		drought, frost, wind
steedmanii	Tree to 12m	300	12-1; yellow		drought, frost, coastal, wind
stoatei	Tree to 6m	411	10-3; yellow		drought, frost, coastal, wind
stricklandii	Tree to 11m	250-280	11-1; yellow-green	sandy, clay	drought, frost, saline,
tetragona	mallee to 3m	330-690	11-1; white	sandy	drought, frost, coastal, wind
transcontinentalis	Tree to 25m	250-380	8-11; cream		drought, frost
websteriana	Mallee to 6m	250	4-9; cream	sandy	drought, frost, shallow soil
yalatensis	Mallee or tree to 6m	300	12-2; creamy		coastal
youngiana	Mallee or tree to 11m	200-230	8-1; red, yellow, peach	sand, sandy-loam	drought, frost

Table 3.6. Use in project and distribution information for all species investigated (adapted from Chippendale, 1973; 1988; Brooker and Kleinig, 1990).

Species	Authority	Common name	Natural distribution	Use in Project					Location		
				1	2	3	4	5	A	B	C
anceps	(R.Br. ex Maiden) Blakely	Kangaroo Island Mallee	W.A., S.A., Williams, northeast to Bendering, southeast to Stirling Ranges, east to coastal and subcoastal S.A., east to Murray Bridge 32°-37°S, 116°-142°E	*						*	
caesia subsp. *caesia*	Benth.	Gungurru	W.A., central, northern and eastern wheatbelt 31°-33°S, 116°-119°E			*					*
eremophila	(Diels) Maiden		W.A., widespread in wheatbelt and goldfields of southwest to east of Zanthus to south coastal plains 29°-34°S, 115°-124°E			*			*		
erythrocorys	F. Muell.	Illyarrie	W.A.; restricted distribution in western coastal areas from Cockleshell Gully in south to northeast of Dongarra 29°-31°S, 110°-117°E	*							
erythronema var. *erythronema*	Turcz.	Red flowered Mallee	W.A., east towards Southern Cross, southern wheatbelt 31°-33°S, 116°-119°E		*						*
erythronema var. *marginata*	Benth.	White Mallee	W.A., northern distribution compared to above, northern wheatbelt 30°-32°S, 116°-119°E		*						*
forrestiana subsp. *forrestiana*	Diels	Fuchsia Gum	W.A., scattered distribution in southern subcoastal areas from northeast of Ravensthorpe, eastwards to Mt Beaumont 32°-34°S, 121°-123°E					*			*
forrestiana subsp. *dolichorhyncha*	Brooker	Fuchsia Gum	W.A., scattered distribution south of Salmon Gums, occurs separate to above 32°-33°S, 121°-122°E	*						*	
gardneri	Maiden	Blue Mallet	W.A., scattered distribution, south of Williams to Ravensthorpe Range, also disjunct occurrence near Cadoux 32°-34°S, 116°-120°E		*	*			*		*
gillii	Maiden	Curly Mallee	N.S.W., S.A., restricted and disjunct distribution in Northern Flinders Range and Barrier Range north of Broken Hill 31°-33°S, 137°-139°E			*			*	*	
gomphocephala	A. DC.	Tuart	W.A., Jurien in north to Ludlow in south, coastal dunes and sub coastal plains 32°-37°S, 116°-142°E		*				*		*
kruseana	F. Muell.	Bookleaf Mallee	W.A., restricted distribution east and southeast from Kalgoorlie, from Cardunia Rock north of Karonie to Binyarinyinna Rock and east of Higginsville, north and northeast of Norseman 31°-33°S, 122°-124°E			*			*		
leptophylla	syn. *foecunda* Schauer		N.S.W., S.A., Vic., W.A., dry country of southern Australia from east Goldfileds to central N.S.W. and Vic., also Mt Lofty foothills, S.A. 27°-37°S, 123°-147°E	*						*	

Continued

Table 3.6. Continued

Species	Authority	Common name	Natural distribution	Use in Project					Location		
				1	2	3	4	5	A	B	C
lesouefii	Maiden	Goldfields Blackbutt	W.A., widespread in the Central and Southern Goldfields, from about Leonora and Lake Minigwal in the north, towards Cundeelee in the east, south to the Norseman area and east to the Fraser Range 28°-33°S, 121°-124°E	*						*	
macrocarpa	Hook	Mottlecah	W.A., scattered but widespread occurrence in western part of wheatbelt, from Piawaning in the north to Wagin Kulin in the south, with an outlier northwest of Badgingarra on the northern sandplain 28°-34°S, 115°-117°E			*	*		*	*	
oldfieldii	F. Muell.	Oldfield's Mallee	W.A., widespread distribution from the sandplains north of Kalbarri National Park and northeast of Wannoo, southeastwards to the Northern Wheatbelt and eastwards to the Great Victoria Desert 26°-32°S, 112°-127°E			*			*		
orbifolia	F. Muell.	Round leaved Mallee	N.T., S.A., W.A., scattered and widespread distribution in Northern Goldfields, extending eastwards to northwest S.A. and Central Australia 22°-26°S, 126°-134°E and 28°-32°S, 118°-120°E			*			*		
polyanthemos	Schauer	Red Box	N.S.W., Vic., central and Southern tablelands, from Grampian Ranges east to Great Dividing Range and intervening plains 32°-38°S, 143°-148°E			*			*		
pruinosa	Schauer	Silver Box	N.T., W.A., Qld., 14°-22°S, 125°-148°E			*			*		
pyriformis	Turcz.	Dowerin Rose, Pear-fruited Mallee	W.A., scattered distribution in the northern wheatbelt from Morawa in the north to Dowerin in the south, more rarely north of Geraldton 27°-32°S, 115°-117°E				*		*		*
sideroxylon subsp.*sideroxlyon*	Cunn. Ex. Woolls	Red Ironbark, Mugga	N.S.W., Qld., Vic., widespread on western slopes and plains of N.S.W., extending sporadically into S.E. Qld, and west of Sydney towards Blue Mountains and down to Wodonga 29°-37°S, 144°-150°E			*			*		
socialis	F. Muell. Ex. Miq.	Red Mallee	N.S.W., Vic., S.A., W.A., widespread distribution 20°-37°S, 115°-147°E			*			*	*	
steedmanii	C. Gardner	Steedman's Gum	W.A., pure stands, 80 km east of Hyden 32°-33°S, 119°-120°E			*			*		
stoatei	C. Gardner	Scarlet Pear Gum	W.A., restricted distribution east and northeast of Ravensthorpe, to south of Pyramid Lake 33°-34°S, 120°-121°E	*				*	*	*	*

Continued

Table 3.6. Continued

Species	Authority	Common name	Natural distribution	Use in Project					Location		
				1	2	3	4	5	A	B	C
stricklandii	Maiden	Strickland's Gum	W.A., scattered distribution south and east of Coolgardie and Kalgoorlie towards Norseman, and east towards Zanthus, and one disjunct occurrence between Menzies and Diemals 30°-33°S, 121°-124°E		*				*		*
tetragona	(R. Br) F. Muell.	Tallerack	W.A.; sandplains of southwest; disjunct occurrence in north to Eneabba and south from Pingarin to Esperance 32°-35°S, 116°-123°E	*				*	*	*	
websteriana	Maiden	Webster's Mallee	W.A., Goldfields 29°-33°S, 121°-123°E			*			*		
yalatensis	Boomsma		S.A., scattered and widespread distribution in western S.A. from Elliston and Hinck's Conservation Park on southern Eyre Peninsula, westwards around southern part 31°-34°S, 125°-136°E	*						*	
youngiana	F. Muell.	Large-fruited Mallee	S.A., W.A., scattered and widespread distribution from north of Kalgoorlie eastwards, north of the Nullarbor Plain and throughout the Great Victoria Desert 26°-33°S, 121°-136°E			*	*	*	*	*	*

1 = Growth trials (Chapter 4)
2 = *E.* 'Urrbrae Gem' analysis (Chapter 5 & 6)
3 = *Bisectaria* and *Adnataria* (Chapter 8)
4 = *Macrocarpae* (Chapter 7)
5 = Postharvest trials (Chapter 9)

A = Waite Arboretum, Urrbrae, S.A.
B = Laidlaw Plantation, Urrbrae, S.A.
C = Monarto Woodland, Monarto, S.A.

Figure 3.1. *Eucalyptus* species location maps.

1. *E. anceps*

2. *E. caesia* subsp. caesia

3. *E. eremophila*

4. *E. erythrocorys*

5. *E. erythronema* var. *erythronema*

6. *E. erythronema* var. *marginata*

7. *E. forrestiana* subsp. *forrestiana*

8. *E. forrestiana* subsp. *dolichorhyncha*

9. *E. gardneri*

10. *E. gillii*

11. *E. gomphocephala*

12. *E. kruseana*

13. *E. leptophylla*

14. *E. lesouefii*

15. *E. macrocarpa*

16. *E. oldfieldii*

17. *E. orbifolia*

18. *E. polyanthemos*

19. *E. pruinosa*

20. *E. pyriformis*

21. *E.* sideroxylon

22. *E. socialis*

23. *E. steedmanii*

24. *E. stoatei*

25. *E. stricklandii*

26. *E. tetragona*

27. *E. transcontinentalis*

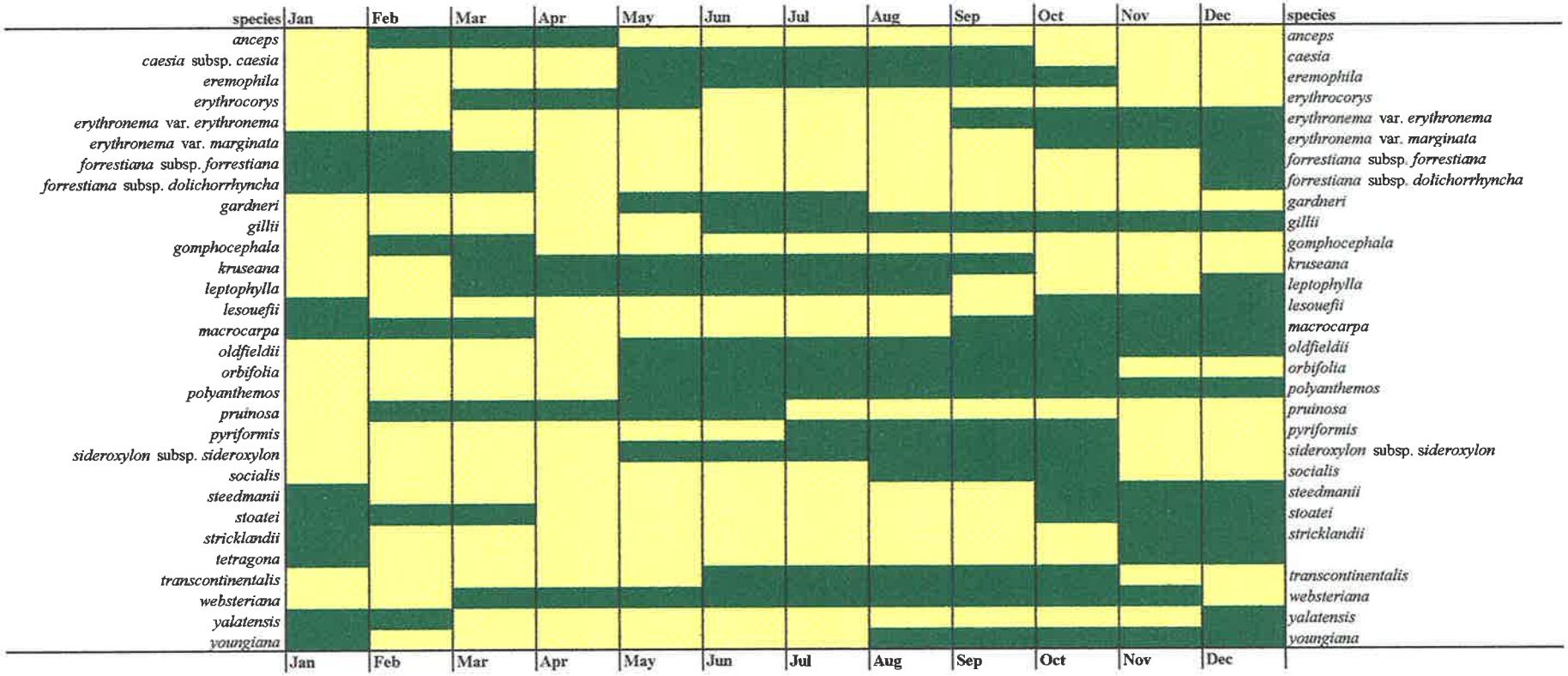
28. *E. websteriana*

29. *E. yalatensis*

30. *E. youngiana*

From: Chippendale, (1988): Flora of Australia Volume 19, *Myrtaceae*, *Eucalyptus, Angophora*. Australian Government Printing Service, Canberra (1988).

Figure 3.2. Flowering times of species used



(data on flowering times adapted from Gardner, 1990; Brooker and Kleinig, 1990; Chippendale, 1988)

3.3. Selection criteria for species chosen

E. anceps

Desirable characters: floriferous; small, coloured buds; mallee habit.

Project use: selection for superior forms for floriculture (Chapter 4).

Horticultural use: garden plant.

E. caesia subsp. *caesia*

Desirable characters: small, weeping habit; dark red stems; powdery new growth, buds and fruit; soft pink flowers; small leaves; attractive bark.

Project use: pollen donor for hybridisation to more vigorous species to introduce desirable characters (Chapter 8).

Horticultural use: garden plant, floriculture Plate 3.1

E. eremophila

Desirable characters: clusters of buds with long opercula, opening to pink or yellow flowers with sparse, wide spreading anthers.

Project use: pollen donor for hybridisation to introduce desirable characters (Chapter 8).

Horticultural use: garden tree if size can be reduced; moderately showy. Plate 3.1

E. erythrocorys

Desirable characters: terminal flowers with bright red cross-shaped operculum; vivid yellow stamens and bright green buds; variation in tree habit and size, as well as flower size flowering period.

Project use: selection for superior cut flower and garden forms (Chapter 4).

Horticultural use: possible pollen donor for hybridisation with *E. tetragona* (the only other closely related species with horticultural potential), garden plant, floriculture. Plate 3.1

E. erythronema var. *erythronema*

Desirable characters: many dark red buds opening to yellow to dark red flowers; attractive habit and trunk; reported to be a highly promiscuous parent.

Project use: female parent of *E.* 'Urrbrae Gem', used in molecular and morphological analysis (Chapters 5 and 6).

Horticultural use: pollen donor or female parent in floricultural crossing programs, or development as a garden tree. Plate 3.2

E. erythronema var. *marginata*

Desirable characters: as above

Project use: sister taxa to female parent of *E.* 'Urrbrae Gem', used in molecular and morphological analysis (Chapters 5 and 6).

Use: as above Plate 3.2

E. forrestiana subsp. *forrestiana*

Desirable characters: pendulous bright red buds with showy hypanthium; short operculum; yellow stamens; small habit; glossy leaves; some variation.

Project use: selection for superior cut flower and garden forms, postharvest testing (Chapters 4 and 9).

Horticultural use: garden or pot plant, or floriculture. Plate 3.1

E. forrestiana subsp. *dolichorhyncha*

Desirable characters: as above, but with longer operculum.

Project use: as above

Horticultural use: as above

E. gardneri

Desirable characters: masses of green buds and yellow flowers; grey-blue foliage.

Project use: pollen donor for vigour, floriferousness and foliage (Chapter 8).

Horticultural use: garden plant. Plate 3.2

E. gillii

Desirable characters: small roundish grey foliage; numerous small white flowers; good coppicing ability.

Project use: pollen donor to impart vigour, floriferousness and foliage (Chapter 8).

Horticultural use: foliage plant. Plate 3.2

E. gomphocephala

Desirable characters: attractive flowers and juvenile foliage.

Project use: putative male parent of *E.* 'Urrbrae Gem' (Chapters 5 and 6).

Horticultural use: large garden tree. Plate 3.3

E. kruseana

Desirable characters: small habit; green flowers; small round grey-blue-purple leaves; variable form.

Project use: pollen donor to impart foliage and habit characters (Chapter 8).

Horticultural use: foliage production, floriculture. Plate 3.3

E. leptophylla

Desirable characters: compact habit; bright yellow or red buds.

Project use: selection for superior cut flower and garden forms (Chapter 4).

Horticultural use: floriculture or garden plant. Plate 3.3

E. lesouefii

Desirable characters: attractive buds of various colours and levels of glaucousness; floriferous; precocious; variable tree habit.

Project use: selection for superior cut flower and garden forms (Chapter 4).

Horticultural use: breeding with closely related smaller species, floriculture. Plate 3.3

E. macrocarpa

Desirable characters: large, bright red flowers; glaucous leaves, buds, stems and fruit.

Project use: female parent and pollen donor in breeding programmes (Chapters 7 and 8).

Horticultural use: breeding with closely related smaller species; garden plant or floriculture. Plate 3.4

E. oldfieldii

Desirable characters: small mallee;, medium round yellow flowers, green buds.

Project use: pollen donor to impart tree habit and flower size (Chapter 8).

Horticultural use: garden plant or floriculture. Plate 3.4

E. orbifolia

Desirable characters: medium-sized, round, glaucous buds with creamy stamens; heart shaped leaves; long flowering season; attractive bark; variation within species.

Project use: pollen donor or female parent to impart above characters (Chapter 8).

Horticultural use: floriculture or garden plant. Plate 3.4

E. polyanthemos

Desirable characters: interesting grey-green foliage; numerous terminal small white buds; attractive round juvenile foliage.

Project use: pollen donor to investigate limits to breeding capability, impart terminal flower position (Chapter 8).

Horticultural use: large garden tree. Plate 3.4

E. pruinosa

Desirable characters: large terminal clusters of yellow flowers; good coppicing ability.

Project use: pollen donor to impart terminal flower position (Chapter 8).

Horticultural use: foliage production, floriculture.

E. pyriformis

Desirable characters: considerable variation within species for flower shape, size and colour, as well as tree habit.

Project use: breeding with closely related species to impart vigour and floral characters (Chapter 7).

Horticultural use: breeding, floriculture or garden plant. Plate 3.5

E. sideroxylon subsp. *sideroxylon*

Desirable characters: attractive pendulous flowers and silvery-grey juvenile foliage.

Project use: pollen donor for flower colour, juvenile foliage (Chapter 8).

Horticultural use: large garden tree. Plate 3.5

E. socialis

Desirable characters: considerable variation within species for leaf size and shape, as well as tree habit and level of glaucousness.

Project use: pollen donor or female parent (Chapter 8).

Horticultural use: foliage production, garden plant.

E. steedmanii

Desirable characters: attractive yellow buds and flowers; small tree habit.

Project use: pollen donor (Chapter 8).

Horticultural use: floriculture or garden plant Plate 3.5

E. stoatei

Desirable characters: pendulous bright red buds with showy hypanthium; blunt operculum; yellow stamens; small habit; glossy leaves; some variation.

Project use: selection for floriculture or as a garden plant, postharvest testing (Chapters 4 and 9).

Horticultural use: garden or pot plant or floriculture. Plate 3.5

E. stricklandii

Desirable characters: masses of yellow buds on glaucous new growth forming many months prior to flowering.

Project use: putative male parent of *E.* 'Urrbrae Gem" (Chapters 5 and 6).

Horticultural use: garden plant or floriculture. Plate 3.6

E. tetragona

Desirable characters: glaucous stems, buds, flower and fruit; Christmas flowering, small tree habit;, within species variation.

Project use: selection for superior forms for floriculture and garden plants, postharvest testing (Chapters 4 and 9).

Horticultural use: breeding with closely related species, such as *E. erythrocorys*, garden plant or for floriculture. Plate 3.6

E. transcontinentalis

Desirable characters: attractive small yellow buds and small round juvenile foliage.

Project use: pollen donor to impart above characters (Chapter 8).

Horticultural use: floriculture.

E. websteriana

Desirable characters: small grey-green round to heart-shaped leaves; small glaucous buds opening to cream flowers; interesting bark; small, neat habit; variation within species; very similar to *E. orbifolia.*

Project use: pollen donor or female parent for above characters (Chapter 8).

Horticultural use: floriculture or garden plant.

E. yalatensis

Desirable characters: compact habit; blue foliage with contrasting yellow or red stems; many yellow or red buds opening to cream flowers.

Project use: selection for garden plant or for floriculture (Chapter 4).

Horticultural use: garden plant or floriculture. Plate 3.6

E. youngiana

Desirable characters: large flowers with variability in flower and bud size, shape and colour.

Project use: breeding with closely related species for above characters; selection for garden plants and floriculture, postharvest testing (Chapters 4, 7, 8 and 9).

Horticultural use: garden plant or floriculture. Plate 3. 6

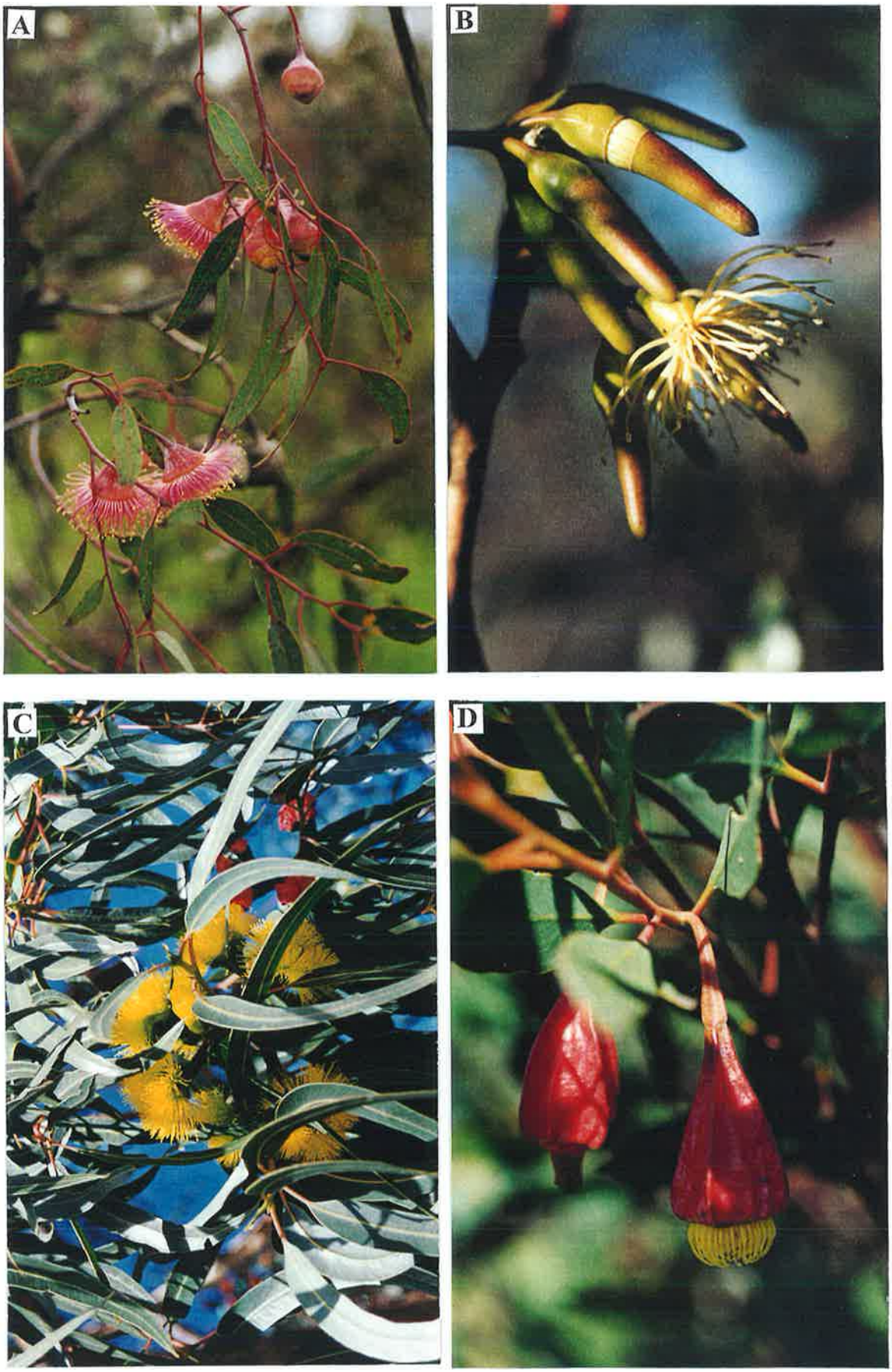


Plate 3.1.
A: *E. caesia* subsp. *caesia;* B: *E. eremophila;*
C: *E. erythrocorys;* D: *E. forrestiana* subsp. *forrestiana*

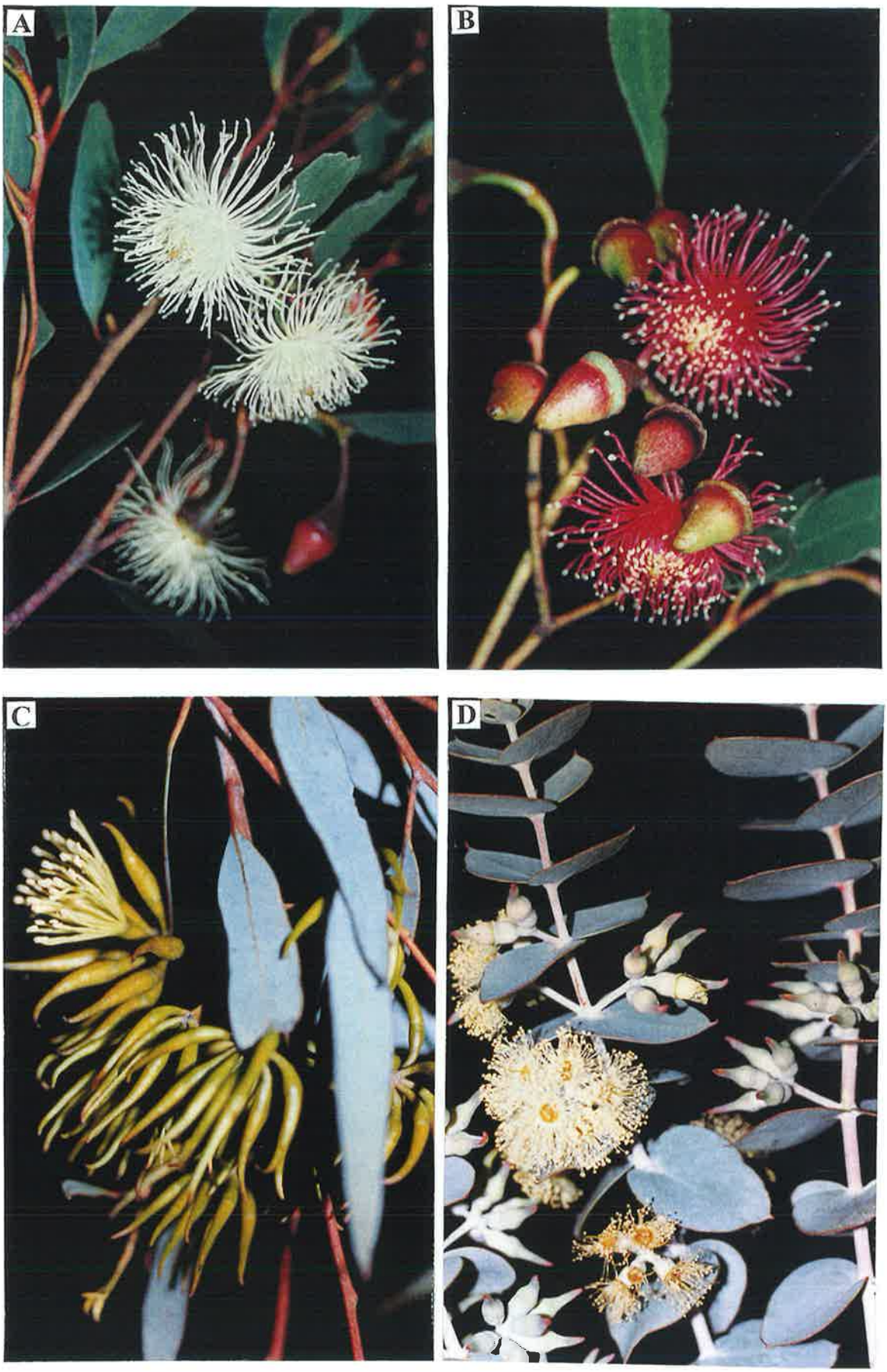


Plate 3.2.
A: *E. erythronema* var. *erythronema;* B: *E. erythronema* var. *marginata;*
C: *E. gardneri;* D: *E. gillii*

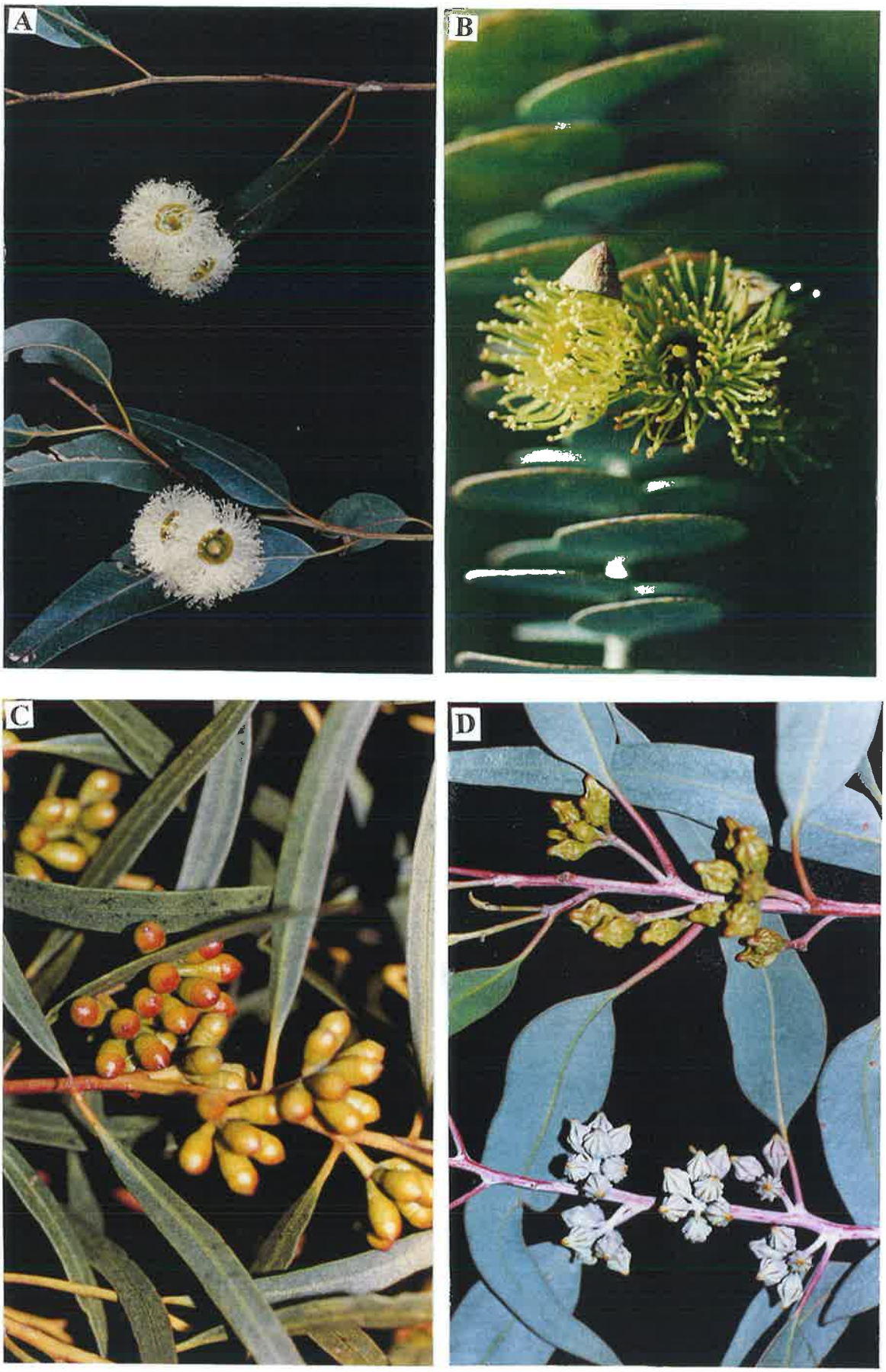


Plate 3.3.
A: *E. gomphocephala;* B: *E. kruseana;* C: *E. leptophylla;* D: *E. lesouefii*

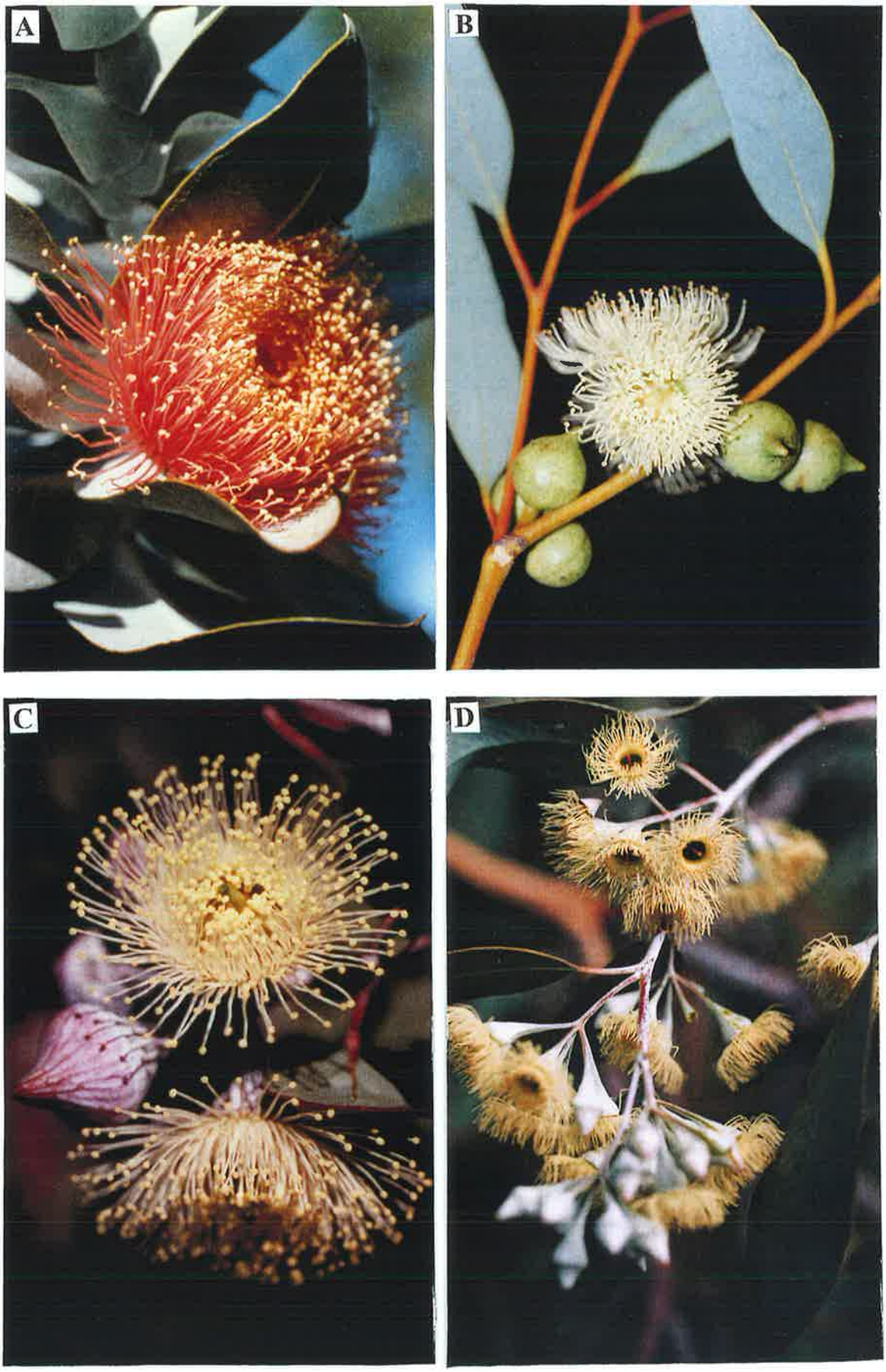


Plate 3.4.
A: *E. macrocarpa;* B: *E. oldfieldii;* C: *E. orbifolia;* D: *E. polyanthemos*

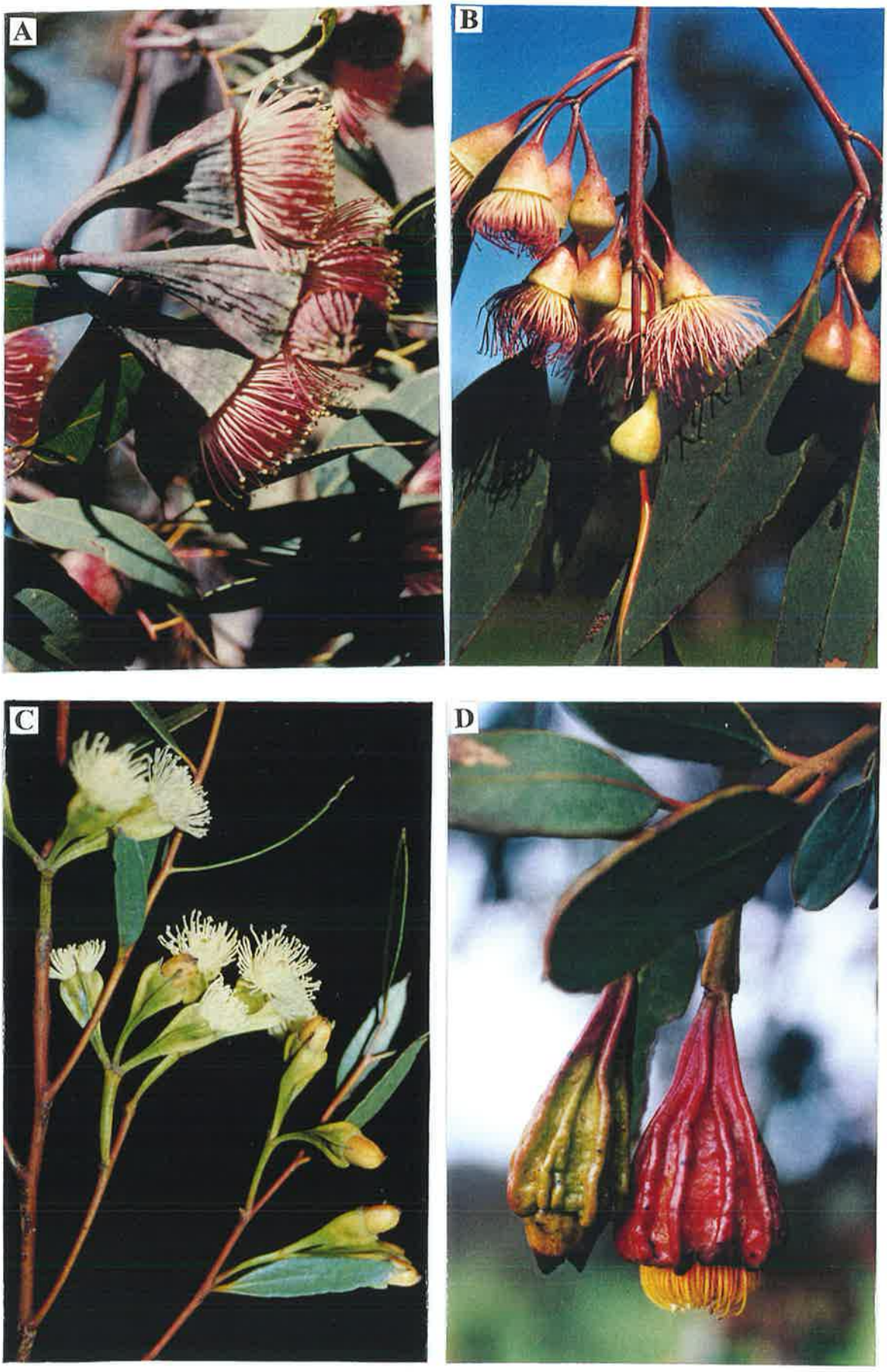


Plate 3.5.
A: *E. pyriformis*; B: *E. sideroxylon*; C: *E. steedmanii*; D: *E. stoatei*

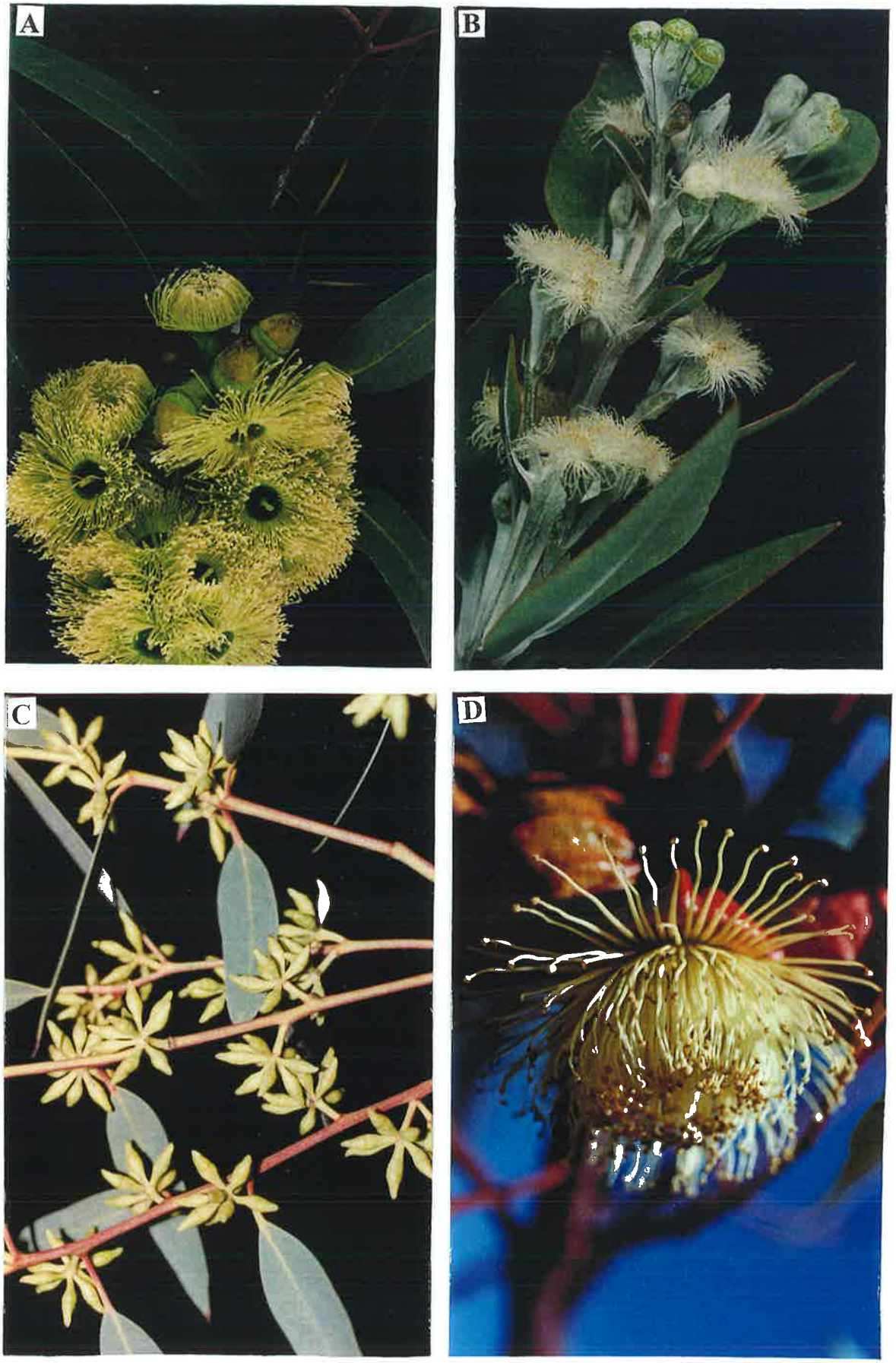


Plate 3.6.
A: *E. stricklandii*; B: *E. tetragona*; C: *E. yalatensis*; D: *E. youngiana*

3.4. Location of material

3.4.1. The Waite Arboretum

The Waite Arboretum (a reference collection of trees and shrubs cultivated for scientific, horticultural and scientific purposes) was established in 1924, along with the Waite Agricultural Research Institute, on land bequeathed to the University of Adelaide by Peter Waite (Gardner, 1990). The Arboretum consists of 27 ha of public land, located in the foothills of Adelaide (34°58′S 138°38′E) 100-110m above sea level. The average annual rainfall is 625 mm, falling mostly in winter, and the area has a mild climate with little or no frosts and warm dry summers. The soil type varies across the area, consisting mainly of red brown earths, with 25 cm or more of a fine sandy loam topsoil and prismatic structured clay subsoil (Litchfield, 1952). The soil is generally free from gravel or stone, with a pH in the surface horizon of 5-8. A small area consists of mosaics of red brown earths and lime enriched black earths, with a pH of 6-9.

Genera from similar micro climes are planted throughout the grounds, with the *Eucalyptus* and *Corymbia* genera represented by over 800 specimens from 370 species. The various species from these genera are native to all states, and cover a range of Series not usually occurring in close proximity in their endemic locations. The mild climate also encourages long flowering seasons. The potential exists for interspecific hybridisation to occur naturally, as reproductive barriers like temporal and spatial isolation are minimised. The Waite grounds have extensive plantings around the perimeter of Australian genera, including *Eucalyptus*, *Acacia*, *Banksia*, *Melaleuca*, *Dryandra*, *Hakea*, *Chamelaucium* and *Casuarina*, the majority of which were planted around 1990.

3.4.2. The Monarto Woodland

The Monarto Woodland (Figure 3.3), planted between 1974 and 1979, is located on the eastern margin of the South Mt. Lofty Ranges, South Australia (35°58'S 139°25'E) (see Chittleborough *et al,*. 1976). The majority of the Woodland is situated along the Bremer escarpment (planted in 1974) and eastwards to Murray Bridge along the South Eastern Freeway (planted 1975-77). The climate in the region is described as being of a Mediterranean type (Bureau of Meteorology, 1975) with hot dry summers and cool winters with most of the rainfall occurring during the cooler periods. Rainfall across the area varies, from <300 mm in the north east, to 420 mm along the Bremer escarpment, but can be irregular. Prevailing winds vary with season: May-October winds are strong and from the northwest to southwest, November-April winds are lighter and from the southeast or northeast. Intense rainfall can occur during the spring months.

The soils of this area are of reddish brown loamy sand or sandy loam, shallow and very low in organic matter. At around 15 cm there is a sharp break to a red-brown clay layer, 10-20cm thick. Below this is a yellow-brown, highly calcareous layer , grading to bedrock between 50-100 cm deep. Slight variations are present in this general soil type, frequently with shallower layers of topsoil and clay, resulting in soil with low water holding capacity. The lower lying areas are of differing soil types, one being a fine sandy loam or loam, greyish-brown in colour, shallow, with a highly calcareous subsoil of pale brown clay loam and a schist bedrock. The other soil type exists on the gently sloping plains near Monarto South, consisting of clayey sands or sandy clays to 10 cm and dark reddish brown in colour that may crack in summer. The sub layer is a dark reddish brown medium clay, with carbonate visible form 30 cm grading to a heavy sandy clay at around 100cm. This soil has good water holding capacity.

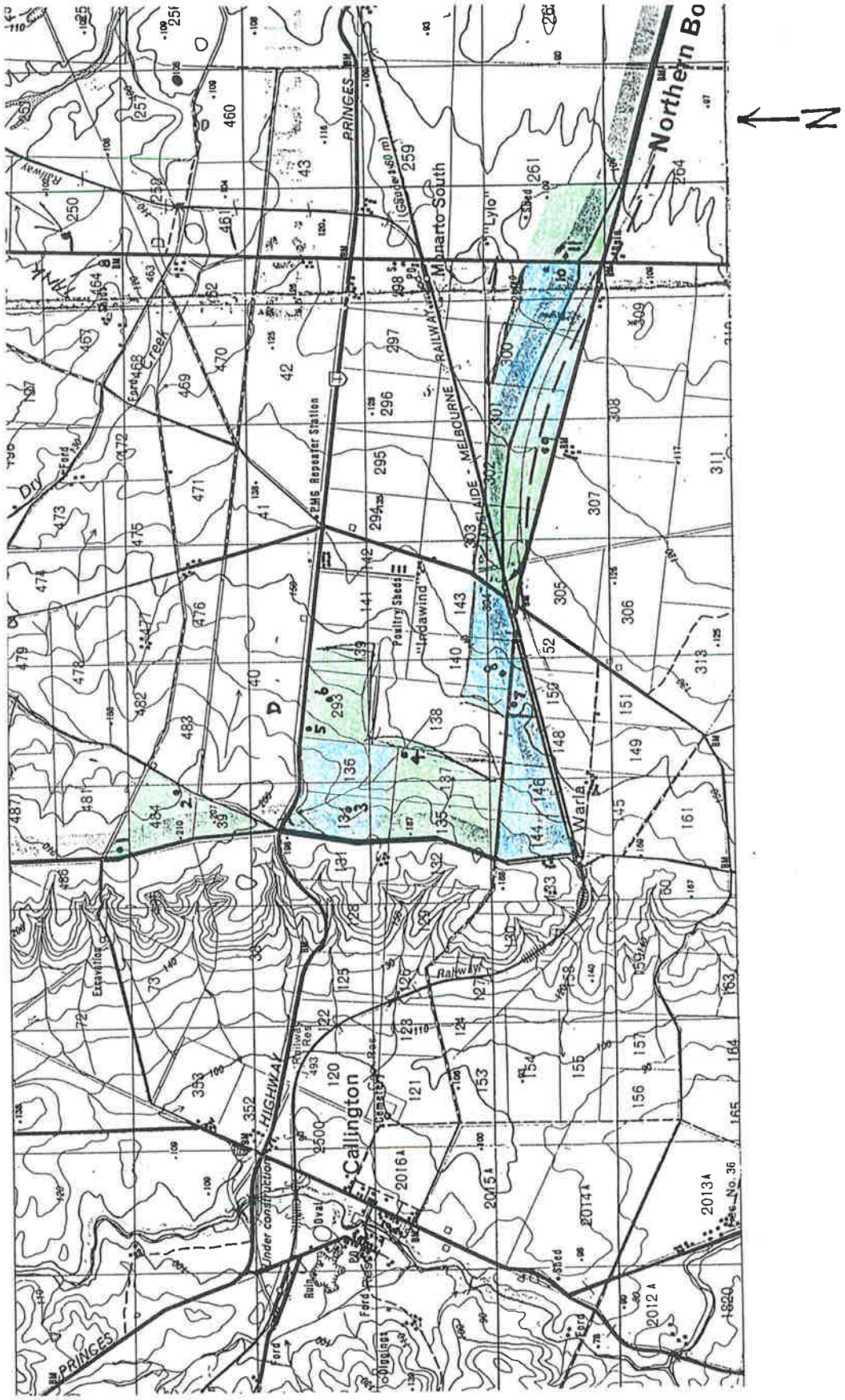
During 1974, 1975 and 1977, rows of varying numbers of plants of a single species were established and monitored to enable the assessment of different species under the local conditions. Genera planted were *Acacia, Agonis, Atriplex, Brachychiton, Callistemon, Callitris, Casuarina, Eremophila, Eucalyptus, Hakea, Leptospermum, Melaleuca, Myoporum, Pinus,* and *Tamarix.* No records of provenance of seed were available.

Specimens from the Monarto Woodland came from Plots 3, 7, 8 and 10 (Figure 3.3). Prior to planting, the ground was ripped to a depth of 50 cm. Each planting site was 'bowled' and mulch, consisting of a square of black plastic sheeting, put over the bowl. The tree was planted in the centre of the 'bowl' and watered in. Watering continued during years 1 and 2, and some inter-row ploughing was carried out, however further maintenance was restricted due to labour costs. Currently, the area is minimally managed, with fire prevention maintenance occurring during spring.

3.4.3. The Laidlaw Plantation

In 1997, the section of land containing all Eucalypts and acacias planted by students of the Department of Horticulture, Viticulture and Oenology as part of the native plant breeding program was dedicated in the name of Donald Hope Laidlaw AO, in recognition of his work for the Playford Memorial Trust Scholarship in Horticulture. The Laidlaw Plantation (Plate 3.7) is the site for the eucalypt bud and flower species trials. The soil type and climate are the same as the Waite Arboretum.

Figure 3.3. The Monarto Woodland, Callington, South Australia. Areas planted highlighted, plant material from plots 3, 7, 8 and 10 (blue).



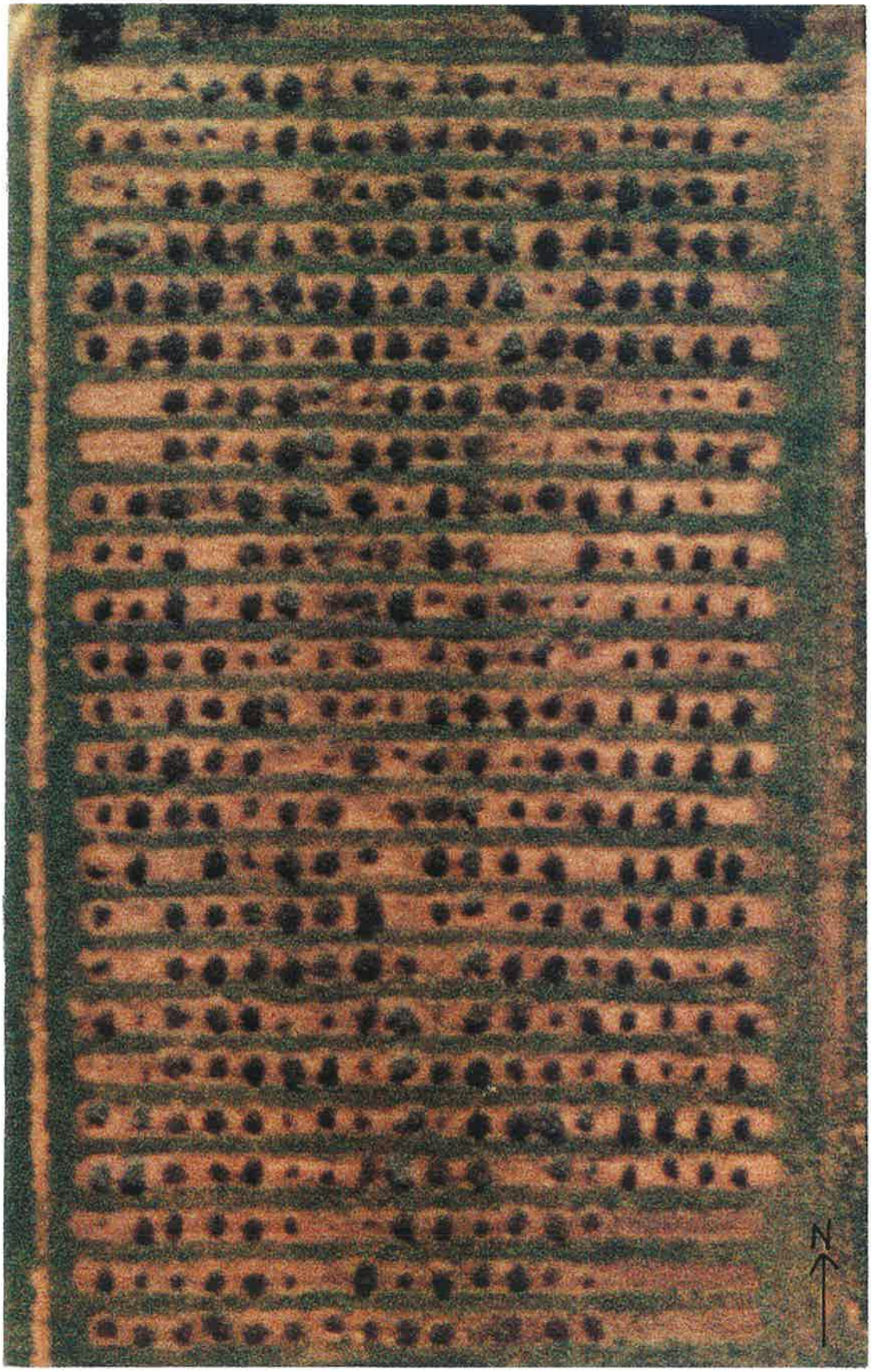
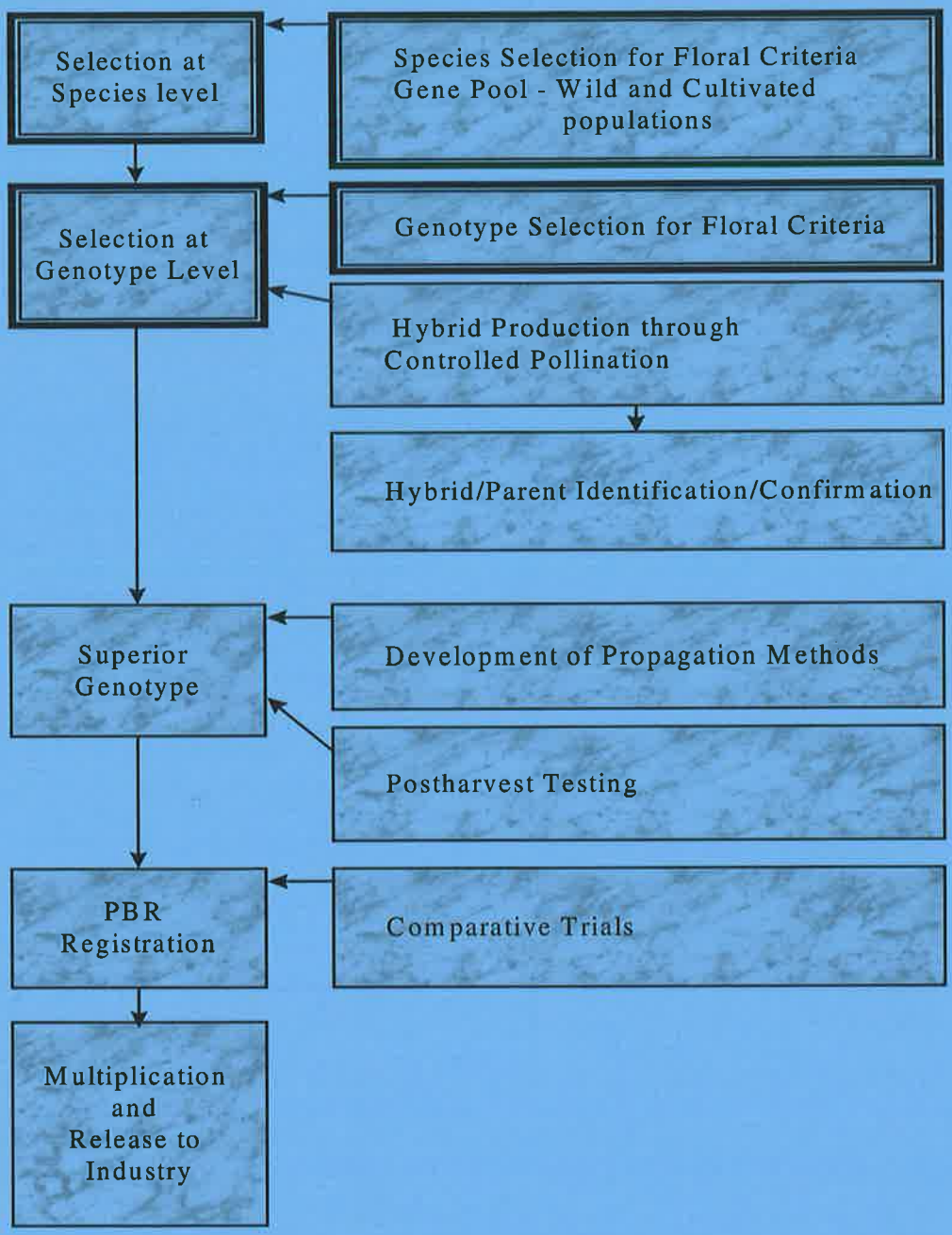


Plate 3.7.
The Laidlaw Plantation: *Eucalyptus* cut flower and bud section, October, 1998, corresponds to Figure 4.1. Scale 1:400



Ornamental eucalypt breeding cycle flow chart.

Areas covered by this chapter are highlighted.

Chapter Four

Selection within species for cut bud and flower production

4.1. Abstract

Nine *Eucalyptus* species, recommended by the Australian cut flower industry as species requiring further development, were grown at the Waite Campus in a randomised complete block design with regular irrigation and fertiliser application. Measurements of tree height and trunk diameters, as well as observations of general growth, health and presence of buds and flowers were made every three months. Analysis of the data showed *E. lesouefii* to be the fastest growing species in both height and trunk diameter, while *E. yalatensis* was the slowest in height and *E. youngiana* the slowest in trunk diameter. The remaining species were variable. Insect and disease attack were observed on *E. anceps*, *E. youngiana, E. stoatei* and *E. forrestiana*, with potentially serious consequences. All species had produced buds by the end of the trial, a little over four years after germination, with the majority producing buds within three years. Seventeen plants from three species showed superior characters across all primary selection criteria, specifically bud size, shape and colour, precocity and floriferousness, and merit further testing for secondary criteria with the aim of selection of named cultivars. These included individuals of the following species: plants numbered 2.2, 2.3, 3.1 and 5.2 from *E. forrestiana* subsp. *dolichorhyncha*; plants numbered 1.3, 1.4, 2.3, 2.4, 3.1, 3.3, 4.1, 4.4 and 5.1 from *E. lesouefii*; and plants numbered 1.2, 2.1, 3.1 and 5.4 from *E. tetragona*.

4.2. Introduction

Horticultural plant breeding relies on the selection of superior individuals or populations, based on gross morphological or agronomic characters, from either wild or cultivated populations (Woodson, 1991; von Hentig, 1995). Individuals exhibiting extremes of the desired characters are noted, with seed or vegetative material collected for further study. The individual may be grown under a range of conditions to test its response to environmental factors. Selection at species level requires a range of genotypes to be grown and assessed for the desired characters, with genotypes with superior attributes selected for further testing.

Development of eucalypts for forestry (Cossalter, 1996; Davidson, 1996; Matheson *et al.*, 1996) or ornamental horticulture uses a combination of selection from established plantations and from natural populations. The stock can be grown in seed orchards for further seed production, or vegetative propagation can be trialed. Plantations provide an opportunity for controlled selection: seed from selected parents are grown under specific, often optimal, conditions. This enables those individuals most suited to the conditions, or exhibiting other desirable characters (depending on end use), to be measured, and their characters quantified and compared.

Selection at species level of eucalypts for cut flower and bud production, requires that species meet floral criteria, specifically relating to bud and flower characters. Bud criteria range from 5 mm to 100 mm in diameter, generally with an inverse correlation between bud size and number per stem. They should be brightly coloured (red, orange, burgundy, green) with shaped, textured or ridged operculum and/or hypanthium. Open flower criteria include bright colours, or contrasting with bud, stem or leaf colour. The fruit are also of interest, as are the leaves and stems.

Selection at genotype level takes selection further, dividing the criteria into primary and secondary levels. Primary level selection criteria are separated into three main categories: flowering, where the focus is on floriferousness, precocity, buds (shape, colour and position on the stem) and flowers (colour); plant form, covering general plant habit (mallee or tree), stems (number, colour, strength and orientation), leaves (size, colour and shape) and growth rate; and plant health, encompassing the plant's response to attack by common insect pests and diseases. Selection at the secondary level focuses on the response of the plant to cultivation (pruning, harvesting, including time to regrowth of commercial length stems with buds or flowers, watering and fertiliser), postharvest testing (sucrose levels and cold storage) and propagation (cuttings, grafting and tissue culture).

Genotypes that exhibit exceptional characters for some criteria, but poor characters for others, could be incorporated in inter and intraspecific breeding programs. The resulting hybrid offspring may combine the exceptional characters of both parents, producing a plant superior to both. Other genotypes may be more suited to production as garden specimens or pot plants.

The aim of this study was to grow a range of species recommended as suitable for the cut flower and bud industry under similar conditions, and to observe and record their growth and development over a three year period. From these data, selection of species and primary selections of genotypes could be made. Those individuals exhibiting exceptional characters in the areas of flowering, plant form and plant health could go on to selection at the secondary level.

4.3. Materials and methods

4.3.1 Plant Material

Nine species of eucalypts recommended by the Australian native cut flower industry were planted in the Laidlaw Plantation in October 1996. The species planted were *E. anceps, E. erythrocorys, E. forrestiana* subsp. *dolichorhyncha, E. leptophylla, E. lesouefii, E. stoatei, E. tetragona, E. yalatensis*, and *E. youngiana.* A tenth species, *E. uncinata*, was included, however as the trial progressed the plants of this species bore little resemblance to true to type seedlings, and so it was removed from the analysis. Plants were grown from seed collected by Nindethana Seed Company, Western Australia, from unspecified provenances. As the provenance of seed was unknown, it could not be determined whether the trees would be wholly representative of the variation within each species. Despite this, the level of variation expressed within each species in the trial was sufficient that it may applied to the species as a whole.

4.3.2. Planting design

Twenty plants from each species were planted in a randomised complete block design, consisting of five replications of four plants from each species (Figure 4.1). Rows were spaced 3.5 m apart, with trees planted 2.5 m apart, with two buffer rows to the north, east and west and three buffer rows to the south.

4.3.3. Pre-planting treatment

The soil type is uniform across the plot consisting of red brown earth, with 25 cm or more of a fine sandy loam topsoil and prismatic structured clay subsoil generally free from gravel or stone, with a pH in the surface horizon of 5-8. As the plot had previously been used for pasture and cropping, the area was deep ripped to a depth of 0.5 m along the rows, the whole area was rotary hoed to a depth of 0.3m to break up the soil.

4.3.4. Irrigation

Irrigation was supplied by 2 L/h pressure compensating drippers (Netafim), once monthly for 12 hours for the first summer after planting, every six weeks the second summer after planting, and every eight weeks the third summer after planting.

4.3.5. Fertiliser

The trees were mulched around the base with oat straw for the first 12 months. Blood and Bone (Pivot) was applied at the rate of 100 gm per tree every six months.

4.3.6. Weed control

A pre-emergent herbicide, Surflan (Agro Sciences), was applied post planting, with applications of Roundup (Monsanto) along the rows each winter to control spring weed growth. The plantation was mown when required.

4.3.7. Plant measurements

At three monthly intervals each tree was measured for height and trunk diameter (10 cm above ground), and lignotuber diameter (if present). Records of the presence of new growth, the appearance of flower buds and flowers, insect pests and diseases were noted at this time. Data from each individual within a species was compared to identify superior genotypes, while averages from each species were analysed using Analysis of Variance (Genstat 5 Release 4.2. (PC/Windows NT, 1997, Lawes Agricultural Trust, Rothamsted Experimental Station) with L.S.D. used where appropriate.

4.3.8. Weather data

Rainfall and evaporation, hours of sunshine and solar radiation, and maximum and minimum temperatures were recorded daily at the Waite Agricultural Research Institute Weather

station. The monthly averages of these data over 36 months, from March 1996 to March 1999, are displayed in Figures 4.2. to 4.4.

Figure 4.1. The Laidlaw Plantation: layout of eucalypt cut bud and flower section as a randomised complete block design of five sections each containing four plants of each species, with two or three buffer rows. Rows and irrigation run East - West. Figure corresponds to Plate 3.7.

N ↑	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	2	2	7	7	3	3	6	6	5	5	4	4	6	6
2	2	2	7	7	3	3	6	6	5	5	4	4	6	6
3	7	7	5	2	10	8	9	6	4	1	3	7	2	2
4	7	7	5	2	10	8	9	6	4	1	3	7	2	2
5	3	3	5	2	10	8	9	6	4	1	3	7	3	3
6	3	3	5	2	10	8	9	6	4	1	3	7	3	3
7	3	3	4	10	1	5	7	2	6	3	9	8	4	4
8	3	3	4	10	1	5	7	2	6	3	9	8	4	4
9	4	4	4	10	1	5	7	2	6	3	9	8	5	5
10	4	4	4	10	1	5	7	2	6	3	9	8	5	5
11	2	2	5	4	3	7	10	9	8	1	2	6	7	7
12	2	2	5	4	3	7	10	9	8	1	2	6	7	7
13	3	3	5	4	3	7	10	9	8	1	2	6	2	2
14	3	3	5	4	3	7	10	9	8	1	2	6	2	2
15	2	2	6	3	1	5	9	8	10	4	7	2	5	5
16	2	2	6	3	1	5	9	8	10	4	7	2	5	5
17	2	2	6	3	1	5	9	8	10	4	7	2	3	3
18	2	2	6	3	1	5	9	8	10	4	7	2	3	3
19	5	5	9	4	2	1	3	10	7	8	6	5	4	4
20	5	5	9	4	2	1	3	10	7	8	6	5	4	4
21	7	7	9	4	2	1	3	10	7	8	6	5	7	7
22	7	7	9	4	2	1	3	10	7	8	6	5	7	7
23	3	3	3	3	2	2	3	3	2	2	3	3	1	1
24	3	3	3	3	2	2	3	3	2	2	3	3	1	1
25	4	4	4	4	4	4	4	4	4	4	4	4	4	4

Key: number indicates species

1 = *E. anceps*
2 = *E. erythrocorys*
3 = *E. forrestiana* subsp. *dolichorhyncha*
4 = *E. leptophylla*
5= *E. lesouefii*
6 = *E. stoatei*
7= *E. tetragona*
8 = *E. uncinata*[a]
9 = *E. yalatensis*
10 = *E. youngiana*

[a] All *E. uncinata* plants were removed from the analysis as not true to type.

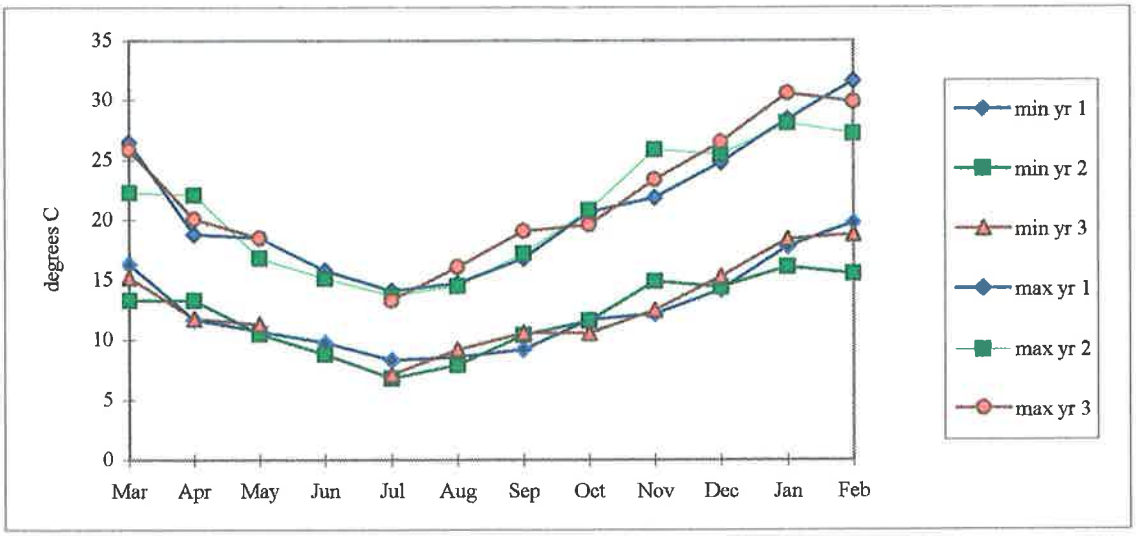


Figure 4.2. Monthly averages of minimum and maximum air tempteratures at the Waite Agricultural Research Institute weather station March '96 to March '99

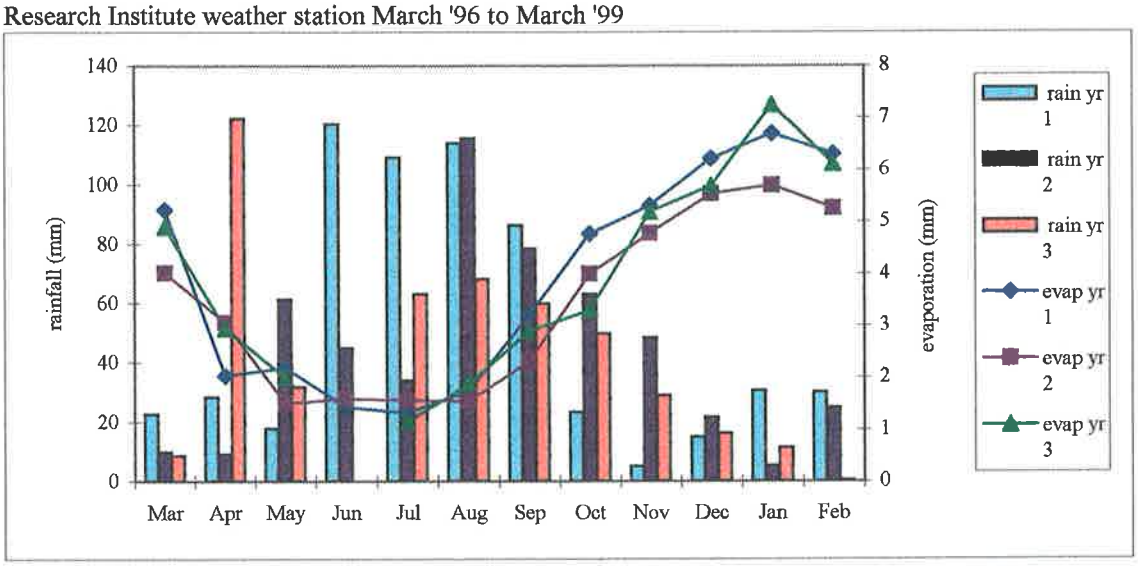


Figure 4.3. Monthly averages of rainfall and evaporation at the Waite Agricultural Research Institute weather station March '96 to March '99

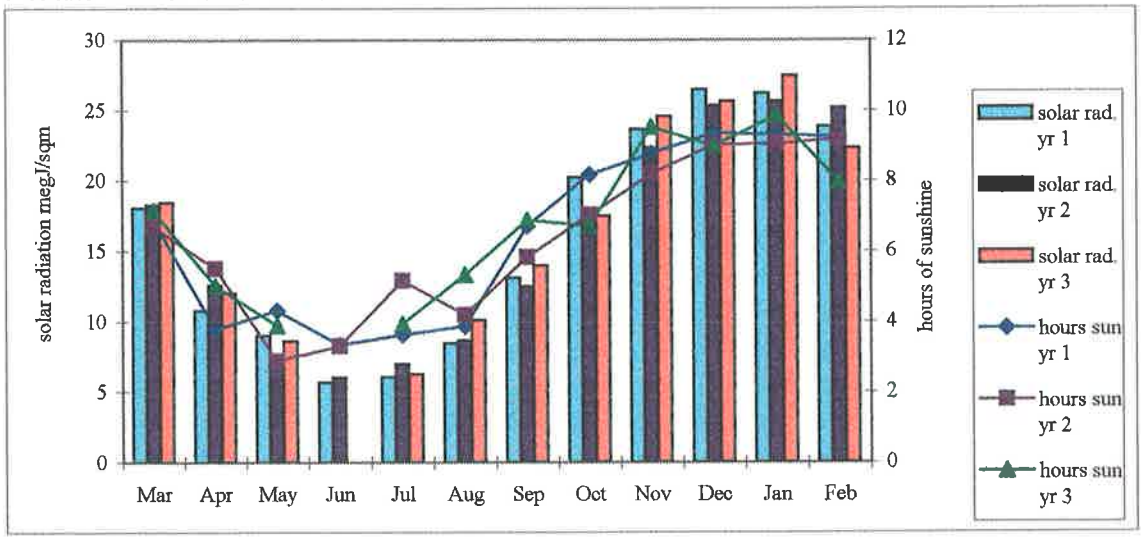


Fig. 4.4. Monthly averages of hours of sunshine and solar radiation at the Waite Agricultural Research Institute weather station March '96 to March '99

4.4. Results

4.4.1. Between species comparison

4.4.1.1. *Growth*: The averages of the three monthly tree height and trunk diameter for each species are summarised in Table 4.1. *E. lesouefii* grew at the fastest rate for both tree height (79.3 mm/month) (Figure 4.5) and trunk diameter (2.1 mm/month) (Figure 4.6), and achieved the greatest height (mean 3125 mm) (Figure 4.7) and trunk diameter (66.9 mm) (Figure 4.8) in the 30 month trial. *E. erythrocorys* recorded the second best results for growth in all four categories. At the other end of the spectrum, *E. yalatensis* produced the lowest rate of vertical growth (39.3 mm/month) and the shortest height (1617 mm), while *E. youngiana* showed the least trunk thickening (46.0 mm at 1.3 mm/month). *E. tetragona* produced a thick trunk of intermediate height, and all other species were consistent in their ordering, with little difference between species.

Table 4.1. Summary of data for nine species in the Laidlaw Plantation.

	Tree height[x]			**Trunk diameter[x]**		
Species	**Final**	**range**	**rate**	**Final**	**range**	**rate**
E. anceps	1805^{d}	650-2650	42.1^{d}	50.0^{cd}	26.8-62.3	1.5^{cd}
E. erythrocorys	2777^{b}	1900-3650	74.7^{a}	64.3^{a}	46.5-74.2	2.0^{a}
E. forrestiana	2054^{d}	1200-3300	44.3^{cd}	55.5^{bc}	36.8-69.7	1.7^{bc}
E. leptophylla	1617^{e}	750-2250	40.2^{d}	51.8^{cd}	32.0-69.8	1.5^{cd}
E. lesouefii	3125^{a}	1800-4000	79.3^{a}	66.9^{a}	52.3-82.5	2.1^{a}
E. stoatei	2085^{cd}	1400-2650	53.5^{bc}	52.8^{cd}	35.4-68.7	1.6^{cd}
E. tetragona	1853^{d}	800-2700	46.1^{cd}	62.3^{ab}	46.0-99.8	1.9^{ab}
E. yalatensis	1658^{e}	1300-2200	39.3^{d}	49.2^{cd}	38.9-69.0	1.5^{cd}
E. youngiana	2363^{c}	1500-2800	61.9^{b}	46.0^{d}	33.5-71.3	1.3^{d}
F pr:	<0.001		<0.001	<0.001		<0.001
S.E.M.	103.1		3.4	2.6		0.1
L.S.D (5%)	288.0		9.4	7.2		0.2

[x]Data are averages 20 plants for each species, planted in 5 blocks (as replications).
Different superscripts indicate significant differences in a column at P<0.05.
Anova table Appendix 1.1

4.4.1.2. *Flowering:* Time to first flowering varied both within and between species (Table 4.2). Almost half the *E. lesouefii* specimens had produced macroscopic buds by the first spring after planting, less than two years after germination, along with three *E. forrestiana* trees. The majority of plants within each species had produced their first buds by the second spring after planting, or less than three years after germination. Only *E. erythrocorys* took longer, flowering in the third summer after planting (four years after germination).

4.4.1.3. *Pest susceptibility*: The presence of pest and disease problems on each tree were recorded at each measurement period. Insect pests were sawflies (*Perga* spp.), found in clumps on *E. lesouefii* in late spring, although they did not appear to be feeding on this species, and leaf miners (*Perthida* spp.), feeding on the young new growth of *E. tetragona*. The most common pests, and possibly most damaging from a production perspective, were the constant presence of ants and scale (*Eriococcus confusus*), and the associated sooty mould. *E. anceps* were highly affected at planting, becoming less susceptible as they grew. Specimens of *E. forrestiana, E. stoatei* and *E. youngiana* became affected during the summer months, with some plants highly susceptible, while others were not affected.

4.4.2. Selection of superior individuals within species

Over the 30 months of the trial, different levels of variation within species became evident. Some variation is valuable at the selection stage, as genotypes with more desired characters can be identified. Individuals within species were allocated to four categories, based on the primary selection criteria (Table 4.3). Individuals in category I merit further testing for secondary selection criteria. Individuals in category II and III could be incorporated into breeding programs; offspring produced would require re- assessment for primary criteria. Individuals in category IV show no ornamental merit in this trial and should be disregarded.

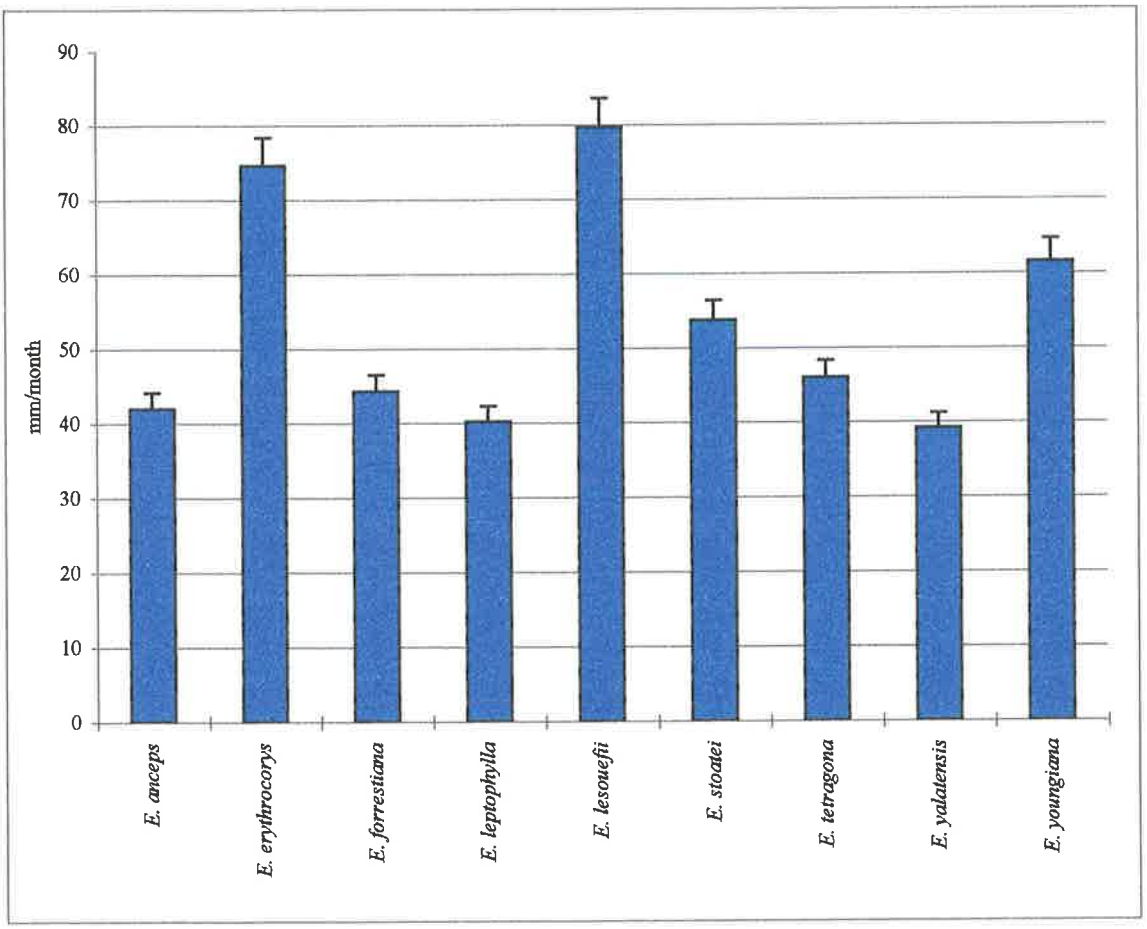


Figure 4.5. Growth rate (height) per month for nine species.

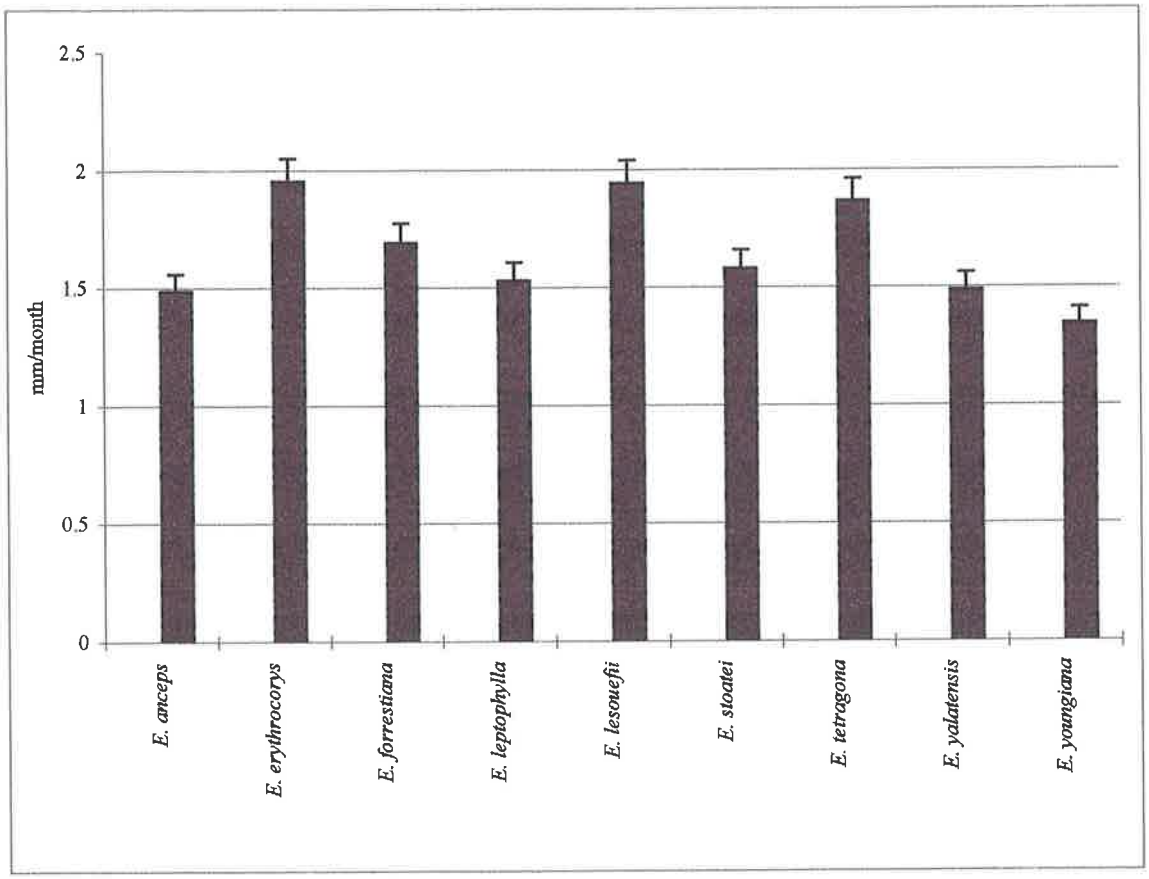


Figure 4.6. Growth rate (trunk) per month for nine species.

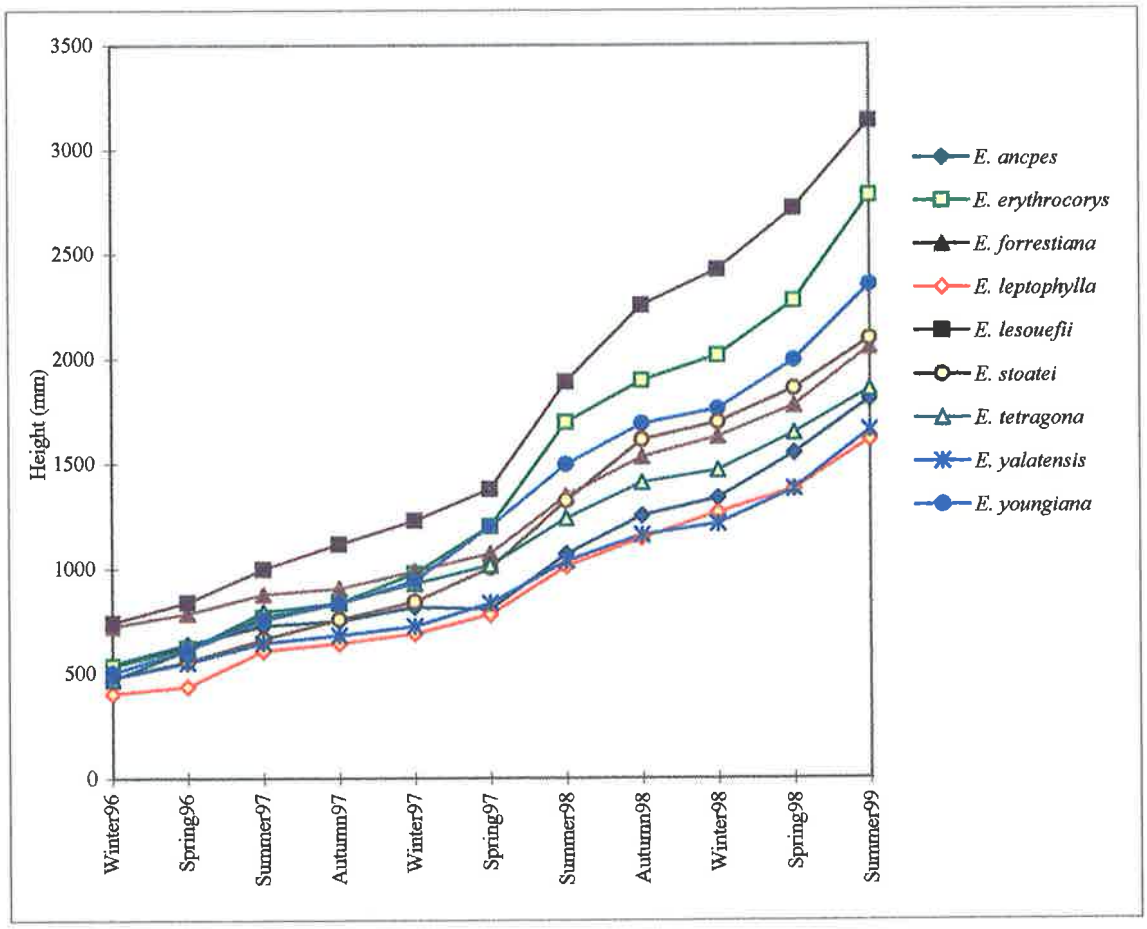


Figure 4.7. Progressive tree heights for nine species.

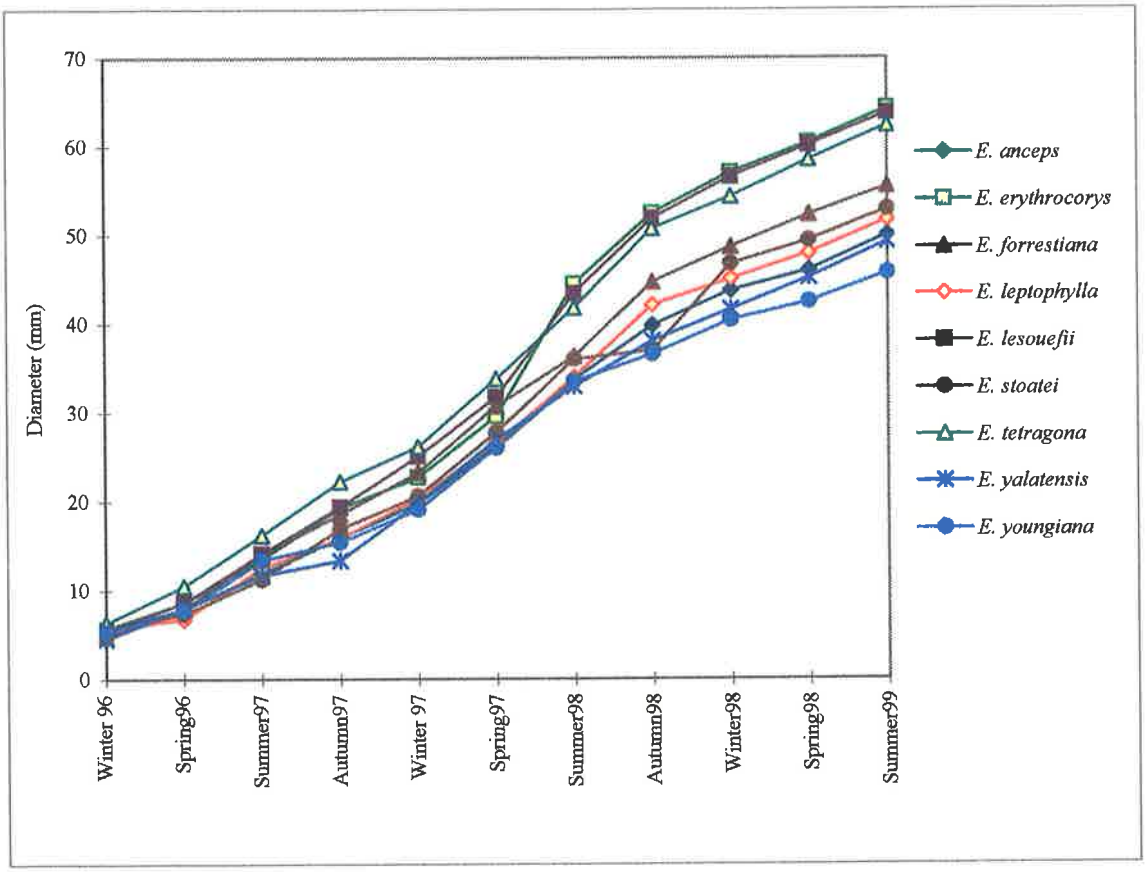


Figure 4.8. Progressive trunk diameters for nine species

Table 4.2. Season of appearance of macroscopic buds for nine species in the Laidlaw Plantation.

Species	Winter 96	Spring 96	Summer 97	Autumn 97	Winter 97	Spring 97	Summer 98	Autumn 98	Winter 98	Spring 98	Summer 99	total[b]
E. anceps						1	3	2	2	2	2	12/20
E. erythrocorys											5	5/20
E. forrestiana subsp. *dolichorhyncha*		3[a]	1		1	1	5	3	1	1		16/20
E. leptophylla			4			13	1	1	1			20/20
E. lesouefii		9	3		1	2				2		19/20
E. stoatei					2				2	2		6/20
E. tetragona						17						17/20
E. yalatensis						7	1	1	2	2	2	18/20
E. youngiana						8	3			3	2	16/20

[a] Number of plants showing new buds in that season.
[b] Total number of plants for that species showing buds in duration of trial.

Table 4.3. Allocation of individuals within species to four primary selection categories.

Plant number	*E. anceps*	*E. erythrocorys*	*E. forrestiana* subsp. *dolichorhyncha*	*E. leptophylla*	*E. lesouefii*	*E. stoatei*	*E. tetragona*	*E. yalatensis*	*E. youngiana*
1.1	IV	III	IV	III	III	III	II	III	III
1.2	IV	III	IV	III	II	IV	I	III	II
1.3	IV	III	II	III	I	II	II	III	II
1.4	IV	III	III	III	I	III	II	III	III
2.1	IV	III	II	III	II	II	I	III	II
2.2	IV	III	I	III	III	II	III	III	II
2.3	IV	III	I	III	I	III	III	III	III
2.4	IV	III	II	III	I	IV	III	III	IV
3.1	IV	III	I	III	I	IV	I	III	III
3.2	IV	II	IV	III	II	II	III	III	III
3.3	IV	III	IV	III	I	II	II	III	II
3.4	IV	II	II	III	II	III	IV	II	IV
4.1	IV	III	III	III	I	IV	II	II	III
4.2	IV	II	IV	III	IV	IV	III	III	IV
4.3	IV	III	II	III	IV	III	II	III	IV
4.4	IV	III	IV	III	I	II	III	II	IV
5.1	IV	II	IV	III	I	IV	III	III	IV
5.2	IV	III	I	III	II	II	III	III	III
5.3	IV	II	II	III	II	III	II	II	III
5.4	IV	III	IV	III	IV	IV	I	III	III

I = Genotypes/plants excel in all primary selection criteria, straight on to secondary selection

II = Genotypes/plants superior in most primary selection criteria, require intraspecific hybridisation to refine genotypes

III = Genotypes/plants more suited to amenity selection or interspecific hybridisation

IV = Genotypes/plant not suitable for commercial development

Most within species variation was seen in bud and flower colour, as well as time of flowering and positioning of flowers. For example, *E. youngiana* plants showed either red or yellow flowers, with hypanthium size ranging from 40 to 100 mm in diameter. The buds of *E. lesouefii* varied slightly in size and greatly in bud colour - from glossy orange to highly glaucous purple. Out of the twenty *E. lesouefii* plants measured, 12 different genotypes were observed, while very little variation was apparent between *E. stoatei* plants. This highlights the degree of variation within some species and the need for selection at genotype level prior to commercial release.

The 17 plants/genotypes placed in category I may now undergo selection at the secondary level. The plants should undergo rigorous postharvest testing, to assess vase life under a range of different conditions. They should be assessed for suitability to clonal propagation, to enable reproduction of the plant without loss of desirable characters. Finally the plants should be tested for their response to production techniques, such as pruning and harvesting.

4.4.3. Summary of each species

E. anceps Plants growing for this species varied in height and growth rate, but maintained a mallee appearance. The presence of flower buds was first recorded in spring '97, with the buds taking at least twelve months to mature and flower. Flower buds were first produced in leaf axils at the growing tip, but as the branch overgrew, the mature buds were located within the canopy. After the trial, *E. anceps* was found not suitable for further testing, with all plants in category IV. Plate 4.1.

E. erythrocorys Plants for this species grew quickly with single trunks. Appearance of flower buds was first recorded in summer '99, with buds taking less than three months to reach maturity and flower. Flower buds were axillary or terminal, but found at the top of the

tree. *E. erythrocorys* shows promise for further development, however the long period to flowering and the large size of the plants are undesirable characters. Five plants were placed in category II, requiring within species breeding and further assessment, with the remaining fifteen plants in category III. Plate 4.1.

E. forrestiana subsp. *dolichorhyncha* All plants from this species grew slowly, with uniform shape and single trunks. Flower buds first appeared in spring '96, maturing within twelve months. Flower buds were initially terminal, as the branch overgrew, more buds developed in each leaf axil, giving each branch many buds at different stages of maturity. The buds changed colour from green to bright red prior to flowering. Four plants from this species were suitable for further testing, as they showed superior primary characters. A further five plants were placed in category II and two in category III. The remaining plants were considered unsuitable for as they suffered badly from infestations of scale and sooty mould. Plate 4.2.

E. leptophylla All plants from this species grew slowly in height, but spread out to form low, wide rounded plants. Buds first appeared in summer'97, and took up to twelve months to reach maturity. Buds formed terminally, but as the branch overgrew mature buds were located within the canopy. Plants form this species were considered highly ornamental, but of little use for cut flower and bud production, due to short stems and over growing. All plants were in category III. Plate 4.1.

E. lesouefii Wide variation was observed within this species for tree height and habit, as well as flower bud shape, colour and position. The first appearance of buds was recorded in spring '96, with buds taking up to twelve months to mature and flower. Buds were initially positioned at the end of each branch, but the branches overgrew, leaving the

mature buds within the canopy. Eight plants were considered worthy of further testing (category I), six in category II and two in category III, with the remaining three plants not suitable. Plate 4.3.

E. stoatei Plants grew moderately fast with uniform shape. Buds first appeared winter '97, taking over twelve months to mature. Buds were initially positioned at the end of each branch, but the branches overgrew, leaving the mature buds within the canopy. Seven plants from this species were placed in category II, requiring within species breeding and further assessment, the main detraction being the non-terminal placement of buds, however this may change as the plants age. Plate 4.1.

E. tetragona Plants from this species grew slowly, with uniform shape but varying height. The plants quickly became top-heavy and leaned over. Buds first appeared in spring '97, maturing and flowering within three months. Buds were terminal. Four plants showed exceptional characters during this study, and were placed in category I. A further seven plants have potential, but may be more suited to within species breeding. Plate 4.4.

E. yalatensis All plants grew into low, rounded mallees. Buds first appeared in spring '97, maturing within twelve months. Buds were initially positioned at the end of each branch, but the branches overgrew, leaving the mature buds within the canopy. All plants were considered highly ornamental, with four plants requiring within species breeding and re-assessment prior to further testing for cut bud or flower production. The remaining plants were placed in category III. Plate 4.5.

E. youngiana Plants grew quickly, with a single trunks or growing from a lignotuber with three or four trunks. The first buds appeared in spring '97, taking up to twelve months to

mature, with additional buds maturing to flower in less than six months. Buds varied in position, commonly low on the branch. Five plants were placed in category II, with potential for breeding and selection for cut buds and flowers. Nine plants would be suitable for ornamental production, with the remaining six plants suffering severely from scale. Plate 4.5.

4.4.4. Further testing

Other species with ornamental potential, not included in this study, but which merit further testing are listed in Table 4.4.

Table 4.3. *Eucalyptus* species with ornamental merit suitable for further testing.

E. orbifolia	*E. macrocarpa*	*E. corrugata*	*E. burracoppinensis*	*E. ewartina*
E. websteriana	*E. rhodantha*	*E. synandra*	*E. oxymitra*	*E. pachyphylla*
E. preissiana	*E. pyriformis*	*E. campaspe*	*E. cocinna*	*E. lane-poolei*
E. kruseana	*E. erythronema*	*E. tetraptera*	*E. megacarpa*	*E. drummondii*
E. gillii	*E. stricklandii*	*E. pruinosa*	*E. eudesmoides*	*E. kingsmillii*
E. crucis	*E. caesia*	*E. rameliana*	*E. nutans*	*E. coronata*
	E. clelandii	*E. oldfieldii*	*E. leptopoda*	*E. sessilis*

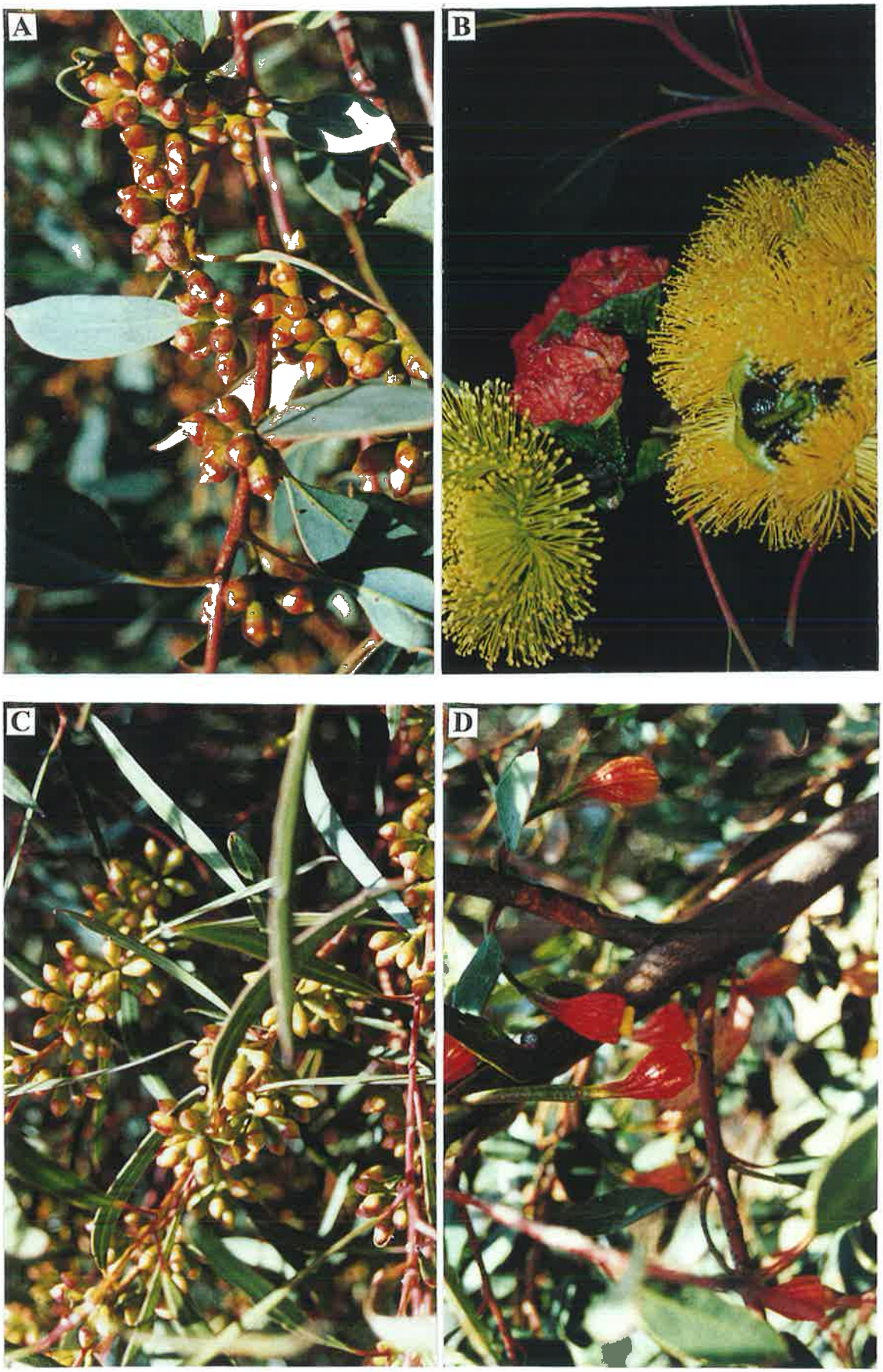


Plate 4.1.
A: *E. anceps* (3.3); B: E. *erythrocorys* (5.1);
C: *E. leptophylla* (Plant 1.4); D: *E. stoatei* (Plant 2.2)

Plate 4.2.
A: *E. forrestiana* subsp. *dolichorhyncha* (2.2); B: *E. forrestiana* subsp. *dolichorhyncha* (2.2); C: *E. forrestiana* subsp. *dolichorhyncha* (3.1); D: *E. forrestiana* subsp. *dolichorhyncha* (3.1).

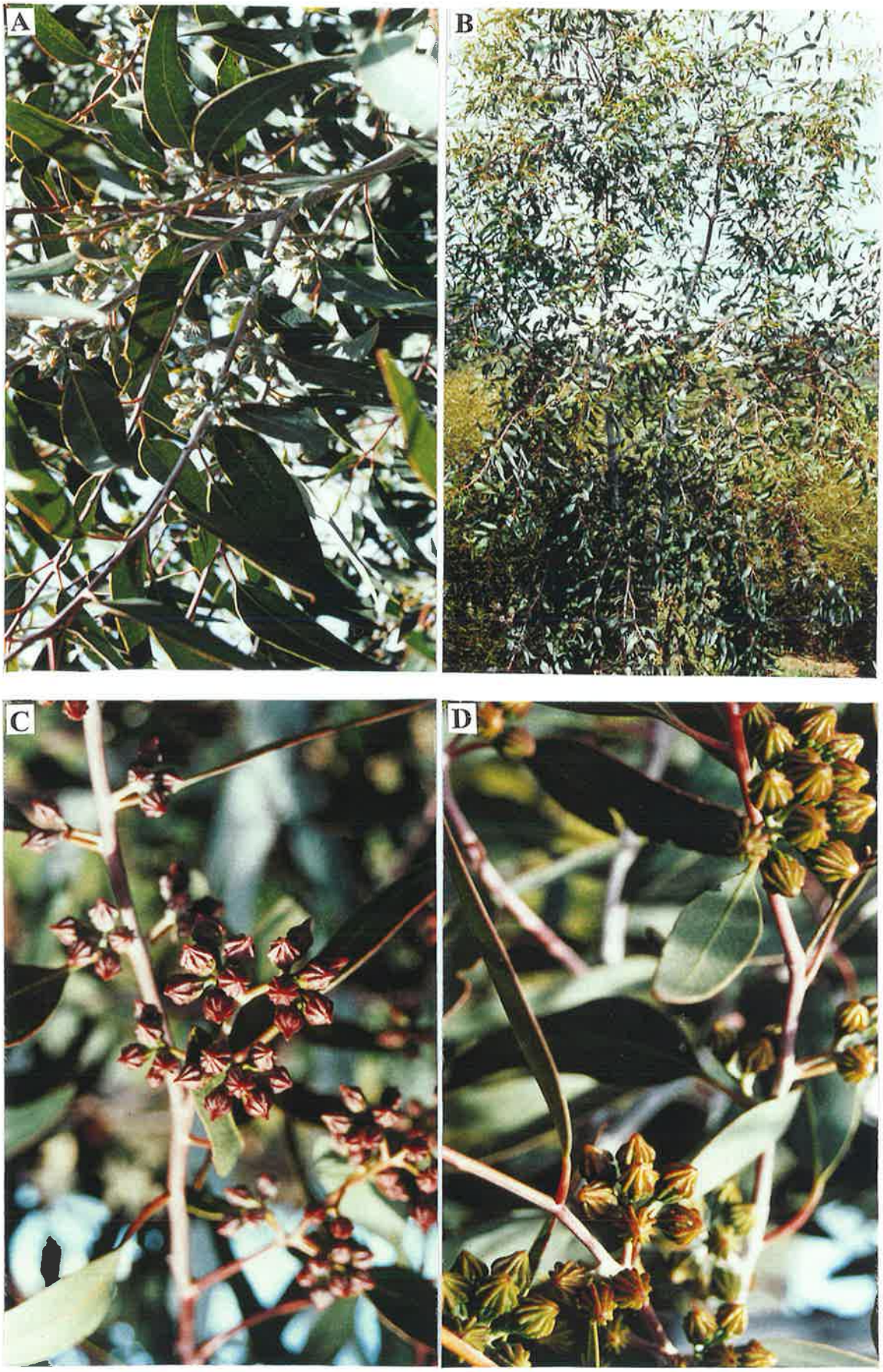


Plate 4.3.
A: *E. lesouefii (1.4);* B: *E. lesouefii* (3.3);
C: *E. lesouefii* (4.4); D: *E. lesouefii* (5.1).

Plate 4.4.
A: *E. tetragona* (1.2); B: *E. tetragona* (2.1)
C: *E. tetragona* (3.1); D: *E. tetragona* buds (Plant 5.4).

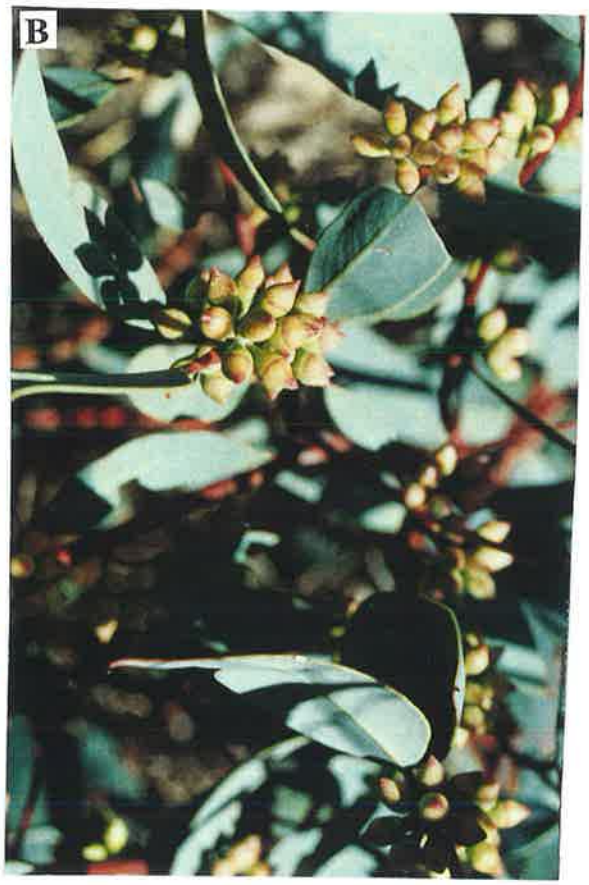


Plate 4.5.
A: *E. yalatensis* (4.1); B: *E. yalatensis* (4.4)
C: *E. youngiana* (5.2).

4.5. Discussion

The nine species trialled in this study were recommended by the Australian cut flower industry as species with merit for cut bud and flower production. Both species and individual genotypes within species were on trial for their suitability for cut flower and bud production. Limits of space required that 20 plants per species were grown, selected from seed grown material of unknown provenance. In recognition of the small population size and potential for limited genetic variability within the trial, it was not considered to be a full trial for selection of plants for seed production, but rather an example of how a full trial might show variability and potential for genetic gains based on selection.

The results of the trial showed that while some species, such as *E. tetragona* and *E. forrestiana* subsp. *dolichorhyncha*, have considerable merit, others, such as *E. anceps*, do not, under the conditions of the trial. These species may grow differently in other climates and soil types. The trial showed the variation in morphology that may be seen in seed grown material, highlighting the need for clonal propagation of selected forms. The observed variation within species of bud (consisting of peduncle, pedicle, hypanthium and operculum) size and colour, as well as flower (stamen) colour, gives positive evidence that selection of superior genotypes is required prior to the release of eucalypt species for both floriculture and amenity horticulture, to ensure the most economically and commercially viable variety is grown (Sedgley, 1998).

The growth rates and final recorded heights and trunk diameters reflect the natural habits of each species: *E. lesouefii* is a single stemmed tree reaching 18 m in height; *E. erythrocorys*, *E. forrestiana* and *E. stoatei* also have single trunks, but generally are small trees up to 5 m in height; *E. tetragona*, *E. leptophylla* and *E. youngiana* can be mallees or small trees (from 3 to 11 m); and *E. yalatensis* and *E. anceps* are mallees up to 6 m. The mallee type species used in

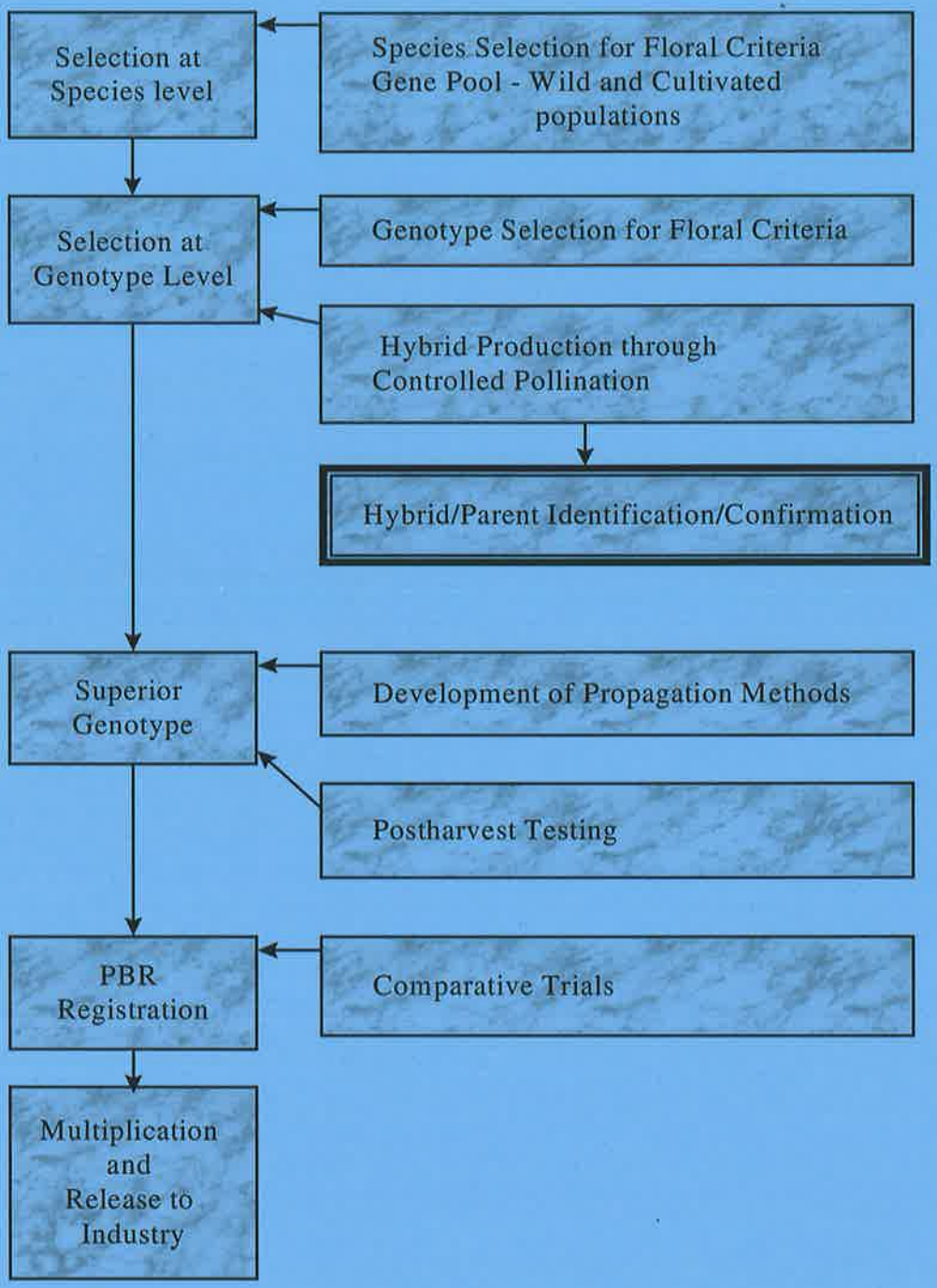
this trial were as wide as they were high, with multiple trunks, thus trunk diameter for these species was generally narrower than that of the single stemmed tree species.

Information on growth rates has important implications for selection of superior genotypes of cut flower species. Tall fast growing species, such as *E. lesouefii* and *E. erythrocorys*, will require different management and spacing compared to low growing, multi stemmed mallee types, such as *E. yalatensis* and *E. leptophylla.*

4.6. Conclusion

Species suitable for cultivation under the trial conditions were: *E. erythrocorys*, *E. forrestiana* subsp. *dolichorhyncha, E. leptophylla*, *E. lesouefii, E. stoatei, E. tetragona, E. yalatensis* and *E. youngiana.*

Superior individuals were identified of some species: plants numbered 2.2, 2.3, 3.1 and 5.2 from *E. forrestiana* subsp. *dolichorhyncha*; plants numbered 1.3, 1.4, 2.3, 2.4, 3.1, 3.3, 4.1, 4.4 and 5.1 from *E. lesouefii*; and plants numbered 1.2, 2.1, 3.1 and 5.4 from *E. tetragona.*



Ornamental eucalypt breeding cycle flow chart.

Areas covered by this chapter are highlighted.

Chapter Five

Eucalyptus 'Urrbrae Gem':

Parentage determination through RAPD analysis

5.1. Abstract

The parentage of the ornamental hybrid *Eucalyptus* 'Urrbrae Gem' was investigated using RAPD-PCR, and analysed using hierarchical and non-hierarchical distance methods. The female parent was known to be *E. erythronema* var. *erythronema*, but previous opinion placed the male parent as either *E. stricklandii* or *E. gomphocephala*, based on adult morphological characters. Samples of DNA from different individuals within each species were amplified with six different 10-mer primers to produce RAPD fragments. These were used to generate a UPGMA dendrogram based on similarity, and an ordination derived by multi-dimensional scaling (MDS) and a minimum spanning tree (MST) to show the relative dissimilarities between the individuals tested. The dendrogram divided the samples into three clusters, with the hybrid slightly closer to *E. stricklandii* than to *E. gomphocephala.* The MDS ordination and MST placed the hybrid between *E. erythronema* var. *erythronema* and *E. stricklandii*, supporting *E. stricklandii* as the male parent.

5.2. Introduction

E. 'Urrbrae Gem' is a spontaneously occurring interspecific hybrid (Kelly, 1969; Gardner, C.A., 1979). The single specimen was discovered in 1936 by the then head gardener, F. A. Cousins, growing within seed collected from a young *E. erythronema* Turcz. var. *erythronema* specimen (Gardner, unpublished, 1987). The seedling differed in leaf morphology, so Cousins kept it separate and planted it in the Waite Arboretum. The male

parent was identified as *E. gomphocephala* DC by L. Pryor (Parks and Gardens, Canberra), based on observation of bud, flower, leaf and fruit morphology (Gardner, unpublished, 1987). This was later amended to *E. stricklandii* Maiden, with characters such as leaf size and shape, flowering times, fruit peduncle and pedicel shapes and operculum size and shape, and proximity to pollen donors being specifically noted as diagnostic (Pryor, unpublished, 1992). The presence of pith glands in *E.* 'Urrbrae Gem' was considered another diagnostic feature by Brooker (unpublished, 1991), as they are absent in *E. gomphocephala* and present in both *E. erythronema* and *E. stricklandii* (Brooker and Kleinig, 1990).

E. 'Urrbrae Gem' is now a medium sized tree with a single trunk of varying colour, and a wide-open canopy of glossy green leaves. The specimen is over 60 years old, and still growing well, despite the close proximity of a mature Bunya Bunya Pine. The bark varies from white to pinkish brown, depending on the time of year. The spectacular clusters of reddish blossoms appear in early summer and remain through to late summer. The umbels have up to seven greenish-yellow buds, appearing up to 18 months before anthesis. Open pollinated seed from the tree is viable, however, seedlings generally exhibit poor form and low vigour. A number of seedlings from *E.* 'Urrbrae Gem' have been planted in the Waite Arboretum over the last 50 years, however, only one adult specimen remains. Aerial rooting was attempted in 1958, apparently with no success (Gardner, unpublished, 1987), as was cutting propagation and tissue culture in 1991 (Mc Laughlin, 1991).

The study to identify the parents was problematic, as previous studies of adult morphology had indicated that both *E. stricklandii* and *E. gomphocephala* shared adult morphological characters with the hybrid. Furthermore, due to the length of time involved, none of the original parent material was available for analysis. Representative specimens from each species involved were required to generate DNA fingerprints indicative of the genetic makeup

of each for comparison to the hybrid. Other eucalypt species growing nearby at the time were discounted as possible pollen donors/parent species, either on the basis of their floral and leaf morphology, or due to their flowering period not overlapping with that of *E.* 'Urrbrae Gem' or *E. erythronema.* The ornamental appeal of the tree makes it an ideal candidate for horticultural development, but prior to registering it under the International Convention of the Union of New Plant Varieties (UPOV), both parents should be known. A thorough study of the hybrid, the female parent species and both potential male parent species, was required to clarify paternal lineage prior to further development.

Recently, random amplified polymorphic DNA (RAPD) (Williams *et al.*, 1990) has been used in a number of eucalypt studies, including investigating genetic distance (Chen and De Filippis, 1996), pedigree analysis (Vaillancourt *et al.*, 1998), generating genetic linkage maps (de Silva and Grattapaglia, 1997; Brondani and Grattapaglia, 1997), and identification of parentage of hybrids (Rossetto *et al.*, 1997). In view of the superiority of *E.* 'Urrbrae gem' for ornamental horticulture over the known or putative parents, this study aimed to use RAPD analysis to determine parentage. The parents could then be included in the University of Adelaide *Eucalyptus* improvement program (Wirthensohn *et al.*, 1999).

5.3. Materials and Methods

5.3.1. Plant material

The original female parent tree and the possible male parent trees had been removed from the Arboretum prior to the commencement of the present study so that none of the original parent material was available for DNA fingerprinting. Alternative specimens from different provenances were sourced to be representative of the species involved. The original female parent specimen, planted in 1931, was removed from the Arboretum in 1983. The possible

male parent specimens of *E. gomphocephala* in the Arboretum were located nearby (Figure5.1) and were both planted in 1928. Others were planted in 1934 and 1937. The majority died in the 1940s, with the last in 1996 (Table 5.1). *E. stricklandii* was also present close to the female parent, and was planted in 1931 (Figure 5.1) and removed in 1969. Thus none of the original parent material was available for DNA fingerprinting. Representative trees from each of *E. erythronema* var. *erythronema* (five trees), *E. stricklandii* (five trees), *E. gomphocephala* (five trees), *E. erythronema* var. *marginata* (three trees) and *E.* 'Urrbrae Gem' (one tree) were selected for DNA extraction. The 19 individuals used were sourced from the Waite Arboretum, the Waite Campus plantings and the Monarto Woodland, and are listed in Table 5.2. Taxonomy follows the informal classification of Pryor and Johnson (1971) and all species are from subgenus *Symphyomyrtus* sect. *Bisectaria*.

Table 5.1. *Eucalyptus* species located near the female parent of *E.* 'Urrbrae Gem', Waite Arboretum, 1955. Applies to Figure 5.1.

Number	Species	Planted	Removed
1867	*E.* 'Urrbrae Gem'	1936	
157	*E. calophylla*	1928	
172	*E. calophylla*	1928	
178	*E. calophylla*	1928	
198	*E. calophylla*	1928	
42	*E. camaldulensis*	original	
28	*E. cladocalyx*	1915	
41	*E. cladocalyx*	1915	
27	*E. conica*	1931	
104	*E. erythronema* var. *erythronema*	1931	1983
23	*E. diversifolia*	1928	
188	*E. ficifolia*	1928	
33	*E. gomphocephala*	1934	1940
36	*E. gomphocephala*	1934	1942
38	*E. gomphocephala*	1937	1961
44	*E. gomphocephala*	1928	1942
48	*E. gomphocephala*	1928	1996
636	*E. leucoxylon*	original	
34	*E. maculata*	1928	
30	*E. megacarpa*	1928	
40	*E. moluccana*	1928	
32	*E. sideroxylon*		
46	*E. sideroxylon*		
43	*E. stricklandii*	1931	1969
26	*E. terminalis*	1928	

Figure 5.1. The Waite Arboretum (1955): section of arboretum showing layout of plantings near the female parent of *E.* 'Urrbrae Gem'. Applies to Table 5.1.

E. erythronema *E. gomphocephala*
E. stricklandii Other *Eucalyptus* species
Right side is Claremont Avenue, running East-West.

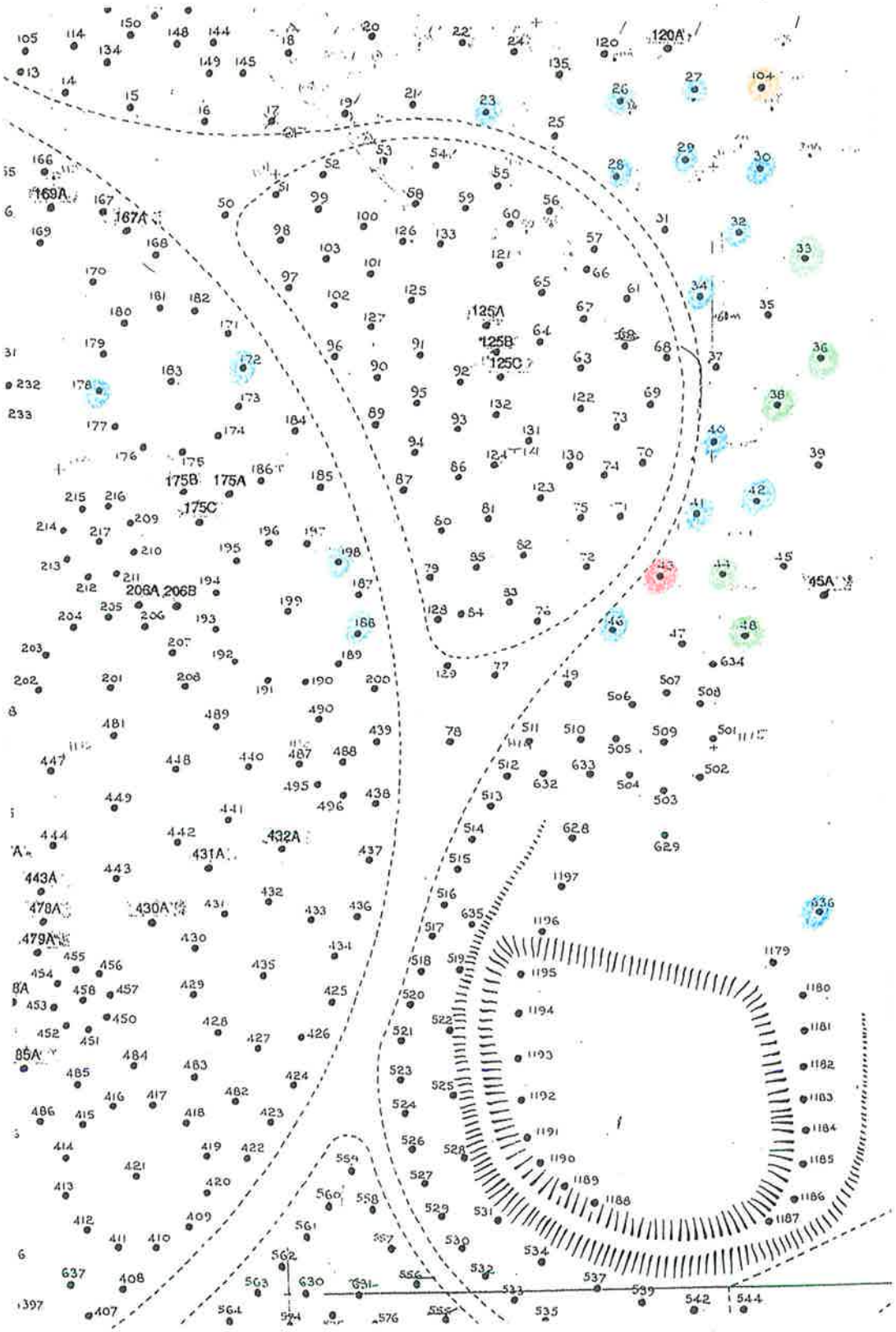


Table 5.2. Individuals used in the *E.* 'Urrbrae Gem' analysis. Taxonomy follows the informal classification of Pryor and Johnson (1971) and all species are from subgenus *Symphyomyrtus,* sec. *Bisectaria.*

Species	Code #	Study	Specimen #	Location	Seed source; provenance
E. 'Urrbrae Gem' F1	F1	M S A	1867	WaA	WaA tree 104; unknown
E. 'Urrbrae Gem' open pollinated	F1A	S A	67	WaA	WaA tree 1867; unknown
Series Erythronemae					
E. erythronema var. *marginata*	*Eem*2	M A	24	WaA	A. Southcott, W.A.; unknown
E. erythronema var. *marginata*	*Eem*3	M S	7.2	MoW	unknown; unknown
E. erythronema var. *marginata*	*Eem*4	M S A	1841A	WaA	unknown; unknown
E. erythronema var. *erythronema*	*Eee*5	M S CP A	7.1	MoW	unknown; unknown
E. erythronema var. *erythronema*	*Eee*6	M A	7.A	MoW	unknown; unknown
E. erythronema var. *erythronema*	*Eee*7	M CP S A	7.2	MoW	unknown; unknown
E. erythronema var. *erythronema*	*Eee*8	M CP S A	7.3	MoW	unknown; unknown
E. erythronema var. *erythronema*	*Eee*9	M A	7.4	MoW	unknown; unknown
Series Grossae					
E. stricklandii	*Es*10	M CP S A	1614	WaA	F. Law Smith, S.A.; unknown
E. stricklandii	*Es*11	M S A	2B	WaI	unknown; unknown
E. stricklandii	*Es*12	M S A	2C	WaI	unknown; unknown
E. stricklandii	*Es*13	M S A	3X	WaI	unknown; unknown
E. stricklandii	*Es*14	M S A	3Y	WaI	unknown; unknown
Series Cornutae					
E. gomphocephala	*Eg*15	M CP S A	1611	WaA	Woods & For, S.A.; unknown
E. gomphocephala	*Eg*16	M	8.1	MoW	unknown; unknown
E. gomphocephala	*Eg*17	M S A	8.2	MoW	unknown; unknown
E. gomphocephala	*Eg*18	M	8.3	MoW	unknown; unknown
E. gomphocephala	*Eg*19	M S A	8.4	MoW	unknown; unknown
Series Reduncae					
E. gardneri	*Ega*20	A	2025	WaA	W.A. For. Dept.; unknown
E. gardneri	*Ega*21	A	10.4	MoW	unknown; unknown

M = specimens used in RAPD analysis (Chapter 5)
CP = specimens used in controlled pollination (Chapter 6)
S = specimens used in seedling morphology (Chapter 6)
A = specimen used in adult morphology (Chapter 6).

5.3.2. DNA extraction

Young, fully expanded leaf material was collected, petioles and midribs discarded, and the laminae immediately stored at -20°C until required (up to 4 weeks). DNA was extracted using the method of Doyle and Doyle (1990), modified as described by Steenkamp *et al.*

(1994) and Wirthensohn *et al.* (1999). Approximately 2.0 g of frozen leaf lamina was ground to a fine powder in liquid nitrogen in a chilled mortar and pestle. The ground leaf tissue was mixed in 9 ml pre-warmed 3% CTAB containing 1% mercaptoethanol and 4% PVP-40T, and incubated at 65°C for thirty minutes, with inversions every ten minutes. After incubation, the tubes were placed on ice and extracted once with twice volume of choloform/isoamyl alcohol [24:1 w:v] by gently mixing for thirty minutes on a spinning wheel, followed by centrifugation (swinging rotor type) at 3000 rpm for thirty minutes at room temperature. The upper aqueous phase was transferred to a clean tube using a wide bore pipette, a 2/3 volume ice-cold isopropanol was added to precipitate the DNA, and the tubes were stored at -20°C for sixty minutes to enhance precipitation. The mix was centrifuged at 4000 rpm for twenty minutes (room temperature) to collect DNA. The upper aqueous phase was removed, and the DNA pellet washed for sixty minutes in 5 mL wash buffer [76% ethanol and 10 mM NH_4Ac] until clean, centrifuged again for ten minutes at 4000 rpm (room temperature) then the wash buffer removed. One mL cold TE buffer [10 mM Tris-HCl, 1 mM EDTA (pH 7.40] was added to the DNA, which was allowed to dissolve prior to adding 2 µL of 10 mg/ml RNAse and the DNA solution incubated at 37°C for 30 minutes. The solution was transferred to a 50 mL plastic tube, 2 mL TE buffer and 1 mL 7.5M NH_4Ac added and placed on ice for twenty minutes (to precipitate proteins), then centrifuged at 10 000 rpm for twenty minutes at 4°C. The upper aqueous phase was transferred to a clean, sterile glass corex tube and 2 volumes of ice-cold ethanol added, mixed slowly, placed on ice for twenty minutes, then centrifuged at 8000 rpm at 4°C for 10 minutes, allowing the DNA to collect on the sides of the tube. The ethanol was poured off, and the tube inverted and allowed to air dry. The DNA was dissolved in 1 mL TE buffer and placed in 1.5 mL plastic centrifuge tubes for storage at -20°C until required. DNA concentration was estimated by visual assessment of band intensities, compared to olive (*Olea europea*) genomic DNA of known concentration (Mekuria *et al.*, 1999) and adjusted to 20 ng/µL. DNA samples, plus the olive DNA, were placed on a 1.0%

agarose gel (Seakem GTG FMC BioProducts) in 1 x TBE buffer (Sambrook *et al.*, 1989) and run for thirty minutes at 80 milliamp. Gels were stained with ethidium bromide for twenty minutes and photographed under UV light with Polaroid 667 film. The DNA bands were compared to the intensities of the bands of known concentrations, and each sample diluted to 20 ng/mL.

5.3.3. RAPD analysis

The optimised PCR reactions consisted of 1 x Taq buffer (Gibco-BRL, Gaithersburg), 3 mM $MgCl_2$, 200 μM each of dGTP, dATP, dCTP, dTTP (Promega, Madison), 1 μM 10-mer oligodeoxy ribonucleotide primer (Operon Technologies, Alameda), 1 unit Taq polymerase (Gibco-BRL, Gaithersburg), 0.5 μL T_4 Gene 32 Protein (Pharmacia Biotech, Upsala) and 20 ng of genomic DNA in a 25 μL volume. One drop of PCR grade paraffin oil was overlaid each on mixture. DNA amplification was performed on a PTC-100™ Programmable Thermal Controller (MJ Research, Watertown), following the protocol used by Wirthensohn *et al.* (1999): initial denaturation at 94°C for two minutes, followed by 41 cycles of 94°C for one minute, 36°C for one minute, 72°C two for minutes, and a final extension at 72°C for five minutes.

DNA amplification fragments were separated by electrophoresis on 2% agarose gels (Seakem GTG FMC BioProducts, Rockland) using 1 x TBE buffer (Sambrook et al. 1989). Gels were stained with ethidium bromide and photographed under UV light with Polaroid 667 film. A negative control of all PCR reagents, but omitting DNA, was added in each run to test for contamination.

Eighty primers were evaluated for their suitability in a pilot survey (series OPA, OPB, OPC and OPD, Operon Technologies, Alameda) with one representative DNA sample for each of

the three species groups. From this survey, six primers were selected that showed clear polymorphisms and gave reproducible banding patterns (OPD-06 ACCTGAACGG, OPD-10 GGTCTACACC, OPD-11 AGCGCCATTG, OPD-14 CTTCCCCAAG, OPD-16 AGGGCGTAAG, OPD-19 CTGGGGACTT). The selected primers were tested on all 19 samples using duplicate PCRs and two gel runs of each separate PCR to test for reproducibility. Bands that were consistently amplified over all reactions were scored.

The gels were scanned directly under UV light using Cream™ for Windows™ Version 1 Revision A (Kem-En-Tec A/S, 1995) and the image saved in Windows™ bitmap format for analysis using DNA Simdex 2.0 (Archer, 1996). Within lanes on the gel, band mobilities were calculated relative to the mobility of the 600 bp band marker from the DNA ladder (Ready-Load™ 100 bp DNA Ladder, Gibco BRL, Gaithersburg) to compensate for differences in absolute mobilities within and between gels. RAPD fragments were scored as present (1) or absent (0) for all individuals, and the data recorded in a binary matrix.

5.3.4. Hierarchical distance analysis

Genetic similarities among all pairs of individuals was estimated using the hierarchical distance method of simple matching coefficient, SMC, (Sokal and Michener, 1958). Cluster analysis was performed on the estimated similarity using the unweighed pair group method with arithmetic average (UPGMA) and the resulting clusters were expressed as a dendrogram using the SAHN algorithm in the computer program NTSYS-pc (Exeter Software) (Rohlf, 1993).

A similarity matrix was calculated using the simple matching coefficient (SMC). The SMC algorithm is preferred over Nei's genetic distance index (Nei and Li, 1979) as the group of individuals from the three different species are highly heterozygous, and with most RAPD

markers, heterozygous individuals cannot be distinguished from homozygous dominant individuals (Bartolozzi *et al.*, 1998). The similarity coefficients from the simple matching algorithm were used to cluster individuals (SAHN) using UPGMA, and a dendrogram constructed. This method has been used to study genetic variation in garlic (Bradley *et al.*, 1996), beans (Skroch *et al.*, 1995) and collards (Farhnam, 1996); also to study intra and inter-specific variation in *Lens* (Abo-elwafa *et al.*, 1995); and for identification and characterisation of woody perennial cultivars of almond (Bartozzi *et al.*, 1998), peach (Warburton and Bliss, 1996), grapevine (Loureiro *et* al., 1998) and citrus (Federici *et al.*, 1998). SMC has also been used in fingerprinting studies of *Eucalyptus globulus* clones (Nesbitt *et al.*, 1997) and *Eucalyptus nitens* ramets (Vaillancourt *et al.*, 1998).

5.3.5. Non-hierarchical distance analysis

The PATN computer analysis program (Belbin, 1994) was used for the non-hierarchical distance multivariate analysis with multidimensional scaling. An association matrix between the objects (ASOs) was first calculated using Gower's metric, a standardised Manhattan Metric, which generated a matrix showing dissimilarity between individuals or operational taxonomic units (OTUs). Gower's metric, when used on presence/absence data, corresponds to the simple matching coefficient (Belbin, 1994), and considers a (0,0) as evidence of homology, and provides nearly equivalent information to the (1,1) comparisons.

Multi-dimensional scaling (MDS) takes the matrix of ASO dissimilarities and orders the individuals in two or three dimensions such that it is the ordering of the input dissimilarities and dissimilarities in the 2 or 3 dimensional ordination space which is maintained. Semi-strong hybrid multi-dimensional scaling (SSH MDS) was used to ordinate ASO dissimilarities. SSH MDS aims to find the minimum value of stress (i.e. distortion of the points away from their original interdistance dissimilarity, and a value less than 20% is

considered satisfactory (Belbin, 1994). The stress level was recorded, and the lowest dimensionality with stress less than 20% adopted. The resulting ordination points were plotted as a 3 dimensional scatter plot (Sigmaplot® 4.0 for Windows®, SPSS), to enable clearer visualisation of the groups of individuals in relation to each other.

Multidimensional scaling (MDS) and ordination of RAPD data enables visualisation of potential patterns of grouping. Many studies have shown that ordination methods involving hybrids generally place the hybrid between clusters of individuals containing the parent species (see McDade, 1997), and when using MDS and ordination, hybrids generally fall between their parents. Principal component analysis (PCA), based on similarity coefficients, is the most common method used, and is documented on studies of beans (Skroch *et al.*, 1995), plum cultivars (Ortiz *et al.*, 1997) and lentils (Sharma *et al.,* 1995). PCA is also documented for use on eucalypts: Sale *et al.* (1996) studied differentiation between two *Eucalyptus* species (*E. risdonii* and *E. amygdalina*) and their hybrids, Costa de Silva and Grattapaglia (1997) studied the relatedness of *E. urophylla* elite clones, and Nesbitt *et al.* (1995) studied variation between different subspecies of *E. globulus.* However, PCA is affected by non-linear or heterozygous data (Shi, 1993) and Belbin (1994) states that SSH is preferred to principal component analysis and other multi-dimensional scaling programs. Nicolle and Conran (1999) used SSH to study variation in the *E. flocktoniae* complex and Cayzer *et al.* (1999) used it to revise *Bursaria* (Pittosporaceae).

Minimum Spanning Tree (MST) analysis is an option of PATN that enables the generation of a set of lines (from the ASO matrix) that minimally interconnect all the objects in the analysis by their relative distances in multidimensional space (provided that all objects have at least one connection, there are no circuits or loops and the network or tree is minimal length) (Belbin, 1994). MST is complementary to ordination, and individuals that are linked directly

are more similar than those not directly linked, or those linked by connection. Because the numbers are 'distances', not actual lengths, they can only be used to indicate links and connections, instead of definite directions or real scales. The ASO matrix was used to generate MST linkages between all objects in the analysis.

5.4. Results

5.4.1. RAPD analysis

PCR, using six primers on 19 individual samples of DNA, produced a total of 561 RAPD fragments of which 27.5% were monomorphic, 57.4% were polymorphic and 15.2% unique to the different species groups (Table 5.3). As this study examined three different species groups, monomorphic bands were those present in all individuals within a group and polymorphic were those bands present in at least one, but not all, individuals within a group. Unique bands were those present in at least one individual in a group and not present in any other groups. Only four bands (1.9%) were present in all individuals tested. With the exception of *E.* 'Urrbrae Gem', all other taxa showed similar numbers of monomorphic, polymorphic and unique bands. Further observation of the data matrix indicated that most bands were present in combinations that included *E. erythronema* var. *erythronema* and *E. erythronema* var. *marginata*, and either *E. stricklandii* or *E. gomphocephala*, or both. Bands common to *E. stricklandii* and *E. gomphocephala* rarely appeared. *E.* 'Urrbrae Gem' showed 60 scorable bands; of these 22 were present in all other species, 14 were present in the hybrid and both varieties of *E. erythronema*, and three were unique to the hybrid. Eight bands were common to the *E.* 'Urrbrae Gem', both varieties of *E. erythronema* and *E. stricklandii*. Three bands were common to the *E.* 'Urrbrae Gem' and *E. stricklandii* only. Two bands were common to the *E.* 'Urrbrae Gem' and *E. gomphocephala*. Five bands were common to the *E.* 'Urrbrae Gem', *E. stricklandii* and *E. gomphocephala* but neither variety of *E. erythronema*.

Table 5.3. Species used in the RAPD analysis. Pooled primer data showing the number of monomorphic, polymorphic and unique bands for each group.

Species	Grp	# trees in samples	Monomorphic bands[b]	Polymorphic bands[c]	Unique bands[d]
E. 'Urrbrae Gem'[a]	1	1	60		3
E. erythronema var. *marginata*	3	5	26	80	17
E. erythronema var. *erythronema*	2	3	25	87	12
E. stricklandii	4	5	17	90	27
E. gomphocephala	5	5	26	65	26
total		19	154	322	85

[a] *E.* 'Urrbrae Gem' is a single individual, thus all bands belong to each category.
[b] Monomorphic bands: present in all individuals in a group.
[c] Polymorphic bands: present in some individuals in a group.
[d] Unique bands: present in one group only.

Bands common to *E. stricklandii* and *E.* 'Urrbrae Gem' occurred at approximately 620 bp for primer OPD-11 and at 450 bp and 510 bp for primer OPD-14 (Plate 5.1). Other primers, OPD-10 and OPD-19, are shown on Plate 5.2.

5.4.2. Hierarchical distance analysis

The dendrogram showed three clusters (Figure 5.2). The five genotypes of *E. gomphocephala* formed a cluster distinct from all other genotypes with a genetic similarity of 65% to the other two clusters. The five genotypes of *E. stricklandii* also formed a cluster with a similarity of 70% to the third cluster that contained the five genotypes of *E. erythronema* var. *erythronema*, three of *E. erythronema* var. *marginata* and *E.* 'Urrbrae Gem'.

Within the genotypes of *E. gomphocephala*, the maximum genetic distance was about 18%, suggesting that this species has a relatively narrow gene pool in the areas from which the samples were collected. Four of the samples of *E. stricklandii* also show a relatively small degree of genetic variability, although one that was collected from the Waite Arboretum showed a 25% genetic distance from the others. In contrast, *E. erythronema* showed high

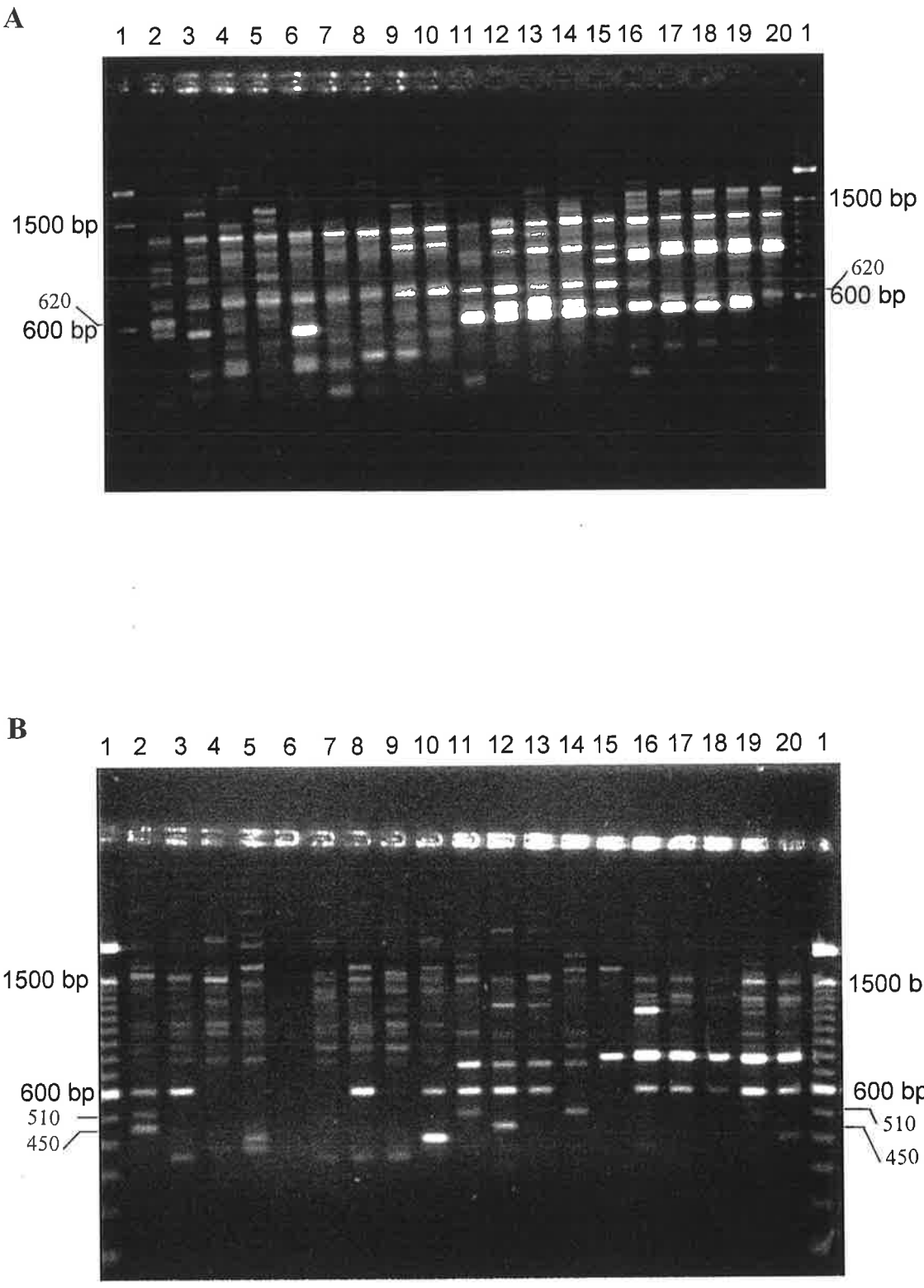


Plate 5.1.
Agarose gels of RAPD primers A: OPD-11 and B: OPD-14. Lane 1= 100 bp ladder, Lane 2 = *E.* 'Urrbrae Gem', Lane 3-5 = *E. erythronema* var. *marginata* 2 to 4, Lane 6-10 = *E. erythronema* var. *erythronema* 5 to 9, Lane 11-15 = *E. stricklandii* 10 to 15, Lane 16-20 = *E. gomphocephala* 16 to 20, Lane 21 = 100 bp ladder.

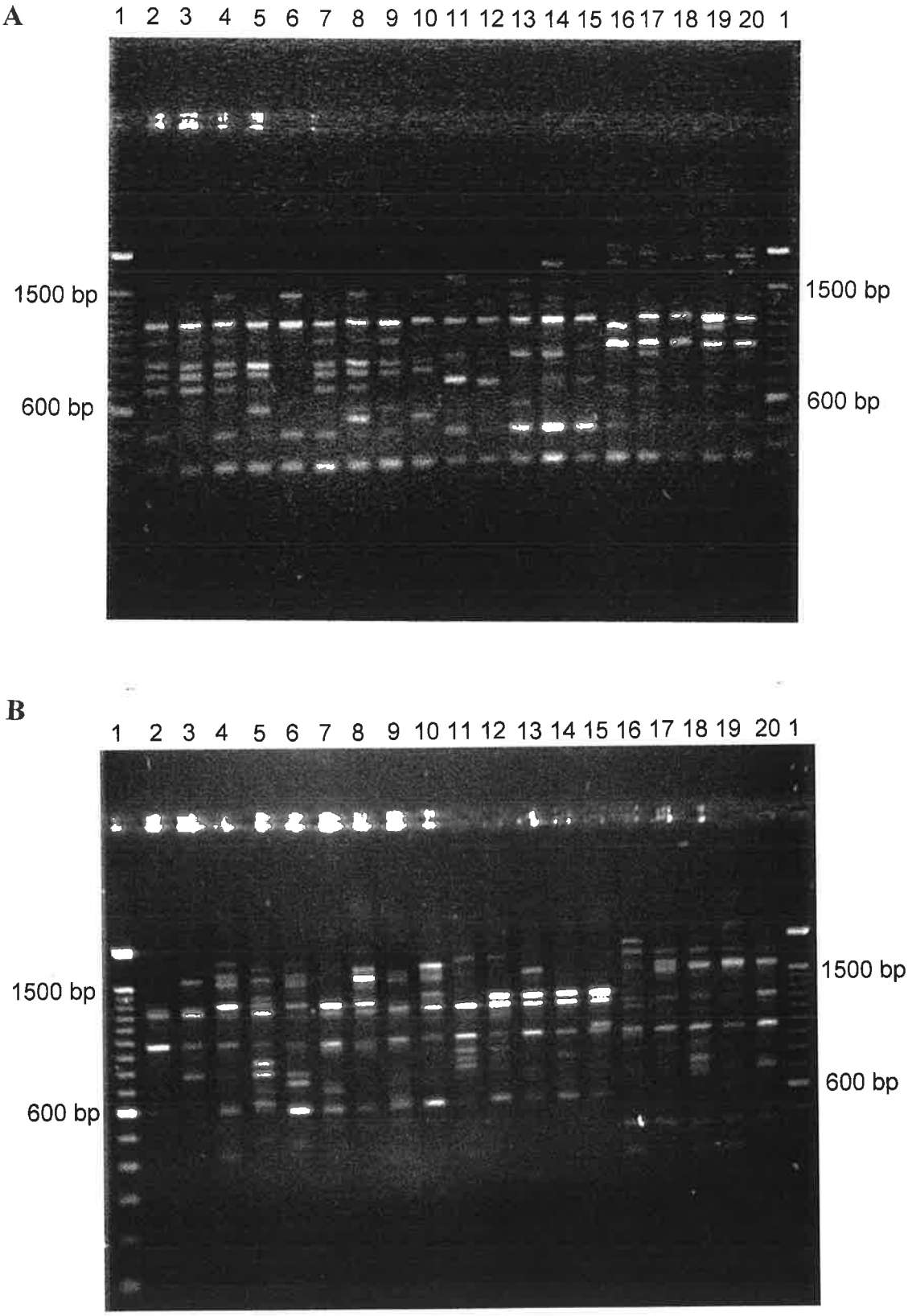


Plate 5.2.
Agarose gels of RAPD primers A: OPD-10 and B: OPD-19. Lane 1= 100 bp ladder, Lane 2 = *E.* 'Urrbrae Gem', Lane 3-5 = *E. erythronema* var. *marginata* 2 to 4, Lane 6-10 = *E. erythronema* var. *erythronema* 5 to 9, Lane 11-15 = *E. stricklandii* 10 to 15, Lane 16-20 = *E. gomphocephala* 16 to 20, Lane 21= 100 bp ladder.

genetic diversity. The three genotypes of var. *marginata* showed a maximum genetic distance of 32%. Within the genotypes of *E. erythronema* var. *erythronema*, *Eee*1 was distinct at a similarity of 73%, whereas the other four were more closely linked at greater than 80% similarity. *E.* 'Urrbrae Gem' clustered with the eight genotypes of *E. erythronema*, with a genetic similarity of 74%, and was placed closer to *E. stricklandii* than to *E. gomphocephala.*

5.4.3. Non-hierarchical distance analysis

The SSH ordination of all specimens into three dimensions (Figure 5.3) showed five closely associated groups of points- *E.* 'Urrbrae Gem' (one), E. *erythronema* var. *erythronema* (five), *E. erythronema* var. *marginata* (three), *E. stricklandii* (five), and *E. gomphocephala* (five). The stress level for the diagram is 0.0849, or 8.5%, which is lower than the maximum acceptable value of 20%.

The MST analysis connects the individuals to their most similar neighbour, with the MST values indicating the relative 'distances' between individuals, and the ordering of the connection indicating individuals with the most similarity. The first connection of *E.* 'Urrbrae Gem' was to *E. stricklandii*, with a MST value of 0.2133, and the second connection was to *E. erythronema* var. *erythronema,* with a MST value of 0.2417.

From these values, it appeared that *E. stricklandii* and *E.* 'Urrbrae Gem' were more similar than *E.* 'Urrbrae Gem' and *E. erythronema* var. *erythronema.* The *E. gomphocephala* cluster linked to *E. stricklandii*, with a MST value of 0.2559, indicating it was dissimilar to *E.* 'Urrbrae Gem'. The average within group MST values were 0.1923 for *E. erythronema* var. *erythronema* and *E. erythronema* var. *marginata*, 0.1801 for *E. stricklandii* and 0.1185 for *E. gomphocephala.* While within group MST values were high in the former and lower in the latter, they were still less than the between group MST values, indicating that the clusters are

Figure 5.2. Dendrogram of RAPD data showing genetic similarity between *E.* 'Urrbrae Gem' (EUGem), *E. erythronema* var. *erythronema* (Eee), *E. erythronema* var. *marginata* (Eem), *E. stricklandii* (Es) and *E. gomphocephala* (Eg), using six primers, the simple matching coefficient and UPGMA clustering.

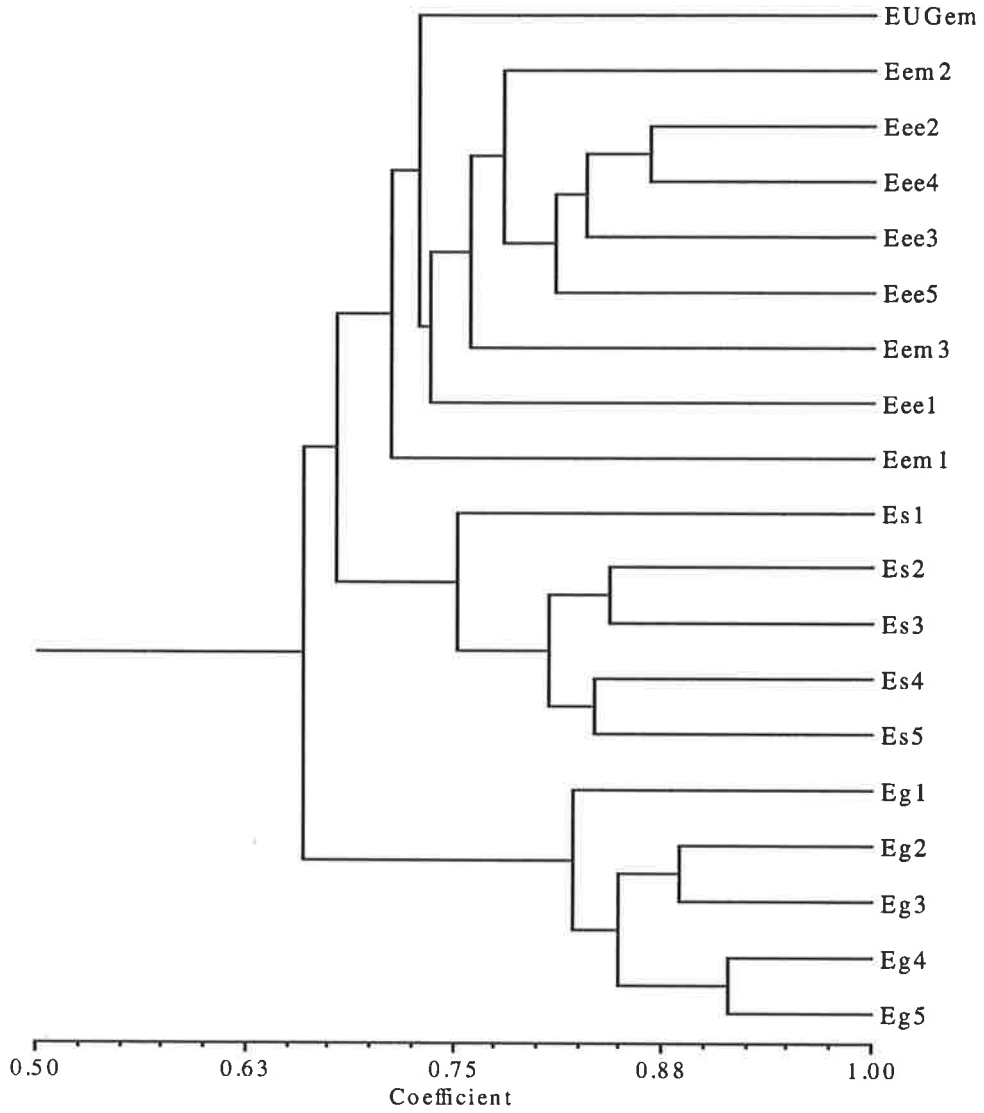
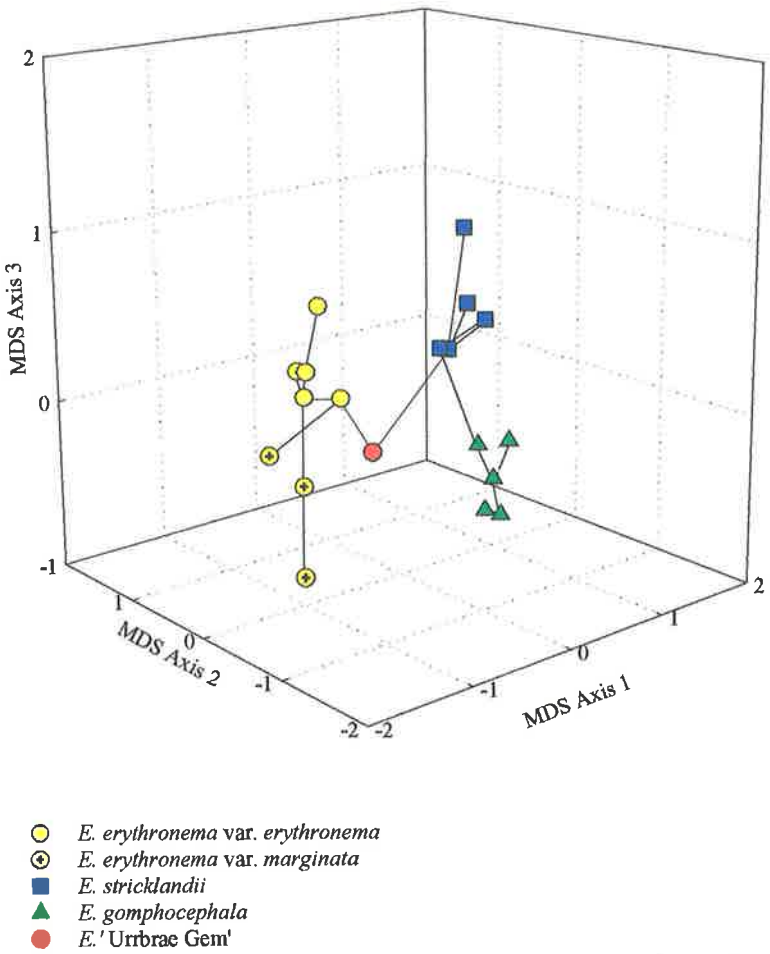


Figure 5.3. MDS ordination with MST linkages of RAPD data from six primers, for *E.* 'Urrbrae Gem', *E. erythronema* var. *erythronema*, *E. erythronema* var. *marginata*, *E. stricklandii* and *E. gomphocephala*.



clearly distinct.

5.5. Discussion

This study has shown that *E. stricklandii* was the probable male parent of *E.* 'Urrbrae Gem, based on analysis of RAPD data by UPGMA clustering and multidimensional scaling. The data generated from the RAPD analysis were analysed using two distance methods: hierarchical, using the simple matching coefficient (SMC) and UPGMA to produce dendrograms, and non-hierarchical, multi-dimensional scaling (MDS) and minimum spanning tree (MST) to produce ordinations and linkages. Distance methods use similarity (or distance) values that summarise character data using pairwise comparisons, either between taxa or characters. Such methods have long been employed in systematics and other biological disciplines to summarise data and compare taxa (McDade, 1997).

The RAPD analysis produced few bands common to all species tested, with most bands being monomorphic, polymorphic or unique to species groups. The hybrid showed a number of bands common to *E. erythronema* (both varieties) and *E. stricklandii*, and relativley few common to *E. stricklandii* only, with three unique bands. These three bands may have been present in the banding patterns for the actual parent plants, but not others for that species. The variation in banding patterns within each species, and the fact that the actual parent individuals of the hybrid could not be located, could explain the presence of unique hybrid bands and the low level of common bands between the hybrid and the parent species. Other factors that could explain the presence of band unique to the hybrid are the reassortment of sequences during hybridisation and the presence of PCR artifacts (Lamboy, 1994).

The dendrogram constructed for the similarity data shows three separate clusters, with each of

the species groups being clearly distinguished. The hybrid, *E.* 'Urrbrae Gem', clustered with individuals from the known female parent species. Hybrids are frequently intermediate between parents, and a study by McDade (1997) noted that distance methods are consistent in placing hybrids between parents, or close to the most similar species. McDade (1997) also showed that the hierarchical distance method using UPGMA will place a hybrid in the cluster with one or both of the known parental species. This analysis indicated that *E.* 'Urrbrae Gem' was more similar to *E. stricklandii* than to *E. gomphocephala.*

Further confirmation was sought, using a non-hierarchical method of analysis, so the RAPD data were analysed by multivariate analysis, with SSH and showing MST linkage, to generate a three dimensional ordination of the ASO matrix. The semi-strong hybrid multidimensional ordination allowed greater visualisation of the clusters of individuals. The three dendrogram operational taxonomic units groups are clearly visible, with *E. erythronema* further separating into the two varieties - var. *erythronema* and var. *marginata*, and all individuals for the *E. stricklandii* and *E. gomphocephala* groups clustering into their respective taxa. *E.* 'Urrbrae Gem' was placed between the female parent species and *E. stricklandii.* The MST values show that *E.* 'Urrbrae Gem' was directly linked to *E. stricklandii* first, then to *E. erythronema* var. *erythronema*, and was not directly linked to *E. gomphocephala.*

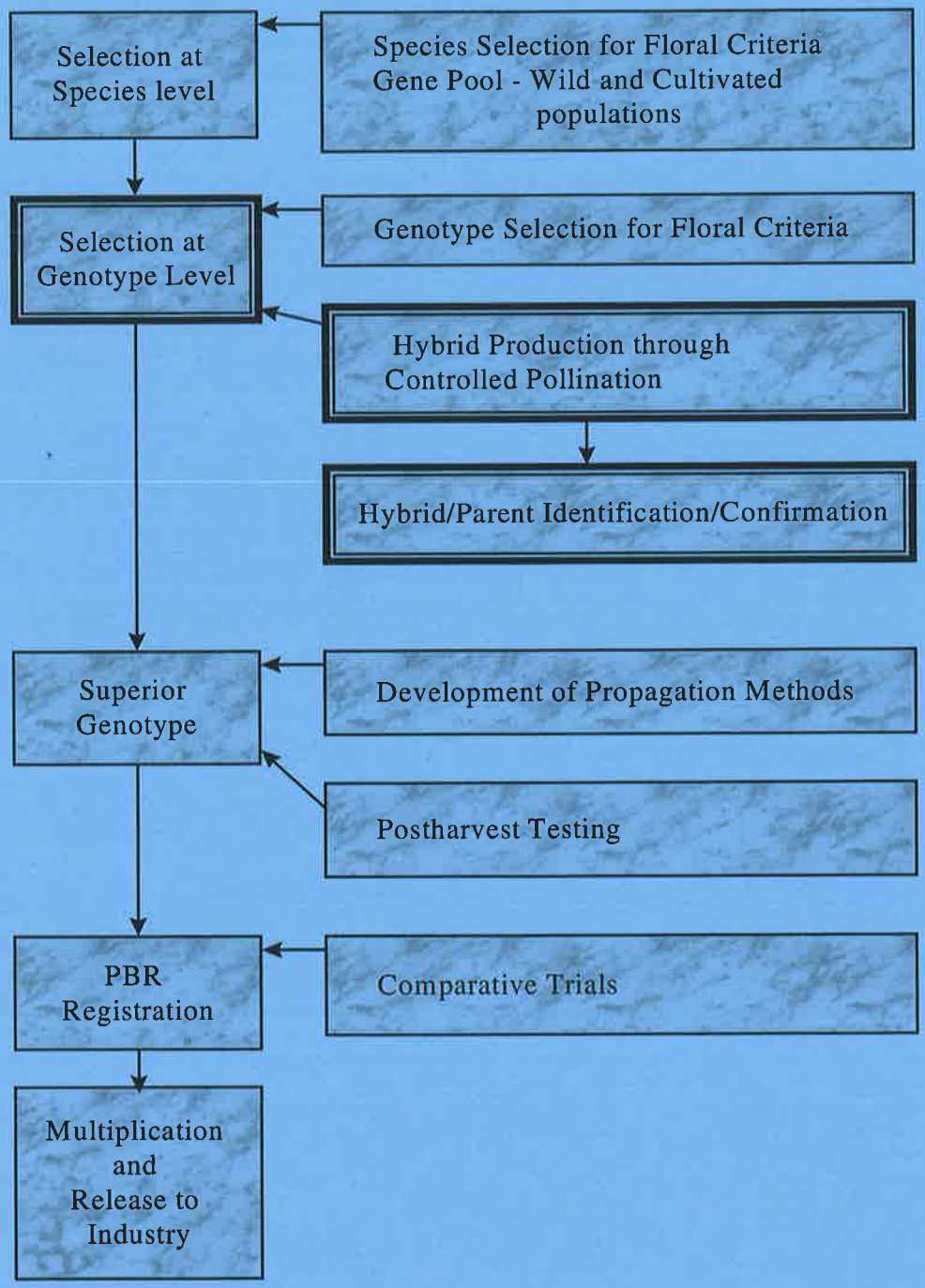
The hypothesis for this study was that the male parent of '*E.* Urrbrae Gem' either *E. stricklandii* or *E. gomphocephala.* The placement of the hybrid with the female parent - *E. erythronema* var. *erythronema* - in the case of the UPGMA clustering method, and the placement of the hybrid between the female parent and *E. stricklandii*, in the case of the multidimensional scaling ordination, supports *E. stricklandii* as the most probable male parent.

Since the discovery of RAPD-PCR by Williams *et al.* (1990) and Welsh and McClelland (1990), the technique has been used in the determination of genetic variation and linkage maps between species and cultivars from a range of plant genera, including *Eucalyptus*.

It has been noted frequently that RAPD-PCR is not without its limitations in systematic studies, such that there are inherent problems in the use of RAPDs as a diagnostic tool for determining relationships between species, populations and hybrids. There is the inference that RAPD analysis should not be used on its own; and that topographies generated, through analyses such as UPGMA, should be compared with alternative topographies. It has also been suggested that alternative methods (such as isozymes or RFLPs) or alternative diagnostic methods (such as morphological character analysis) might be useful to support RAPD results (Arnold and Emms, 1998). Gillies and Abbott (1998) argue that RAPDS are not useful indistinguishing between distantly related genera, only in very similar ones. This said, there are inherent problems with all DNA sequencing techniques (Wolf and Liston, 1998) and RAPDs were deemed a sufficiently descriptive technique to determine the parental status of *E.* 'Urrbrae Gem', given that every effort was made to remove experimental error, and that the species involved were related closely. Further studies to support these data were carried out on adult morphological characters, as well as manipulated hybridisations and seedling morphological character analysis (Chapter 6).

5.6. Conclusion

The conclusion to this study is that RAPD data, when analysed with hierarchical and non-hierarchical distance methods, placed the interspecific hybrid *E.* 'Urrbrae Gem' between *E. stricklandii* and the female parent species, *E. erythronema* var. *erythronema*, strongly suggesting that *E. stricklandii* is the male parent of *E.* 'Urrbrae Gem'.



Ornamental eucalypt breeding cycle flow chart.

Areas covered by this chapter are highlighted.

Chapter Six

Eucalyptus 'Urrbrae Gem': Parentage determination through morphological analysis

6.1. Abstract

Eucalyptus 'Urrbrae Gem' is an attractive amenity tree which is believed to be a natural F1 hybrid with *E. erythronema* var. *erythronema* the known female parent. Previous observations based on adult morphology led to suggestions that either *E. gomphocephala* or *E. stricklandii* could be the male parent. Multivariate analysis of 54 adult morphological characters placed *E.* 'Urrbrae Gem' between *E. erythronema* var. *erythronema* and *E. stricklandii,* with minimum distance values of 0.21 and 0.27 respectively. Controlled pollinations between *E. erythronema* var. *erythronema* and both *E. stricklandii* and *E. gomphocephala* were undertaken, and the resulting seedlings were compared to each other, to open pollinated seedlings of the putative parent species, and to open pollinated seedlings of the F1 and 2nd generation *E.* 'Urrbrae Gem' trees. Based on 14 morphological characters, seedlings clustered into eight groups, with *E. erythronema* var. *erythronema* x *E. stricklandii* seedlings most similar to open pollinated seedlings of the F1 and 2nd generation *E.* 'Urrbrae Gem' trees. In contrast, *E. erythronema* var. *erythronema* x *E. gomphocephala* seedlings clustered into a number of groups, with some close to *E. erythronema* var. *erythronema* seedlings and others to *E. gomphocephala* open pollinated seedlings. The results indicated that *E. stricklandii* is the male parent of *E.* 'Urrbrae Gem'.

6.2. Introduction

The aim of this study was to determine which of *E. stricklandii* or *E. gomphocephala* was the most likely male parent of *E.* 'Urrbrae Gem' based on morphological characters The study was conducted in two parts; firstly, adult morphological characters of the candidate species were measured and compared with the hybrid; and secondly, seedlings generated from controlled pollinations between the known female parent and each of the possible male parents, were compared with open pollinated seedlings of each of the parent species, as well as open pollinated seedlings from *E.* 'Urrbrae Gem' and the 2nd generation *E.* 'Urrbrae Gem' tree.

6.3. Materials and methods

6.3.1. Plant Material

Mature flowering trees of the F1 (one) open pollinated 2nd generation (one) *E.* 'Urrbrae Gem', *E. erythronema* var. *marginata* (three), *E. erythronema* var. *erythronema* (five), *E. stricklandii* trees (five), *E. gomphocephala* (three) and *E. gardneri* (two), were sourced from the Waite Arboretum, the Waite Campus grounds and the Monarto Woodland. Individuals from each taxa are listed in Table 5.1. *E. gardneri* was included as it is a closely related species to *E. erythronema* var. *erythronema*, providing a reference for the degree of difference that might be expected between related species.

Species descriptions - adapted from Brooker and Kleinig (1990)

E. erythronema Turcz. var. *erythronema* (Red flowered mallee) (Plate 3.4) is a small mallee, of the eastern end of the southern wheatbelt of Western Australia. The trunk varies in colour, from white to purplish to red, with an open canopy of glossy green narrowly lanceolate leaves. The inflorescences are usually 7-flowered, with down-curved peduncles and long

curved pendulous pedicels, hypanthium obconical, and operculum conical. Flowers can be bright red to creamy white, occurring from October to January. *E. erythronema* var. *marginata* (Benth.) Domin (plate 3.3) is closely related to *E. erythronema* Turcz. var. *erythronema*, differing in the slightly more northern distribution, consistently 3-flowered umbels, slightly ribbed buds and fruit, and the flared rim at the top of the hypanthium. The buds of both species appear on the tree several months before flowering, and the previous season's fruit falls when mature.

E. gomphocephala DC. (Tuart) (Plate 3.6) is a small to medium tree of the western limestone coastal dunes and subcoastal plains of south west Western Australia. The bark is rough and dark grey on most of the tree, with branchlets having smooth yellow bark. The leaves are glossy, light green, lanceolate to falcate, and thin. The inflorescences are usually 7-flowered, with broad, flat peduncles and short stout pedicels. The buds are mushroomed shaped and yellow, with the hemispherical operculum slightly wider than the hypanthium. The creamy flowers open from January to April. Buds appear a few months prior to flowering, and the fruit remains on the tree for a few seasons when mature.

E. stricklandii Maiden (Strickland's Gum) (Plate 3.6) occurs around the goldfields of Western Australia. The bark is dark grey to black, rough, being flaky or hard and tessellated on the lower part of the trunk, variable from white to grey over copper above, with shiny, reddish or yellow, glaucous branchlets. The leaves are lanceolate, glossy, green and thick. Inflorescences are 7-flowered, with a broad, flattened peduncle and little or no pedicel, operculum conical, hypanthium campanulate The yellow buds open to show yellow - green flowers from November to March. Buds appear many months prior to flowering, and fruit remain for many months when mature.

E. gardneri Maiden (Blue Mallet) (Plate 3.2) is a mallee of scattered distribution in south west Western Australia. The trunk has smooth light coloured bark, with imperfectly shedding flakes, and the adult leaves are dull, intensely blue-green to purplish. The inflorescences are more than 7-flowered, with a flattened peduncle. The yellow-red buds are small and horn-shaped, with pale-yellow green flowers opening in March to November.

E. 'Urrbrae Gem' (Plate 6.1) has a smooth trunk of varying colours similar to *E. erythronema* var. *erythronema*, shedding seasonally. The open canopy consists of narrow lanceolate to falcate shining dark green leaves. The inflorescences contain up to seven yellowish buds, each with a conical operculum and an obconical to campanulate hypanthium with a slight lip, a flattened peduncle and a 3-sided short pedicel. The buds appear up to 18 months prior to flowering and open from late spring to early summer, with pink-red stamens.

Plate 6.1.
Flowers of *E.* 'Urrbrae Gem' (photo courtesy of Dr. J. Gardner).

6.3.2. Adult morphology

Sixty binary, continuous or ordered multistate characters were measured on fresh material for each tree (Table 6.1.). One hundred measurements were made for each character; ranging from 20 measurements from five individuals from each species, or 5 x 20 measurements from sub-samples of the single available individual (in the case of the F1 and 2nd generation specimens of *E.* 'Urrbrae Gem').

6.3.3. Controlled pollination

The controlled pollination method followed that of van Wyk (1977) and Moncur (1995). Pollen was collected from single trees of *E. stricklandii* and *E. gomphocephala* flowers prior to anthesis at operculum lift. Flowers at this stage were cut from the tree and taken to the laboratory. The anthers were removed with forceps and spread out on filter paper over silica gel for 24-48 hours to promote dehiscence. After drying, the pollen and anthers were removed from the stamens and placed in 1.5 ml plastic tubes and labeled. The tubes were placed inside larger vials containing 1 cm silica gel and stored at -20°C until required. A small amount of pollen was placed in another 1.5 ml vial for regular use and stored at 4°C between pollinations. This 'working' pollen was replaced every ten days from stored reserves. Viability of the pollen was checked after 12 months storage by germination on Agar medium for 24 hours; all samples showed a minimum of 50% viability.

E. erythronema var. *erythronema* flowered from August to November, with peak flowering in October. Controlled pollination took place over four weeks during this peak flowering period. Flower buds at anthesis were emasculated by removing all anthers; all flowers on the tree at this stage were emasculated, and those already open were removed. Pollen was applied with a small paint brush to the stigma, and the umbel tagged. This step was repeated every five days, with every emasculated flower having repeated pollinations, until all flowers in the

umbel had been pollinated at least twice. Pollinated flowers were not isolated , as additional empirical information could be gathered on the interaction and competition between applied pollen and pollen applied through natural vectors on emasculated flowers. Information on the effectiveness of the controlled pollination technique without bagging was also sought, in light of recent work by Harbard et al. (1999). Pollinated flowers were not randomised on the tree.

Capsules were harvested eight months after pollination and stored separately in paper bags. They were placed in a warm dry room at 30°C until open (up to four weeks). Seed was removed from each capsule, counted and weighed, then stored in a cool dry dark place until planting. A total of two hundred seeds were randomly selected from different capsules from each plant for each cross: seeds were soaked for 24 hours in 3000 ppm gibberellic acid to aid germination (Ostrowska et al., 1998), then four seeds planted per 25 mm pot, containing soil (sand and pinebark in a 1:2 ratio) and covered with a thin layer of vermiculite, and the forty pots within each tray covered with plastic to retain humidity. The plastic was removed when the majority of seeds in the tray had germinated. Dates of germination were recorded, and the shape of the cotyledons noted seven days after emergence when both cotyledons were free from the seed coat. Seedlings were grown in a glasshouse during Spring, under conditions of natural daylength and temperatures of 18±3°C to 25±3°C. Liquid fertiliser (Aquasol®) was applied at fortnightly intervals from 21 days after germination. Seed from parent species was sourced from commercial seed companies and supplemented with open pollinated seed from the actual parent trees.

6.3.4. Seedling morphology

The surviving seedlings from both crosses were measured at three months of age for 17 binary, continuous or ordered multistate characters at the fifth fully expanded leaf pair after the cotyledons (Table 6.1). Sixty-nine seedlings from *E. erythronema* var. *erythronema* x *E.*

stricklandii (*EeEs*), and 93 from *E. erythronema* var. *erythronema* x *E. gomphocephala* (*EeEg*) were measured, along with 65 open pollinated seedlings from each of the parent species, with the exception of *E. erythronema* var. *erythronema* and *E. erythronema* var. *marginata*, for which 45 and 20 were measured, respectively. Open pollinated seed from the F1 and 2nd generation trees of *E.* 'Urrbrae Gem' was included in the analysis. As the pollinated flowers were not isolated and the female trees were growing in a multi-species environment, the possibility existed that seed from the controlled crosses and open pollinated seed may have contained contaminants such as intraspecific crosses (selfs or outcrosses) or interspecific crosses with other species.

6.3.5. Data Analysis

The PATN analysis package (Belbin, 1994) was used to analyse the morphological character data for both adults and seedlings using hierarchical (UPGMA) and non-hierarchical (multidimensional scaling, minimum spanning tree and principal canonical correlation) analysis to determine the relationships between the hybrid *E.* 'Urrbrae Gem' and the putative parent species. The morphological characters were standardised using Gower's metric, a standardised Manhattan Metric (see Chapter 5).

In addition to allowing dendrogram groups to be identified and characterised, the Group Definition option of PATN (GDEF) enables large numbers of individuals to be averaged into groups of equally dissimilar individuals, and the means of these groups re-analysed to provide a more succinct picture of the overall pattern. This option was used on the seedling morphology study, due to the large numbers of seedlings measured. The groups statistics option (GSTA) was used to interpret each dendrogram group in terms of characters, with the Kruskal-Wallis *H*-statistic used as a non-parametric analysis of variance to determine whether the ranked groups means are significantly different from one another for each character

(Nicolle and Conran, 1999).

Principal Canonical Correlation (PCC) analysis was used to determine whether there were significant correlations between the semi-strong hybrid ordination OTU coordinates and the individual characters scores for these OTUs. This enabled the ordination to be interpreted in terms of the characters causing the clusters and for clusters/taxa to be defined more clearly. The correlation coefficient (r^2) for each character against the spread fo the OTUs in the ordination space was calculated, and the significance of the correlation was Bonferroni adjusted to allow for the multiple comparisons (Nicolle and Conran, 1999). When a character is significantly correlated to the MDS coordinate for the individual, that character will be descriptive for that taxon.

Table 6.1. Characters used in phenetic analysis (seedling and adult) with codes.

Seedling	*- continuous*	
LLE	Leaf length	(mm)
LWI	Leaf width	(mm)
LPL	Leaf petiole length	(mm)
Seedling	*- binary*	
LEA	Leaf	concolorous (0) discolorous (1)
LSM	Leaf smoothness	smooth (0) rough (1)
LPS	Leaf petiolate/sessile	sessile (0) petiolate (1)
LPO	Leaf position	opposite (0) alternate (1)
STE	Stem	single (0) branching (1)
SSM	Stem smoothness	smooth (0) rough (1)
OGP	Oil glands prominent	inconspicuous (0) prominent (1)
Seedling	*- ordered multistate*	
LSH	Leaf shape	lanceolate (0) falcate (1) elliptic (2) ovate (3)
LST	Leaf tip shape	acute (0) mucronate (1) mucronulate (2) obtuse (3)
LSB	Leaf base shape	attenuate (0) oblique (1) obtuse (2) cordate (3)
LSW	Leaf surface wax	shining (0) dull (1) glaucous (2)
LCO	Leaf colour	light green (0) green (1) blue green (2)
SSH	Stem shape	square (0) angular(1) round (2)
LMP	Leaf margin proximity	close (0) medium (1) far (2)
Adult	*inflorescence - continuous*	
HLE	Hypanthium length	(mm)
HWI	Hypanthium width	(mm)
OLE	Operculum length	(mm)
OWI	Operculum width	(mm)
PLE	Peduncle length	(mm)
PWI	Peduncle width	(mm)
PDE	Peduncle depth	(mm)
PELE	Pedicel length	(mm)
PEWI	Pedicel width	(mm)
PEDE	Pedicel depth	(mm)
Adult	*fruit -continuous*	
FDI	Fruit diameter	(mm)
FLE	Fruit length	(mm)
FPL	Fruit peduncle length	(mm)

Continued

Table 6.1. Continued

FPW	Fruit peduncle width	(mm)
FPD	Fruit peduncle depth	(mm)
FPEL	Fruit pedicel length	(mm)
FPEW	Fruit pedicel width	(mm)
FPED	Fruit pedicel depth	(mm)
Adult	*miscellaneous -continuous*	
NBU	Number of buds in umbel	
LAL	Leaf petiole angle	° left
LAR	Leaf petiole angle	° right
LWI	Leaf width	(mm)
LLE	Leaf length	(mm)
LPL	Leaf petiole length	(mm)
LAT	Leaf lamina thickness	(mm)

Continued

Table 6.1. continued

Adult	*fruit - binary*	
PCU	Peduncle curvature	erect (0) pendulous (1)
Adult	*inflorescence - binary*	
BAN	Bud angular	not angular (0) angular (1)
BSM	Bud smooth	smooth (0) warty (1)
HYR	hypanthium ring	absent (0) present (1)
OAP	Operculum apex shape	rounded hemispherical (0) acutely conical (1)
OAT	Operculum attachment	abutting (0) enclosing (1)
OBS	Operculum base shape	not overhanging (0) overhanging (1)
OSC	Operculum base scar	wider at base (0) wider at apex (1)
Adult	*miscellaneous - binary*	
LPS	Leaf petiolate/sessile	sessile (0) petiolate (1)
LPO	Leaf position	opposite (0) alternate (1)
PEC	Leaf petiole colour	orange (0) red (1)
SSW	Stem smooth/warty	smooth (0) warty (1)
SCO	Stem colour	orange (0) red (1)
PGS	Pith glands stem	absent (0) present (1)
PGF	Pith-like glands at base of fruit	absent (0) present (1)
OGP	Oil glands	inconspicuous (0) prominent (1)
Adult	*inflorescence - ordered*	*multistate*
BWA	Bud wax	shiny (0) dull (1) glaucous(2)
HSH	Hypanthium shape	obconical (0) slightly campanulate (1) campanulate (2)
OSH	Operculum shape	beaked (0) conical (1) horn-shaped (2) hemispherical
BCO	Bud colour	yellow-green (0) orange (1) dark red (2) brown (3)
PSH	Peduncle shape	absent (0) flattened (1) round (2)
PESH	Pedicel shape	absent (0) flattened (1) round (2)
Adult	*fruit - ordered*	*multistate*
FSH	Fruit shape	conical (0) slightly campanulate (1) campanulate (2)
FWA	Fruit wax	shiny (0) dull (1) glaucous (2)
FRR	Fruit ribbing	smooth (0 slight ribbing (1) strong ribbing (2)
VPO	Valve position	shrunken (0) level (1) exserted (2)
DPO	Disc position	descending (0) level (1) ascending (2)
FCO	Fruit colour	green (0) orange (1) red brown (2) brown (3)
Adult	*miscellaneous - ordered*	*multistate*
LSH	Leaf shape	lanceolate (0) falcate (1) elliptic (2) ovate (3)
LSB	Leaf base	attenuate (0) oblique (1) obtuse (2) cordate (3
LST	Leaf tip	acute (0) mucronate (1) mucronulate (2) obtuse (3)
LSW	Leaf surface wax	shining (0) dull (1) glaucous (2)
LCO	Leaf colour	light green (0) green (1) blue green (2)
STW	Stem wax	shining (0) dull (1) glaucous (2)
FLC	Flower colour	red (0) pink red (1) cream (2) greenish yellow (3)

6.4. Results

6.4.1. Adult morphology analysis

Sixty morphological characters were measured, although leaf shape (LSH), leaf base shape (LSB), leaf tip shape (LST), leaf surface wax (LSW), leaf petiolate/sessile (LPS) and leaf position (LPO) were found to be invariant and were removed, leaving 54 characters for analysis. For simplicity, the five representative points for the F1 and open pollinated 2nd generation *E.* 'Urrbrae Gem' trees were averaged after full analysis, resulting in one point for each existing tree on the dendrogram and the ordination.

The UPGMA dendrogram (Figure 6.1) separated the four species, two sub-species and F1 and open pollinated 2nd generation *E.* 'Urrbrae Gem' into five clusters. Individuals within clusters were very similar to each other. The F1 and open pollinated 2nd generation *E.* 'Urrbrae Gem' were most closely associated with each other in cluster 1, and then with cluster 2, including the varieties of *E. erythronema.* Cluster 3 contained *E. gardneri*, cluster 4 *E. stricklandii* and cluster 5 *E. gomphocephala.* Neither putative parent was associated closely with the F1 and open pollinated 2nd generation *E.* 'Urrbrae Gem' in the dendrogram.

The semi-strong hybrid ordination of all specimens into two dimensions (Figure 6.2) follows the dendrogram with five clusters of points. The F1 and open pollinated 2nd generation *E.* 'Urrbrae Gem' were placed centrally and relatively close to each other (MST value 0.13). The *E. erythronema* var. *erythronema* and *E. erythronema* var. *marginata* groups were connected via MST value 0.07, with the former close to the open pollinated 2nd generation *E.* 'Urrbrae Gem'(MST value 0.21). Both possible male parent species are represented as outlying groups, both closest to the F1 *E.* 'Urrbrae Gem', but with *E. stricklandii* closer than *E. gomphocephala* (MST distances of 0.27 and 0.34 respectively). *E. gardneri* was an outlying group nearest the F2 (MST 0.28). The stress value for the ordination is 10.3%, well within

the 20% distortion level generally deemed to be acceptable (Belbin, 1994). Additional analysis of group means showed that the centroid MST linkages connect the F1 and 2nd generation *E.* 'Urrbrae Gem" to the female parent species group (MST 0.30), to the *E. stricklandii* group (MST 0.39), to the *E. gardneri* group (MST 0.40) and finally to the *E. gomphocephala* group (MST 0.42), further clarifying the connections between groups.

PCC analysis of all adult characters shows clear correlations between the plotted OTUs and taxon groups and the characters used to derive them (Figure 6.3.). Table 6.2 lists all adult characters with their correlation coefficients and significance levels. All taxon groups are almost equidistant from the origin, indicating extremes of very high or very low values for those morphological characters that define them. The female parent, *E. erythronema*, shared two unique characters, peduncle shape (PSH) and peduncle curvature (PCU), while *E. erythronema* var. *marginata* showed one unique character, that of hypanthium ring (HYR). Neither the F1 or open pollinated 2nd generation *E.* 'Urrbrae Gem' showed any unique characters. *E. stricklandii* had powdery wax present on all young stems, and the characteristics of pedicel shape, length, width and depth (PESH, PELE, PEWI, PEDE) and fruit pedicel width and depth (FPEW, FPED). *E. gomphocephala* lacked bud angularity (BAN), had shiny fruit (FWA), an operculum enclosing the hypanthium (OAT), a rounded hemispherical operculum apex (OAP), an overhanging operculum base (OBS) and an operculum scar widest at its base (OSC). All of these characters were unique to the species. *E. gardneri* is defined by absence of pith-like glands at the base of the flower (PGF), and comparatively small values for hypanthium width (HWI), operculum width (OWI), fruit diameter and length (FDI, FLE) and fruit peduncle depth (FPD). The F1 *E.* 'Urrbrae Gem' and both possible male parents all shared warty buds (BSM), the F1 *E.* 'Urrbrae Gem' and *E. stricklandii* both possessed green fruit (FCO) and warty stems (SSW), and the F1 *E.* 'Urrbrae Gem' and *E. gomphocephala* shared orange petiole colour (PEC). The F1 and 2nd generation

E. 'Urrbrae Gem" and *E. stricklandii* trees shared the character of dull bud wax (BWA) with each other and *E. gardneri*. There were no characters that were uniquely common to both the F1 and F2 *E.* 'Urrbrae Gem' trees and either variety of the female parent, although fruit pedicel depth (FPED), leaf width (LWI) and leaf petiole length (LPL) were similar and very different from the putative male parents. The continuous characters measured for the F1 hybrid *E.* 'Urrbrae Gem' were most often intermediate in value between *E. erythronema* (both varieties) and *E. stricklandii*, however, as the continuous characters measured for *E.* gomphocephala were of values close to that of the F1, the significance of many of these characters were lost. Key descriptive characters, such as bud and fruit colour, showed strong similarities between the hybrid and *E. stricklandii*.

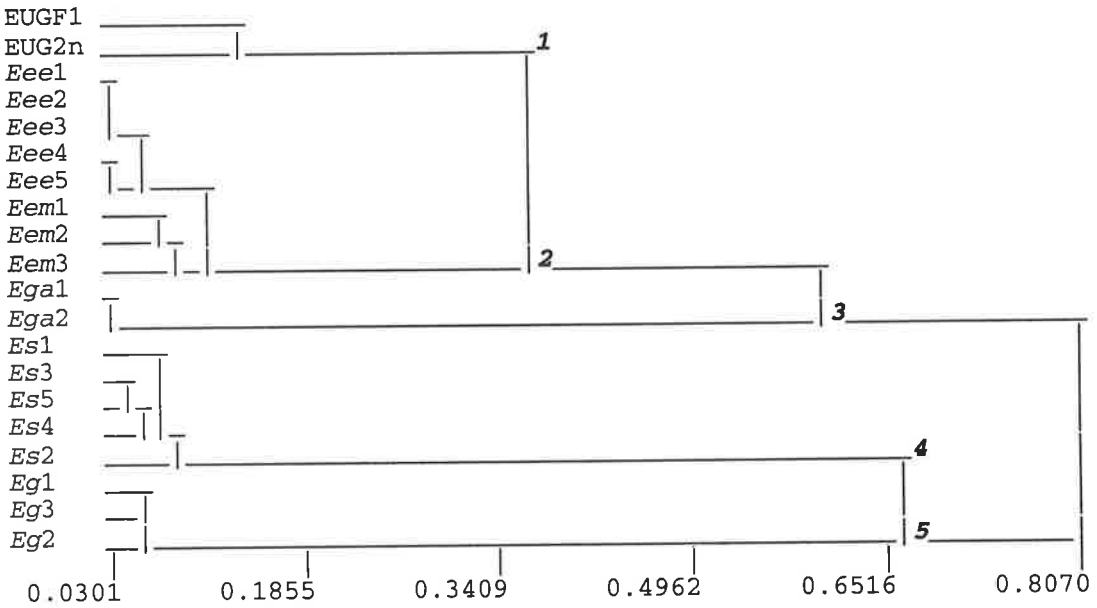


Figure 6.1. UPGMA dendrogram of 20 adult individuals measured. Scale represents standardised Manhattan distance (Gower metric) based on 54 characters measured (Table 6.2). The dendrogram shows five clusters: cluster 1 - F1 *E.* 'Urrbrae Gem' (EUGF1) and 2nd generation *E.* 'Urrbrae Gem' (EUG2n); cluster 2 - *E. erythronema* var. *erythronema* (*Eee* trees 1-5) and *E. erythronema* var. *marginata* (*Eem* trees 1-3); cluster 3 - *E. gardneri* (*Ega* trees 1-2); cluster 4 - *E. stricklandii* (*Es* trees 1-5); and cluster 5 - *E. gomphocephala* (*Eg* trees 1-3).

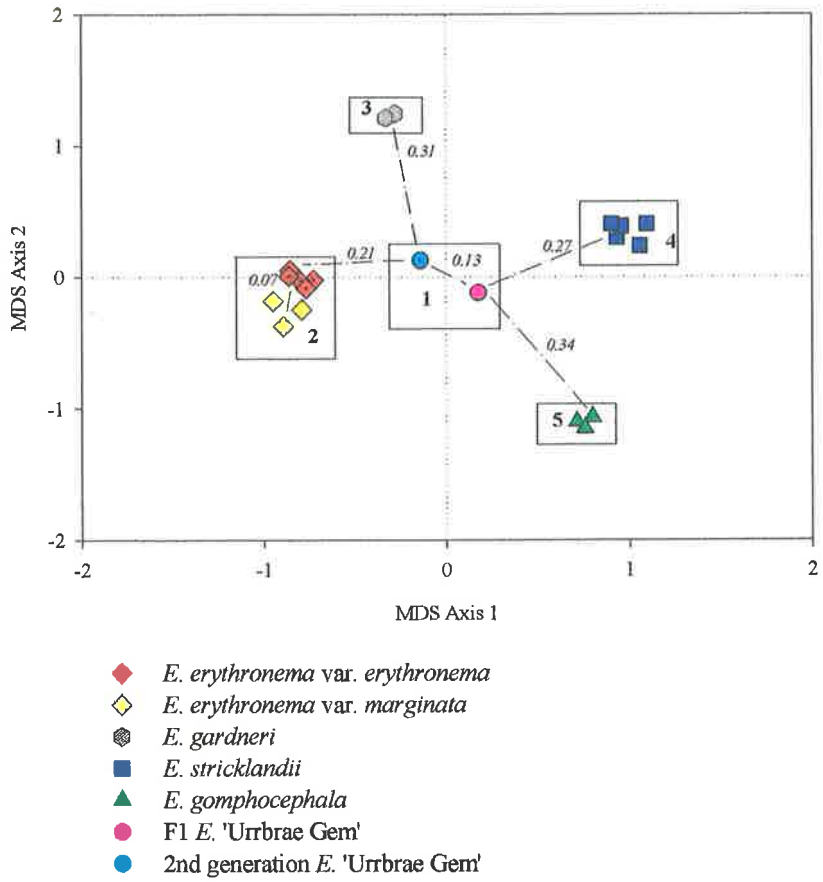


Figure 6.2. Semi-strong hybrid multidimensional scaling of 21 individuals into their five taxonomic groups based on 54 adult characters. Distances and linkages are results from a minimum spanning tree analysis of the data.

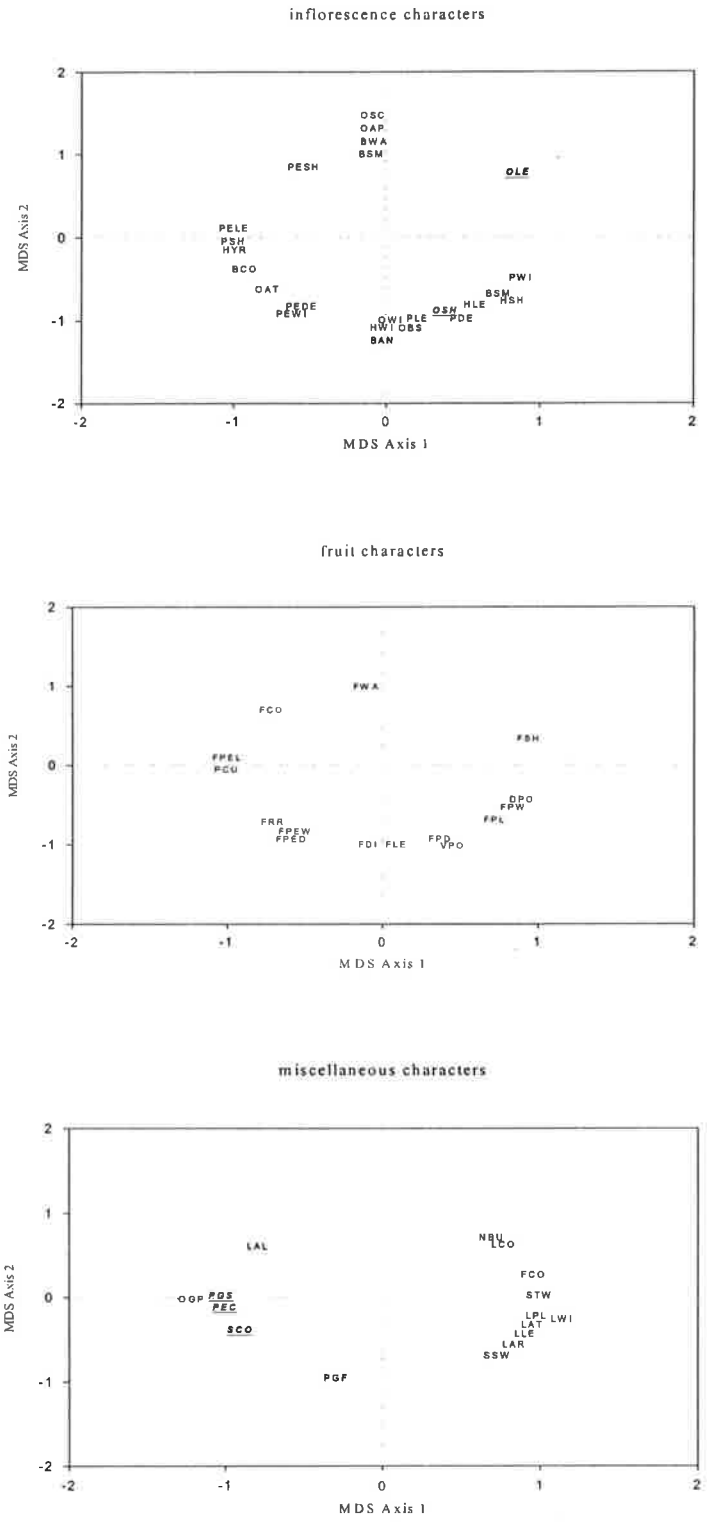


Figure 6.3. Principal canonical correlation plot for the SSH ordination (Figure 6.2) based on the adult characters measured - inflorescence, fruit, and miscellaneous, all vector lengths 1. Character codes are those detailed in Table 6.1., italics indicate characters not significantly correlated with the SSH OTU coordinates, characters from Table 6.2.

Table 6.2. Adult morphological characters (Table 6.1.) of *E.* 'Urrbrae Gem' and putative relatives (from Table 5.1), with correlation coefficient[a] (r^2) of the individual character vectors in the ordination space, Kruskal-Wallis *H* statistic[b] and probability (*P*). Measurements in mm or ° presented as range (mean), when $r^2 > 0.66$ significant at $P < 0.05$, NA = not applicable as character invariant and not included in analysis.

Character	F1 *E.* 'Urrbrae Gem'	F2 *E.* 'Urrbrae Gem'	*E. erythronema* var. *erythronema*	*E. erythronema* var. *marginata*	*E. stricklandii*	*E. gomphocephala*	*E. gardneri*	r^2	*H*	*P*
Hypanthium length	9.6-15.1 (11.9)	6.6-9.6 (8.2)	5.4-9.9 (7.3)	6.5-10.5 (8.4)	10.8-16.9 (13.0)	8.7-12.9 (10.4)	3.6-5.5 (4.4)	0.81	16.24	0.0027
Hypanthium width	9.4-12.8 (11.1)	7.4-9.9 (8.9)	7.9-10.3 (8.8)	7.8-12.8 (10.2)	9.8-13.8 (11.6)	9.3-12.9 (11.3)	1.8-2.5 (2.3)	0.75	13.39	0.0082
Operculum length	8.6-12.4 (10.9)	7.1-10.6 (10.1)	10.6-14.9 (12.1)	10.7-16.6 (13.1)	10.6-17.9 (13.2)	10.9-13.5 (12.2)	9.7-16.6 (13.3)	0.22	6.21	0.1838
Operculum width	8.6-12.4 (10.9)	8.1-10.2 (9.3)	7.8-10.9 (8.9)	6.7-11.3 (9.5)	7.3-13.5 (11.2)	12.9-16.2 (14.5)	1.6-2.3 (2.1)	0.92	15.69	0.0035
Peduncle length	11.8-28.0 (21.6)	12.5-21.3 (17.3)	9.9-23.2 (18.1)	13.8-25.6 (19.8)	15.6-30.9 (20.9)	15.6-32.8 (21.1)	9.8-19.8 (15.6)	0.67	9.99	0.0405
Peduncle width	3.3-10.1 (5.4)	2.6-5.1 (3.6)	1.5-3.8 (2.2)	1.7-3.4 (2.5)	6.9-15.8 (11.1)	5.2-10.0 (8.3)	1.9-3.5 (2.9)	0.95	16.77	0.0021
Peduncle depth	3.8-5.8 (4.7)	2.3-3.5 (2.8)	1.8-3.0 (2.3)	1.7-4.0 (2.5)	3.1-5.2 (3.7)	4.0-5.5 (4.6)	1.2-2.0 (1.6)	0.89	14.59	0.0056
Pedicel length	2.0-6.3 (3.4)	4.2-7.9 (6.3)	7.6-14.5 (12.1)	8.7-16.5 (14.1)	0	1.0-2.1 (1.7)	3.2-5.6 (4.3)	0.95	17.59	0.0015
Pedicel width	2.3-6.6 (3.9)	2.1-5.1 (3.3)	1.7-4.4 (2.8)	1.9-3.7 (2.6)	0	3.6-6.2 (5.0)	1.0-1.8 (1.3)	0.86	17.75	0.0014
Pedicel depth	2.8-3.8 (3.3)	2.5-3.2 (2.9)	1.9-3.0 (2.3)	1.9-2.8 (2.2)	0	4.0-4.9 (4.4)	1.0-1.8 (1.3)	0.84	17.80	0.0013
Fruit diameter	11.2-12.9 (12.3)	10.8-13.0 (11.9)	12.1-13.8 (13.2)	10.3-15.0 (12.7)	12.6-16.9 (14.4)	13.0-16.0 (14.7)	4.4-6.3 (5.2)	0.74	15.41	0.0039
Fruit length	11.5-13.5 (12.8)	10.3-12.1 (11.5)	11.5-14.2 (12.4)	10.8-16.4 (14.5)	14.5-18.8 (16.3)	13.1-18.0 (15.4)	6.0-8.0 (7.1)	0.77	15.95	0.0031
Fruit peduncle length	16.6-25.3 (21.9)	14.8-20.8 (17.8)	12.6-24.0 (15.7)	15.4-25.9 (18.4)	17.1-32.1 (23.7)	14.0-29.2 (20.6)	12.8-17.3 (15.2)	0.81	13.85	0.0078
Fruit peduncle width	3.4-7.5 (5.2)	3.2-4.9 (4.1)	2.4-3.8 (3.3)	2.5-4.2 (3.4)	7.8-16.9 (11.7)	3.2-9.9 (8.8)	2.8-3.6 (3.2)	0.92	16.37	0.0026
Fruit peduncle depth	2.7-4.3 (3.5)	2.6-4.2 (3.3)	2.3-3.5 (2.5)	2.4-4.0 (3.1)	2.8-6.5 (4.7)	6.8-12.4 (8.7)	1.3-2.0 (1.6)	0.86	16.41	0.0025
Fruit pedicel length	2.0-4.6 (3.3)	4.1-6.9 (5.5)	7.2-11.9 (8.8)	4.5-25.9 (13.8)	0	0.2-1.2 (0.6)	2.6-3.6 (3.0)	0.90	17.60	0.0015
Fruit pedicel width	3.2-6.5 (4.3)	3.4-5.5 (4.1)	3.2-5.1 (3.8)	3.5-6.3 (4.4)	0	6.4-9.5 (7.9)	1.5-2.1 (1.7)	0.87	17.76	0.0014
Fruit pedicel depth	2.6-4.7 (3.5)	3.0-3.9 (3.3)	2.2-3.5 (3.2)	2.9-5.1 (3.6)	0	5.3-8.7 (6.9)	1.4-1.7 (1.6)	0.86	17.11	0.0018
Leaf petiole angle	50-90 (75.2)	60-90 (76.4)	50-90 (79.5)	60-90 (75.7)	60-90 (71.3)	50-90 (72.6)	50-90 (76.8)	0.72	9.99	0.0407
Leaf petiole angle	90-130 (104.8)	90-120 (103.6)	90-130 (100.5)	90-120 (104.3)	90-120 (108.7)	90-130 (107.4)	90-130 (103.2)	0.69	9.18	0.0569
Leaf width	11.8-20.4 (14.9)	9.7-17.0 (12.7)	8.9-17.5 (12.4)	6.2-17.6 (8.8)	22.0-50.3 (33.0)	17.6-30.9 (22.4)	11.0-18.4 (14.9)	0.94	15.70	0.0035

Continued

Table 6.2. Continued

Character	F1 *E.* 'Urrbrae Gem'	F2 *E.* 'Urrbrae Gem'	*E. erythronema* var. *erythronema*	*E. erythronema* var. *marginata*	*E. stricklandii*	*E. gomphocephala*	*E. gardneri*	r^2	*H*	*P*
Leaf length	90.0-125.0 (106.3)	67.0-115.0 (90.2)	44.2-77.9 (60.5)	49.8-87.2 (69.0)	103.2-192.1 (150.8)	84.0-152.0(118.5)	55.9-92.5 (74.2)	0.89	16.81	0.0017
Leaf petiole length	12.1-21.2 (15.2)	6.0-13.3 (10.3)	4.9-12.2 (7.0)	5.1-10.6 (8.4)	25.1-43.7 (33.6)	13.0-23.0 (18.1)	7.9-17.5 (12.0)	0.92	17.31	0.0017
Leaf lamina thickness	0.3-0.5 (0.43)	0.3-0.4 (0.37)	0.3-0.5 (0.38)	0.1-0.4 (0.26)	0.4-0.7 (0.55)	0.2-0.5 (0.31)	0.2-0.3 (0.23)	0.57	15.43	0.0039
Number of buds in umbel	3-7 (4.8)	1-7 (3.6)	1-5 (2.6)	1-4 (2.5)	3-7 (5.7)	1-7 (3.9)	7-11 (9.6)	0.83	16.81	0.0021
Bud shape	angular	angular	angular	angular	angular	not angular	angular	0.82	19.00	0.0008
Bud surface	warty	smooth	smooth	smooth	warty	warty	smooth	0.94	17.08	0.0019
Hypanthium ring	absent	absent	absent	present	absent	absent	absent	0.52	19.00	0.0008
Operculum apex shape	acutely conical	acutely conical	acutely conical	acutely conical	acutely conical	rounded	acutely conical	0.83	19.00	0.0008
Operculum attachment	abutting	abutting	abutting	abutting	abutting	hemispherical enclosing	abutting	0.83	19.00	0.0008
Operculum base shape	not overhanging	not overhanging	not overhanging	not overhanging	not overhanging	overhanging	not overhanging	0.83	19.00	0.0008
Operculum base scar	wider at apex	wider at apex	wider at apex	wider at apex	wider at apex	wider at base	wider at apex	0.83	19.00	0.0008
Leaf petiolate/sessile	petiolate	petiolate	petiolate	petiolate	petiolate	petiolate	petiolate	NA		
Leaf position	alternate	alternate	alternate	alternate	alternate	alternate	alternate	NA		
Leaf petiole colour	orange	orange	red	red	red	orange	orange	0.26	19.00	0.0008
Stem smooth/warty	warty	smooth	smooth	smooth	warty	smooth	smooth	0.96	17.08	0.0019
Stem colour	red	orange	red	red	red	orange	orange	0.24	16.74	0.0022
Pith glands stem	present	present	present	present	present	absent	absent	0.28	19.00	0.0008
Pith-like glands at base of fruit	present	present	present	present	present	present	absent	0.67	19.00	0.0008
Oil glands	prominent	prominent	prominent	prominent	inconspicuous	inconspicuous	inconspicuous	0.87	19.00	0.0008
Bud wax	dull	dull	shiny	shiny	dull	shiny	dull	0.92	19.00	0.0008
Hypanthium shape	slightly campanulate	slightly campanulate	obconical	obconical	campanulate	campanulate	obconical	0.99	19.00	0.0008
Operculum shape	conical	conical	conical	conical	conical	hemispherical	horn-shaped	0.55	19.00	0.0008

Continued

Table 6.2. Continued

Character	F1 *E.* 'Urrbrae Gem'	F2 *E.* 'Urrbrae Gem'	*E. erythronema* var. *erythronema*	*E. erythronema* var. *marginata*	*E. stricklandii*	*E. gomphocephala*	*E. gardneri*	r^2	*H*	*P*
Bud colour	yellow-green	yellow-green	dark red	dark red	yellow-green	orange	yellow-green	0.92	19.00	0.0008
Peduncle shape	flattened	flattened	round	round	flattened	flattened	flattened	0.89	19.00	0.0008
Pedicel shape	flattened	flattened	flattened	flattened	absent	flattened	round	0.62	19.00	0.0008
Peduncle curvature	erect	erect	pendulous	pendulous	erect	erect	erect	0.89	19.00	0.0008
Fruit shape	slightly campanulate	slightly campanulate	conical	conical	campanulate	campanulate	obpyriform	0.84	19.00	0.0008
Fruit wax	dull	dull	dull	dull	dull	shiny	dull	0.83	19.00	0.0008
Fruit ribbing	slight	slight	slight	strong	slight	slight	none	0.61	19.00	0.0008
Valve position	exserted	level	level	level	exserted	exserted	shrunken	0.89	19.00	0.0008
Disc position	descending	descending	descending	descending	ascending	level	descending	0.91	19.00	0.0008
Fruit colour	green	green	red brown	red brown	green	orange	brown	0.79	19.00	0.0008
Leaf shape	lanceolate	lanceolate	lanceolate	lanceolate	lanceolate	lanceolate	lanceolate	NA		
Leaf base	attenuate	attenuate	attenuate	attenuate	attenuate	attenuate	attenuate	NA		
Leaf tip	mucronulate	mucronulate	mucronulate	mucronulate	mucronulate	mucronulate	mucronulate	NA		
Leaf surface wax	dull	dull	dull	dull	dull	dull	dull	NA		
Leaf colour	green	green	green	green	blue green	green	blue green	0.96	19.00	0.0008
Stem wax	dull	dull	dull	dull	glaucous	dull	dull	0.82	19.00	0.0008
Flower colour	pink red	pink red	red	red	greenish	cream	greenish	0.89	17.45	0.0016

Correlation coefficient[a] : showing the fit (r^2) of the individual character vectors in the ordination space.

Kruskal-Wallis *H* statistic[b] : the non parametric analysis of variance to determine whether the ranked group means are significantly different from one another for each character.

6.4.2. Controlled pollination seed data

A total of 161 *E. erythronema* var. *erythronema* flowers were pollinated with *E. stricklandii* pollen resulting in a 34.2% pollination success rate. This compares with the 23.7% pollination rate success rate for *E. erythronema* var. *erythronema* x *E. gomphocephala* cross (Table 6.3.), with an average of 8.6 seeds (*E. stricklandii*) and 4.4 (*E. gomphocephala*) seeds produced for every flower pollinated. Mean seed weight for each cross was similar, and, on average, weighed less than seed from either male parent or the female parent. From the 200 seeds planted for each cross, 69 *E. erythronema* var. *erythronema* x *E. stricklandii* (34.5% germination rate) and 93 *E. erythronema* var. *erythronema* x *E. gomphocephala* (46.5% germination rate) seedlings were measured. All cotyledon leaves of open pollinated parent species seedlings were Y shaped, and were divided into three types representing each parent species: *E. erythronema* var. *erythronema* cotyledons were thin, *E. stricklandii* were medium and *E. gomphocephala* were wide. Cotyledons of seedlings from controlled pollinations bore strong resemblance to male parent species cotyledons in all cases.

6.4.3. Seedling morphology

Seventeen characters were measured initially, with leaf surface wax (LSW), leaf petiolate or sessile (LPS) and stem single or branching (STE) recorded as uniform and subsequently deleted, leaving 14 characters for analysis.

Open pollinated *E. erythronema* seedlings, three months after germination, had petiolate, alternating, medium sized lanceolate leaves, with acute tips and attenuate bases. The leaf surface was rough and concolorous, with prominent oil glands. Open pollinated *E. stricklandii* seedlings, of the same age, had large, alternating, lanceolate to ovate leaves, with

Table 6.3. Seed data from *E. erythronema* var. *erythronema* x *E. stricklandii* and *E. erythronema* var. *erythronema* x *E. gomphocephala* controlled pollinations and open pollinations of putative parent species.

female	male	# flrs poll.	# caps harv.	# caps with seed	% caps. harv. with seed[b]	%flrs poll. prod. seed[a]	total # seed	mean # seeds per flr poll.[c]	mean # seeds per caps harv.[d]	mean # seeds per cap with seeds[e]	min # seed per cap	max # seed per cap	mean weight seed per cap (mg)	min weight seed (mg)	max weight (mg)
Eee 7.A.[1]	*Eg*[2]	13	4	2	50.00	15.39	18	1.39	4.5	9.0	9	9	0.402	0.377	0.426
	Es[3]	11	0	0	0.00	0.00	0	0.00	0.0	0.0	0	0	0.000	0.000	0.000
Eee 7.2.[1]	*Eg*[2]	132	53	35	66.04	26.52	665	5.04	12.55	19.0	3	55	0.337	0.209	0.597
	Es[3]	112	78	49	62.82	43.75	1305	11.65	16.73	26.63	4	71	0.316	0.200	0.532
Eee 7.3.[1]	*Eg*[2]	45	18	8	44.45	17.78	150	3.34	8.34	18.75	4	37	0.332	0.201	0.485
	Es[3]	38	22	6	27.27	15.79	83	2.18	3.77	13.83	2	36	0.343	0.249	0.415
total	*Eg*[2]	190	75	45	60.00*	23.68*	833	4.38*	11.11*	18.52*	3	55	0.357	0.201	0.597
total	*Es*[3]	161	100	55	55.00*	34.16*	1388	8.62*	13.88*	25.36*	4	71	0.334	0.200	0.532
Eee 7.A.[1]	open		65				278		4.27	4.28			0.436		
Eee 7.2.[1]	open		75				1171		15.61	24.04			0.401		
Eee 7.3.[1]	open		84				276		3.29	3.65			0.448		
Eee[1]	open		224				1725		7.70*				0.428**		
Eg[2]	open						40						2.27		
Es[3]	open						40						1.08		

[a] # capsules with seed / # flowers pollinated x 100/1
[b] # caps with seeds / capsule harvested x 100/1
[c] total number of seeds / total flowers pollinated
[d] total number of seeds / capsules harvested
[e] total number of seeds / total capsules harvested with seed
[1] *E. erythronema* var. *erythronema* Plot 7 tree A, 2 or 3
[2] *E. gomphocephala*
[3] *E. stricklandii*
* mean

petioles, mucronate tips and obtuse or oblique bases. The leaves were discolorous, smooth, blue green with variably conspicuous oil glands. The leaves of open pollinated *E. gomphocephala* seedlings were petiolate, alternate and ovate, with mucronate tips, however the bases were cordate rarely obtuse. The small leaves were discolorous, smooth, light green with inconspicuous oil glands. The leaves of seedlings from open pollinated F1 and 2nd generation *E.* 'Urrbrae Gem' trees were variable, with characters similar to either *E. erythronema* var. *erythronema* or *E. stricklandii*. Seedling characters from both controlled crosses were intermediate between parent species (Plate 6.2), with the main diagnostic features between the taxa studied leaf length and width (Table 6.4).

Seventeen characters were measured with leaf surface wax, leaf petiolate or sessile and stem single or branching recorded as uniform and deleted from the analysis leaving 14 characters (Table 6.4). The UPGMA dendrogram (Figure 6.4) separated the seedlings into eight clusters based on 20 group means of similar individuals. All *E. erythronema* var. *erythronema* x *E. stricklandii* seedlings and the majority of the *E. erythronema* var. *erythronema* x *E. gomphocephala* seedlings clustered separately from the maternal species (Tables 6.5).

The seedling semi-strong hybrid ordination (Figure 6.5) showed the relative positions of the eight clusters from the dendrogram in two dimensions (stress = 19.4%), with connecting lines representing minimum spanning tree linkages between clusters. Cluster 1, containing six *E. erythronema* var. erythronema x *E. stricklandii* seedlings, connected to cluster 2 at 0.15, which included the majority of *E. erythronema* var. *erythronema* x *E. stricklandii* seedlings (54) and one open pollinated F1 *E.* 'Urrbrae Gem' seedling. Cluster 2 connected to cluster 3 at 0.19, which comprised 65 open pollinated seedlings of *E. stricklandii* and three *E. erythronema* var. *erythronema* x *E. gomphocephala* seedlings. Cluster 1 also connected to cluster 4 at 0.15, which contained 64 and 65 open pollinated seedlings of F1 and 2nd

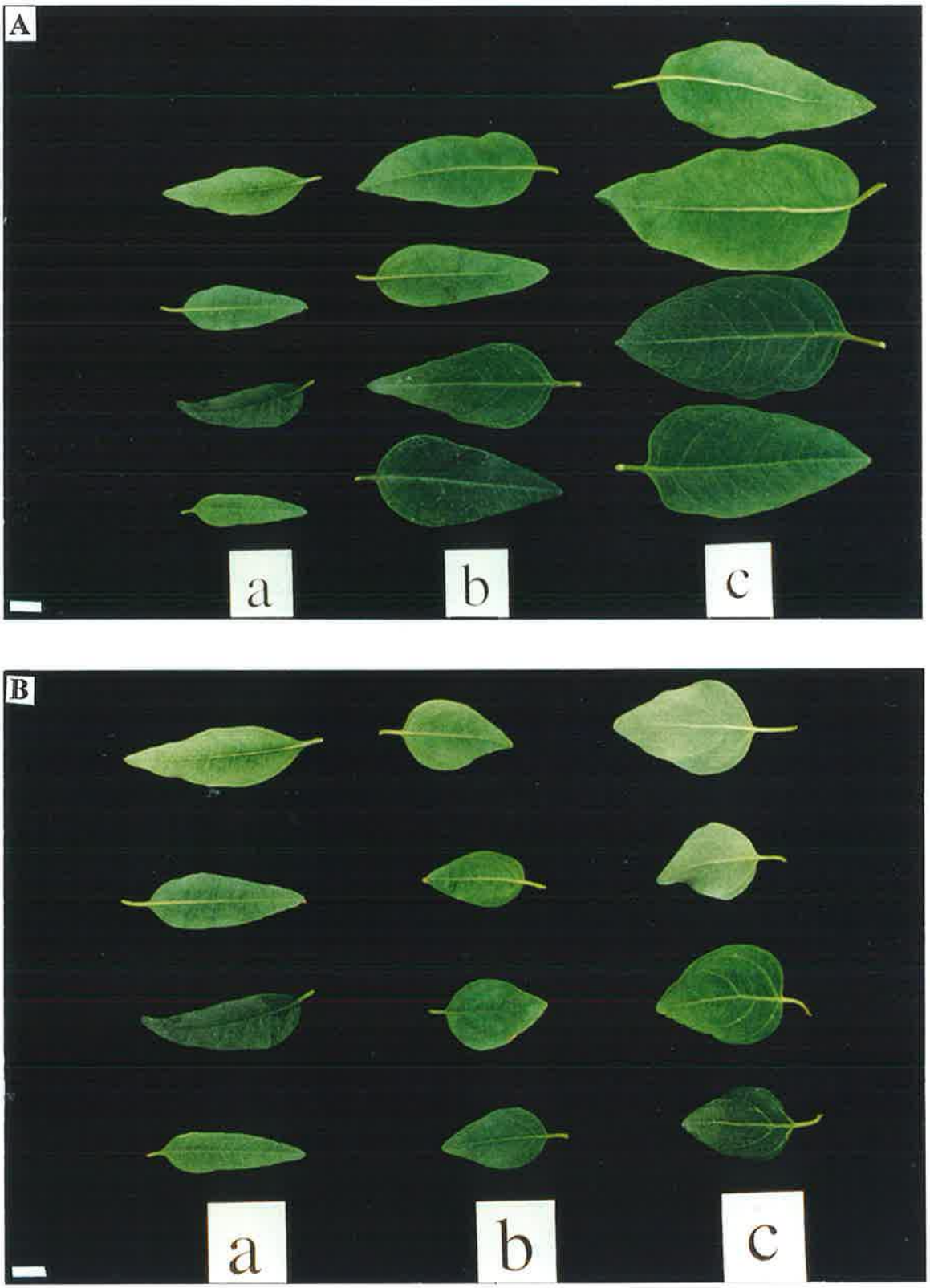


Plate 6.2.
Seedling leaves of A: a) open pollinated *E. erythronema* var. *erythronema*, b) *E. erythronema* var. *erythronema* x *E. stricklandii* and c) open pollinated *E. stricklandii*, and B: a) open pollinated *E. erythronema* var. *erythronema*, b) *E. erythronema* var. *erythronema* x *E. gomphocephala* and c) open pollinated *E. gomphocephala*, three months after germination. Top two rows show lower surfaces, lower two rows show upper surfaces. Bar represents 1 cm.

Table 6.4. Seedling morphological characters of *E.* 'Urrbrae Gem' (from Table 6.1) and putative relatives with correlation coefficient[a] (r^2), Kruskal-Wallis *H* statistic[b] and probability (*P*). Measurements in mm or ° presented as range (mean), r^2 >0.61 significant at $P<0.05$, NA = not applicable as character invariant and not included in analysis.

Characters	F1 *E.* 'Urrbrae Gem' open pollinated seedlings	F2 *E.* 'Urrbrae Gem' open pollinated seedlings	*E. erythronema* var. *erythronema* and var. *marginata* open pollinated seedlings	*E. erythronema* var. *erythronema* x *E. stricklandii*	*E. stricklandii* open pollinated seedlings	*E. erythronema* var. *erythronema* x *E. gomphocephala*	*E. gomphocephala* open pollinated seedlings	r^2	*H*	*P*
Leaf shape	lanceolate or falcate or ovate	lanceolate	lanceolate	lanceolate or elliptic or ovate	lanceolate or ovate	lanceolate or ovate	ovate	0.92	11.58	0.0090
Leaf tip	acute	acute	acute	mucronate, sometimes acute	mucronate	acute or mucronate or obtuse	mucronate	0.87	5.52	0.1372
Leaf base	attenuate, sometimes obtuse	attenuate, sometimes obtuse	attenuate	attenuate or oblique or obtuse	obtuse or oblique	attenuate or oblique or obtuse	obtuse or cordate	0.80	5.99	0.1117
Leaf length	30.5-105.0 (61.5)	45.6-129.0 (79.3)	38.1-89.5 (60.3)	57.0-128.0 (80.7)	63.5-124.3 (92.4)	27.0-112.5 (49.9)	15.3-72.3 (53.0)	0.73	8.05	0.0450
Leaf width	8.1-37.4 (21.1)	12.5-37.2 (22.2)	9.8-39.5 (18.7)	22.5-57.9 (37.6)	34.0-73.5 (47.6)	13.0-50.0 (28.3)	17.5-63.2 (40.7)	0.81	10.67	0.0137
Leaf surface wax	dull	dull	dull	dull	dull	dull	dull	NA		
Leaf surfaces	concolorous	concolorous	concolorous	concolorous	discolorous	discolorous	discolorous	0.56	5.38	0.1461
Leaf smoothness	smooth	rough	rough	smooth	smooth	smooth	smooth	0.55	4.49	0.2135
Leaf petiolate/sessile	petiolate	petiolate	petiolate	petiolate	petiolate	petiolate	petiolate	NA		
Leaf petiole length	5.2-15.2 (8.6)	4.9-20.1 (9.3)	5.6-21.6 (8.2)	5.7-17.0 (10.5)	8.6-23.2 (15.5)	3.2-15.2 (8.7)	8.4-21.5 (13.5)	0.77	12.40	0.0061
Leaf position	alternate	alternate	alternate	alternate, sometimes opposite	alternate	alternate, sometimes opposite	alternate	0.76	7.03	0.0709
Leaf colour	blue green	blue green	green	blue green	blue green	green	light green or green	0.55	5.56	0.1239
Leaf margin prominence	close, medium or far	medium or far	medium or far	medium or far	far or medium	medium or far	far	0.53	3.20	0.3617
Stem shape	angular	square	square	angular	angular	angular	round or angular	0.55	16.83	0.0008
Stem	single	single	single	single	single	single	single	NA		
Stem smoothness	smooth or rough	smooth or rough	smooth or rough	not smooth	not smooth	smooth or rough	smooth	0.60	6.02	0.1108
Oil glands prominent	prominent	prominent	prominent	inconspicuous or prominent	inconspicuous or prominent	inconspicuous or prominent	inconspicuous	0.70	19.00	0.0003

Correlation coefficient[a] : showing the fit (r^2) of the individual character vectors in the ordination space

Kruskal-Wallis *H* statistic[b] : the non parametric analysis of variance to determine whether the ranked group means are significantly different from one another for each character.

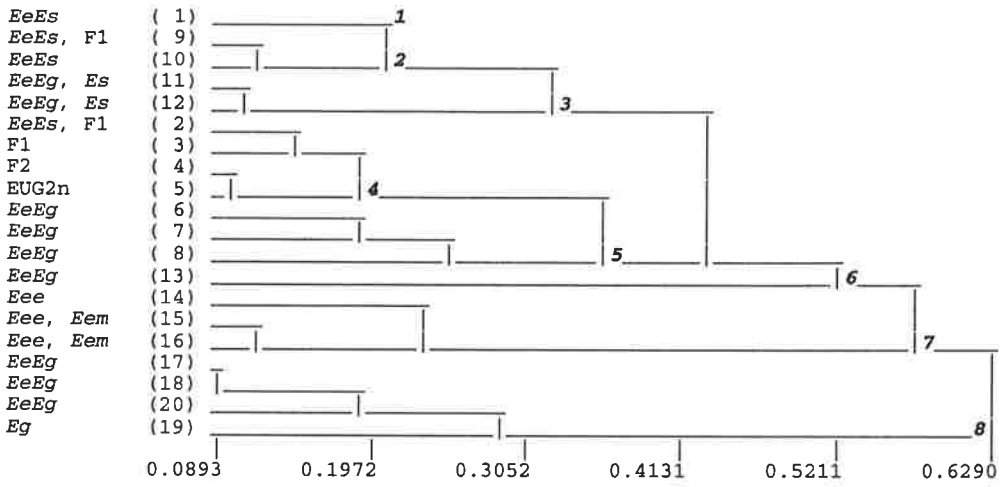


Figure 6.4. UPGMA dendrogram representing 486 seedlings of *E. erythronema* var. *erythronema* x *E. stricklandii* (*EeEs*), *E. erythronema* var. *erythronema* x *E. gomphocephala* (*EeEg*), open pollinated *E. erythronema* var. *erythronema* (*Eee*), open pollinated *E. erythronema* var. *marginata* (*Eem*), open pollinated *E. stricklandii* (*Es*), open pollinated *E. gomphocephala* (*Eg*), open pollinated F1 *E.* 'Urrbrae Gem' (F1) and open pollinated 2nd generation *E.* 'Urrbrae Gem' (EUG2n). The dendrogram identifies eight clusters (1-8) based on 20 group (in parentheses) means. Scale represents standardised Manhattan distance (Gower metric) based on 14 characters (Table 6.4).

Table 6.5. Number of seedlings of *E.* 'Urrbrae Gem' and putative relatives in each dendrogram cluster (Figure 6.4).

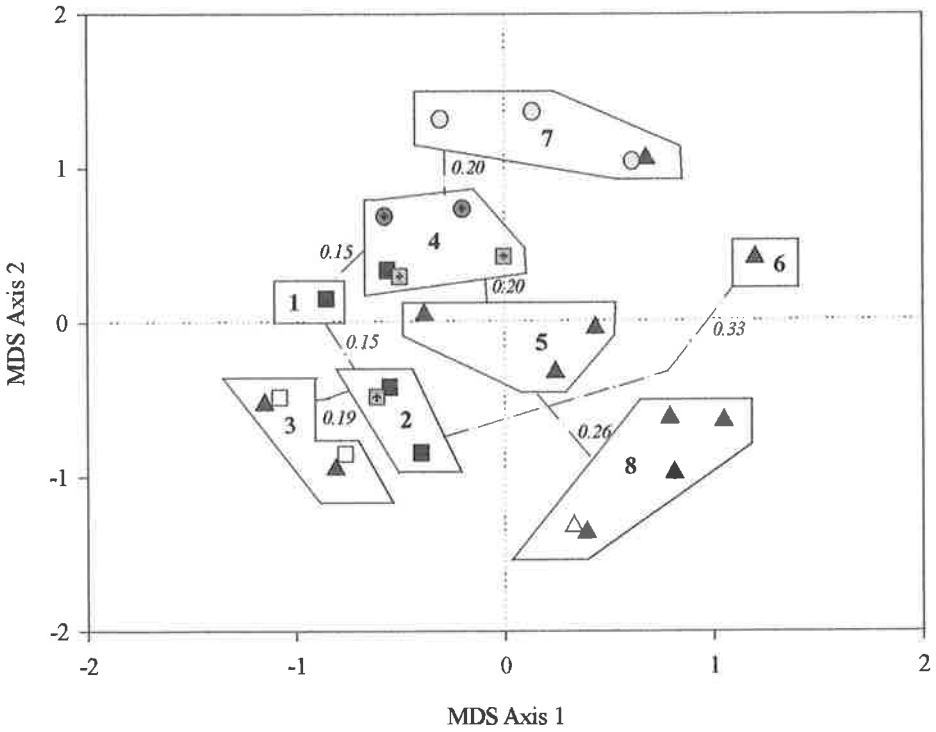
Cluster	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
E. erythronema var. *erythronema* open pollinated seedlings							45	
E. erythronema var. *marginata* open pollinated seedlings		1					20	
F1 *E.* 'Urrbrae Gem' open pollinated seedlings				64				
2nd generation *E.* 'Urrbrae Gem' open pollinated seedlings				65				
E. erythronema var. *erythronema* x *E. stricklandii*	6	54		9				
E. stricklandii open pollinated seedlings			65					
E. erythronema var. *erythronema* x *E. gomphocephala*			3		16	2	2	70
E. gomphocephala open pollinated seedlings								65

generation *E.* 'Urrbrae Gem' respectively, plus the remainder of the *E. erythronema* var. *erythronema* x *E. stricklandii* seedlings (9).

The majority of *E. erythronema* var. *erythronema* x *E. gomphocephala* seedlings (70) were in cluster 8, with the 65 open pollinated seedlings from *E. gomphocephala.* Cluster 8 connected to cluster 5 at 0.26, which contained 16 *E. erythronema* var. *erythronema* x *E. gomphocephala* seedlings. This group connected to cluster 4 at 0.20. The remaining two *E. erythronema* var. *erythronema* x *E. gomphocephala* seedlings were separated as cluster 6, which was connected to cluster 2 at 0.33. Cluster 7 included the open pollinated seedlings of *E. erythronema* vars. *erythronema* and *marginata*, and was connected to cluster 4 at 0.20.

Principal canonical correlation analysis of seedling characters (Figure 6.6) showed significant correlations between most taxon groups and characters (Table 6.4). There were no defining characters for the hybrid seedling groups, but the open pollinated F1 *E.* 'Urrbrae Gem' were more similar to the *E. erythronema* var. *erythronema* x *E. stricklandii* seedlings than to any other group. Leaves of the seedlings produced by controlled pollination were intermediate in appearance between those of the open pollinated seedlings of the parents at the same age.

The results of this study indicate that progeny from a controlled cross between *E. erythronema* var. *erythronema* and *E. stricklandii* are more similar to the open pollinated seedlings from the F1 and 2nd generation *E.* 'Urrbrae Gem', than to the progeny of a cross between *E. erythronema* var. *erythronema* and *E. gomphocephala.* It is possible to identify hybrids between these species from differences between seedling leaves at the cotyledonary leaf stage and at three months of age.



■ *E. erythronema* var. *erythronema* x *E. stricklandii*
▩ F1 *E.* 'Urrbrae Gem' open pollinated seedlings
□ *E. stricklandii* open pollinated seedlings
● 2nd generation *E.* 'Urrbrae Gem' open pollinated seedlings
▲ *E. erythronema* var. *erythronema* x *E. gomphocephala*
○ *E. erythronema* var. *erythronema* and var. *marginata* open pollinated seedlings
△ *E. gomphocephala* open pollinated seedlings

Figure 6.5. SSH Multidimensional scaling of 20 groups means of 486 seedlings using all juvenile characters. Distances and linkages are results from a minimum spanning tree analysis of the data.

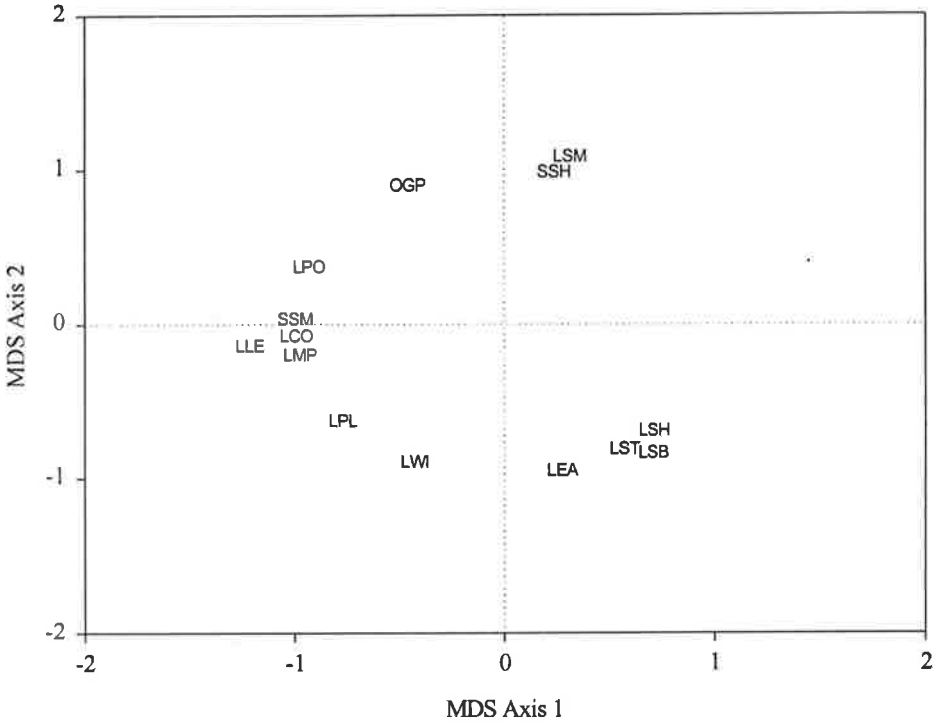


Figure 6.6. Principal canonical correlation plot for the semi-strong hybrid ordination (Fig. 6.5) based on the seedling characters measured, all vector lengths 1. Character codes are those detailed in Table 6.1., characters listed in Table 6.4.

6.5. Discussion

Interspecific hybridisation within the genus *Eucalyptus* reflects the hierarchy of taxonomic affinities (Griffin *et al.*, 1988). Natural, or spontaneous, hybridisation does occur, particularly in areas where the geographical barriers to hybridisation have been removed, however the occurrence of such hybrids is often poorly documented (Griffin *et al.*, 1988; Hopper, 1995). Several suspected F1 hybrid adult trees have been studied in detail, by examining adult morphological characters and seedling morphological characters of progeny (Pryor, 1950; Hopper *et al.*, 1978) as well as investigating chemical traits (Grayling and Brooker, 1996). Natural hybrids can occur as single individuals some distance from the male parent (Ashton and Sandiford, 1988), single individuals near the junction of two species (Potts and Reid, 1983), or as hybrid swarms at the junction of the parent species' range (Potts and Reid, 1985). The effect of areas such as arboreta on the potential for spontaneous hybridisation has not yet been documented, but as species are removed from their natural distribution and planted near others normally geographically isolated, the rate of hybridisation may increase. Manipulated hybridisation is a tool used frequently in tree improvement programs, and is quite successful in eucalypts (Eldridge *et al.*, 1993).

In a review of interspecific hybridisation patterns in *Eucalyptus*, Griffin *et al.* (1988) listed five interspecific hybrids involving two of the species investigated in this study. Naturally occurring crosses between *E. erythronema* var. *erythronema* and *E. eremophila* (from sect. *Bisectaria* ser. *Erythronemae* and sect. *Bisectaria* ser. *Occidentales*), *E. gomphocephala* and *E. cornuta* (from sect. *Bisectaria* ser. *Cornutae* and sect. *Bisectaria* ser. *Cornutae*), and *E. gomphocephala* and *E. wandoo* (from sect. *Bisectaria* ser. *Cornutae* and sect. *Bisectaria* ser. *Reduncae*) were recorded in Eucalist (Chippendale and Wolfe, 1984). Hybrids occurring as manipulated or spontaneous were also reported by Griffin *et al.* (1988), between *E. erythronema* var. *erythronema* and *E. torquata* (from sect. *Bisectaria* ser. *Erythronemae* and

sect. *Dumaria* ser. *Torquatae*) (Chippendale and Wolf, 1984; Gardner, 1979), *E. gomphocephala* and *E. cornuta* (from sect. *Bisectaria* ser. *Cornutae*) (Jacobs 1979), and *E. gomphocephala* and *E. occidentalis* (from sect. *Bisectaria* ser. *Cornutae* and sect. *Bisectaria* ser. *Occidentales*) (Jacobs, 1979). The final species, *E. stricklandii* (sect. *Bisectaria* ser. *Grossae*) had no reported hybrids from that study.

Determination of the parentage of the spontaneous F1 hybrid, *E.* 'Urrbrae Gem', followed some of the criteria for defining a putative eucalypt hybrid (Hopper *et al.*, 1978; Hopper, 1995). The criteria included evidence of intermediate features (morphological, anatomical and physiological) between the putative hybrid and the parent species, and a close resemblance between suspected and manipulated hybrids; and of secondary consideration, phenotypic segregation and impaired reproductive capabilities of hybrids relative to the paternal individuals (i.e. F2 breakdown).

To investigate the criteria of intermediate morphology and hybrid resemblance, 60 adult and 17 seedling characters were measured. Morphological characters are frequently used to identify species and hybrids. In eucalypts, morphological characters have been used to investigate differences between species, within species, between provenances, complexes and intergrades, as well as to study geographical variation within species. A diverse range of characters is measured from cotyledons and leaves (from seedlings and adults), inflorescences, buds and fruit, as well as the bark and overall tree habit (Doran and Burgess, 1993; Jordan *et al.,* 1993; Chappill and Ladiges, 1996). In some cases environmental attributes are taken into account, such as associated soil type and rainfall data (Nicolle and Conran, 1999), as are characters such as leaf waxes (Hallam and Chambers 1970), trichomes (Ladiges 1984) and oil composition (Dunlop *et al.,* 1997). For identification of F1 hybrids, the characters measured are often specific to the species involved. For example, Hopper *et al.*

(1978) determined the relationship between *E. preissiana* and *E. buprestium* and their possible hybrid *E. chrysantha* using adult morphological characters specifically selected to provide the best species discrimination.

The results of the MDS analysis of adult characters placed the F1 *E.* 'Urrbrae Gem' centrally on the ordination axis, and most closely associated with the open pollinated 2nd generation *E.* 'Urrbrae Gem'. The two varieties of the female parent species were linked very closely to each other and less closely to the open pollinated 2nd generation *E.* 'Urrbrae Gem'. This indicates that the analysis was robust enough to show the difference between two varieties, even though they possessed few differing morphological characters, and that the open pollinated 2nd generation population was segregating back to the female parent, indicating possible back crossing to the original female parent species. The two postulated male parent species were both equally close to the F1 *E.* 'Urrbrae Gem', however, the MST values showed that *E. stricklandii* was closer to the F1 *E.* 'Urrbrae Gem' than *E. gomphocephala*, with additional analysis of group means confirming this.

On the assumption that a hybrid will often exhibit intermediate morphology (Hopper *et al.* 1978; Hopper, 1995), hybrids should be placed between clusters of individuals from the parental species in an ordination plot. McDade (1997) discussed MDS and similar non-hierarchical graphical methods, and their use in hybrid parental analysis, noting that a hybrid will be placed either with one of the parents or between them, and that calculation of a distance matrix should enable the identification of the closest (parent) taxa. Our study placed *E.* 'Urrbrae Gem' directly between *E. erythronema* var. *erythronema* and *E. stricklandii*, with which it shares the two shortest MST distances, indicating that it is intermediate between them. The positions and MST distances of the remaining species (*E. gomphocephala* and *E. gardneri*) to the F1, are not as close and thus these are less likely parental candidates.

The PCC analysis for adult characters showed that there are few characters uniquely shared by the hybrid and either of the possible male parents, and that the F1 and 2nd generation hybrids have intermediate scores for nearly all of the characters measured. The fact that *E. stricklandii* was only marginally closer to the F1 *E.* 'Urrbrae Gem' than *E. gomphocephala*, may explain the original confusion in opinion on the identity of the male parent.

The criteria of secondary consideration; that is phenotypic segregation and impaired reproductive capabilities of hybrids relative to the paternal individuals (i.e. F2 breakdown), were studied on an empirical level only due to limited numbers of plants. The 2nd generation *E.* 'Urrbrae Gem' tree used throughout the study is the single surviving adult specimen of a number of seedlings from *E.* 'Urrbrae Gem' (F1) to be planted in the Waite Arboretum over the last 50 years. While open pollinated seed from the F1 and 2nd generation *E.* 'Urrbrae Gem is viable, seedlings generally exhibit poor form, increased phenotypic segregation and low vigour. This was observed whilst germinating open pollinated seed from both the F1 and 2nd generation *E.* 'Urrbrae Gem" during this study.

Controlled or manipulated hybridisation has been used in numerous studies investigating hybridisation in eucalypts, to refute or support the identity of a hybrid through field studies and progeny tests of natural and manipulated hybrid combinations (Pryor, 1950, 1951a, 1951b, 1952, 1954, 1956, 1957). The standard technique of controlled pollination for eucalypts varies with researchers, but generally, the flowers are emasculated prior to anthesis, bagged to exclude external pollen, pollinated manually some days later, and left to develop. Research has found that emasculation of flower buds can discourage pollinators such as bees (Hodgson, 1976), bagging can have a detrimental effect on the survival of the pollinated flowers (Pryor, 1951a), and that successful pollination can be achieved with neither bagging (Tibbits, 1989; Beardsell *et al.*, 1979) or emasculation (Beardsell *et al.*, 1979). Flowers in

this study were emasculated byt not bagged in order to observe the proficiency of controlled pollination without isolation.

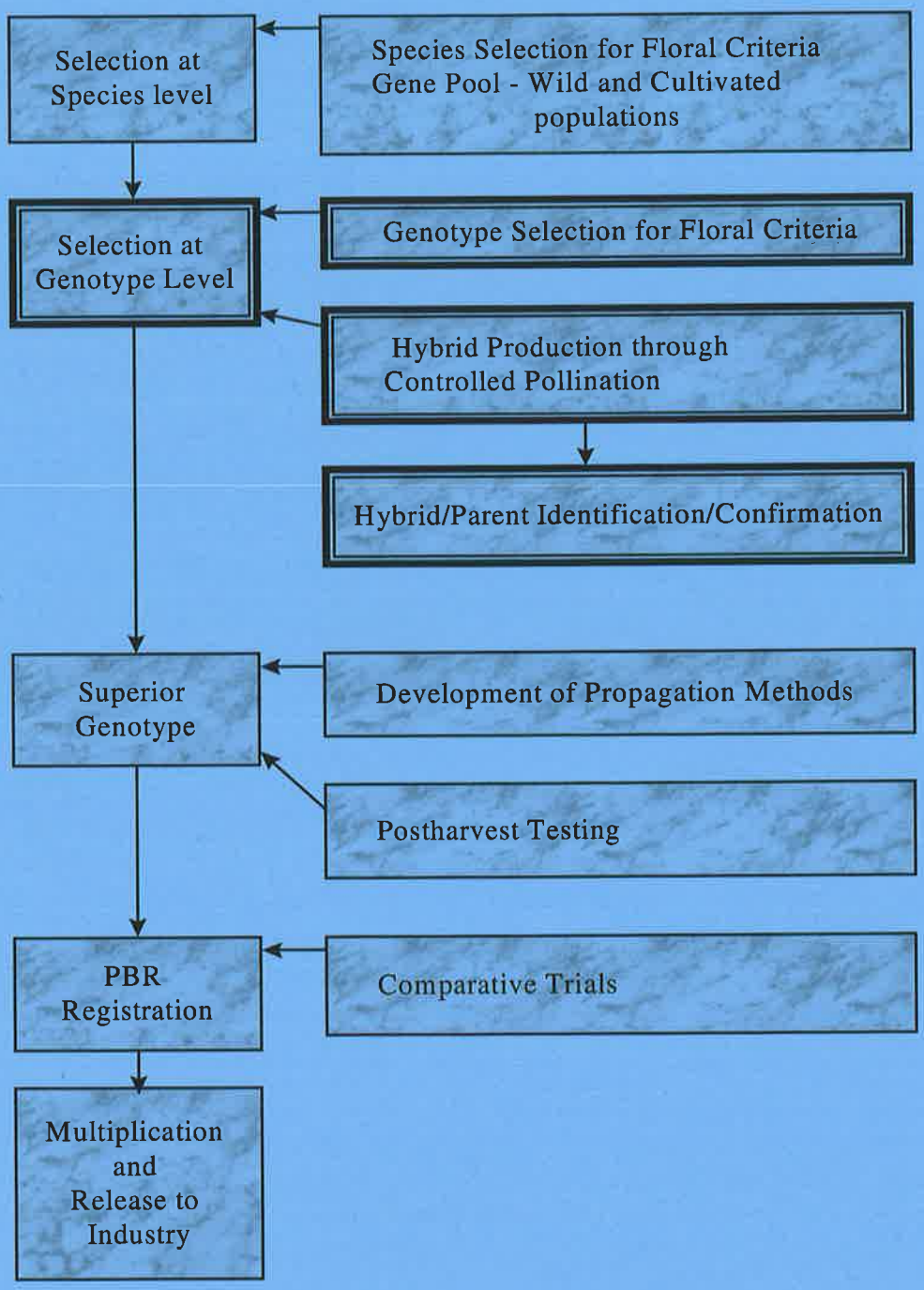
Pollen and pistil physiology also play an important part in hybridisation success, and should be considered in the pollination protocol. Pollen from different species varies in its level and rate of germination and tube growth (Potts and Marsden-Smedley, 1989). Pollen grains may take up to two days to germinate if the stigma is not receptive (Hodgson, 1976), and individual grains may germinate at different rates (Heslop-Harrison and Heslop-Harrison, 1985). In addition, there is variability between species in time to stigma receptivity after anthesis, from two days for *E. macarthurii, E. cinerea* and *E. occidentalis* (Davis, 1968) to 13 days for *E. urnigera* (Savva *et al.*, 1988). Cauvin (1988) demonstrated that cutting of the style, to remove the stigma, could have applications to breeding programs, as it meant that it was no longer necessary to wait for stigma receptivity prior to pollination. Recent work by Harbard *et al.* (1999), where the style is cut longitudinally, was found to increase seed set in *E. globulus*. In both cases, masculation, cutting and pollination could be completed in one step; the chances of contamination were reduced; and the problem of the length of style in relation to pollen tube length could be overcome. This last point has been considered to be the cause of some previous failed interspecific hybridisation attempts between long styled and short styled species, as it was suggested that pollen tube length is correlated to style length. These include *E. caesia* x *E. sideroxylon* and *E. caesia* x *E. leucoxylon* (Beardsell *et al.*, 1979), *E. caesia* x *E. pulverulenta* (Pryor, 1956) and *E. nitens* x *E. globulus* (Tibbits, 1988; 1989; Gore *et al.*, 1990) where the reciprocal was successful. In each case, the cross was successful only when the style from the male species flower was longer than that of the female species. In both cases in this study, the style and stigma of *E. erythronema* var. *erythronema* are very similar in length and size to those of *E. stricklandii* and *E. gomphocephala*.

Controlled pollination between *E. erythronema* var. *erythronema* and *E. stricklandii* produced more seed per flower pollinated that the cross between *E. erythronema* var. *erythronema* and *E. gomphocephala.* The seed collection data indicated that there may be more of an effect of female tree, rather than male species, on seed production, indicating maternal inheritance. Maternal inheritance in interspecific hybridisation of eucalypts has been identified (Tibbits, 1989). However, the cotyledon shapes of the seedlings for each cross were similar to the cotyledon shapes of the open pollinated seedlings of the respective male parent. Large numbers of seedlings were obtained for measurement, despite seedling mortality rates of 65 and 53% for *E. erythronema* var. *erythronema* and *E. stricklandii*, and *E. erythronema* var. *erythronema* and *E. gomphocephala,* respectively.

In the present study, progeny from controlled pollinations between *E. erythronema* var. *erythronema* and *E. stricklandii*, and *E. erythronema* var *erythronema* and *E. gomphocephala*, were measured for a range of seedling leaf and stem characters three months after germination. *E. erythronema* var. *erythronema* x *E. stricklandii* seedlings were intermediate between the parent species, with morphology similar to that of the open pollinated seedlings of F1 and 2nd generation *E.* 'Urrbrae Gem'. The majority of the *E. erythronema* var. *erythronema* x *E. gomphocephala* seedlings grouped with open pollinated *E. gomphocephala* seedlings, supporting their hybrid status, and isolating them from the open pollinated seedlings of F1 and 2nd generation *E.* 'Urrbrae Gem'. A small number of *E. erythronema* var *erythronema* x *E. gomphocephala* seedlings was placed very close to the open pollinated seedlings of F1 and 2nd generation *E.* 'Urrbrae Gem'. This may reflect early seedling variability, or may be due to past hybridisation events within this cross fertile group of species. It is notable that large numbers of hybrid seedlings were obtained, despite seedling mortality rates of 65 and 53% for *E. erythronema* var. *erythronema* x *E. stricklandii* and *E. erythronema* var. *erythronema* x *E. gomphocephala* respectively.

6.6. Conclusion

This study, through comparison of adult and seedling morphological characters, has strongly suggested that *E. stricklandii* is the probable male parent of the hybrid *E.* 'Urrbrae Gem'. The adult morphological characters study placed the hybrid intermediately between clusters of *E. erythronema* var. *erythronema* and *E. stricklandii* seedlings. Morphological character analysis of seedlings from controlled pollinations indicated similarities between *E. erythronema* var. *erythronema* x *E. stricklandii* hybrid seedlings and the open pollinated seedlings from the F1 and 2nd generation trees of *E.* 'Urrbrae Gem', while seedlings from crosses between *E. erythronema* var. *erythronema* x *E. gomphocephala* are not similar. This study has also shown that adult and seedling morphology studies are valuable tools in plant improvement programs. They are cheap to conduct and can be applied to large numbers of plants. The data can be used to indicate profitable hybridisation matrices for the generation of novel lines for ornamental horticulture.



Ornamental eucalypt breeding cycle flow chart.

Areas covered by this chapter are highlighted.

Chapter Seven

Interspecific hybridisation: *E. macrocarpa*, *E. pyriformis* and *E. youngiana*

7.1. Abstract

Interspecific hybridisation between three *Eucalyptus* species with horticultural merit was undertaken to investigate the likelihood of success of such crosses. *E. macrocarpa*, *E. pyriformis* and *E. youngiana* are closely related species from Western Australia, with slightly differing geographic distributions. Crosses were conducted between species with all combinations producing fertile seed. There was no effect of male parent, with interspecific crosses as successful as intraspecific crosses, but there were difference in fertility between female trees within a species. Hierarchical and non-hierarchical analysis of each cross indicated a high degree of interspecific hybridisation, with the majority of seedlings measured clustering separately from either parent. The 166 seedlings from the interspecific cross seed, when measured for a range of leaf and stem characters at three different nodes, show strong evidence of intermediacy between parents, with 1.2% grouped with the male parent, 94.6% clustered between the parent species, and the remaining 4.2% with the female parent. The success of this program in producing both large numbers of seed and large numbers of hybrids indicated that it is possible that these species will hybridise naturally in the same location. It also demonstrated the potential of controlled pollination, between closely related species of similar floral morphology, as a method to produce hybrids for further evaluation.

7.2. Introduction

The international cut-flower and nursery industries are constantly searching for new and novel products. A number of *Eucalyptus* species show floricultural potential, with coloured buds of varying sizes and shapes, which open to flowers of a range of colours. Three such species with potential are *E. macrocarpa* Hook (Plate. 7.1), *E pyriformis* Turcz. (Plate 7.1) and *E. youngiana* F. Muell. (Plate 7.1), all of which have buds and flowers greater than 40 mm in diameter, ranging in colour from yellow to deep red, flowering from early spring to late summer. These species also show merit as garden plants, as they are small growing trees or mallees, with interesting tree forms, long flowering seasons and attractive seed capsules.

All three taxa occur naturally in Western Australia: distributions of *E. macrocarpa* and *E. pyriformis* range from Geraldton to Perth, with *E. pyriformis* more northerly. *E. youngiana* occurs further east, from near Kalgoorlie to the north western part of South Australia (Figure 7.1).

E. macrocarpa, *E. pyriformis* and *E. youngiana* are closely related taxonomically, in *E.* series Curviptera (Chippendale, 1988), also known as Subgenus Symphyomyrtus, Section Bisectaria, Series Macrocarpae (Pryor and Johnson, 1971). A later revision (Brooker and Hopper, 1993) divided *E.* ser. Curviptera (Chippendale, 1988) into two subseries - subser. Inflexae (stamens inflexed) and subser. Curviptera (stamens erect, oblique or variously curved). Natural hybrids involving these species have been reported between *E. macrocarpa* and *E. pyriformis*, *E. pyriformis* and *E. youngiana* and *E. drummondii* and *E. macrocarpa* (a very rare and almost sterile hybrid known as *E.* x *carnabyi*) (Griffin *et al.*, 1988; Hopper, 1995) and it has been suggested that natural hybridisation between these species may occur where their distributions overlap.

The technique of manipulated hybridisation, or controlled pollination, can be a useful tool in plant breeding to generate hybrids that may show improved characteristics, and the possibility exists to combine species that are incapable of breeding through geographical or temporal isolation (Tibbits, 1988). Manipulated hybridisation has also been used to test the probability of natural hybridisation (Pryor, 1950, 1951, 1952, 1954, 1956, 1957).

All three species have characters that make them suitable for floriculture or garden plants, however, each also has undesirable characters. *E. macrocarpa* has sessile buds, flowers and leaves, as well as being slow growing with poor form, but the large red flowers and heavily glaucous leaves are very attractive. *E. pyriformis* has pendulous buds and flowers, but has a range of bud and flower colours and sizes as well as good tree form. *E. youngiana* has good vigour and form, as well as a range of flower colours, but has a tendency to hold fruit for a long time, resulting in heavy branches that frequently break. The combination of desirable characters, such as flower colour, glaucousness, tree form and vigour, in seedlings from crosses between these species, may enhance their ornamental merit, resulting in a plant more commercially viable than its parents.

The aim of this study was to determine the likelihood of success of controlled hybridisations between these three species, and to generate hybrids for evaluation for ornamental merit.

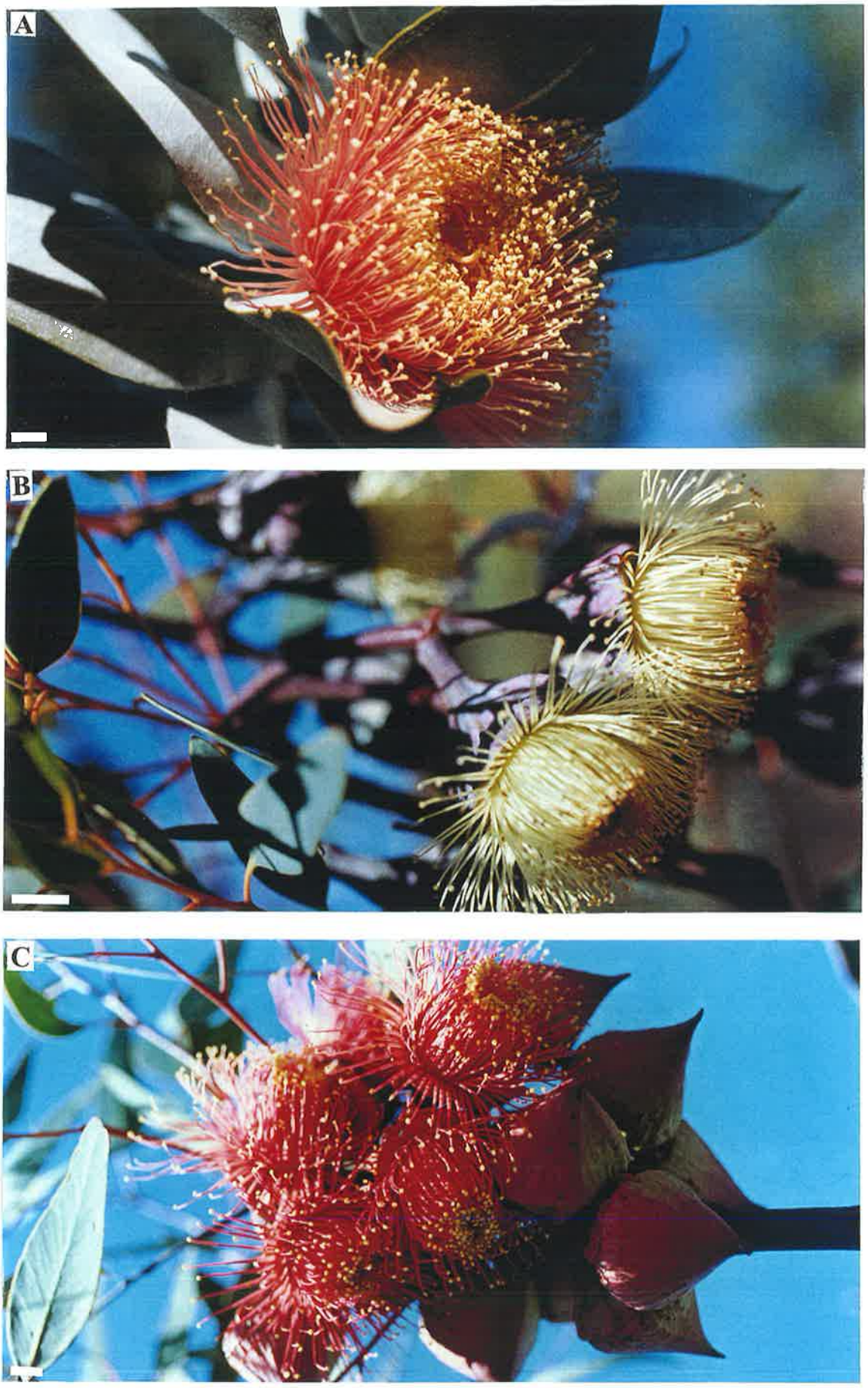
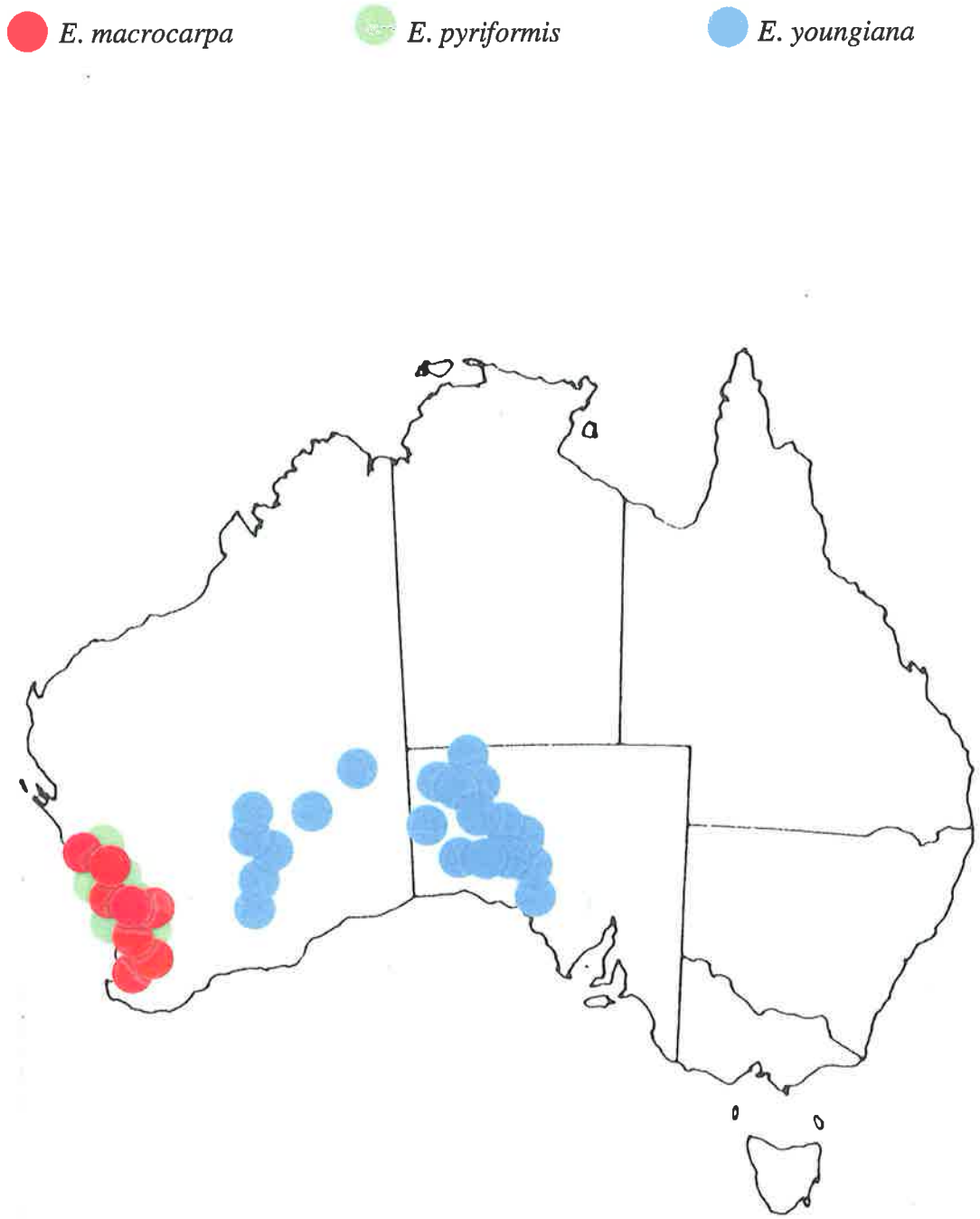


Plate 7.1.
A: *E. macrocarpa* flower; B: *E. pyriformis* flower; C: *E. youngiana* flower. Bar = 1cm.

Figure 7.1. Map of natural distribution of *E. macrocarpa, E. pyriformis* and *E. youngiana.*



7.3. Materials and methods

7.3.1. Plant Material

The fourteen adult plants used are listed in Table 7.1. Plants were sourced from the Waite Arboretum and the Monarto Woodland. Plants were selected for their different bud and flower colours. Interspecific crosses between *E. macrocarpa, E. pyriformis* and *E. youngiana* were made, as well as intraspecific crosses. Open pollinated capsules were collected from these trees for comparison with seed from controlled pollinations.

7.3.2. Controlled pollination

The pollination technique follows that of van Wyk (1977) and Moncur (1995), and described in detail in Chapter 6, section 6.3.3. Equal amounts of pollen from different plants of the same species were mixed for use, reducing the chance of pollinations with low fertility or related pollen. Between four and forty flowers per tree per cross were pollinated on each tree of *E. pyriformis*, depending on the number of flowers available, with all flowers pollinated and pollinations distributed evenly between crosses. All flowers on *E. macrocarpa* were pollinated, up to fourteen per cross, with pollinations distributed evenly between crosses. Up to forty flowers were pollinated for each cross on each *E. youngiana* tree with all other flowers being removed. Flowers were emasculated but not isolated, nor were they randomised to the tree (see section 6.3.3.). Capsules were harvested 8-10 months after pollination, and each capsule was placed separately in a paper bag. The capsules were stored in boxes over silica gel, in a dry room at 30°C until open (c. 6 weeks). Seed was extracted and counted, and seedlots of individual capsules weighed. Seeds were planted and grown as described in Chapter 6, section 6.3.3., with 200 seeds randomly selected for each cross, dates of germination recorded and seedlings grown on under glasshouse conditions. In addition, seed of the parent species from various commercial seed sources was grown for comparison; this proved to be highly variable.

7.3.3. Seed data analysis

Differences in the mean number of seeds produced per flower pollinated, mean seed weight for all seeds of that cross and germination rates were calculated and compared using a randomised complete block design, general analysis of variance and least significant difference where appropriate (Genstat 5 Release 4.2.).

Table 7.1. Plants used in controlled pollination matrix from subgenus *Symphyomyrtus*, sect. *Bisectaria*, ser. *Macrocarpae* (Pryor and Johnson, 1971).

	Number[a]	Code[b]	Location[c]	Use[d]	Characters[e]	Seed source
E. macrocarpa	1632	Em1	WaA	F M	SM; large red flowers, glaucous leaves	WA Forestry Dept., 1952
	1952		WaA	M	SM; small to medium red flowers, glaucous leaves	Nind., 1963
	1848		WaA	M	SM; small red flowers, glaucous leaves	Eastern Park, 1963
	1838		WaA	M	SM; red flowers, glaucous leaves	Seed Index A1261, 1962
	Nind, RPS[f]			S		
E. pyriformis	1926A	Ep1	WaA	F M	SM; red flowers, green buds	Seed from 1926A, WaA
	1813C	Ep2	WaA	F M	ST; yellow flowers, green buds	Woods and Forest, 1961
	8.9.2	Ep3	MoW	F	SM; yellow flowers, green buds	unknown
	8.9.4	Ep4	MoW	F	SM; yellow flowers, purple buds	unknown
	8.27.2	Ep5	MoW	F	SM; peach flowers, purple buds	unknown
	Nind			S		
E. youngiana	8.54.2	Ey2	MoW	F M	ST; red flowers	unknown
	8.54.4	Ey4	MoW	F M	ST; peach flowers	unknown
	8.54.5	Ey5	MoW	F	ST; red flowers	unknown
	8.54.7	Ey7	MoW	F	ST; yellow flowers	unknown
	8.54.8		MoW	M	ST; yellow flowers	unknown
	1569		WaA	M	ST; yellow flowers	D. Symons 1984
	Nind, RPS			S		

[a] Number: number of plant in plantation
[b] Code: number given to plant in crossing program (female trees only)
[c] Location: WaA = Waite Arboretum, MoW = Monarto Woodland
[d] Use: use of plants in program (F = female parent, M = male parent, S = seedling morphology only)
[e] Character: SM = small mallee (to 4 m, multiple trunk), ST = small tree (to 4 m, single trunk), desirable character of plant.
[f] Seed source: Nind = Nindethana seeds W.A., RPS= Royston Petrie seeds N.S.W.

7.3.4. Seedling morphology

All surviving seedlings from controlled pollinations were measured three months after germination for thirteen leaf characters on fully expanded leaves at the 3rd, 5th and 7th nodes above the cotyledons and for three stem characters (Table 7.2). In addition, open pollinated seed from the female parent trees and seed from commercial sources was grown to provide a representative sample of seedling morphology of each species. Between 40 and 80 seedlings, selected at random from each parent species depending on survival rates, were measured also at three months after germination. Data were averaged to generate five representative individuals for each species, and these individuals used in the analysis for comparison with the seedlings from the controlled pollinations. The characters of leaf petiole/sessile (LPS) and stem structure (STE) were removed from all crosses prior to analysis as they were identical for all individuals measured.

Table 7.2. Characters and codes used in phenetic analysis (seedling), measured at nodes 3, 5 and 7, three months after germination.

Code	Character	Description
LSH#	Leaf shape	linear (0) lanceolate (1) elliptic (2) ovate (3) orbicular (4)[a]
LST#	Leaf tip	acuminate (0) acute (1) mucronate (2) obtuse (3)
LSB#	Leaf base	attenuate (0) obtuse (1) cordate(2)
LLE#	Leaf length	(mm)
LWI#	Leaf width	(mm)
LSW#	Leaf surface wax	shining (0) dull (1) glaucous (2) very glaucous (3)
LEA#	Leaf sides	concolorous (0) discolorous (1)
LSM#	Leaf surface	smooth (0) not smooth (1)
LPS#	Leaf attachment	sessile (0) petiolate (1)
LPL#	Leaf petiole length	(mm)
LPO#	Leaf position	opposite (0) alternate (1)
LCO#	Leaf colour	light green (0) green (1) grey green (2) blue green (3) dark green (4)
LMI#	Leaf margin	smooth (0) partially jagged (1) jagged (2)
STE#	Stem arrangement	single (0) branching (1)
SSH#	Stem shape	round (0) angular (1)
SSM#	Stem surface	smooth (0) not smooth (1)

[a]linear (L:4W) lanceolate (2W<L<4W) elliptic (more extended than lanceolate) ovate (L<2W) orbicular (L:W)

7.3.5. Data analysis

The PATN multivariate analysis package (Belbin, 1994) was used to analyse the morphological character data for the seedlings, using hierarchical (Gower UPGMA) and non-hierarchical (multidimensional scaling, minimum spanning tree and principal canonical correlation) analysis to determine the relationships between the seedlings and their parents.

7.4. Results

7.4.1. Controlled pollination seed data

7.4.1.1. Between species comparison

All controlled pollinations between the three species produced seed (Table 7.3). The lowest number of seeds per capsule was produced from the intraspecific cross, and for *E. macrocarpa* and *E. youngiana* the highest number of seeds was produced from the open pollinated cross. No obvious trends were revealed when comparisons of mean number of seeds per flower pollinated to mean weight of seeds (mg) (Figure 7.2) and percent germination of seed to mean seed weight (Figure 7.3) were represented graphically.

There were no significant differences between the male species in the mean number of seeds produced per flower pollinated, or percent seed germinated. There were differences between female parent species in mean seed weight (Table 7.3) with *E. macrocarpa* having the heaviest seed (3.10 mg), *E. pyriformis* the lightest (2.21 mg) and *E. youngiana* seed intermediate (2.52 mg).

Table 7.3. Summary of flower, capsule, seed and seedling data for interspecific, intraspecific and open pollination of *E. macrocarpa*, *E. pyriformis* and *E. youngiana*.

Female species	Cross	Number of flowers pollinated	Number of capsules with seed	Total seed	[1]Mean number of seeds per flower pollinated	[2]Mean seed weight (mg)	Number of seeds planted	Number of seeds germinated	[3]Percent seeds germinated	Number of seedlings measured
E. macrocarpa	*E. pyriformis*	10	4	187	18.7	3.26	186	11	5.9	5
E. macrocarpa	*E. youngiana*	14	5	230	16.4	2.90	200	36	18.0	20
E. macrocarpa	*E. macrocarpa* (intra)	14	3	69	4.9	3.41	64	20	31.3	16
E. macrocarpa	open	10	8	409	40.9	2.86	200	53	26.5	
E. pyriformis	*E. macrocarpa*	110	55	1451	13.2	2.28	200	62	31.0	36
E. pyriformis	*E. youngiana*	100	56	2029	20.3	2.18	200	66	33.0	28
E. pyriformis	*E. pyriformis* (intra)	88	73	1450	13.1	2.29	200	63	31.5	28
E. pyriformis	open	54	30	903	16.7	2.16	200	85	42.5	
E. youngiana	*E. macrocarpa*	135	81	1330	9.9	2.51	200	74	37.0	39
E. youngiana	*E. pyriformis*	128	68	1241	9.7	2.57	200	81	40.5	38
E. youngiana	*E. youngiana* (intra)	120	67	1076	9.0	2.40	200	16	8.0	6
E. youngiana	open	80	76	3124	39.1	2.51	200	63	31.5	
E. macrocarpa					20.5	3.10			20.4	
E. pyriformis					16.5	2.21			34.5	
E. youngiana					15.9	2.52			29.3	
L.S.D.					23.30	0.292			17.71	

[1] total number of seed from all capsules of that cross divided by the total number of flowers pollinate for that cross.
[2] weight of all seeds in a capsule divided by the number of seeds in that capsule.
[3] number of seeds germinated divided by the number of seeds planted, multiplied by 100.

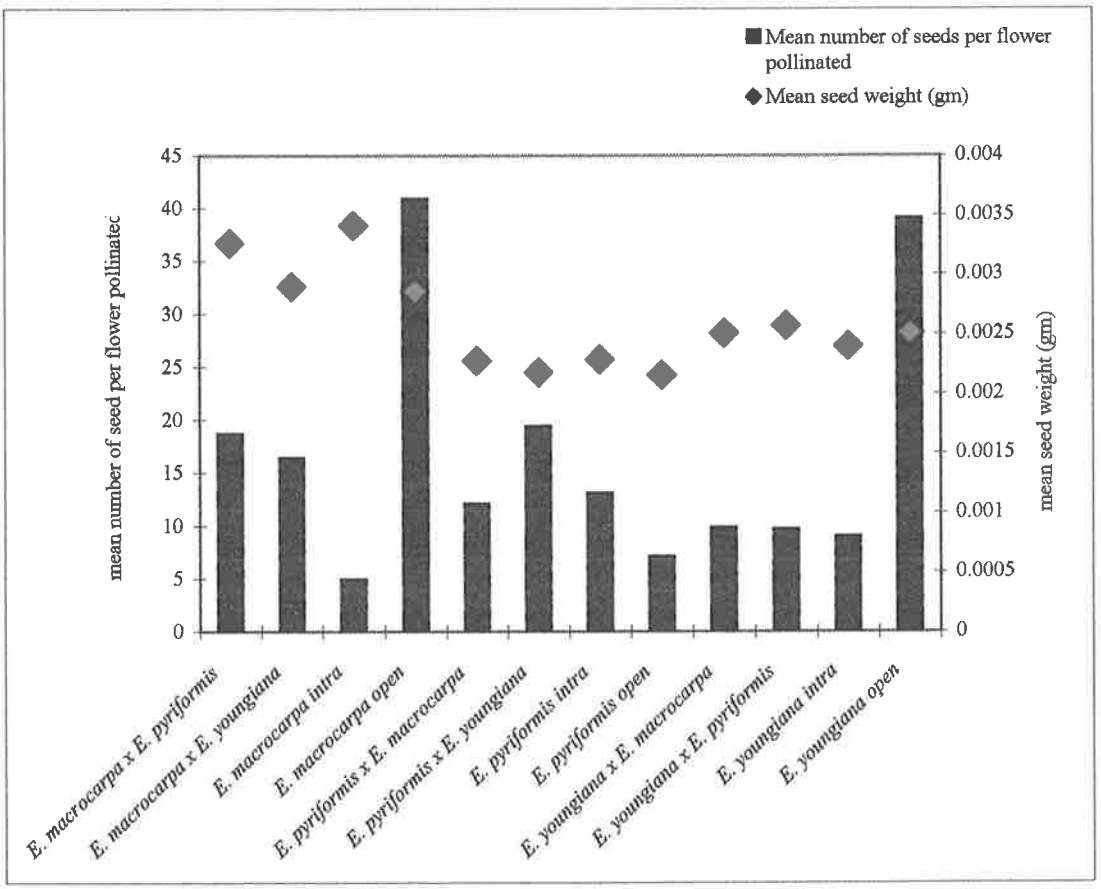


Figure 7.2. Mean number of seeds per flower pollinated and mean seed weight (mg)

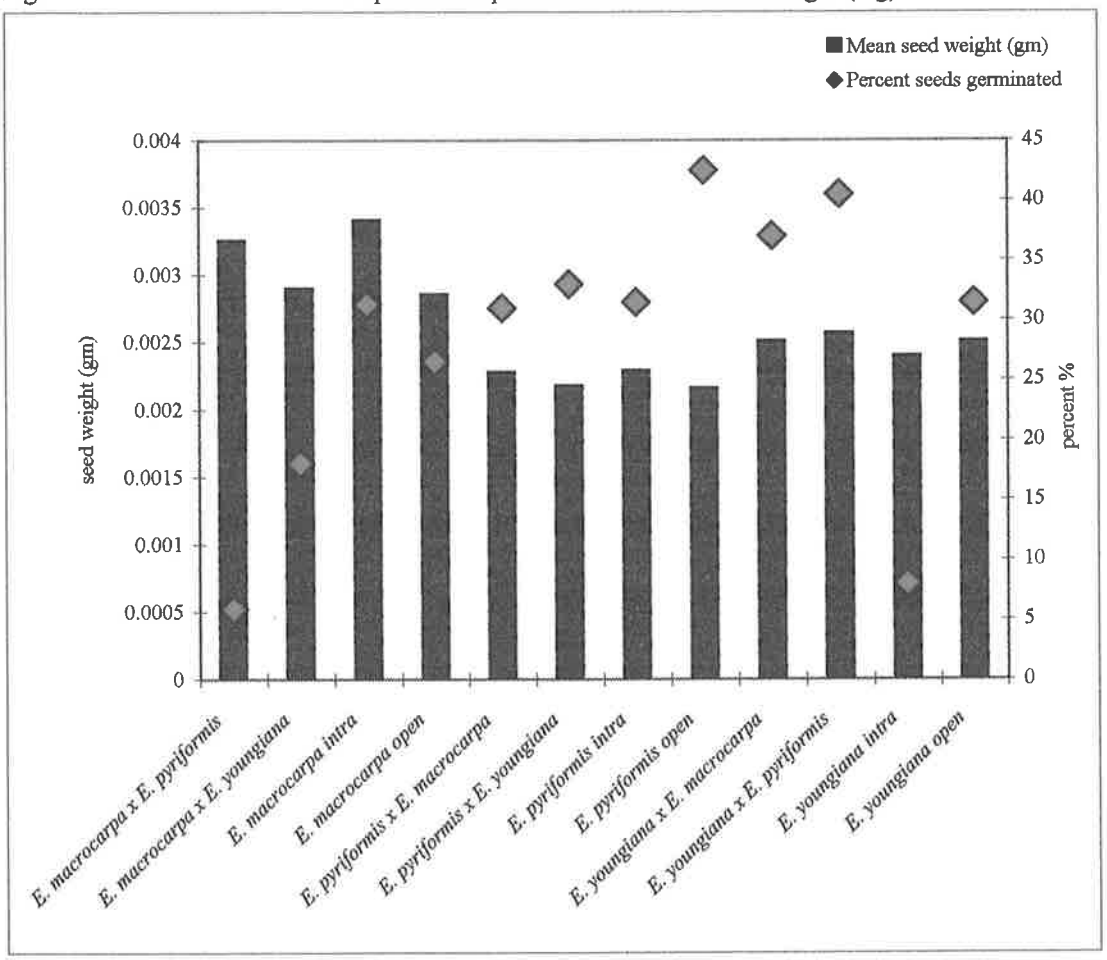


Figure 7.3. Comparison of mean seed weight (mg) and percent germination of seed

7.4.1.2. Within species comparison

E. macrocarpa as female parent

While four *E. macrocarpa* trees were available as pollen donors, only one was available for use as a female due to limited flowering. A total of 38 flowers on this tree were hand pollinated, using pollen from *E. pyriformis*, *E. youngiana*, or *E. macrocarpa*. Open pollinated seed was collected from capsules from the previous flowering season. The data are summarised in Table 7.4, with no statistical analysis possible as only one tree was available as a female parent.

Data showed that open pollinated flowers produced the greatest number of seeds per flower pollinated, the two interspecific crosses produced similar amounts, while the intraspecific cross produced less. Weight of seeds from each cross was not greatly different. For hand pollinated flowers, fewer than 50% of flowers pollinated produced seed, regardless of the cross. Of the seeds planted, germination percentages for each cross were very low, particularly in the *E. macrocarpa* x *E. pyriformis* cross, where only 11 seeds out of 186 germinated and only five survived to three months. Highest germination rates were recorded following open pollination.

E. pyriformis as female parent

There were no significant differences between male species on the mean number of seeds produced per flower hand pollinated, with open pollination the least successful cross (Table 7.5). Mean seed weight and percent seeds germinated were not affected by male species (Table 7.5), but were affected by female trees, as were the number of seeds per flower pollinated. *E. pyriformis* 8.27.2. (Ep5) produced the most seed, the heaviest seed and the second highest germination rate of the five female trees tested, while *E. pyriformis* 8.9.2. (Ep3) produced the least seed, the lightest seed and the second lowest germination rate.

Table 7.4. Effect of male parent on controlled pollination of *E. macrocarpa.*

Cross	Female tree	Number of flowers pollinated	Number of capsules with seed	Total seed	[1]Mean number of seeds per flower pollinated [2](range)	[3]Mean seed weight (mg) (range)	Number of seeds planted	Number of seeds germinated	Percent seed germinated[4]	Number of seedlings 3 months after germination
E. pyriformis	Em1	10	4	187	18.7 (5-88)	3.26 (2.76-4.23)	186	11	5.9	5
E. youngiana	Em1	14	5	230	16.4 (22-84)	2.90 (2.10-3.96)	200	36	18.0	20
E. macrocarpa - controlled intraspecific	Em1	14	3	69	4.9 (8-47)	3.41 (2.63-3.08)	64	20	31.3	16
Open	Em1	10	8	409	40.9 (20-77)	2.86 (3.16-3.63)	200	53	26.5	46

[1] total number of seed from all capsules of that cross divided by the total number of flowers pollinate for that cross.
[2] Range of number of seeds in capsules for that cross – minimum to maximum
[3] weight of all seeds in a capsule divided by the number of seeds in that capsule.
[4] number of seeds germinated divided by the number of seeds planted, multiplied by 100/1.

Table 7.5. Effect of male parent on controlled pollination of *E. pyriformis*. Superscripts denote significant differences in a column at $P<0.05$, numerical superscripts as for Table 7.3.

Cross	Female tree	Number of flowers pollinated	Number of capsules with seed	Total seed	[1]Mean number of seeds/flower pollinated [2](range)	[3]Mean seed weight (mg) (range)	Number of seeds planted	Number of seeds germinated	Percent seeds germinated[3]	Number of seedlings 3 months after germination
E. macrocarpa	Ep1	4	2	29	7.3 (5-24)	2.15 (2.12-2.17)	16	6	37.5	4
	Ep2	12	8	127	10.6 (2-31)	2.04 (1.72-2.32)	32	7	21.9	3
	Ep3	38	17	198	5.2 (1-53)	1.86 (1.58-2.54)	48	6	12.5	3
	Ep4	16	6	262	16.4 (7-88)	2.51 (1.94-3.37)	24	10	41.7	9
	Ep5	40	22	835	20.9 (1-88)	2.83 (2.01-3.61)	80	33	41.3	17
	Total	110	55	1451	12.1[a] (1-88)	2.28[a] (1.58-3.61)	200	62	31.0[a]	36
E. youngiana	Ep1	4	2	63	15.8 (16-47)	2.10 (1.91-2.29)	8	3	37.5	2
	Ep2	11	11	231	21.0 (10-46)	2.23 (1.82-3.12)	40	26	65.0	14
	Ep3	31	10	193	6.2 (1-53)	2.08 (1.48-2.71)	32	5	15.6	1
	Ep4	14	6	334	23.9 (11-53)	1.59 (1.29-2.62)	24	1	4.2	1
	Ep5	40	27	1208	30.2 (1-117)	2.84 (1.26-4.07)	96	31	32.3	10
	Total	100	56	2029	19.4[a] (1-117)	2.18[a] (1.26-4.07)	200	66	30.9[a]	28
E. pyriformis	Ep1	7	5	123	17.6 (10-36)	2.19 (1.89-2.53)	28	8	28.6	3
- controlled	Ep2	5	3	16	3.2 (8-8)	2.33 (2.31-2.35)	8	4	50.0	1
intraspecific	Ep3	19	11	4	0.21 (4-4)	1.18 (1.10-1.28)	4	0	0.0	0
	Ep4	17	16	354	20.8 (1-52)	2.71 (2.18-3.58)	68	5	7.4	5
	Ep5	40	38	956	23.9 (1-39)	3.03 (2.13-4.57)	92	46	50.0	19
	Total	88	73	1453	13.1[a] (1-52)	2.29[a] (1.10-4.57)	200	63	27.2[a]	28
Open	Ep1	9	6	135	15.0 (4-50)	212 (1.70-2.54)	20	9	45.0	1
	Ep2	10	6	42	4.2 (1-32)	227 (2.06-2.40)	12	8	66.7	4
	Ep3	11	1	1	0.1 (1-1)	1.18 (1.18-1.18)	8	5	62.5	4
	Ep4	7	2	43	6.1 (20-23)	2.36 (2.17-2.54)	16	1	6.3	0
	Ep5	17	15	682	10.1 (1-110)	2.88 (2.11-3.59)	144	62	43.1	22
	Total	54	30	903	7.1[b] (1-110)	2.16[a] (1.18-3.59)	200	85	44.7[a]	31
L.S.D.					11.1	0.47			24.9	

Table 7.6. Effect of male parent on pollination of *E. youngiana*. Superscripts denote significant differences in a column at $P<0.05$, numerical superscripts as for Table 7.3.

Cross	Female tree	Number of flowers pollinated	Number of capsules with seed	Total seed	[1]Mean number of seeds per flower pollinated [2](range)	Mean seed weight (mg)[3] (range)	Number of seeds planted	Number of seeds germinated	Percent seeds germinated[3]	Number of seedlings 3 months after germination
E. macrocarpa	Ey2	36	11	494	13.7 (5-131)	2.63 (2.16-2.97)	40	4	10.0	0
	Ey4	30	19	105	3.5 (1-16)	3.04 (1.99-3.65)	28	21	75.0	7
	Ey5	32	24	203	6.3 (1-33)	2.90 (1.04-4.12)	48	34	70.8	21
	Ey7	37	27	528	14.3 (1-105)	1.47 (0.58-1.98)	84	15	17.9	10
	Total	135	81	1330	9.9[b] (1-131)	2.51[a] (0.58-4.12)	200	74	43.4[a]	38
E. pyriformis	Ey2	36	11	652	18.1 (11-133)	2.65 (2.26-3.23)	64	5	7.8	0
	Ey4	30	28	173	5.8 (1-14)	3.13 (1.98-3.96)	68	38	55.9	15
	Ey5	32	17	364	11.4 (1-94)	3.00 (2.29-4.69)	36	34	94.4	21
	Ey7	30	12	52	1.7 (1-43)	1.49 (1.05-1.97)	32	4	12.5	3
	Total	128	68	1241	9.7[b] (1-133)	2.57[a] (1.05-4.69)	200	81	42.7[a]	39
E. youngiana	Ey2	37	28	635	17.2 (4-61)	2.47 (1.81-3.26)	112	2	1.8	0
- controlled	Ey4	35	24	208	5.9 (1-58)	2.20 (0.98-3.72)	40	2	5.0	0
intraspecific	Ey5	15	9	223	14.9 (3-51)	3.35 (0.37-4.60)	48	12	25.0	6
	Ey7	33	6	10	0.3 (1-3)	1.57 (1.00-2.42)	0	0	0.0	0
	Total	120	67	1076	9.0[b] (1-61)	2.40[a] (0.37-4.60)	200	16	8.0[a]	6
Open	Ey2	20	19	635	12.2	3.59	48	20	41.6	13
	Ey4	20	20	208	35.0	2.54	48	7	14.6	2
	Ey5	20	18	223	84.4	2.26	48	17	35.4	3
	Ey7	20	19	10	24.8	1.65	52	19	36.5	10
	Total	80	76	1076	39.1[a]	2.51[a]	200	63	32.0[a]	28
L.S.D.					27.0	0.74			36.8	

E. youngiana as the female parent

There was no significant effect of male species on the mean number of seeds produced per flower hand pollinated, with open pollinated flowers producing most seed (Table 7.6). Mean seed weight and percent seeds germinated were affected by female tree, with *E. youngiana* 8.54.7 (Ey7) producing the lightest seed with low germination (Table 7.7).

Table 7.7. Comparison of female parent on controlled pollination of *E. macrocarpa*, *E. pyriformis* and *E. youngiana*. Superscripts denote significant differences in a column at *$P<0.05$.

Female species	Female tree	[1]Mean number of seeds per flower pollinated	[2]Mean seed weight per flower pollinated (mg)	Percent seeds germinated[3]
E. macrocarpa	Em1	20.5	3.10	20.4
E. pyriformis	Ep1	13.9^{ab}	2.14^{c}	37.1^{ab}
	Ep2	9.8^{bc}	2.22^{bc}	50.9^{a}
	Ep3	2.9^{c}	1.58^{d}	22.6^{b}
	Ep4	16.8^{ab}	2.29^{b}	14.9^{b}
	Ep5	21.3^{a}	2.90^{a}	41.7^{ab}
	L.S.D.	7.7	0.52	27.8
E. youngiana	Ey2	15.3^{a}	2.83^{a}	15.3^{b}
	Ey4	12.5^{a}	2.73^{a}	37.6^{a}
	Ey5	29.2^{a}	2.88^{a}	56.4^{a}
	Ey7	10.3^{a}	1.54^{b}	16.7^{b}
	L.S.D	26.9	0.74	36.8
All	*E. macrocarpa*	20.5	3.10	20.4
	E. pyriformis	16.5	2.21	34.5
	E. youngiana	15.9	2.52	29.3
	L.S.D	23.3	0.29	17.7

numbered superscripts as for Table 7.3.

Anova table Appendix 1.2.

7.4.2. Seedling morphology analysis

7.4.2.1. Summary of all crosses

Seedling morphological characters measured for all crosses are described in Tables 7.8, 7.9 and 7.10: considerable differences in characters, such as leaf surface wax, petiole length, leaf length and width, were recorded, and indicated potential hybrid status of the seedlings. Characters that are distinctive for taxa in a cross are noted. Seed from a variety of different commercial seed sources were grown as the parent seedling comparators, and the variation displayed within the intraspecifc crosses of *E. macrocarpa* and *E. youngiana* reflects the naturally high levels of variability within these species. In the case of *E. pyriformis*, commercially sourced seed proved inviable, so comparisons were made with open pollinated seed from the female parent trees, resulting in less apparent variation within the intraspecific cross.

The results of the hierarchical and non-hierarchical analyses for each of the nine crosses are summarised in Table 7.11., with 96% of seedlings from interspecific crosses clustering separately from the female parent. Numbers of seedlings measured for each cross ranged from five for *E. macrocarpa* x *E. pyriformis* to 39 for *E. youngiana* x *E. pyriformis*, and reflect the germination rates of seeds planted for each cross.

The Gower UPGMA dendrogram for each interspecific cross produced three main clusters. These clusters represented the female species, the male species, and the F1 hybrid seedlings. In three crosses, some seedlings linked with either the male or female parent species. The low values of dissimilarity on each dendrogram show the relatively high similarities between each species and their seedlings. The dendrograms for the *E. macrocarpa* and *E. pyriformis* intraspecific crosses did not show any distinctive clusters, while the *E. youngiana* intraspecific cross showed two distinct clusters.

Table 7.8. Seedling morphological characters of seedlings from *E. macrocarpa* and putative parents. Measurements in mm or presented as range (mean). [a]Characters unique or highly descriptive for that taxon according to PCC (Figs 7.7, 7.10, 7.13), [b]Characters significantly correlated* (r^2) to variation amoungst individuals (Table 7.12).

Characters	*E. macrocarpa* open pollinated seedlings	*E. pyriformis* open pollinated seedlings	*E. youngiana* open pollinated seedlings	*E. macrocarpa* x *E. pyriformis*	*E. macrocarpa* x *E. youngiana*	*E. macrocarpa* x *E. macrocarpa*
LSH3	lanceolate	ovate, rarely elliptic [ab]	lanceolate, ovate, elliptic [ab]	lanceolate, elliptic	elliptic or ovate	lanceolate
LST3	acute or obtuse [b]	acute or obtuse	acute or obtuse	acute or obtuse	acute or obtuse	acute or obtuse
LSB3	attenuate	attenuate or obtuse [b]	attenuate or obtuse [ab]	attenuate	attenuate or obtuse	attenuate
LLE3	26.1-55.7 (36.8)[a]	19.0-52.3 (38.8)[a]	20.2-42.2 (32.5)	24.8-41.6(34.7)	24.3-44.9 (34.4)	26.5-44.6 (35.7)
LWI3	9.2-22.8 (14.2)	11.3-41.7 (22.9)[a b]	8.1-23.4 (14.6)ab	7.7-13.4 (11.4)	7.6-22.6 (16.2)	6.6-18.4 (14.0)
LSW3	glaucous or very glaucous [ab]	dull, rarely glaucous [b]	dull, rarely shining [b]	dull or glaucous	dull or glaucous	glaucous or very glaucous
LEA3	concolorous or discolorous	discolorous, rarely concolorous [a]	discolorous	discolorous	discolorous	discolorous
LSM3	smooth	smooth	smooth	smooth	smooth	smooth
LPS3	petiolate	petiolate	petiolate	petiolate	petiolate	petiolate
LPL3	2.3-6.3 (4.1)[a]	2.8-10.9 (4.8)	2.5-8.1 (5.1)[a]	3.2-6.3 (4.5)	2.3-6.1 (3.6)	0.5-4.1 (3.0)
LPO3	opposite	opposite, rarely alternate	opposite	opposite	opposite	opposite
LCO3	grey green or dark green [a]	dark green, rarely green [ab]	green, blue green or dark green [b]	dark green	dark green	dark green, rarely grey green
LMI3	partially jagged or jagged [a]	smooth, partially jagged or jagged	smooth [b]	smooth or partially jagged	smooth or partially jagged	partially jagged or jagged
LSH5	elliptic or ovate	ovate, sometimes elliptic	ovate [ab]	elliptic or ovate	elliptic	ovate
LST5	acute, mucronate or obtuse [b]	obtuse, acute or mucronate	obtuse, acute or mucronate [b]	obtuse	acute or obtuse	acute, mucronate or obtuse
LSB5	attenuate	attenuate, rarely obtuse or cordate [ab]	attenuate or obtuse [ab]	attenuate or obtuse	attenuate or obtuse or cordate	attenuate
LLE5	31.7-71.2 (43.7)[ab]	22.8-74.0 (47.2)[a]	22.6-75.8 (43.8)[a]	33.4-46.9 (41.3)	34.3-62.5 (46.8)	26.6-57.7 (46)
LWI5	13.3-42.8 (23.2) [b]	19.8-62.7 (37.3)[a]	13.3-52.7 (27.2)[ab]	13.8-32.1 (22.7)	18.4-47.5 (31.9)	11.4-40.5 (27.5)
LSW5	very glaucous [a]	dull, sometimes glaucous	dull, rarely shining [b]	dull or glaucous	dull or glaucous	dull, glaucous or very glaucous
LEA5	concolorous or discolorous [b]	discolorous or concolorous [ab]	discolorous, rarely concolorous [b]	discolorous	concolorous or discolorous	concolorous or discolorous
LSM5	smooth	smooth, rarely not smooth [a]	smooth [b]	smooth, rarely not smooth	smooth, rarely not smooth	smooth
LPS5	petiolate	petiolate	petiolate	petiolate	petiolate	petiolate

Continued

Table 7.8. Continued

Characters	*E. macrocarpa* open pollinated seedlings	*E. pyriformis* open pollinated seedlings	*E. youngiana* open pollinated seedlings	*E. macrocarpa* x *E. pyriformis*	*E. macrocarpa* x *E. youngiana*	*E. macrocarpa* x *E. macrocarpa*
LPL5	1.3-6.6 (4.2) [b]	2.7-12.0 (7.0)[ab]	2.1-14.5 (6.5)[ab]	2.9-5.3 (4.5)	2.1-7.3 (4.6)	1.0-4.5 (3.9)
LPO5	opposite	alternate or opposite [ab]	alternate or opposite [a]	opposite	alternate or opposite	opposite
LCO5	grey green or dark green	green, sometimes dark green [a]	light green or dark green	green, sometimes light or dark green	green, grey green or dark green	green, grey green, or dark green
LMI5	partially jagged or jagged [a]	partially jagged, smooth or jagged [b]	smooth [b]	partially jagged	partially jagged	partially jagged, smooth or jagged
LSH7	ovate	ovate	elliptic or ovate	ovate	ovate	ovate
LST7	mucronate or obtuse [a]	acute or mucronate [b]	acute, mucronate or obtuse [b]	acute or mucronate	acuminate, acute or mucronate	mucronate or obtuse
LSB7	attenuate or obtuse [ab]	obtuse [ab]	obtuse, rarely attenuate [a]	attenuate or obtuse	attenuate, obtuse or cordate	attenuate or obtuse
LLE7	28.9-85.3 (52.2)[b]	16.5-87.5 (57.1)[a]	33.0-80.0 (56.2)[a]	45.7-61.3 (53.3)	33.4-82.5 (62.8)	37.6-76.3 (63.0)
LWI7	24.8-45.3 (34.7)[b]	18.5-65.9 (43.0)[ab]	15.6-44.8 (30.3)[a]	26.6-43.3 (35.4)	23.8-54.6 (39.9)	22.3-49.0 (40.3)
LSW7	very glaucous [a]	dull or glaucous [b]	dull or shiny [b]	dull	glaucous	very glaucous
LEA7	concolorous	concolorous or discolorous	concolorous or discolorous	concolorous or discolorous	concolorous or discolorous	concolorous
LSM7	smooth	smooth or not smooth [ab]	smooth [b]	not smooth	smooth or not smooth	smooth
LPS7	petiolate	petiolate	petiolate	petiolate	petiolate	petiolate
LPL7	0.1-6.1 (3.4)[b]	3.1-13. 1 (8.4)[ab]	3.9-17.5 (9.6)[ab]	1.0-7.3 (3.4)	0.2-8.3 (5.0)	1.0-4.3 (2.5)
LPO7	opposite	alternate, sometimes opposite [ab]	alternate [ab]	opposite	opposite or alternate	opposite
LCO7	grey green [b]	green, rarely light green [b]	green, blue green or dark green	grey green	green or grey green	green or grey green
LMI7	partially jagged or jagged [a]	partially jagged or smooth [b]	smooth [b]	partially jagged or jagged	partially jagged	smooth or partially jagged
SSH7	angular	round [b]	round [b]	angular	angular	angular
SSM7	not smooth [a]	smooth or not smooth [b]	smooth [b]	smooth or not smooth	smooth or not smooth	not smooth

Correlation coefficient* : showing the fit (r^2) of the individual character vectors in the ordination space.

Table 7.9. Seedling morphological characters of seedlings from *E. pyriformis* and putative parents. Measurements in mm or presented as range (mean). [a]Characters unique or highly descriptive for that taxon according to PCC (Figs 8.16, 8.19, 8.22), [b]Characters significantly correlated* (r^2) to individuals.

Characters	*E. macrocarpa* open pollinated seedlings	*E. pyriformis* open pollinated seedlings	*E. youngiana* open pollinated seedlings	*E. pyriformis* x *E. macrocarpa*	*E. pyriformis* x *E. youngiana*	*E. pyriformis* x *E. pyriformis*
LSH3	lanceolate [b]	ovate, rarely elliptic [ab]	lanceolate, ovate, elliptic	elliptic or ovate	lanceolate, elliptic or ovate	lanceolate, elliptic or ovate
LST3	acute or obtuse [b]	acute or obtuse [b]	acute or obtuse [b]	acute or obtuse	acute or obtuse	acute or obtuse
LSB3	attenuate [b]	attenuate or obtuse [b]	attenuate or obtuse [b]	attenuate or obtuse	attenuate or obtuse	attenuate or obtuse
LLE3	26.1-55.7 (36.8)	19.0-52.3 (38.8)[ab]	20.2-42.2 (32.5)[b]	14.9-44.0 (32.4)	21.0-60.5 (36.7)	15.7-43.7 (34.9)
LWI3	9.2-22.8 (14.2)	11.3-41.7 (22.9)[ab]	8.1-23.4 (14.6)[b]	6.9-28.0 (17.6)	8.1-39.4 (18.5)	7.5-33.6 (21.1)
LSW3	glaucous or very glaucous [ab]	dull, rarely glaucous	dull, rarely shining	dull, sometimes glaucous	dull	dull, rarely glaucous
LEA3	concolorous or discolorous	discolorous, rarely concolorous [ab]	discolorous	discolorous, rarely concolorous	discolorous,	discolorous, rarely concolorous
LSM3	smooth	smooth	smooth	smooth, rarely not smooth	smooth	smooth
LPS3	petiolate	petiolate	petiolate	petiolate	petiolate	petiolate
LPL3	2.3-6.3 (4.1)	2.8-10.9 (4.8)[b]	2.5-8.1 (5.1)[b]	1.8-7.1 (3.5)	2.1-8.1 (4.7)	1.6-7.9 (4.8)
LPO3	opposite	opposite or alternate [a]	opposite	opposite or alternate	opposite or alternate	opposite or alternate
LCO3	grey green or dark green [b]	dark green, rarely green [ab]	green, blue green or dark green	green, grey green or dark green	dark green, rarely green	dark green, rarely green
LMI3	partially jagged or jagged [a]	smooth, partially jagged or jagged [a]	smooth [b]	smooth, partially jagged or jagged	smooth or partially jagged	smooth or partially jagged
LSH5	elliptic or ovate [b]	ovate, sometimes elliptic	ovate [a]	ovate, rarely orbicular	ovate, rarely lanceolate	ovate, rarely orbicular
LST5	acute, mucronate or obtuse [b]	obtuse, acute or mucronate [b]	obtuse, acute or mucronate [a]	acute, rarely obtuse	acute, rarely mucronate or obtuse	acute, mucronate or obtuse
LSB5	attenuate [b]	attenuate, rarely obtuse or cordate [a]	attenuate or obtuse	obtuse, rarely attenuate	obtuse, rarely attenuate or cordate	obtuse, rarely attenuate
LLE5	31.7-71.2 (43.7)	22.8-74.0 (47.2)[ab]	22.6-75.8 (43.8)[b]	31.7-70.1 (45.8)	28.4-82.1 (51.4)	28.0-71.2 (49.2)
LWI5	13.3-42.8 (23.2)[b]	19.8-62.7 (37.3)[ab]	13.3-52.7 (27.2)[b]	23.4-62.9 (34.3)	16.8-56.8 (35.0)	22.6-64.7 (38.9)
LSW5	very glaucous [ab]	dull sometimes glaucous [b]	dull rarely shining [a]	dull or glaucous	shining	dull, rarely glaucous
LEA5	concolorous or discolorous [b]	discolorous or concolorous [ab]	discolorous, rarely concolorous [b]	discolorous or concolorous	discolorous	discolorous, sometimes concolorous
LSM5	smooth [ab]	smooth, rarely not smooth [a]	smooth	smooth, rarely not smooth	smooth, rarely not smooth	smooth
LPS5	petiolate	petiolate	petiolate	petiolate	petiolate	petiolate
LPL5	1.3-6.6 (4.2)[b]	2.7-12.0 (7.0)[ab]	2.1-14.5 (6.5)[b]	2.8-11.1 (4.8)	4.3-13.6 (8.1)	2.1-13.8 (6.8)

Continued

Table 7.9. Continued

Characters	*E. macrocarpa* open pollinated seedlings	*E. pyriformis* open pollinated seedlings	*E. youngiana* open pollinated seedlings	*E. pyriformis* x *E. macrocarpa*	*E. pyriformis* x *E. youngiana*	*E. pyriformis* x *E. pyriformis*
LPO5	opposite [b]	alternate or opposite [a]	alternate or opposite [b]	opposite, rarely alternate	alternate or opposite	alternate or opposite
LCO5	grey green or dark green	green, sometimes light green or dark green [b]	light green or dark green [b]	green, grey green or dark green	dark green, rarely green	green or dark green
LMI5	partially jagged or jagged [a]	partially jagged, rarely smooth or jagged [a]	smooth [b]	partially jagged, rarely smooth or jagged	partially jagged, sometimes smooth	partially jagged, rarely smooth or jagged
LSH7	ovate [a]	ovate	elliptic or ovate	ovate, rarely elliptic	ovate, rarely elliptic	ovate
LST7	mucronate or obtuse	acute or mucronate	acute, mucronate or obtuse [b]	acuminate, acute or obtuse	acute, sometimes obtuse	acute, or obtuse
LSB7	attenuate or obtuse	obtuse [a]	obtuse, rarely attenuate	obtuse, rarely attenuate or cordate	obtuse, rarely attenuate or cordate	obtuse, rarely attenuate
LLE7	28.9-85.3 (52.2)	16.5-87.5 (57.1)[ab]	33.0-80.0 (56.2)[b]	27.1-98.5 (56.3)	35.8-89.6 (56.7)	42.5-78.5 (56.7)
LWI7	24.8-45.3 (34.7)	18.5-65.9 (43.0)[a]	15.6-44.8 (30.3)[b]	17.4-52.4 (37.9)	26.8-62.4 (37.6)	20.7-58.0 (41.8)
LSW7	very glaucous [ab]	dull or glaucous [ab]	dull or shiny [b]	dull, glaucous or very glaucous	dull, sometimes glaucous	dull or glaucous
LEA7	concolorous [b]	concolorous or discolorous [a]	concolorous or discolorous [b]	concolorous or discolorous	concolorous or discolorous	concolorous or discolorous
LSM7	smooth [b]	smooth or not smooth [a]	smooth	smooth or not smooth	smooth	smooth
LPS7	petiolate	petiolate	petiolate	petiolate	petiolate	petiolate
LPL7	0.0-6.1 (3.4)[b]	3.1-13. 1 (8.4)	3.9-17.5 (9.6)[b]	0.5-12.0 (4.5)	5.7-16.2 (10.2)	4.0-13.6 (8.8)
LPO7	opposite [b]	alternate, sometimes opposite [ab]	alternate [a]	alternate or opposite	alternate, rarely opposite	alternate, sometimes opposite
LCO7	grey green [ab]	green, rarely light green	green, blue green or dark green [ab]	green or grey green	green, rarely dark green	green, rarely dark green
LMI7	partially jagged or jagged [b]	partially jagged or smooth [a]	smooth	partially jagged or jagged	partially jagged, rarely smooth or jagged	partially jagged, rarely smooth or jagged
SSH7	angular [ab]	round	round	angular	round	round, rarely angular
SSM7	not smooth [ab]	smooth or not smooth	smooth	not smooth, rarely smooth	smooth, rarely not smooth	smooth, rarely not smooth

Correlation coefficient* : showing the fit (r^2) of the individual character vectors in the ordination space.

Table 7.10. Seedling morphological characters of seedlings from *E. youngiana* and putative parents. Measurements in mm or presented as range (mean). [a]Characters unique or highly descriptive for that taxon according to PCC (Figs 8.25, 8.28, 8.31). [b]Characters significantly correlated* (r^2) to individuals.

Characters	*E. macrocarpa* open pollinated seedlings	*E. pyriformis* open pollinated seedlings	*E. youngiana* open pollinated seedlings	*E. youngiana* x *E. macrocarpa*	*E. youngiana* x *E. pyriformis*	*E. youngiana* x *E. youngiana*
LSH3	lanceolate [b]	ovate, rarely elliptic [b]	lanceolate, ovate, elliptic [ab]	lanceolate, elliptic or ovate	ovate, rarely elliptic or lanceolate	ovate, rarely lanceolate
LST3	acute or obtuse [b]	acute or obtuse [b]	acute or obtuse [b]	acute or obtuse	acute, rarely mucronate or obtuse	acute, rarely obtuse
LSB3	attenuate [b]	attenuate or obtuse [b]	attenuate or obtuse [a]	attenuate, rarely obtuse	attenuate or obtuse	attenuate, rarely obtuse
LLE3	26.1-55.7 (36.8)[b]	19.0-52.3 (38.8)[ab]	20.2-42.2 (32.5)	19.5-40.8 (29.3)	16.9-43.9 (31.2)	29.5-40.6 (25.9)
LWI3	9.2-22.8 (14.2)[ab]	11.3-41.7 (22.9)[ab]	8.1-23.4 (14.6)[b]	7.9-21.1 (12.4)	5.2-32.4(19.0)	17.1-25.6 (20.3)
LSW3	glaucous or very glaucous [ab]	dull	dull, rarely shining	dull	dull	dull
LEA3	concolorous or discolorous	discolorous, rarely concolorous [a]	discolorous [a]	discolorous	discolorous	discolorous
LSM3	smooth	smooth	smooth	smooth	smooth	smooth
LPS3	petiolate	petiolate	petiolate	petiolate	petiolate	petiolate
LPL3	2.3-6.3 (4.1)	2.8-10.9 (4.8)[a]	2.5-8.1 (5.1)[ab]	2.7-6.1 (4.2)	2.9-.6 (5.2)	4.9-8.1 (6.4)
LPO3	opposite	opposite, rarely alternate [b]	opposite	opposite or alternate	opposite, rarely alternate	opposite, rarely alternate
LCO3	grey green or dark green	dark green, rarely green	green, blue green or dark green	dark green, rarely green	dark green, rarely green	dark green, rarely green
LMI3	partially jagged or jagged [ab]	smooth, partially jagged or jagged [b]	smooth	smooth or not smooth	smooth or not smooth	smooth
LSH5	elliptic or ovate [b]	ovate, sometimes elliptic	ovate [a]	ovate	ovate, rarely lanceolate	ovate
LST5	acute, mucronate or obtuse	obtuse, acute or mucronate [b]	obtuse, acute or mucronate	acute, rarely mucronate or obtuse	acute, rarely mucronate or obtuse	mucronate, rarely acute
LSB5	attenuate [b]	attenuate, rarely obtuse or cordate	attenuate or obtuse [a]	attenuate or obtuse	attenuate, rarely obtuse	obtuse
LLE5	31.7-71.2 (43.7)[b]	22.8-74.0 (47.2)[ab]	22.6-75.8 (43.8)[b]	27.9-48.9 (36.7)	24.4-63.6 (41.9)	41.8-61.3 (52.1)
LWI5	13.3-42.8 (23.2)[ab]	19.8-62.7 (37.3)[ab]	13.3-52.7 (27.2)[b]	14.3-34.1 (24.1)	15.4-47.1 (30.7)	22.7-42.0 (33.1)
LSW5	very glaucous [ab]	dull, sometimes glaucous [b]	dull, rarely shining	dull, glaucous or very glaucous	dull or glaucous	dull, rarely shining
LEA5	concolorous or discolorous [b]	discolorous or concolorous	discolorous, rarely concolorous [a]	discolorous	discolorous	discolorous
LSM5	smooth	smooth, rarely not smooth	smooth	smooth	smooth	smooth
LPS5	petiolate	petiolate	petiolate	petiolate	petiolate	petiolate

Continued

Table 7.10. Continued

Characters	*E. macrocarpa* open pollinated seedlings	*E. pyriformis* open pollinated seedlings	*E. youngiana* open pollinated seedlings	*E. youngiana* x *E. macrocarpa*	*E. youngiana* x *E. pyriformis*	*E. youngiana* x *E. youngiana*
LPL5	1.3-6.6 (4.2)[b]	2.7-12.0 (7.0)[ab]	2.1-14.5 (6.5)[b]	2.1-7.4 (4.9)	3.0-13.9 (7.6)	6.8-10.9 (9.7)
LPO5	opposite [b]	alternate or opposite [b]	alternate or opposite [ab]	opposite, rarely alternate	alternate, rarely opposite	alternate, rarely opposite
LCO5	grey green or dark green [b]	green, sometimes dark or light green [a]	light or dark green	grey green or dark green, rarely light green	dark green, sometimes green or grey green	dark green
LMI5	partially jagged or jagged [ab]	partially jagged, sometimes smooth or jagged [b]	smooth	partially jagged, rarely smooth	partially jagged, rarely smooth	smooth
LSH7	ovate	ovate	elliptic or ovate	ovate, rarely elliptic	ovate	ovate
LST7	mucronate or obtuse [ab]	acute or mucronate [b]	acute, mucronate or obtuse	acute or mucronate	acute, sometimes mucronate or obtuse	mucronate, sometimes acute
LSB7	attenuate or obtuse [b]	obtuse [a]	obtuse, rarely attenuate [ab]	obtuse, rarely attenuate	obtuse	obtuse
LLE7	28.9-85.3 (52.2)[b]	16.5-87.5 (57.1)[a]	33.0-80.0 (56.2)[b]	34.1-75.1 (54.8)	32.1-89.5 (52.3)	45.8-65.4 (58.4)
LWI7	24.8-45.3 (34.7)[ab]	18.5-65.9 (43.0)[a]	15.6-44.8 (30.3)[b]	21.0-54.3 (35.7)	25.4-56.9 (38.3)	27.3-40.3(34.8)
LSW7	very glaucous [ab]	dull or glaucous	dull or shining	very glaucous, rarely dull or glaucous	dull or glaucous	shining or dull
LEA7	concolorous [b]	concolorous or discolorous [b]	concolorous or discolorous	discolorous, rarely concolorous	discolorous, sometimes concolorous	concolorous
LSM7	smooth [a]	smooth or not smooth	smooth	smooth, rarely not smooth	smooth	smooth
LPS7	petiolate	petiolate	petiolate	sessile or petiolate	petiolate	petiolate
LPL7	0.0-6.1 (3.4)[b]	3.1-13. 1 (8.4)[a]	3.9-17.5 (9.6)[b]	3.3-15.0 (6.2)	4.9-15.9 (10.0)	8.0-14.8 (11.5)
LPO7	opposite [b]	alternate, sometimes opposite [a]	alternate [a]	opposite or alternate	alternate	opposite
LCO7	grey green	green, rarely light green [ab]	green, blue green or dark green [b]	grey green, rarely dark green	green, grey green or dark green	blue green or dark green
LMI7	partially jagged or jagged [ab]	partially jagged or smooth [b]	smooth	partially jagged, rarely smooth or jagged	partially jagged, sometimes smooth	smooth
SSH7	angular [b]	round	round	angular	round	round
SSM7	not smooth [ab]	smooth or not smooth [a]	smooth	smooth, rarely not smooth	smooth	smooth

Correlation coefficient* : showing the fit (r^2) of the individual character vectors in the ordination space.

Table 7.11. Summary of information from UPGMA dendrograms, MDS ordination and MST distance values for all nine crosses. S = seedlings, M = male, F = female.

Female Male	*E. mac* *E. pyr*	*E. mac* *E. you*	*E. pyr* *E. mac*	*E. pyr* *E. you*	*E. you* *E. mac*	*E. you* *E. pyr*	*E. mac* *E. mac*	*E. pyr* *E. pyr*	*E. you* *E. you*
# S measured	5	20	36	28	38	39	16	28	6
# Dendrogram clusters	3	3	3	3	3	3	1	1	2
# Ss in F cluster	0	0	2	5	0	0	16	28	6
# S in M cluster	0	0	0	2	1	0	0	0	0
# S in separate cluster	5	20	34	21	37	39	0	0	0
# MDS bunch[a]	3	3	3	3	3	3	scattered	scattered	2
SSH stress	8.0%	15.9%	18.1%	15.4%	18.2%	17.3%	17.5%	7.2%	7.2%
Mean MST of F cluster [b]	0.1224	0.1041	0.1564	0.1884	0.1022	0.1014	0.1440	0.1665	0.1825
MST distance F to S[c]	0.2578	0.1888	0.1966	0.1493	0.1348	0.1755	-	-	-
S link to F[dc]	#3	#14	#10	many	#32	#7	many	many	many
Mean MST of M cluster[e]	0.1540	0.1044	0.0854	0.1052	0.1617	0.1110	-	-	-
MST M distance to S[f]	0.3358	0.2017	0.1677	0.1330	0.1749	0.2054	-	-	-
# S links to M cluster[g]	#2	#7	#18	#28	#19	#11	-	-	-
Mean MST of S cluster[h]	0.1837	0.1458	0.1607	0.1233	0.1317	0.1078	0.1848	0.1562	0.2986

[a] 'bunch' refers to a discrete cluster of similar individuals
[b] Mean of all MST values connecting female parent individuals
[c] MST link connecting female bunch to seedlings
[dc] Seedlings (code number from dendrogram) linking to female (code number from dendrogram)
[e] Mean of all MST values connecting male parent individuals
[f] MST link connecting male bunch to seedlings
[g] Seedlings (code number from dendrogram) linking to male (code number from dendrogram)
[h] Mean of all MST values connecting seedlings

The semi-strong hybrid MDS ordinations for each interspecific cross reflected the equivalent UPGMA dendrogram clusters, with seedlings placed intermediately between parents. In the cases of the intraspecific crosses, the points were scattered across the plot, with no apparent pattern.

In most cases, the minimum spanning tree connections between seedlings were variable, connecting individuals seemingly at random, with rarely more than one connection to either parent, while all female or male parent seedlings connect to each other. Mean MST values connecting female parent individuals to each other ranged from 0.1014 to 0.1884 (with an average of 0.1409), mean MST values connecting male individuals ranged from 0.0854 to 0.1617 (average of 0.1203) and mean MST values connecting seedlings ranged from 0.1337 to 0.2986 (average 0.1658). The mean MST values connecting seedlings to females and seedlings to males of 0.1869 and 0.2031, respectively, show that although there is some variation within parent or seedlings groups, the variation between groups of parents and seedlings is, on average, greater that the variation within these groups.

The PCC results for each cross are summarised in Table 7.12, with the correlation coefficient (r^2) for each cross. The invariant nature of some characters for particular crosses was also noted, where the character was the same for each individual measured and so was not descriptive. The intraspecific crosses, *E. macrocarpa* x *E. macrocarpa* and *E. youngiana* x *E. youngiana*, showed the highest number of invariant characters, 11 and 16 respectively, indicating there was little variation within the seedlings for these crosses. The interspecific cross, *E. pyriformis* x *E. macrocarpa*, showed no invariant characters. Characters that were found to be highly descriptive for most crosses were leaf width (LWI), leaf length (LLE), leaf tip shape (LST), leaf surface wax (LSW), leaf petiole length (LPL) and leaf margin shape (LMI).

Descriptions of each character for all crosses are given in tables 7.8, 7.9 and 7.10, where the significantly correlated characters for each cross are noted, as are the characters unique or highly descriptive for each cross from the PCC ordinations. The PCC ordinations allow for identification of the characters causing the clusters, and the clearer definition of clusters/taxa.

Table 7.12. Summary of variant (Y) and invariant (-) characters and significance (*) from correlation coefficient r^2. All measurements at nodes 3, 5 and 7 (e.g. LSH3 = LSH at node 3). Characters LPS(3, 5, 7) and STE (3, 5, 7) removed prior to analysis as invariant for all individuals.

	EmxEp	*EmxEy*	*EmxEm*	*EpxEm*	*EpxEy*	*EpxEp*	*EyxEp*	*EyxEm*	*EyxEy*
r^2	0.74	0.54	0.62	0.46	0.52	0.56	0.44	0.44	0.78
LSH3	Y*	Y*	-	Y*	Y	Y*	Y*	Y*	Y*
LST3	Y	Y	Y*	Y*	Y*	Y*	Y*	Y*	Y*
LSB3	Y*	Y*	-	Y*	Y*	Y*	Y*	Y*	Y
LLE3	Y	Y	Y	Y	Y*	Y*	Y*	Y*	Y
LWI3	Y*	Y*	Y	Y	Y*	Y*	Y*	Y*	Y*
LSW3	Y*	Y*	Y*	Y*	-	Y	-	Y*	-
LEA3	Y	Y	Y	Y	Y	Y*	Y	Y	-
LSM3	-	-	-	Y	-	-	-	-	-
LPL3	Y	Y	Y	Y	Y*	Y*	Y	Y	Y*
LPO3	Y	-	-	Y	Y	Y	Y*	Y	Y
LCO3	Y*	Y*	Y	Y*	Y	Y*	Y	Y	Y*
LMI3	Y	Y*	Y	Y	Y*	Y	Y*	Y*	-
LSH5	Y	Y*	Y	Y*	Y	Y	Y	Y*	-
LST5	Y	Y*	Y*	Y*	Y	Y*	Y*	Y	Y
LSB5	Y*	Y*	Y	Y*	Y	Y	Y	Y*	-
LLE5	Y	Y	Y*	Y	Y*	Y*	Y*	Y*	Y*
LWI5	Y*	Y*	Y*	Y*	Y*	Y*	Y*	Y*	Y*
LSW5	Y*	Y*	Y	Y*	Y	Y*	Y*	Y*	Y
LEA5	Y*	Y*	Y*	Y*	Y*	Y*	Y	Y*	-
LSM5	Y	Y*	-	Y*	-	Y	-	-	-
LPL5	Y*	Y*	Y*	Y*	Y*	Y*	Y	Y*	Y*
LPO5	Y*	Y	-	Y*	Y*	Y	Y*	Y*	Y*
LCO5	Y	Y	Y	Y	Y*	Y*	Y	Y*	Y
LMI5	Y*	Y*	Y	Y	Y*	Y	Y*	Y*	-
LSH7	-	Y	-	Y	Y	-	Y	Y	Y
LST7	Y*	Y*	Y	Y	Y*	Y	Y*	Y*	Y
LSB7	Y*	Y	Y*	Y	Y	Y	Y	Y*	-
LLE7	Y	Y	Y*	Y	Y*	Y*	Y	Y*	Y*
LWI7	Y*	Y	Y*	Y	Y*	Y	Y	Y*	Y*
LSW7	Y*	Y*	Y	Y*	Y*	Y*	Y	Y*	Y*
LEA7	Y	Y	-	Y*	Y*	Y	Y*	Y*	-
LSM7	Y*	Y*	Y	Y*	-	-	-	Y	-
LPL7	Y*	Y*	Y*	Y*	Y*	Y	Y	Y*	Y*
LPO7	Y*	Y*	-	Y*	Y	Y*	Y	Y*	-
LCO7	Y*	Y	Y*	Y*	Y*	Y	Y*	Y	Y*
LMI7	Y*	Y*	Y	Y*	Y	Y	Y*	Y*	-
SSH7	Y*	Y*	-	Y*	-	Y	-	Y*	-
SSM7	Y*	Y*	-	Y*	Y	Y	Y	Y*	-

7.4.2.2. *Summary of each cross*

E. macrocarpa x *E. pyriformis*

The dendrogram (Figure 7.4) grouped the five seedlings from this cross in a separate cluster from the female and male parent seedlings, and the MDS ordination plot placed them in the same distinct groups with the seedling cluster between the parents and closer to the mother (Figure 7.5). The MST values for the connections between the seedlings and the female and male parent seedlings were 0.2578 and 0.3358, respectively, and the mean MST value within the seedling cluster was 0.1387, indicating that while there was some variation between seedlings, and considerable variation between the seedlings and both parents, the seedlings were most similar to each other, then most similar to the female parent. PCC analysis (Figure 7.6.) identified characters, such as LLE, LWI, LSW, LMI and LCO, as distinctive of either the male or female parent taxa. Leaf surface wax (LSW) and variations in length (LLE) and width (LWI) were highly distinctive from an early age (Plate 7.2).

E. macrocarpa x *E. youngiana*

Both the UPGMA dendrogram (Figure 7.7) and the MDS ordination (Figure 7.8) showed the twenty seedlings clustering into one group, distinctly separate from, but intermediate between, either parent. The MST values linking the seedlings to the female and male seedlings were 0.1888 and 0.2017 respectively, and the mean MST value within the seedling cluster was 0.1458, indicating that although there was some variation within the seedlings and between the seedlings and the parents, the seedlings are most similar to each other then most similar to the female parent. PCC analysis (Figure 7.9.) identified characters, such as LLE, LWI, LSW, LMI, LPL and LSB, as distinctive of either the male or female parent taxa. Again, leaf surface wax (LSW) and variations in length (LLE) and width (LWI) were highly distinctive from an early age (Plate 7.2).

E. macrocarpa x *E. macrocarpa*

The dendrogram (Fig 7.10) showed no distinct clustering and no obvious structure. The MDS ordination (Fig 7.11) scattered the individuals across the plot, intermingling *E. macrocarpa* open pollinated seedlings with seedlings. The MST value connecting seedlings and female parents was 0.1432 and the mean MST value between seedlings was 0.1458. This indicated that the variation within the seedling cluster was the same as the variation between the seedlings and the female parent species. Descriptive characters were difficult to define, as this was an intraspecific cross (Figure 7.12.).

E. pyriformis x *E. macrocarpa*

The UPGMA dendrogram (Figure 7.13) of this cross placed 33 of the seedlings in a cluster, one on its own, with the remaining three clustered with the female parent group. The MDS ordination (Fig 7.14) showed the points scattered across the plot, with the majority of the seedlings placed between the parent species groups. The MST values linking the seedlings to the female and male seedlings were moderate, 0.1966 and 0.1677 respectively, with the seedlings connected more closely to the male. The mean MST value within the seedling cluster, 0.1607, indicated some variation within the cluster. Three seedlings, EpxEm3, EpxEm14 and EpxEm19, were shown close to *E. pyriformis*. The MST connections place these seedlings with the female parent, *E. pyriformis*. PCC analysis (Figure 7.15.) identified characters, such as LLE, LWI, LSW, LMI, LCO, LPL and LSB, as distinctive of either the male or female parent taxa. Again, leaf surface wax (LSW) and variations in length (LLE) and width (LWI) were highly distinctive from an early age (Plate 7.3).

E. pyriformis x *E. youngiana*

The UPGMA dendrogram (Figure 7.16) placed five seedlings in a cluster with the female parent, most on their own, and two clustered with the male parent group. The MDS

ordination (Fig 7.17) showed the points scattered across the plot, with the majority of the seedlings placed between the parent species. There were many MST points of connection between the seedlings and the female parent, and the five seedlings from the dendrogram were connected directly to *E. pyriformis*. The male parents were connected to the seedlings by a value of 0.1330, with the two seedlings that clustered with *E. youngiana* in the dendrogram connected closely to EpxEy28, which connects directly to *E. youngiana*. The mean MST value within the seedling cluster was 0.1233. These low values and the lack of clear groupings in the MDS ordination reflect the relative similarities between the seedlings of these two species. PCC analysis (Figure 7.18.) identified characters, such as LLE, LWI, LMI, LCO, LPL and LSB, as distinctive of *E. pyriformis*, and only LPO7 and LCO7 distinctive for *E. youngiana*. Variations in leaf shape (LSH), length (LLE) and width (LWI) were highly distinctive from an early age (Plate 7.3).

E. pyriformis x *E. pyriformis*

The UPGMA dendrogram (Fig 7.19) did not clearly define any groups, and the MDS ordination (Fig 7.20) scattered the points across the plot. The MST value linking the female parent to the seedlings was 0.2000 and the mean MST value for the seedling cluster was 0.1562, indicating some variation within the seedlings. No defining characters were evident from the PCC analysis (Figure 7.21) as this was an interspecific cross, with no distinct groups of female and seedlings individuals.

E. youngiana x *E. macrocarpa*

The dendrogram (Figure 7.22) grouped the 38 seedlings one clusters, with one cluster of female and one of male seedlings. The MDS ordination (Fig 7.23) placed the seedlings between the parent groups. The MST values linking the seedling to the female and male seedlings were 0.1348 and 0.1749 respectively, and the mean MST value within the seedling

cluster was 0.1317, indicating a moderate level of variation within the seedlings and a greater similarity to the female parent than to the male. PCC analysis (Figure 7.24) identified characters, such as LSH, LSB, LWI, LSW, LEA, LPL, LMI, and LPO, as distinctive of either the male or female parent taxa. Again, leaf surface wax (LSW) and variations in length (LLE) and width (LWI) were highly distinctive from an early age (Plate 7.4).

E. youngiana x *E. pyriformis*

The dendrogram (Fig 7.25) grouped 38 seedlings into one cluster, with one cluster of *E. youngiana* and one seedlings and another of *E. pyriformis*. The MDS ordination (Fig 7.26) scattered the individuals across the whole plot, with the majority of seedlings between the parent groups. The MST values for the seedlings to the female and male groups were 0.1755 and 0.2054 respectively, and the mean MST value between the seedlings was 0.1078, indicating that the seedlings are vary similar to each other, and then more similar to the female parent. PCC analysis (Figure 7+.27) identified few characters as distinctive for *E. youngiana* (LPO and LEA, both at node 3), while *E. pyriformis* was identified by such as LLE, LWI, LCO, and LPL. Seedlings of this cross differed in leaf length (LLE) and width (LWI) (Plate 7.4).

E. youngiana x *E. youngiana*

Only six intraspecific seedlings survived to be measured at three months, and the dendrogram (Fig 7.28) clusters them into two groups. The MDS ordination (Figure 7.29) reflected these groupings. The MST value connecting the seedlings with the female parent was 0.1986, and the value within the seedlings was 0.2986, indicating there to be more variation within the seedlings than between the seedlings and the parents. The PCC analysis (Figure 7.30) did not reveal any distinctive characters.

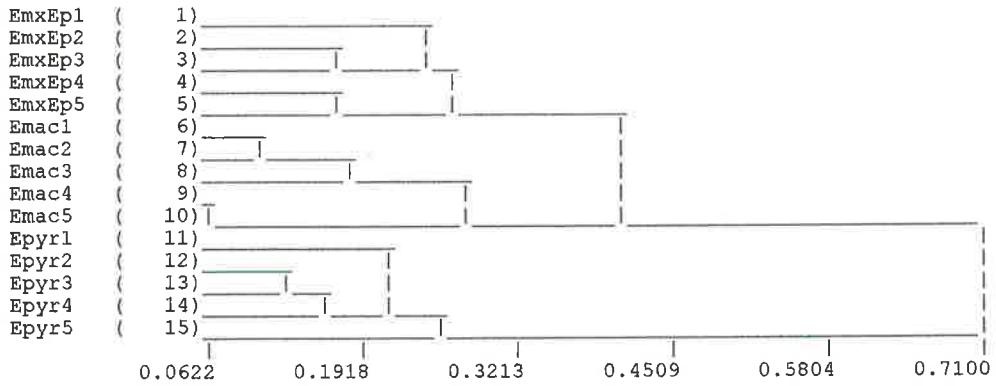


Figure 7.4. Dendrogram of *E. macrocarpa* x *E. pyriformis* seedlings and parent seedlings. *E. macrocarpa* (Emac), *E. pyriformis* (Epyr) *E. macrocarpa* x *E. pyriformis* (EmxEp). Scale represents standardised Manhattan distance (Gower metric) based on variant seedling characters (Table 7.8).

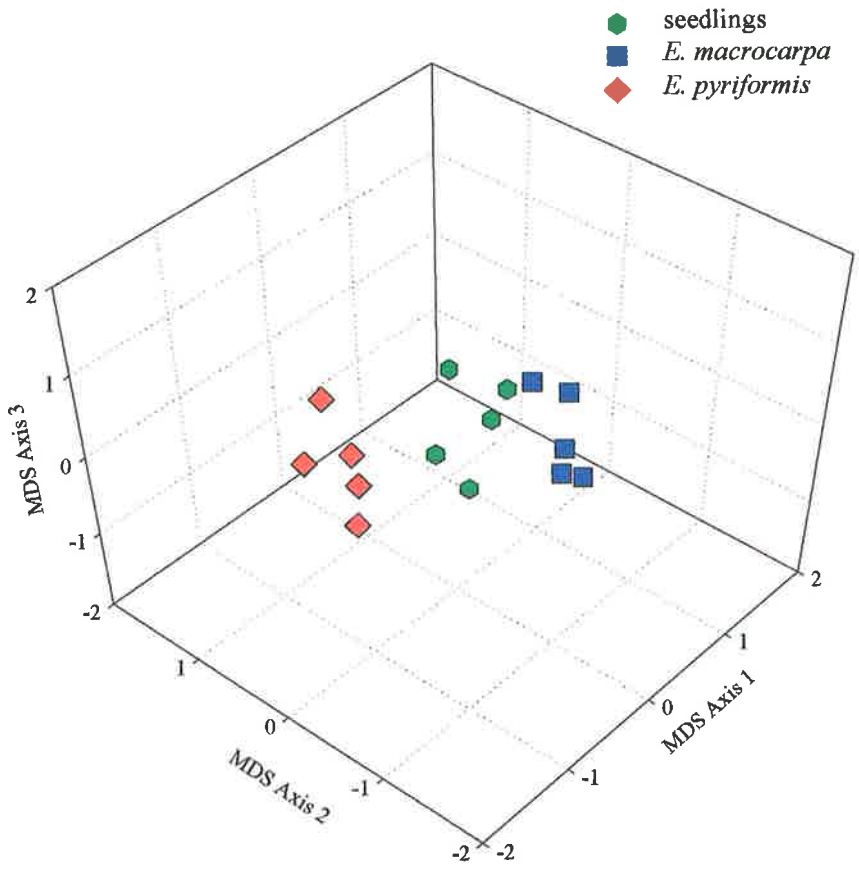


Figure 7.5. Multidimensional ordination of *E. macrocarpa* x *E. pyriformis* seedlings and parents.

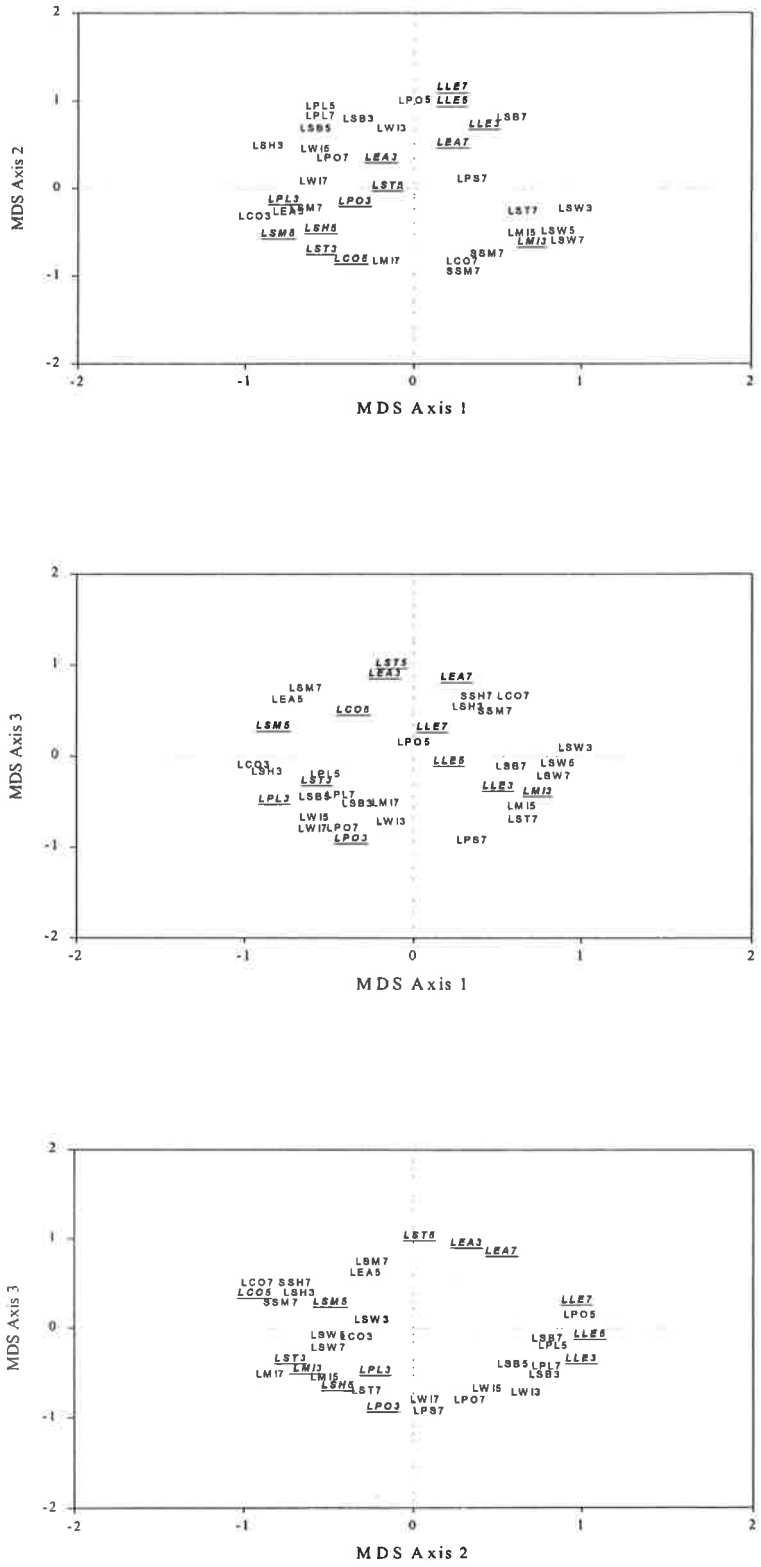


Figure 7.6. Principal component correlation graphs of *E. macrocarpa* x *E. pyriformis* seedlings and parents. Characters in italics and underlined not significantly correlated to MDS ordinations (Table 7.8).

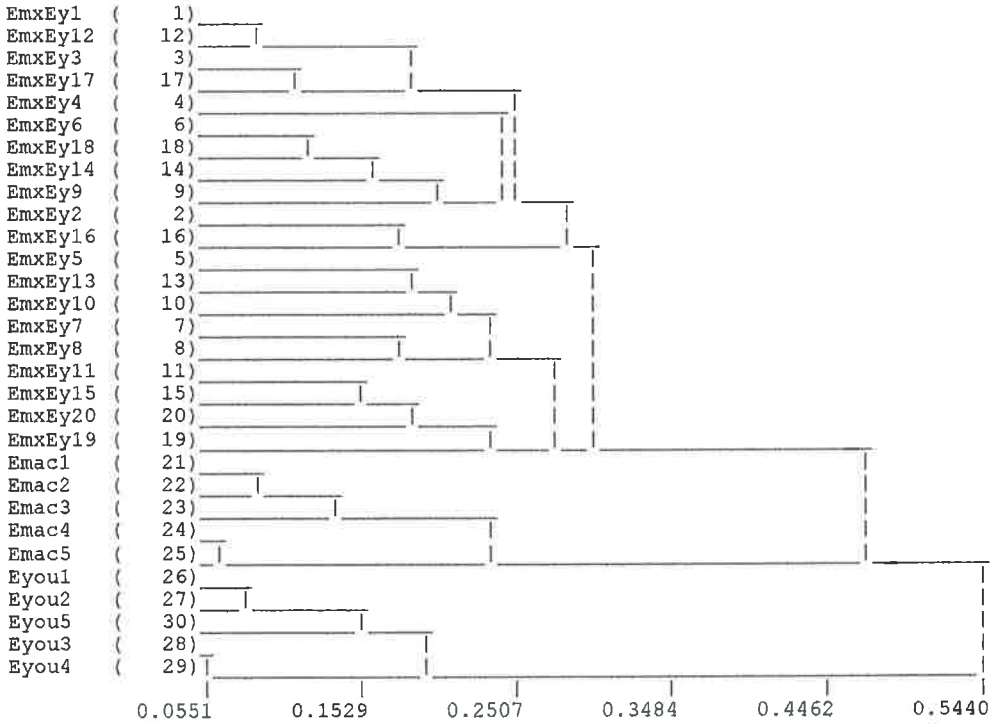


Figure 7.7. Dendrogram of *E. macrocarpa* x *E. youngiana* seedlings and parent seedlings. *E. macrocarpa* (Emac), *E.* (Eyou) *E. macrocarpa* x *E. youngiana* (EmxEy). Scale represents standardised Manhattan distance (Gower metric) based on variant seedling characters (Table 7.8).

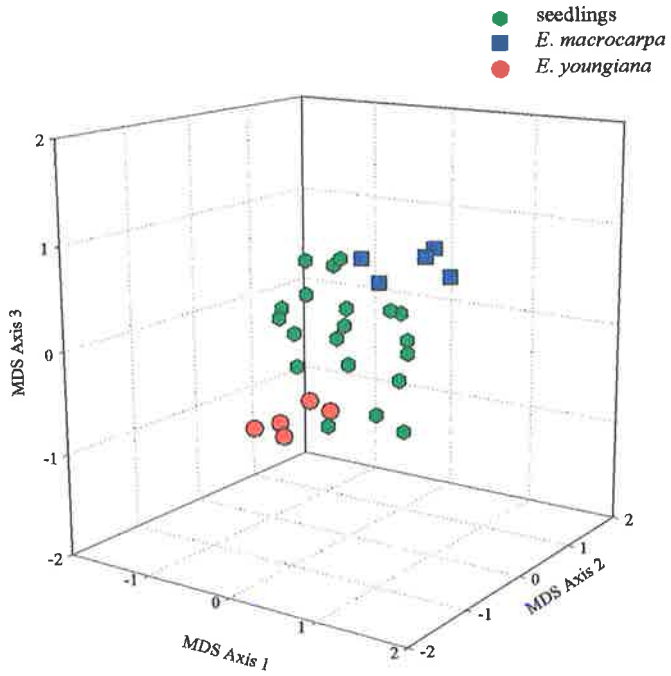


Figure 7.8. Multidimensional ordination of *E. macrocarpa* x *E. youngiana* seedlings and parent seedlings.

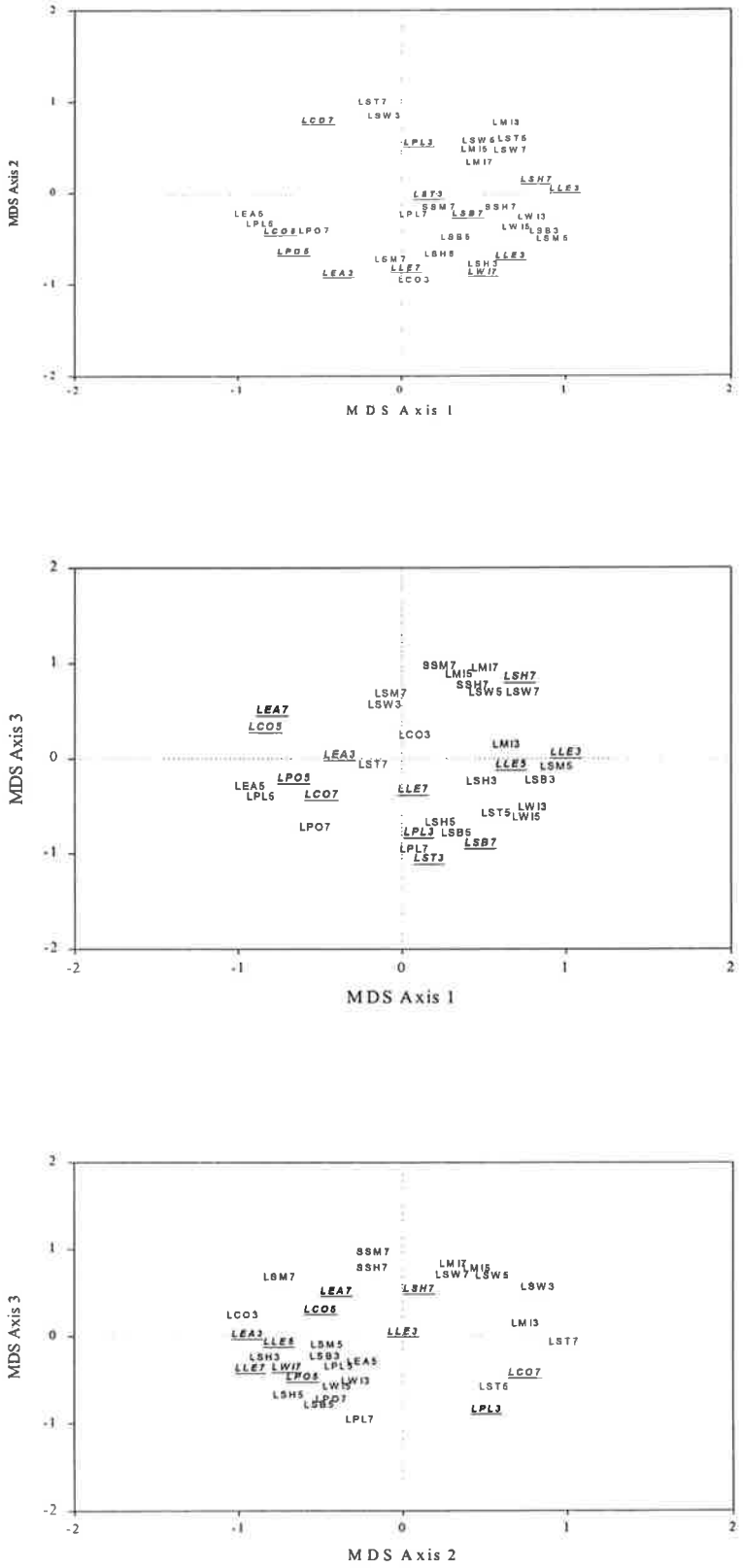


Figure 7.9. Principal component correlation graphs of *E. macrocarpa* x *E. youngiana* seedlings and parents. Characters in italics and underlined not significantly correlated to MDS ordinations (Table 7.8).

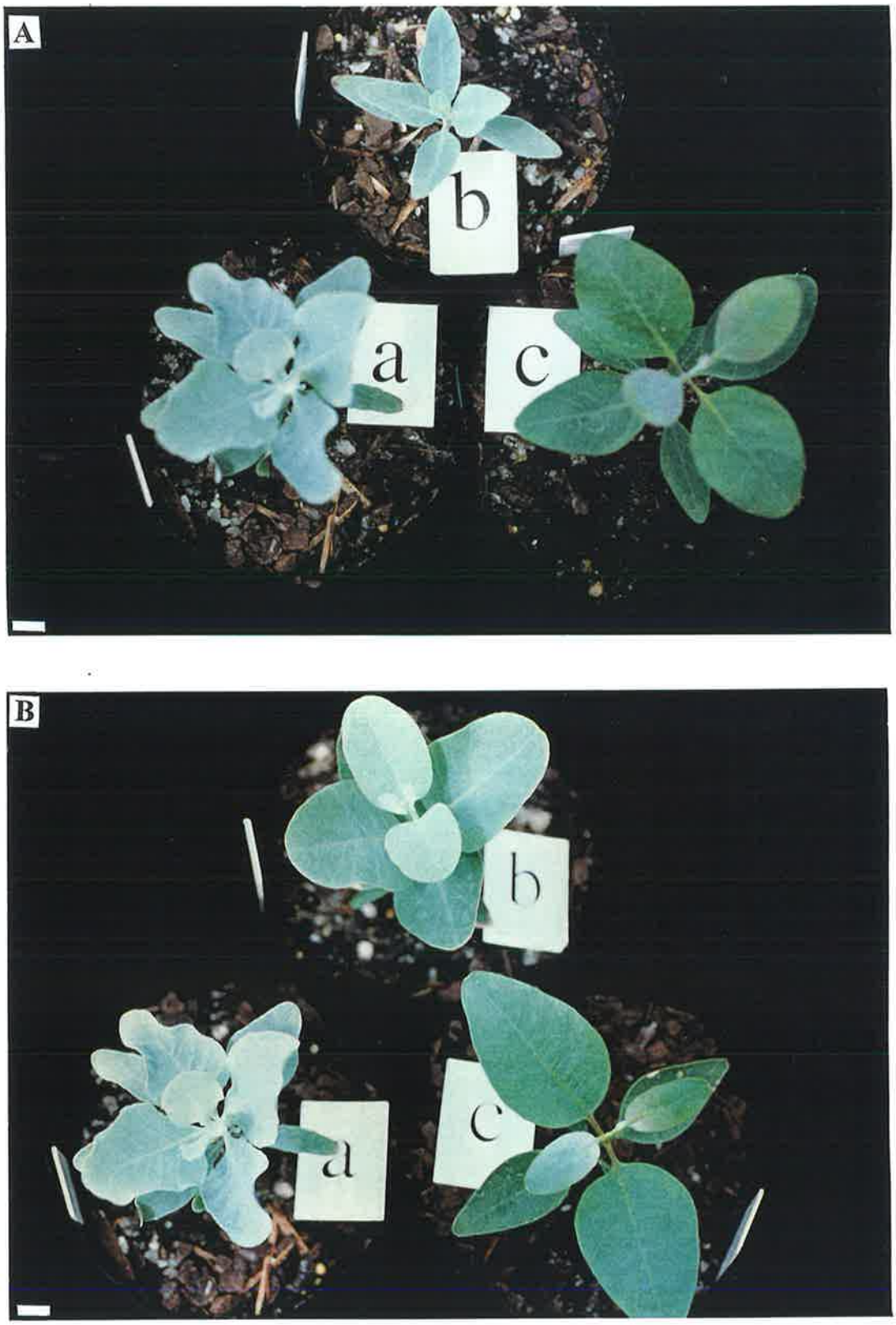


Plate 7.2.
Hybrid seedlings six weeks after germination.
A: a) *E. macrocarpa,* b) *E. macrocarpa* x *E. pyriformis,* and c) *E. pyriformis*;
B: a) *E. macrocarpa,* b) *E. macrocarpa* x *E. youngiana,* and c) *E. youngiana* seedlings.
Bar = 1cm.

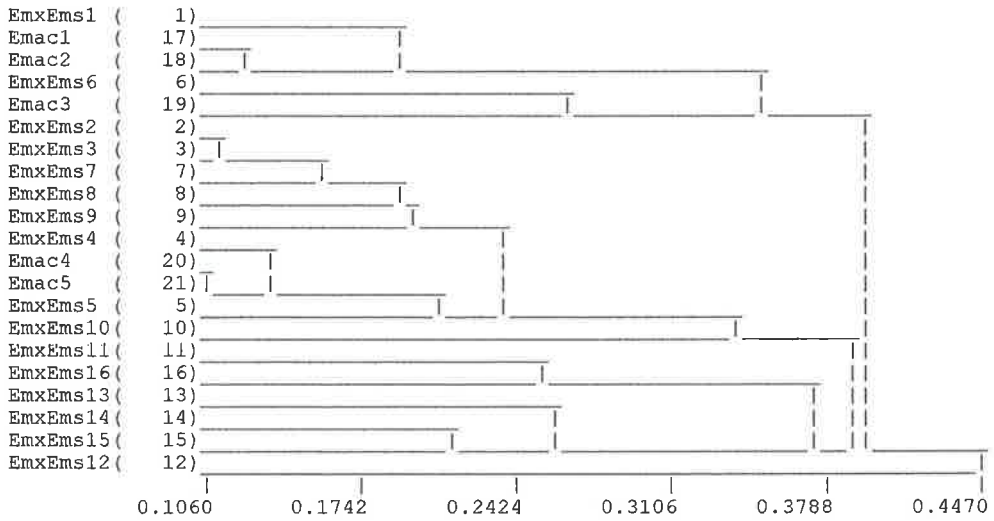


Figure 7.10. Dendrogram of *E. macrocarpa* x *E. macrocarpa* seedlings and parent seedlings. *E. macrocarpa* (Emac), *E. macrocarpa* x *E. macrocarpa* (EmxEms). Scale represents standardised Manhattan distance (Gower metric) based on variant seedling characters (Table 7.8).

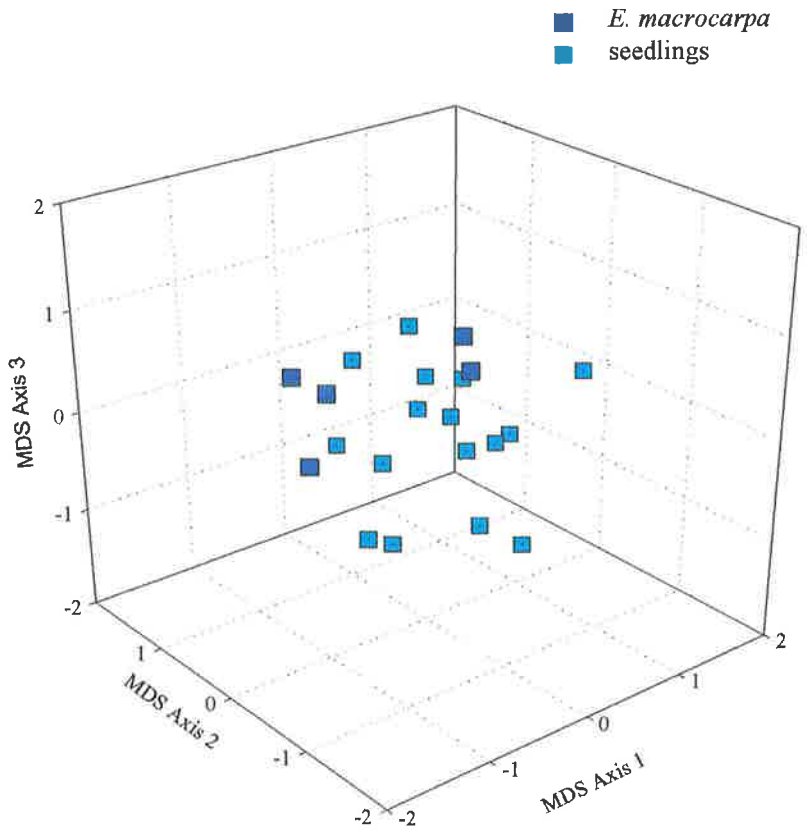


Figure 7.11. Multidimensional ordination of *E. macrocarpa* x *E. macrocarpa* seedlings and parent seedlings.

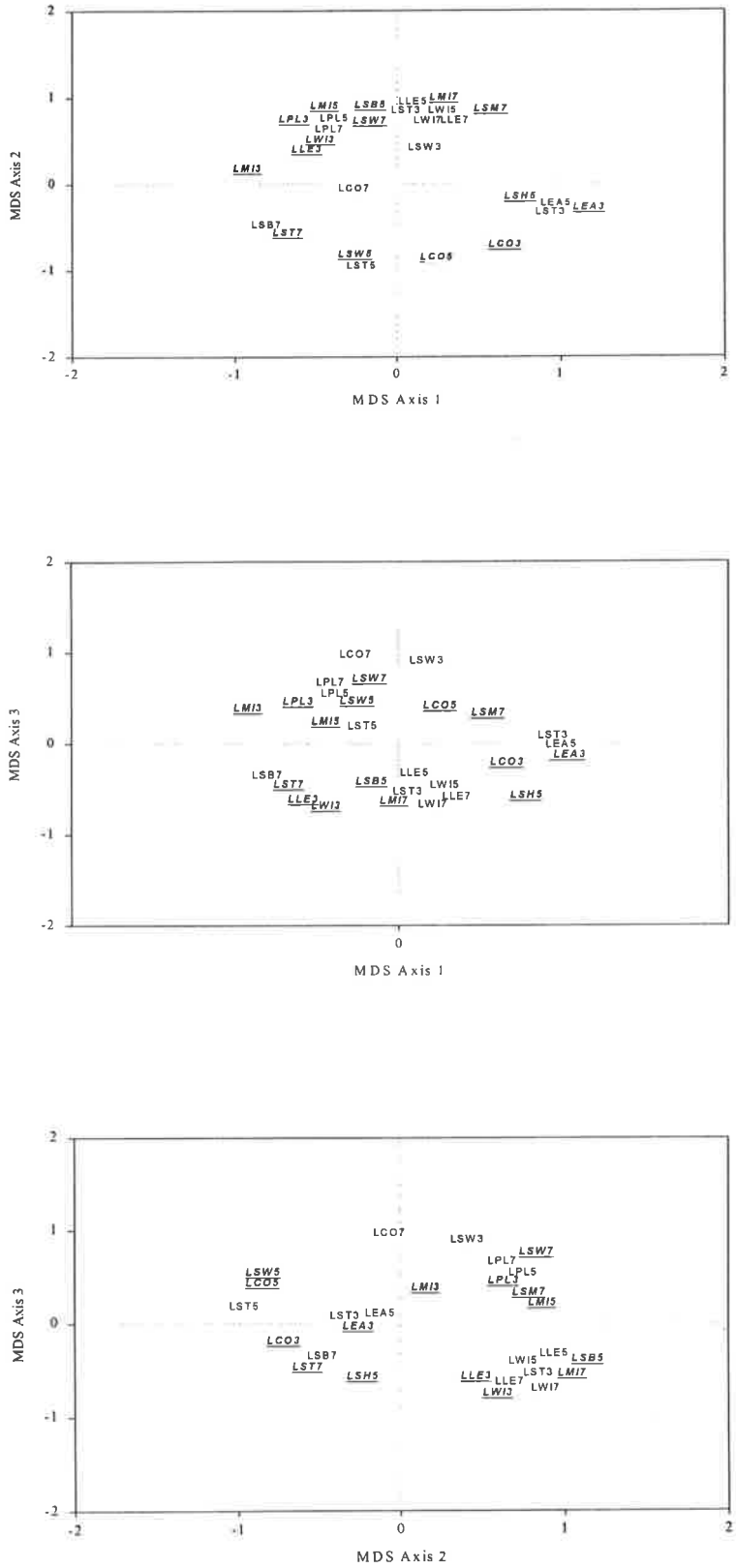


Figure 7.12. Principal component correlation graphs of *E. macrocarpa* x *E. macrocarpa* seedlings and parents. Characters in italics and underlined not significantly correlated to MDS ordinations (Table 7.8).

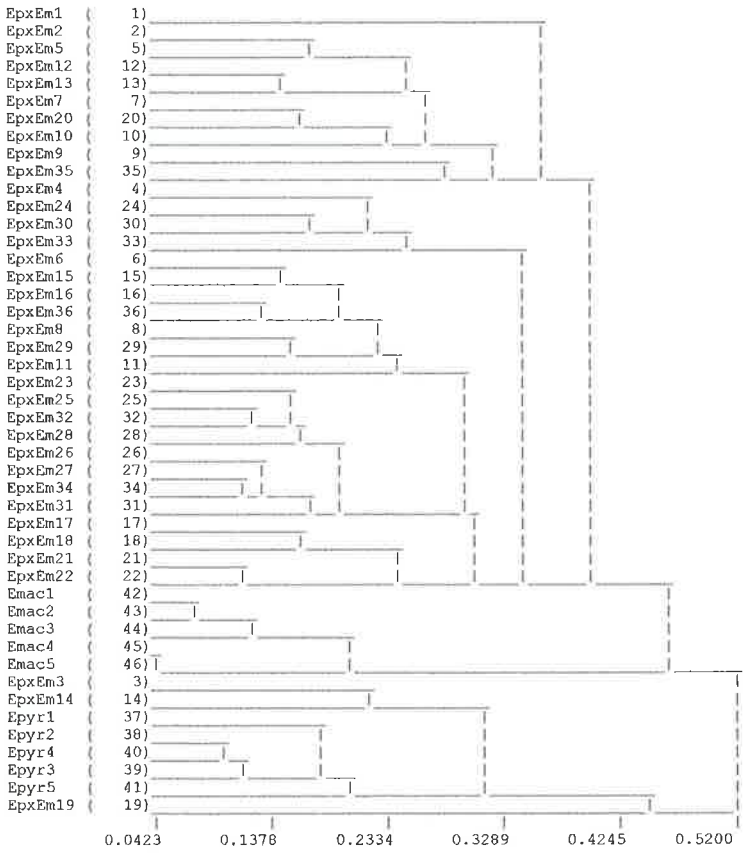


Figure 7.13. Dendrogram of *E. pyriformis* x *E. macrocarpa* seedlings and parent seedlings. *E. macrocarpa* (Emac), *E. pyriformis* (Epyr), *E. pyriformis* x *E. macrocarpa* (EpxEm). Scale represents standardised Manhattan distance (Gower metric) based on variant seedling characters (Table 7.9).

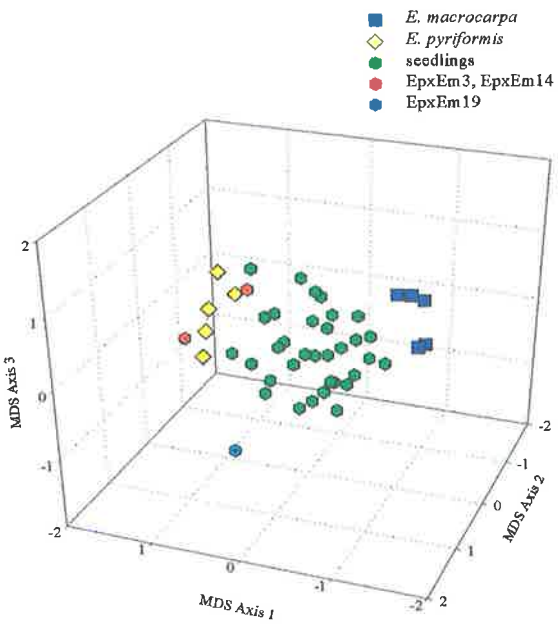


Figure 7.14. Multidimensional ordination of *E. pyriformis* x *E. macrocarpa* seedlings and parent seedlings.

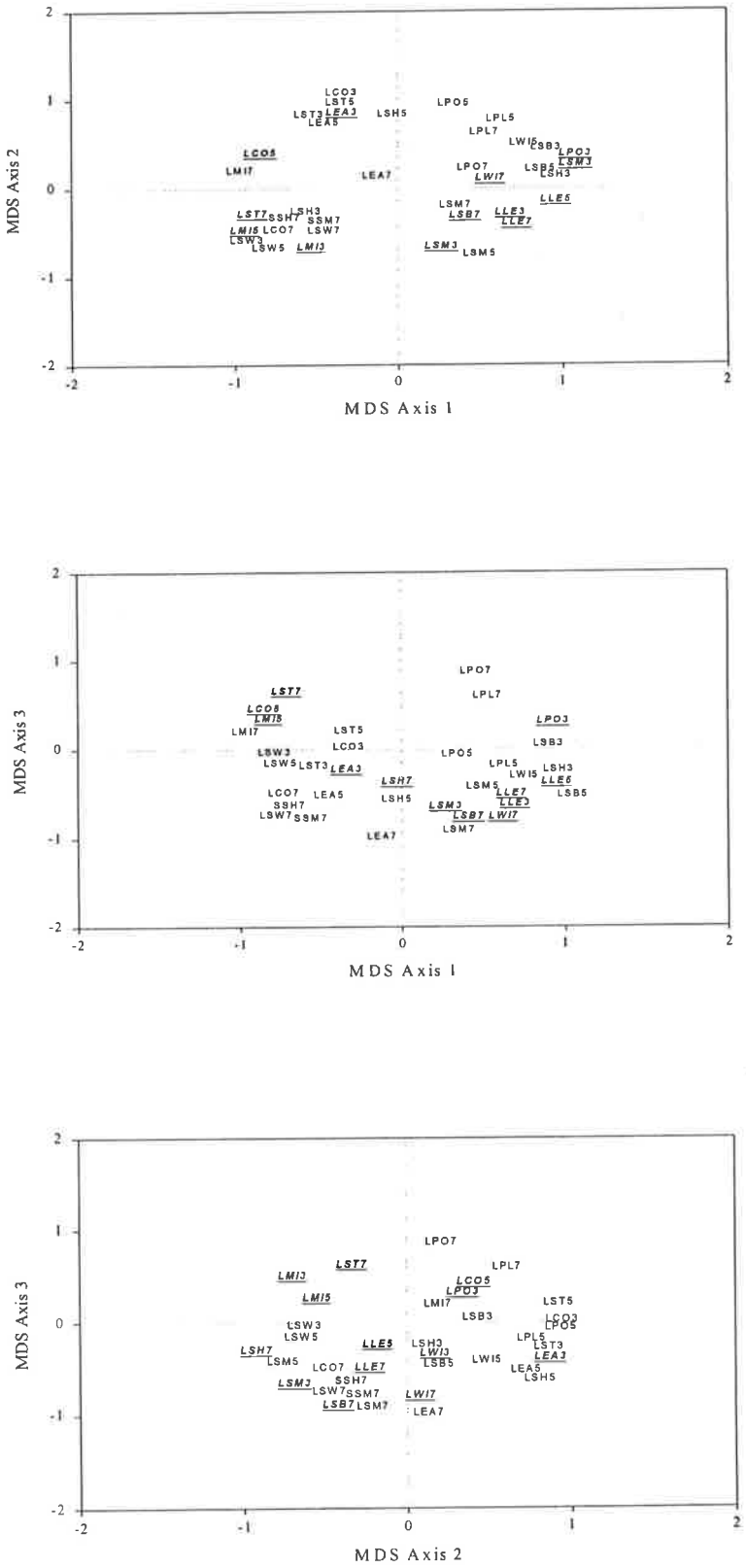


Figure 7.15. Principal component correlation graphs of *E. pyriformis* x *E. macrocarpa* seedlings and parents. Characters in italics and underlined not significantly correlated to MDS ordinations (Table 7.9).

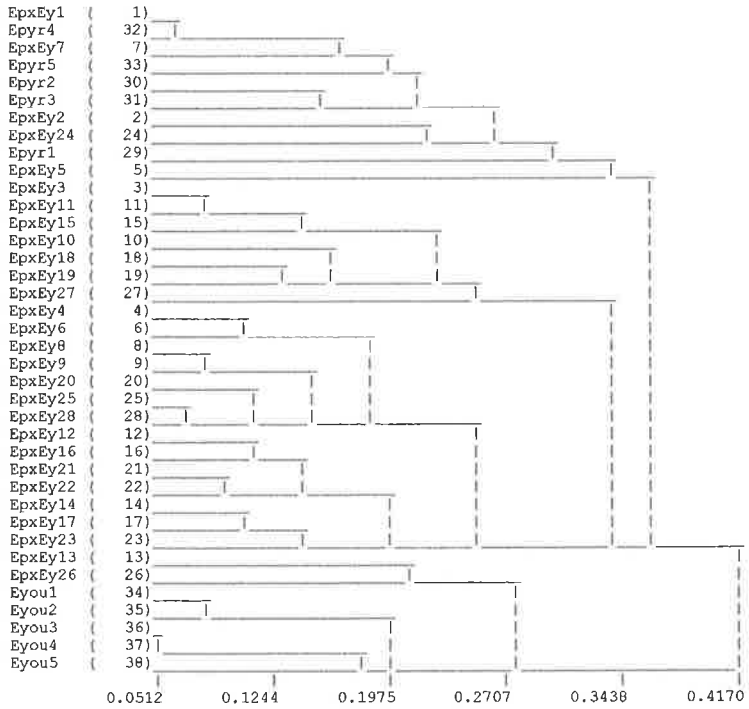


Figure 7.16. Dendrogram of *E. pyriformis* x *E. youngiana* seedlings and parent seedlings. *E. pyriformis* (Epyr) *E. youngiana* (Eyou), *E. pyriformis* x *E. youngiana* (EpxEy). Scale represents standardised Manhattan distance (Gower metric) based on variant seedling characters (Table 7.9).

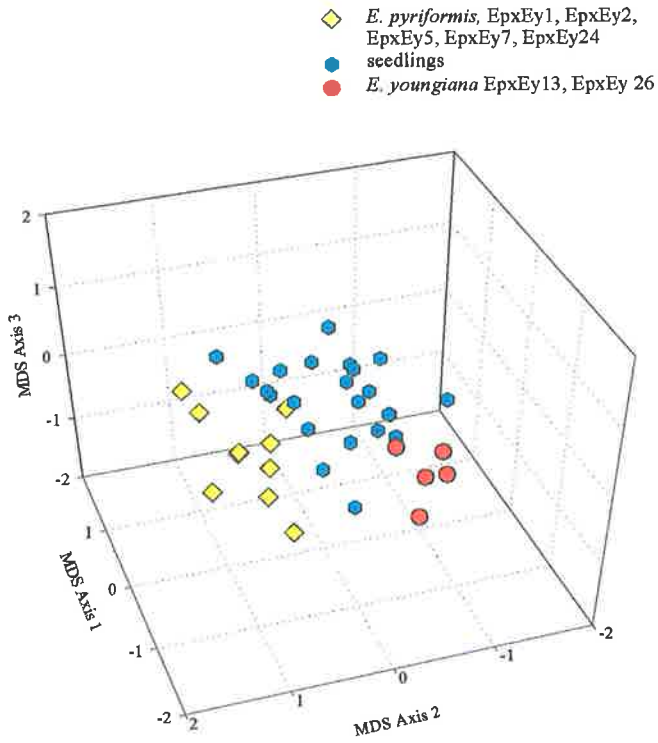


Figure 7.17. Multidimensional ordination of *E. pyriformis* x *E. youngiana* seedlings and parent seedlings.

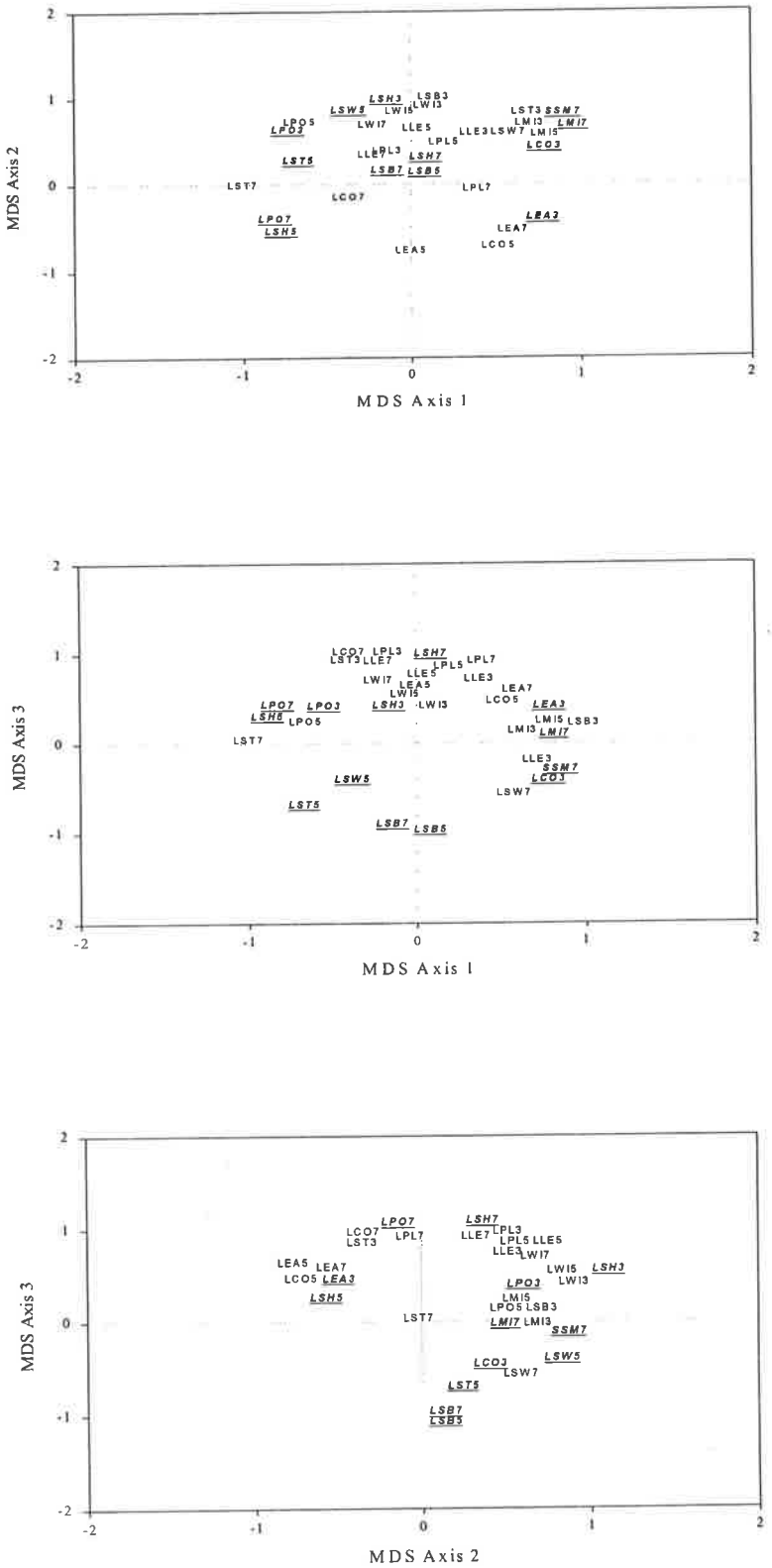


Figure 7.18. Principal component correlation graphs of *E. pyriformis* x *E. youngiana* seedlings and parents. Characters in italics and underlined not significantly correlated to MDS ordinations (Table 7.9).

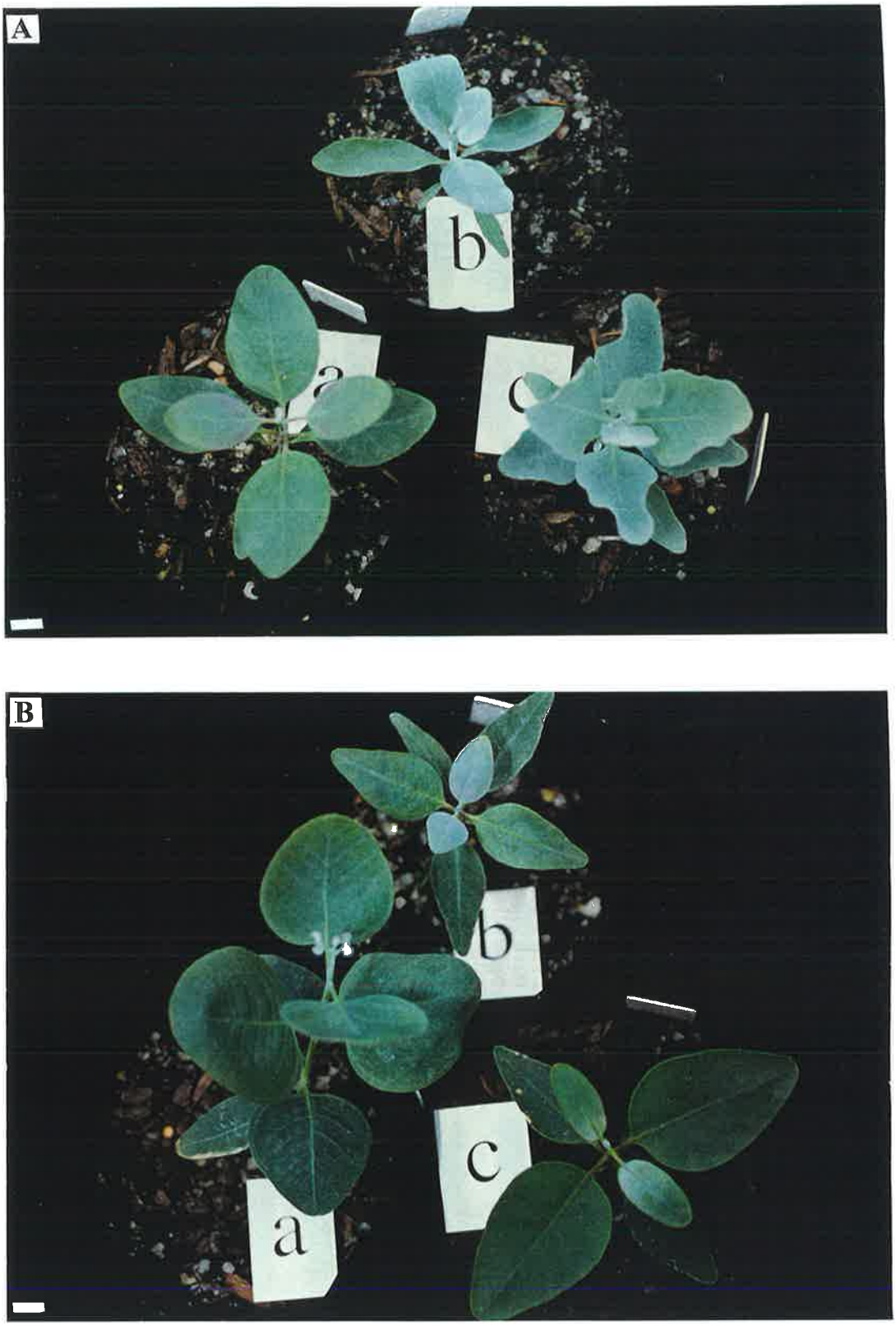


Plate 7.3.
Hybrid seedlings six weeks after germination.
A: a) *E. pyriformis*, b) *E. pyriformis* x *E. macrocarpa,* and c) *E. macrocarpa;*
B: a) *E. pyriformis,* b) *E. pyriformis* x *E. youngiana,* and c) *E. youngiana.*
Bar - 1 cm.

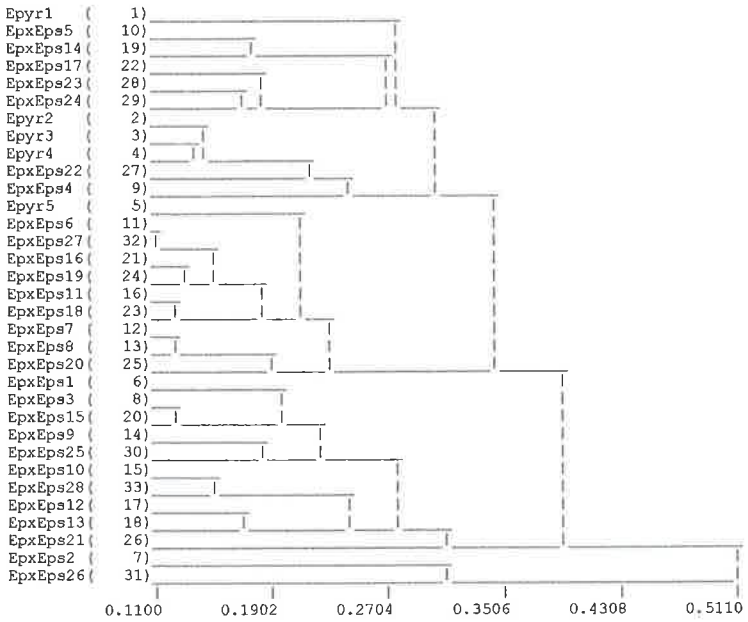


Figure 7.19. Dendrogram of *E. pyriformis* x *E. pyriformis* seedlings and parent seedlings. *E. pyriformis* (Epyr) *E. pyriformis* x *E. pyriformis* (EpxEps). Scale represents standardised Manhattan distance (Gower metric) based on variant seedling characters (Table 7.9).

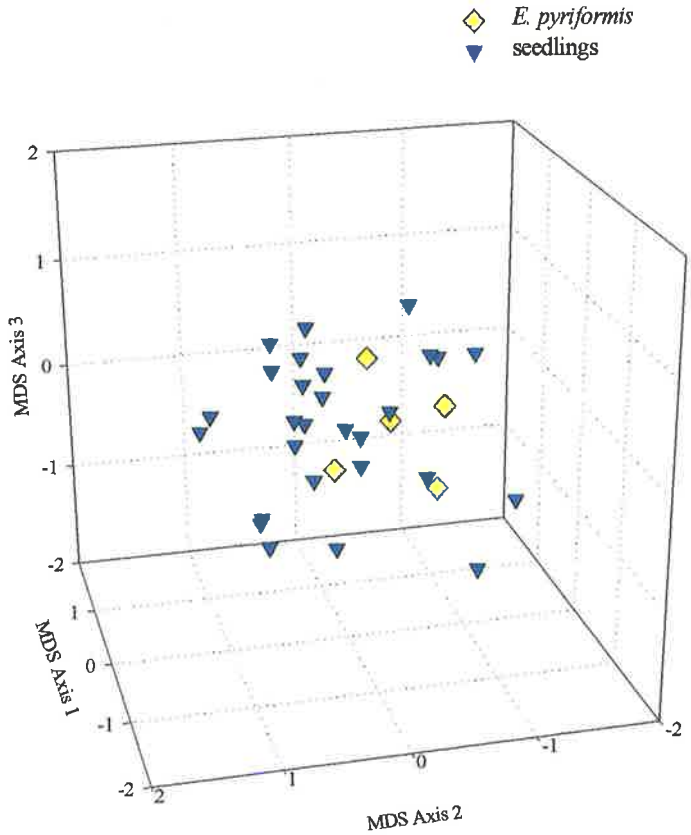


Figure 7.20. Multidimensional ordination of *E. pyriformis* x *E. pyriformis* seedlings and parent seedlings.

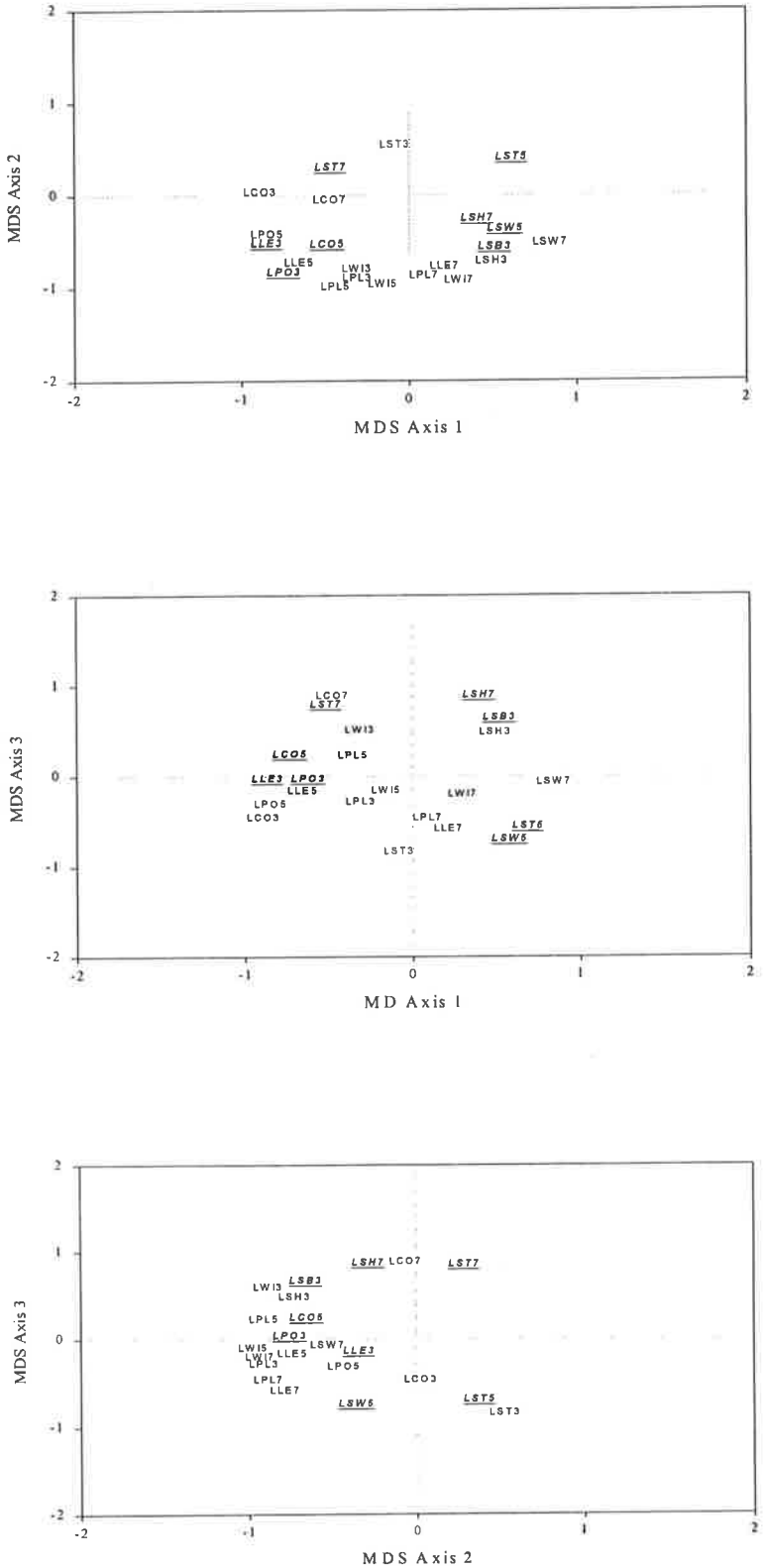


Figure 7.21. Principal component correlation graphs of *E. pyriformis* x *E. pyriformis* seedlings and parents. Characters in italics and underlined not significantly correlated to MDS ordinations (Table 7.9).

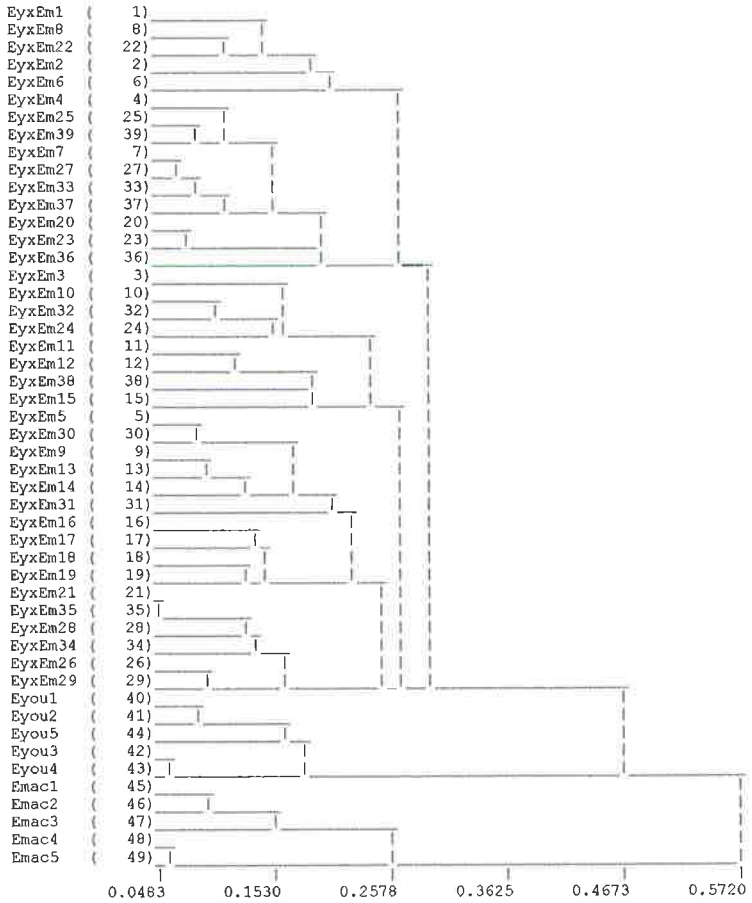


Figure 7.22. Dendrogram of *E. youngiana* x *E. macrocarpa* seedlings and parent seedlings. E. *youngiana* (Eyou), *E. macrocarpa* (Emac), *E. youngiana* x *E. macrocarpa* (EyxEm). Scale represents standardised Manhattan distance (Gower metric) based on variant seedling characters (Table 7.10).

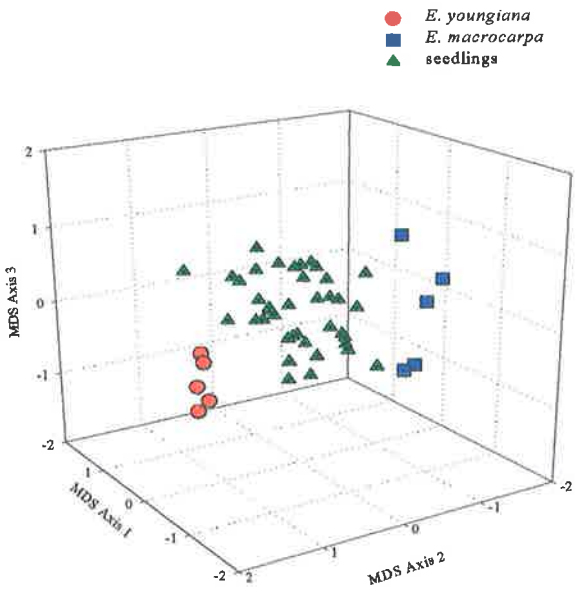


Figure 7.23. Multidimensional ordination of *E. youngiana* x *E. macrocarpa* seedlings and parent seedlings.

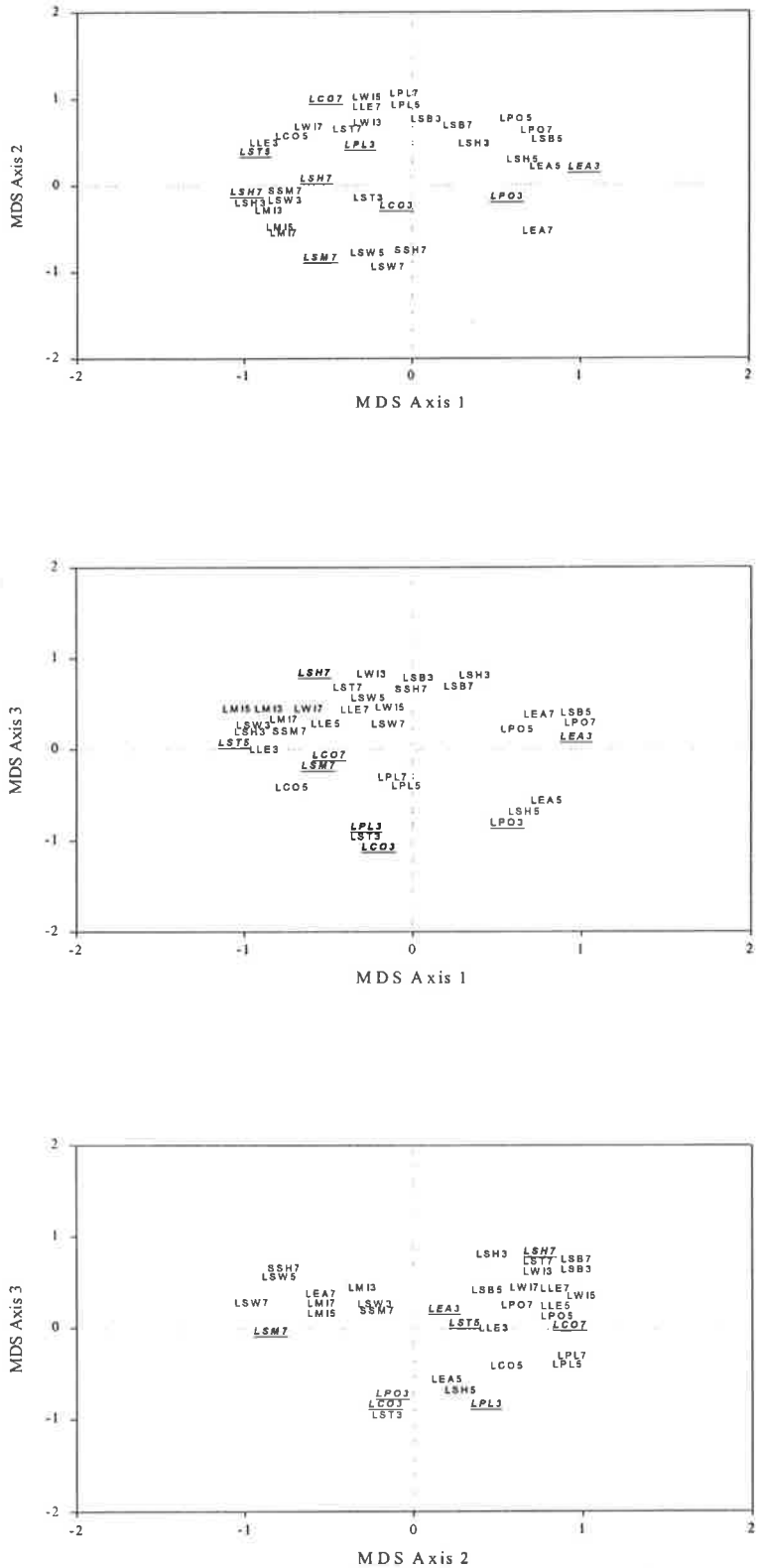


Figure 7.24. Principal component correlation graphs of *E. youngiana* x *E. macrocarpa* seedlings and parents. Characters in italics and underlined not significantly correlated to MDS ordinations (Table 7.10).

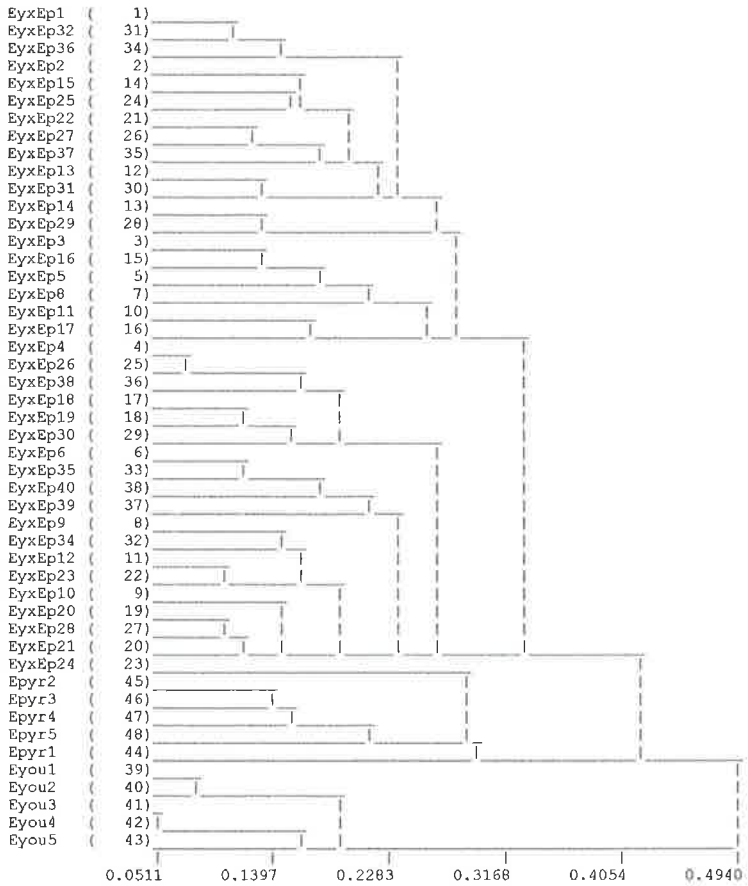


Figure 7.25. Dendrogram of *E. youngiana* x *E. pyriformis* seedlings and parent seedlings. *E. youngiana* (Eyou), *E. pyriformis* (Epyr) *E. youngiana* x *E. pyriformis* (EyxEp). Scale represents standardised Manhattan distance (Gower metric) based on variant seedling characters (Table 7.10).

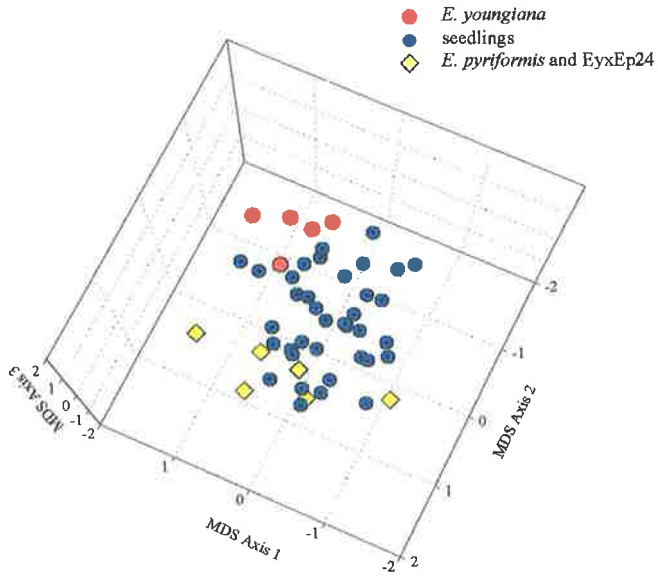


Figure 7.26. Multidimensional ordination of *E. youngiana* x *E. pyriformis* seedlings and parent seedlings.

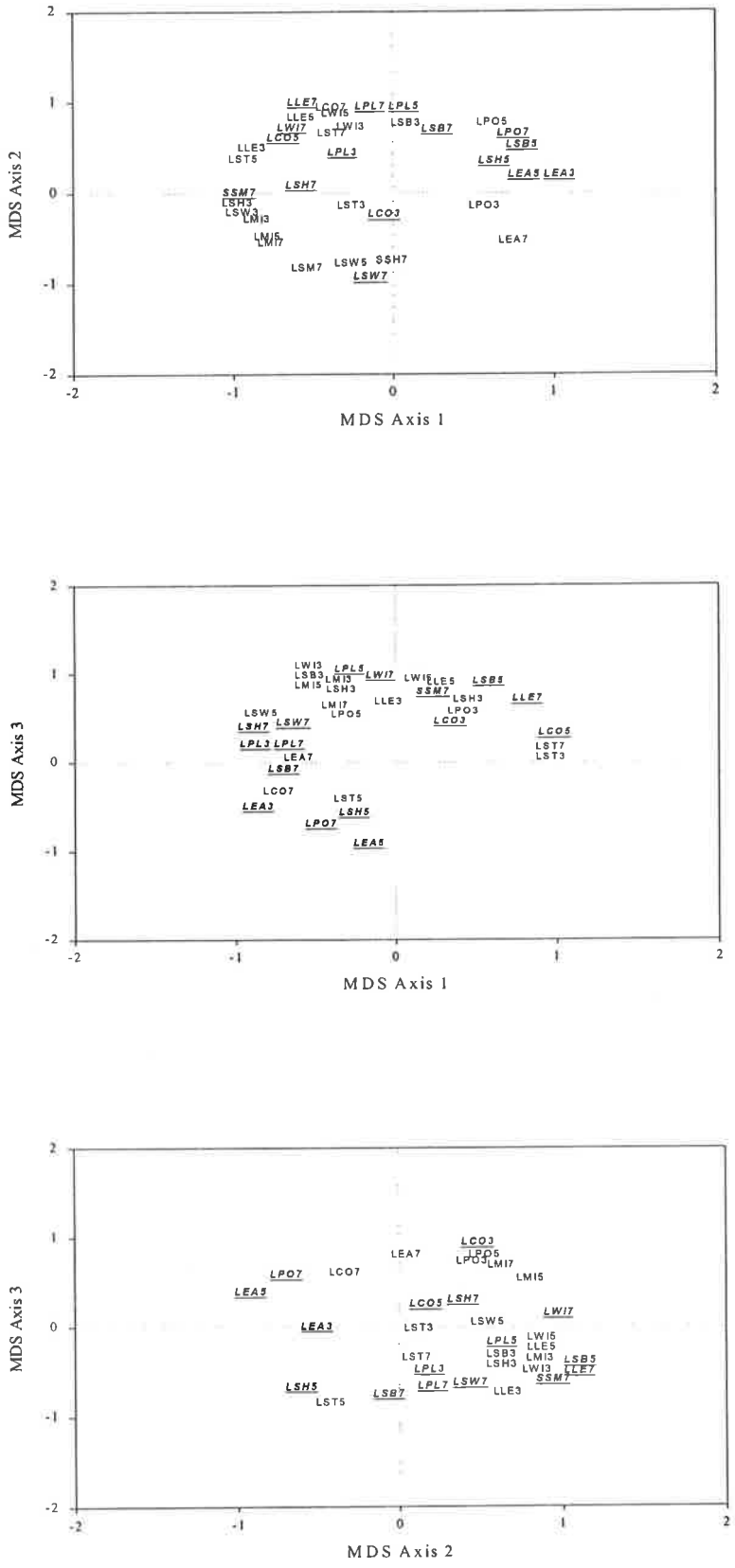


Figure 7.27. Principal component correlation graphs of *E. youngiana* x *E. pyriformis* seedlings and parents. Characters in italics and underlined not significantly correlated to MDS ordinations (Table 7.10).

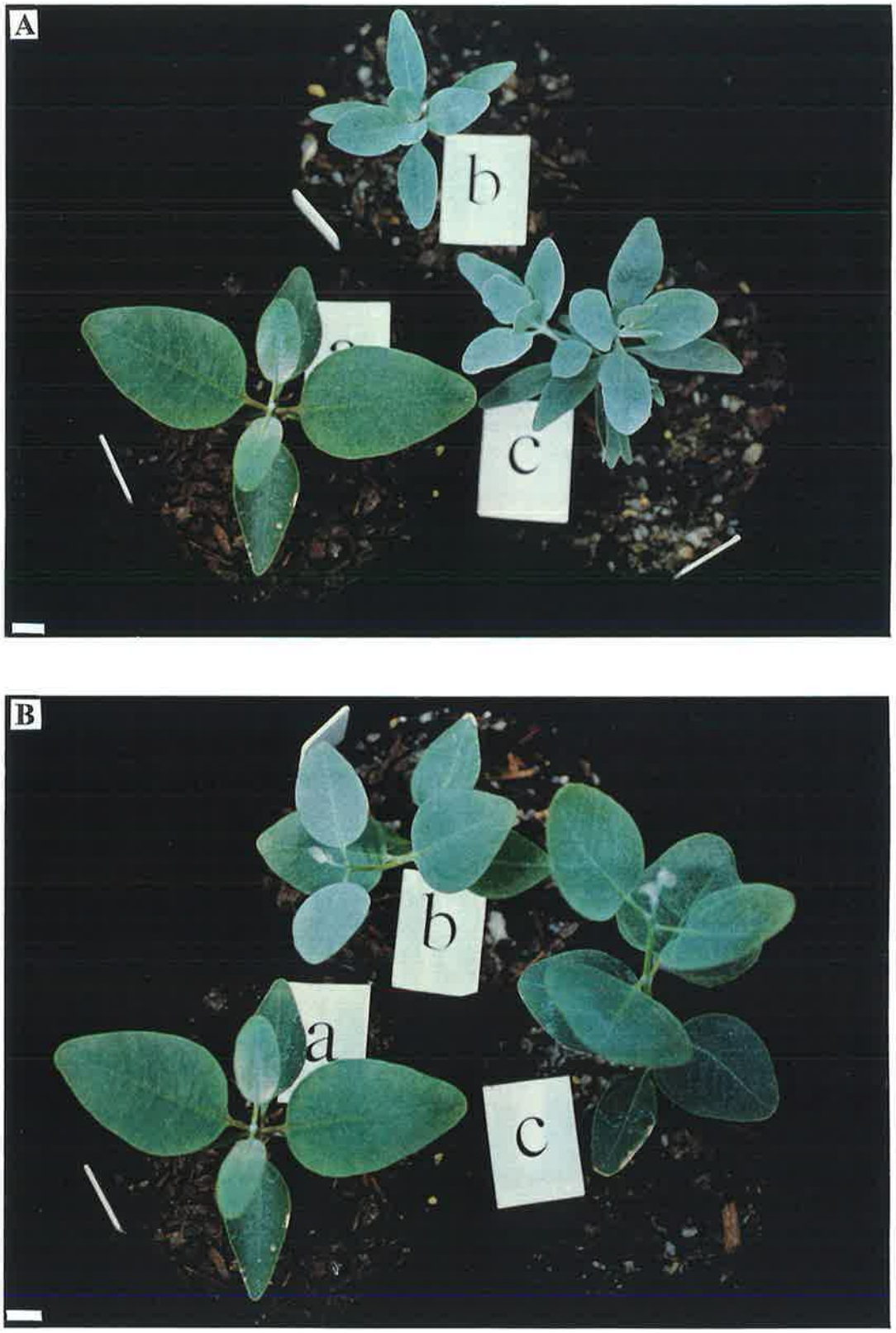


Plate 7.4.
Hybrid seedlings six weeks after germination.
A: a) *E. youngiana,* b) *E. youngiana* x *E. macrocarpa*, and c) *E. macrocarpa*;
B: a) *E. youngiana,* b) *E. youngiana* x *E. pyriformis,* and c) *E. pyriformis.* Bar = 1cm.

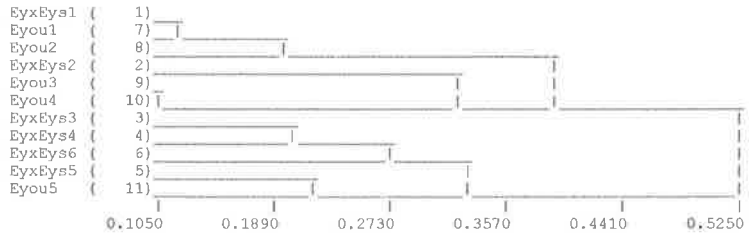


Figure 7.28. Dendrogram of *E. youngiana* x *E. youngiana* seedlings and parent seedlings. *E. youngiana* (Eyou), *E. youngiana* x *E. youngiana* (EyxEys). Scale represents standardised Manhattan distance (Gower metric) based on variant seedling characters (Table 7.10).

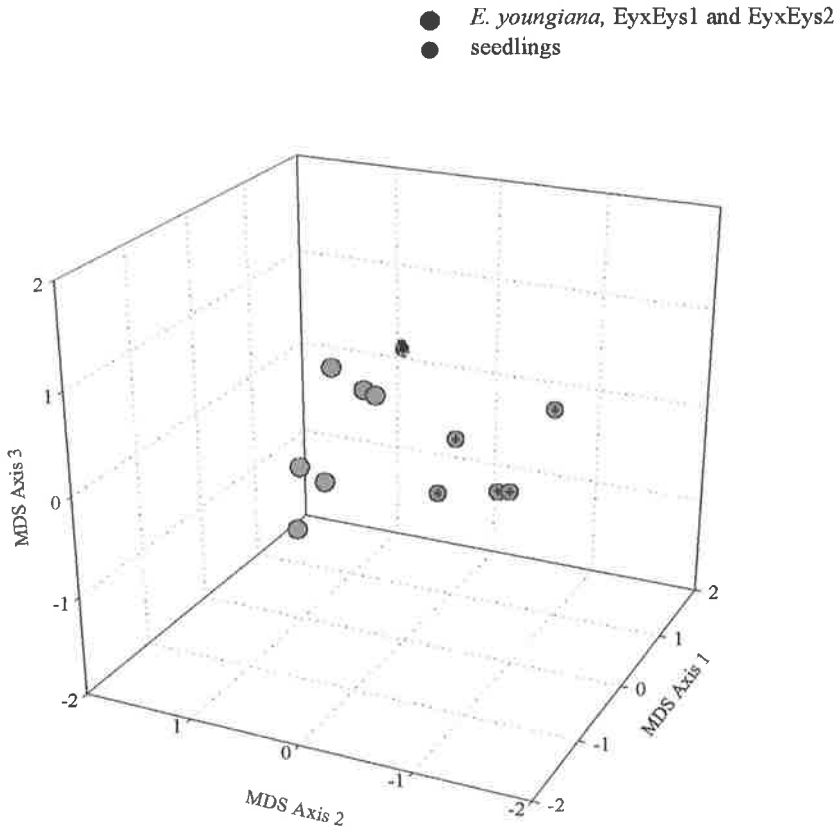
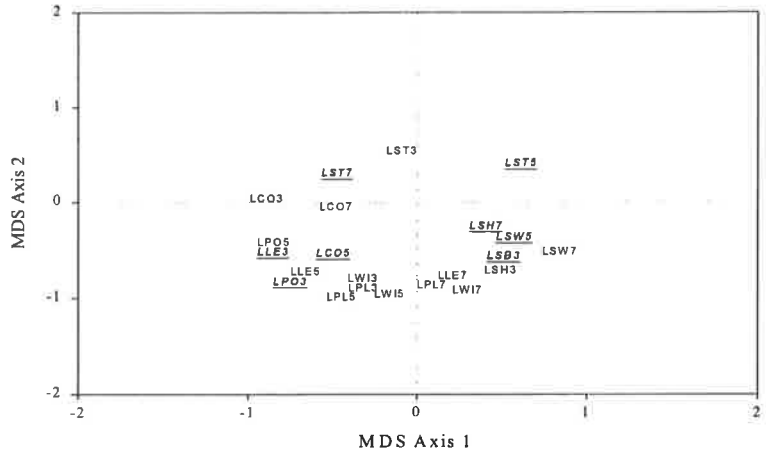
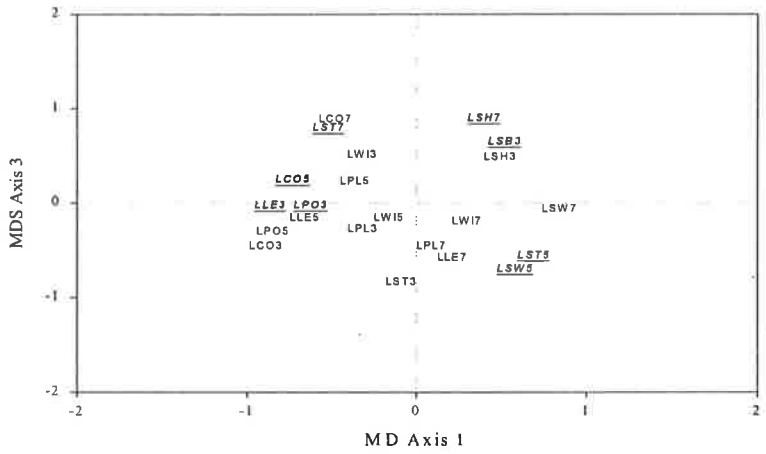


Figure 7.29. Multidimensional ordination of *E. youngiana* x *E. youngiana* seedlings and parent seedlings.





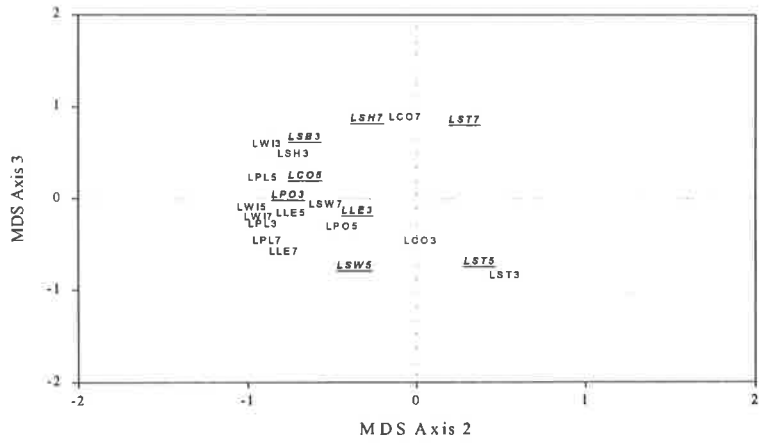


Figure 7.30. Principal component correlation graphs of *E. youngiana* x *E. youngiana* seedlings and parents. Characters in italics and underlined not significantly correlated to MDS ordinations (Table 7.10).

7.5. Discussion

E. macrocarpa, *E. pyriformis* and *E. youngiana* will hybridise successfully under controlled conditions, shown by the results of this study. Analysis with hierarchical and non-hierarchical distance methods placed the majority of interspecific seedlings as distinct clusters in the Gower UPGMA dendrogram and between seedlings of both parents on the MDS ordination, thus supporting their F1 hybrid status.

Analysis of seed data showed that mean number of seeds produced per flower pollinated and mean seed weight generally varied more between female plants than between crosses. Percent seedlings germinated gave an indication of the fertility of each cross or female tree, the results again showed that there was more variation within species than between. This would indicate that with these closely related species of eucalypts, the effect of interspecific crosses was less than the effect of different maternal genotypes on seed production. The effect of maternal influence over number of seed set and seed weight has been recorded in other interspecific pollination trials (Tibbits, 1989). Such an effect can have important implications in breeding programs, as plants with limited reproductive capabilities would not be used as females in a breeding program, unless they exhibited other, more desirable, characters. Seed set is also influenced by the number and size of individual buds in an umbel, while time of flowering and time to fruit maturation are influenced by bud and fruit volume (Keatley and Hudson, 1998, Primack, 1987). All three species in the study had fewer than three large buds per umbel, and took up to eight months for fruit maturation, with generally less than 50% abscission of pollinated flowers. This compares well with *E. nitens*, where 30-50% of flowers from interspecific crosses produced seed (Tibbits, 1989). The same study found that a higher seed set can be realised with hand pollination as opposed to pollination by natural vectors (Tibbits, 1989).

Hierarchical and non-hierarchical analysis of each cross showed a promising trend across all crosses: in that the majority of seedlings measured (96% of interspecific crosses) did not cluster with the female parent. Such clustering of seedlings either between parent species clusters or close to male parent species clusters, is an indicator of hybrid status (McDade, 1997) as was the generally intermediate position of the seedlings in the MDS plots. A few seedlings clustered with the male species, for example, two seedlings from the *E. pyriformis* x *E. youngiana* and one from the *E. youngiana* x *E. macrocarpa* clustered with the male parent; these seedlings will be important to observe as they develop adult characters as they have the highest chance of showing combinations of maternal and paternal characters. Seedlings that clustered with the female parents are more likely to be a result of contamination with female parent pollen, rather than extremes of hybrid distribution. Principal canonical correlation revealed a number of characters that differentiated between seedlings of the three species: glaucousness, leaf length and width, petiole length, leaf margin shape and leaf colour the most descriptive for all species. *E. macrocarpa* was defined by high levels of glaucousness, a trait which was inherited by its seedlings, regardless of whether the species was the male or female parent, and in many seedlings hybrid status could be identified on the basis of that single character. A similar situation of identification of hybrids by single characters was discussed by Tibbits (1988) in a study of *E. nitens*. All parts of the *E. macrocarpa* plant were covered in high densities of long tubes of wax (Wirthensohn and Sedgley, 1996). Inheritance of glaucousness may be simple, requiring one or two genes (Barber, 1955; Cauvin et al, 1987)). Differing leaf length and width was descriptive for all species, as was leaf margin shape and petiole length, while leaf colour was most descriptive for seedlings from crosses with *E. youngiana*.

Several of the twenty-five species within Subgenus Symphyomyrtus Section Bisectaria Series Macrocarpae (Pryor and Johnson, 1971) or *E.* ser. *Curviptera* subseries *Curviptera* (Brooker

and Hopper, 1993) bear strong similarities to *E. macrocarpa*, *E. pyriformis* and *E. youngiana*. *E. rhodantha*, a distinct and rare species from Watheroo, W.A., differs from *E. macrocarpa* in its smaller leaves and 'stalked' flowers, and was originally thought to be a hybrid between *E. macrocarpa* and *E. pyriformis* (Sampson *et al.*, 1990). In addition, *E. rameliana* and *E. impensa* are similar to both *E. macrocarpa* and *E. rhodantha*, but without glaucous leaves and with petioles, differing from each other in leaf colour and petiole length (Brooker and Hopper, 1993). Another species of note is *E. carnabyi*, a very rare possible cross between *E. macrocarpa* and *E. drummondii* (a closely related species in same subseries) with shortly petiolate leaves and three to seven flowers per inflorescence (Hopper, 1995). Incidences of naturally occurring unnamed hybrids, where the distributions of the species overlap, between *E. pyriformis* and *E. macrocarpa* have also been recorded (Griffin *et al.*, 1988; Hopper, 1995). In a study on the allozyme variation at 11 loci in 45 populations of fourteen species of *E.* ser. Curviptera, Sampson *et al.* (1999) found that while phylogenetic analysis of the molecular data supported continued recognition of several species, other species had populations displaying significant divergence in allozyme frequencies. The suggestion has also been raised that *E. youngiana* is a subspecies of *E. pyriformis* (Boomsma, 1972), however the majority of classifications place it as a separate species.

E. macrocarpa, *E. pyriformis* and *E. youngiana* exhibit similar floral morphology: all three have large flowers, where the hypanthium may measure up to 80 mm in diameter with a depth of 40 mm, and the style may measure up to 40 mm in length. Although there is currently no information regarding the stigma morphology of these species, *E. caesia*, from the same section, has a tapered or blunt stigma (Boland and Sedgley, 1986), and the gross morphology of these species indicates that this type of stigma may be constant for each. The styles of each species are of similar length (30 - 40 mm), negating the possibility of unilateral failure of hybridisation due to incompatible style to pollen tube length ratio (Gore *et al.*, 1990).

Differing style to pollen tube length ratios have been reported as a possible cause of failure to hybridise in a number of studies involving *Eucalyptus* species (Pryor, 1956; Beardsell *et al.*, 1979; Tibbits, 1988, 1989; Gore *et al.*, 1990). The study showed that depsite the non-isolation of flowers from contaminating pollen, a relatively high percentage of seedlings were hybrids, indicating that isolation may not be necessary if all remaining flowers are removed.

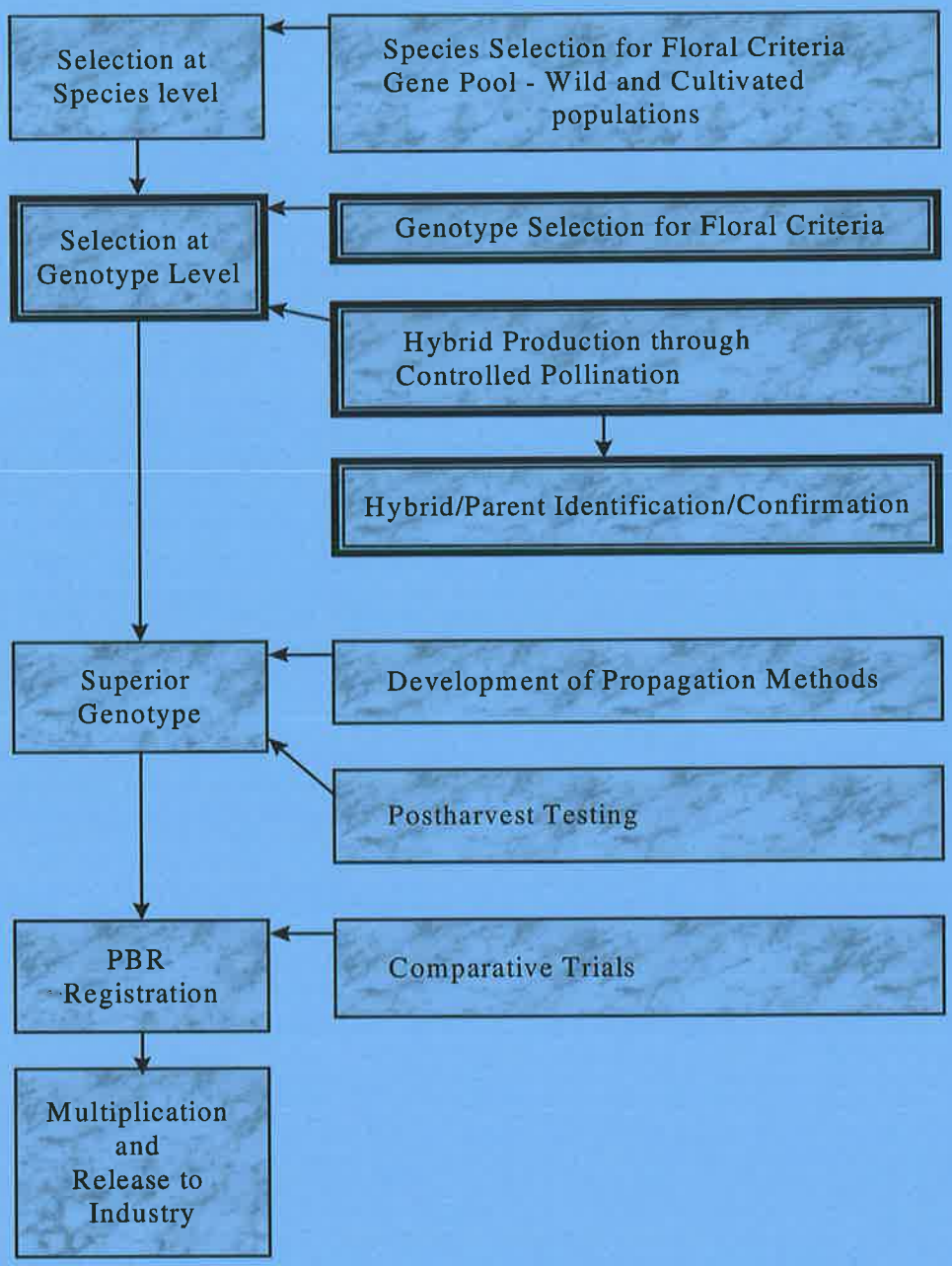
A high level of seedling mortality was observed in both open pollinated seed and seed produced by controlled pollination. Less than 43 % seed germination was recorded for any of the twelve crosses studied, and the number of seedlings surviving to three months of age ranged from 80% for *E. macrocarpa* x *E. macrocarpa* to 37.5% for *E. youngiana* x *E. youngiana*. Despite the high mortality, hybrid seedlings were more vigorous than parental seedlings, and there were few abnormalities observed.

RAPD analysis of the hybrid seedlings and the parent plants involved in this crossing program has confirmed the data from the morphological analysis (Neaylon, 1999), and provided evidence that some of the hybrids are extremes of the hybrid distribution or more likely contaminants. Two putative natural hybrids were included in the RAPD analysis for comparison (Plate 7.5).

Plate 7.5.
A: Putative hybrid between *E. pyriformis* and *E. youngiana* from the Waite Arboretum; B: Putative hybrid between *E. youngiana* and *E. macrocarpa* from the Waite Arboretum.

7.6. Conclusion

The aim of this study was to determine whether *E. macrocarpa*, *E. pyriformis* and *E. youngiana* would hybridise successfully by controlled pollination, generating hybrids. The results indicate that the three species concerned will hybridise, producing viable seed. The 166 seedlings from the interspecific cross seed, when measured for a range of leaf and stem characters at three different nodes, show strong evidence of intermediacy between parents, with 1.2% grouped with the male parent, 94.6% clustered between the parent species, and the remaining 4.2% with the female parent.



Ornamental eucalypt breeding cycle flow chart.

Areas covered by this chapter are highlighted.

Chapter Eight

Interspecific hybridisation: subgenus *Symphyomyrtus*, sections *Bisectaria* and *Adnataria*

8.1. Abstract

Eucalyptus gillii and *E. socialis* were used as the female parents in a controlled pollination program using pollen collected from sixteen species with desirable characters for floriculture. The 425 seedlings produced were measured at three months for fifteen seedling characters, and the results analysed using hierarchical and non-hierarchical methods. The UPGMA dendrograms showed 225 seedlings from *E. gillii* x *E. gardneri*, *E. gillii* x *E. kruseana*, *E. gillii* x *E. oldfieldii*, *E. gillii* x *E. polyanthemos*, *E. gillii* x *E. socialis*, *E. gillii* x *E. transcontinentalis*, *E. socialis* x *E. gardneri*, *E. socialis* x *E. gillii*, *E. socialis* x *E. kruseana*, *E. socialis* x *E. steedmanii* and *E. socialis* x *E. transcontinentalis* clustered with neither the male nor female parent seedlings, suggesting intermediacy. The remaining 200 seedlings, from *E. gillii* x *E. caesia*, *E. gillii* x *E. eremophila*, *E. gillii* x *E. gillii*, *E. gillii* x *E. orbifolia*, *E. gillii* x *sideroxylon*, *E. gillii* x *websteriana*, *E. gillii* x *E. youngiana*, *E. socialis* x *E. caesia*, *E. socialis* x *E. eremophila*, *E. socialis* x *E. macrocarpa*, *E. socialis* x *E. oldfieldii*, *E. socialis* x *E. orbifolia*, *E. socialis* x *E. sideroxylon*, *E. socialis* x *E. socialis*, *E. socialis* x *E. websteriana* and *E. socialis* x *E. youngiana* clustered with the female parent, and could be the result of self pollination. The multivariate analysis supported the UPGMA results, with the ordination point clusters remaining consistent with the dendrogram groupings. Crosses between closely related species showed a higher degree of success than those between distant crosses, as did those between species with similar flower size.

8.2. Introduction

The potential for natural hybridisation between species in the genus *Eucalyptus* L'Herit. has been discussed recently by Ladiges (1997), with respect to genetic isolation between subgenera, sections and some series, and also by Potts and Wiltshire (1997), regarding patterns of hybridisation and its evolutionary significance, and taxonomic, structural and physiological barriers to crossing. The topic was covered by Griffin *et al.* (1988), in their review of the patterns of natural and manipulated hybridisation, where it was determined that taxonomic affinity will greatly influence hybridisation success.

Eucalypts may belong to groups, and Pryor and Johnson (1971) proposed a classification into a hierarchy of groups of subgenera, sections, series, subseries, superspecies, species and subspecies, with groupings based on shared diagnostic morphological characteristics of the constituent taxa. Subgenera are reproductively isolated and the likelihood of a successful cross increases with closeness of taxonomic affinity i.e. intraseries crosses have a greater chance than interseries crosses, which have a greater chance than intersectional crosses (Griffin *et al.*, 1988). Some sections show greater affinity than others to crossing: Ellis *et al.* (1991) showed that *Bisectaria* and *Adnataria* have a higher chance of pollination success than intersectional crosses between *Bisectaria* and *Maidenaria*, *Exsertaria*, *Tingleria*, *Transversaria*, or *Dumaria*. Ellis *et al.* (1991) also found that wide hybridisations are more likely to fail pre-fertilisation than close hybridisations. Hybrids may exhibit heterosis (increased vigour) when compared to parents, including adaptive heterosis when the parents are at an optimal genetic distance, or abnormalities and reduced growth (dwarfing) due to inbreeding depression (with intraspecific crosses that are too close) or heterozygosity (crosses that are too distant) (Eldridge *et al.*, 1993; Martin, 1988). This has implications for improvement through crossing programs: potential crosses must be considered not only for the combination of desirable characters, but also for taxonomic affinities and possible

structural and physiological barriers to hybridisation.

Controlled pollination, or manipulated hybridisation, is a common technique used for the generation of interspecific hybrids for plant improvement and is widely used in the development of eucalypts for forestry (Potts *et al.*, 1992; van Wyk *et al.*, 1989). The technique was seen as a useful method to test natural hybrids, where putative crosses were replicated manually, and the seedlings measured for morphological characters (Pryor, 1950; 1951; 1952; 1956; 1957). Seedlings from manipulated crosses often show intermediate morphology, phenetic segregation in their open pollinated seedlings, and impaired reproductive capabilities compared to the parents (i.e. F2 breakdown) (Hopper *et al.*, 1978; Hopper, 1995).

The aim of the study was three-fold: to investigate controlled pollination as a method to produce hybrids with increased merit as ornamentals; to determine hybrid status through seedling morphological character analysis; and to assess the likelihood of successful crosses between species from different series in section *Bisectaria*, and between species from sections *Bisectaria* and *Adnataria*. Each species was selected for ornamental characters; specifically tree habit and vigour, floriferousness, inflorescence insertion and structure, flower size, shape and colour, leaf size, shape and colour. The taxonomic position of the species in relation to others in the program and accessibility of specimens were also important.

8.3. Materials and Methods

8.3.1. Plant material

The sixteen species used in the study were selected on the basis of one or more desirable ornamental characters, as well as their phylogenetic relationships to the female parent species. The species used are listed in Table 8.1, and were sourced from the Waite Arboretum and the Monarto Woodland.

8.3.2. Controlled pollination

The method of controlled pollination followed that of van Wyk (1977) and Moncur (1995),and described in detail in Chapter 6, section 6.3.3. Pollen was collected from a single representative from each male parent species over a twelve month period, and stored in the freezer at -20°C until required. Pollen was tested for viability prior to pollinations (refer section 6.3.3). Three plants from each of the female parent species were used, with between 50 and 150 flowers pollinated for each cross. Each umbel was tagged, and the pollination step repeated every three days. Every emasculated flower was pollinated at least twice. Flowers were not isolated. Capsules were harvested eight months after pollination, when most were swollen and woody. Each capsule was placed separately in a paper bag, and stored at 30°C until open (c. four weeks). Seed was extracted and each seed weighed and stored separately. Seed was planted, germinated and recorded in the method described in Chapter 6 (Section 6.3.3.).

8.3.3. Seed data analysis

Differences in the mean number of seeds produced per flower pollinated, mean seed weight for all seeds of that cross and germination rates were calculated .

Table 8.1. Species used in controlled pollinations. Based on Pryor and Johnson (1971) informal classification.

Crosses to					
E. gillii	***E. socialis***	Species used as male parents	Section	Series	Desirable characters
3[a]	3	*E. caesia* subsp. *caesia* Benth.	Bisectaria	Macrocarpae	medium pink flowers, glaucous buds, weeping small habit
4	4	*E. eremophila* (Diels) Maiden	Bisectaria	Occidentales	floriferous, pink flowers
3	4	*E. gardneri* Maiden	Bisectaria	Reduncae	blue foliage, highly floriferous
1	2	*E. gillii* Maiden	Bisectaria	Oleosae	grey foliage and buds, highly floriferous, long flowering season
4	4	*E. kruseana* F.Muell.	Bisectaria	Kruseanae	grey round foliage, small habit, long flowering season
3	3	*E. macrocarpa* Hook.	Bisectaria	Macrocarpae	very large red flowers, glaucous buds, leaves and flowers
3	3	*E. oldfieldii* F.Muell.	Bisectaria	Macrocarpae	medium cream flowers, round buds, small habit
3	3	*E. orbifolia* F.Muell.	Bisectaria	Macrocarpae	medium cream flowers, heart shaped leaves, glaucous buds
5	5	*E. polyanthemos* Schauer	Adnataria	Polyanthemae	numerous small terminal flowers, grey leaves
5	5	*E. pruinosa* Schauer	Adnataria	Pruinosae	numerous medium cream terminal flowers
5	5	*E. sideroxylon* Cunn. Ex Wools	Adnataria	Melliodorae	medium pink buds and flowers
2	1	*E. socialis* F.Muell. ex Miq.	Bisectaria	Oleosae	floriferous, glaucous buds, flowers and leaves
4	4	*E. steedmanii* C.Gardner	Bisectaria	Occidentales	yellow square buds and flowers, small neat habit
2	2	*E. transcontinentalis* Maiden	Bisectaria	Oleosae	numerous yellow buds and flowers
3	3	*E. websteriana* Maiden	Bisectaria	Macrocarpae	small cream flowers, heart shaped/round leaves, glaucous buds
3	3	*E. youngiana* F.Muell.	Bisectaria	Macrocarpae	large yellow - red flowers, green - red - purple buds

[a]1 intraspecific cross
2 intraseries cross
3 close interseries cross
4 distant interseries cross
5 intersectional cross

8.3.4. Seedling morphology

Each surviving seedling from controlled pollinations was measured for fifteen continuous, binary or ordered multistate leaf and stem characters on fully expanded leaves at the 3rd, 7th and 10th nodes above the cotyledons, and all measurements were conducted three months after germination (Table 8.2). In addition, open pollinated seed from the female parent trees and open pollinated seed sourced from other populations of the species used, was grown to ensure a broad representation of seedling morphology for each species. Between 40 and 80 seedlings from each parent species were measured three months after germination for the same characters. These scores were averaged to generate five representative individuals for each species, which were used in the analysis for comparison with the seedlings from controlled pollinations. Only leaf data for node 7 were used, as nodes 3 and 10 provided incomplete data sets. The invariant characters were leaf surface wax (LSW), leaf concolorous or discolorous (LEA), leaf smoothness (LSM) and stem smoothness (SSM).

8.3.5. Data analysis

The seedling morphological data were analysed using the PATN analysis package (Belbin, 1994) in order to determine the similarity between seedlings and parent species, using hierarchical (UPGMA) and non-hierarchical (multidimensional scaling, minimum spanning tree and principal canonical correlation) analysis.

Table 8.2. Characters and codes used in phenetic analysis (seedling), measured at node 3, 7 and 10, three months after germination (node 7 was used in the analysis due to incomplete data sets for nodes 3 and 10).

Code	Character	
LSH	Leaf shape	linear (0) lanceolate (1) ovate (2) cordate (3) orbicular (4)
LST	Leaf tip	acuminate (0) acute (1) mucronulate (2) obtuse (3) truncate (4)
LSB	Leaf base	attenuate (0) oblique (1) obtuse (2) cordate (3)
LLE	Leaf length	(mm)
LWI	Leaf width	(mm)
LSW	Leaf surface wax	shining (0) dull (1) glaucous (2) very glaucous (3)
LEA	Leaf surfaces	concolorous (0) discolorous (1)
LSM	Leaf surface	smooth (0) not smooth (1)
LPS	Leaf stalk	sessile (0) petiolate (1)
LPL	Leaf petiole length	(mm)
LPO	Leaf position	opposite (0) alternate (1)
LCO	Leaf colour	light green (0) green (1) grey green (2) blue green (3) dark green (4)
SSH	Stem shape	square (0) angular (1) round (2)
STE	Stem	single (0) branching (1)
SSM	Stem	smooth (0) not smooth (1)

8.4. Results

8.4.1. Seed data analysis

The mean number of seeds per flower was low for all crosses, including open pollinated flowers (Tables 8.3 and 8.4). Statistical analysis was not possible as only one female parent produced seed for both *E. gillii* and *E. socialis*. Seed weights were highly variable within crosses, however there was no correlation between seed weight and germination. There was no correlation between male parent seed weight and seed weight of crosses. All cotyledons resembled those of the female parent, with the exception of five *E. gillii* x *E. polyanthemos* seedlings whose cotyledons resembled those of *E. polyanthemos*, however these seedlings died before true leaves emerged. High levels of seedling mortality occurred in all *E. gillii* crosses soon after germination, while crosses with *E. socialis* as the female showed low seedling mortality.

Table 8.3. Summary of flower, capsule, seed and seedling data for interspecific, intraspecific and open pollination with *E. gillii* as the female parent. Data for one tree only, other two did not set seed.

Male parent	Number of flowers pollinated	Number of capsules with seed	Percent capsule set	Total seed	Mean number of seeds per flower pollinated[1]	Mean seed weight (mg)[2] (range)	Number of seeds planted	Number of seedlings		Percent seeds germinated[3]	Number of seedlings showing similarity to:		Number of seedlings intermediate between parents
								Germ.	Meas.		female parent	male parent	
E. caesia	119	6	5.0	6	0.05	1.45 (0.77-1.85)	6	6	6	100.0	6	0	0
E. eremophila	130	14	10.8	23	0.18	1.25 (0.26-1.72)	23	20	11	87.0	11	0	0
E. gardneri	162	29	17.9	64	0.40	1.23 (0.19-1.71)	64	52	42	81.3	0	0	42
E. gillii (intra)	100	62	62.0	122	1.22	1.11 (0.33-1.71)	122	118	68	96.7	68	0	0
E. kruseana	128	20	15.6	66	0.52	0.98 (0.17-1.49)	66	56	38	84.8	0	0	38
E. macrocarpa	190	8	4.2	12	0.06	0.64 (0.16-1.25)	12	5	0	41.7	0	0	0
E. oldfieldii	139	35	25.2	106	0.76	1.04 (0.09-1.61)	106	62	54	58.5	1	0	53
E. orbifolia	141	36	25.5	135	0.96	1.35 (0.33-2.03)	135	117	10	86.7	9	0	1
E. polyanthemos	100	18	18.0	48	0.48	1.32 (0.91-1.68)	48	21	6	73.8	0	0	6
E. pruinosa	60	0	0	0	0.00	0.00	0	0	0	0	0	0	0
E. sideroxylon	118	1	0.8	1	0.09	1.30	1	1	1	100	1	0	0
E. socialis	110	30	37.3	91	0.83	1.02 (0.10-2.46)	91	53	38	58.2	0	0	38
E. steedmanii	116	1	0.9	2	0.02	0.24 (0.22-0.26)	2	0	0	0.0	0	0	0
E. transcontinentalis	79	26	32.9	64	0.81	1.29 (0.30-1.64)	64	47	35	73.4	10	0	25
E. websteriana	136	4	2.9	13	0.14	0.72 (0.15-1.20)	13	8	1	61.5	1	0	0
E. youngiana	189	16	8.5	39	0.21	0.78 (0.16-1.99)	39	12	4	30.8	4	0	0
open	50	20	40.0	22	0.44	1.33 (0.65-1.65)							
Total	2067	326		814			792	578	314		111	0	203

[1] total number of seed from all capsules of that cross divided by the total number of flowers pollinate for that cross.
[2] weight of all seeds in a capsule divided by the number of seeds in that capsule.
[3] number of seeds germinated divided by the number of seeds planted, multiplied by 100/1.

Table 8.4. Summary of flower, capsule, seed and seedling data for interspecific, intraspecific and open pollination with *E. socialis* as the female parent. Data for one tree only, other two did not set seed.

Male parent	Number of flowers pollinated	Number of capsules with seed	Percent capsule set	Total seed	Mean number of seeds per flower pollinated[1]	Mean seed weight (mg)[2] (range)	Number of seeds planted	Number of seedlings		Percent seed germinated[3]	Number of seedlings showing similarity to:		Number of seedlings intermediate
								Germ.	Meas.		female parent	male parent	
E. caesia	73	20	27.4	26	0.36	0.98 (0.25-1.45)	26	8	6	30.8	6	0	0
E. eremophila	65	10	6.5	18	0.28	0.96 (0.19-1.30)	18	7	7	38.9	7	0	0
E. gardneri	100	31	31.0	49	0.49	0.86 (0.12-1.33)	49	10	5	20.4	2	0	3
E. gillii	63	10	6.3	20	0.32	0.86 (0.13-1.27)	20	5	5	25.0	1	0	4
E. kruseana	62	17	27.4	28	0.45	1.04 (0.21-1.67)	28	10	8	35.7	5	0	3
E. macrocarpa	58	3	5.2	5	0.09	0.76 (0.15-1.15)	5	2	2	40.0	2	0	0
E. oldfieldii	100	29	29.0	55	0.55	0.91 (0.33-1.30)	55	12	10	21.8	10	0	0
E. orbifolia	43	9	20.9	13	0.30	0.95 (0.18-1.47)	13	5	5	38.5	5	0	0
E. polyanthemos	63	28	44.4	49	0.78	0.94 (0.23-1.74)	49	11	9	22.4	9	0	0
E. pruinosa	54	0	0.0	0	0	0.00	0	0	0	0.0	0	0	0
E. sideroxylon	90	10	11.1	21	0.23	1.01 (0.64-1.52)	21	5	3	23.8	3	0	0
E. socialis intra	48	10	20.8	18	0.38	1.08 (0.38-1.76)	18	11	10	61.1	10	0	0
E. steedmanii	25	19	76.0	38	1.52	0.90 (0.21-1.36)	38	15	9	39.5	5	0	4
E. transcontinentalis	44	32	72.7	62	1.41	0.93 (0.17-1.46)	62	12	12	19.4	3	0	9
E. websteriana	100	67	67.0	142	1.42	1.00 (0.25-1.73)	142	20	18	14.2	18	0	0
E. youngiana	78	22	28.2	41	0.53	0.83 (0.23-1.27)	41	2	2	4.8	2	0	0
Open	50	48	96.0	96	2.0	1.18 (0.68-1.35)							
Total	1116	365		681			585	135	111		88	0	23

[1] total number of seed from all capsules of that cross divided by the total number of flowers pollinate for that cross.
[2] weight of all seeds in a capsule divided by the number of seeds in that capsule.
[3] number of seeds germinated divided by the number of seeds planted, multiplied by 100/1.

8.4.2. Seedling morphology analysis

8.4.2.1. E. gillii as the female parent

The results of the hierarchical and non-hierarchical analyses for each of the thirteen successful crosses with *E. gillii* as the female parent are summarised in Table 8.5. Numbers of seedlings measured for each cross varied considerably (Table 8.3), from one seedling only for crosses with *E. sideroxylon* and *E. websteriana*, fewer than twelve for crosses with *E. caesia, E. eremophila, E. orbifolia*, *E. polyanthemos* and *E. youngiana*, and between 35 and 54 for the remaining crosses. The intraspecific cross of *E. gillii* produced 68 seedlings. Crosses with *E. pruinosa*, *E. steedmanii* and *E. macrocarpa* did not produce seedlings. Seedling comparisons were made with open pollinated seed from the female parent tree, resulting in less apparent variation within the intraspecific cross.

On each of the thirteen dendrograms, seedlings and parent species were arranged in two or three clusters. All seedlings from crosses with *E. caesia* (Figure 8.1), *E. eremophila* (Figure 8.2), *E. sideroxylon* (Figure 8.3), *E. websteriana* (Figure 8.4) and *E. youngiana* (Figure 8.5) as the male parent, clustered with *E. gillii.* A portion of seedlings from crosses between *E. gillii* and *E. orbifolia* (9) (Figure 8.6), *E. transcontinentalis* (10) (Figure 8.7) and *E. oldfieldii* (1) (Figure 8.8) clustered with *E. gillii*, with the remaining seedlings from each cross forming distinct groups, suggesting intermediacy. All seedlings from crosses with *E. gardneri* (Figure 8.9), *E. kruseana* (Figure 8.10), *E. polyanthemos* (Figure 8.11) and *E. socialis* (Figure 8.12) clustered between the female and male parent clusters, also suggesting intermediacy. The *E. gillii* x *E. gillii* cross produced the highest number of seedlings, with all seedlings clustering with the female parent (Figure 8.13).

The MDS scatter plots for each cross reflected the dendrogram groupings, with seedlings from crosses with *E. caesia* (Figure 8.14), *E. eremophila* (Figure 8.15), *E. sideroxylon* (Figure

8.16), *E. websteriana* (Figure 8.17) and *E. youngiana* (Figure 8.18) as the male parent, grouped with the female parent, *E. gillii.* Seedlings from crosses with *E. gardneri* (Figure 8.19), *E. kruseana* (Figure 8.20), *E. polyanthemos* (Figure 8.21), and *E. socialis* (Figure 8.22) were positioned between the parents. Seedlings from crosses with *E. oldfieldii* (Figure 8.23), *E. orbifolia* (Figure 8.24) and *E. transcontinentalis* (Figure 8.25) clustered either between both parents or with *E. gillii.* Seedlings from *E. gillii* x *E. gillii* were randomly scattered across the plot (Figure 8.26). The stress values for all SSH analyses were between 1.17 and 10.80%, and well below the accepted 20 % distortion value.

Minimum spanning tree analysis for the crosses between *E. gillii* and *E. oldfieldii*, *E. sideroxylon* and *E. websteriana*, showed the female parent group linked to the male parent group, indicating that the seedlings bear little resemblance to the male parent (MST value: min. 0.5056 max. 0.6058). The other crosses linked the male parent group to the seedlings at a distance, indicating a low level of similarity between them (MST value: min. 0.2979 max. 0.7340). Two exceptions were seedlings from the cross with *E. socialis* (MST value 0.1303), and the cross with *E. transcontinentalis* (0.2258), which were linked relatively closely to the male parent species. The highest MST value linking hybrids to female parents was 0.1035, and the lowest was 0.037, indicating high levels of similarity between all seedlings and *E. gillii.* The same data for the female parent group was used in each analysis, and the mean MST values between female individuals ranged from 0.037 to 0.0867. The mean MST value linking individuals for each male parent group was between 0.0126 and 0.0927, indicating that each group was very uniform. *E. websteriana* and *E. youngiana* were the two exceptions, with values of 0.1210 and 0.1370 respectively, indicating a moderate level of variability within each group. One individual, *E. gillii* x *E. kruseana* #1 was distinct from both female (MST value 0.2248) and male parent groups (0.4574), indicating a high degree of dissimilarity to both parent species. Principal canonical correlation analysis (Table 8.6)

showed most characters to be significantly correlated to the MDS ordination coordinates (Appendix 2.1: Figures 8.57 - 8.69).

Table 8.5. Summary of data for *E. gillii*: Gower UPGMA dendrogram, MDS plot and MST analysis.
S = seedlings from cross; M = male; F = female.

Cross	#S	# Dend clusters	# S in female cluster	# S in male cluster	# S in S cluster	# MDS bunch[a]	MDS SSH stress	Mean MST of female bunch[b]	MST female to S[c]	S link to female[d] S	S link to female[d] F	Mean MST of male bunch[e]	MST male to S[f]	S link to male[g] S	S link to male[g] M	Mean MST of S bunch[h]
E. caesia	6	2	6	0	0	2	1.55%	0.0555	0.0380	4	4	0.0339	0.7340	4	4	0.0107
E. eremophila	11	2	11	0	0	2	1.71%	0.0370	0.0154	many		0.0370	0.6802	6	4	
E. gardneri	42	3	0	0	42	3	9.49%	0.0487	0.0967	31	1	0.0486	0.2979	17	5	0.0267
									0.0934	9	4					
E. gillii (intra)	68	1	68	0	0	scat	7.33%									
E. kruseana	38	3	0	0	38	3	2.97%	0.0533	0.1035	35	2	0.0854	0.4570	1	5	0.0128
E. oldfieldii	54	2	1	0	53	3	6.41%	0.0778	0.0906	1	5	0.0463	0.5056	F1	5	0.0343
									0.1387	3	5			F1	5	
E. orbifolia	10	3	9	0	1	3	2.80%	0.0595	0.0527	10	4	0.0709	0.5211	1	1	0.0628
E. polyanthemos	6	3	0	0	6	3	2.82%	0.0642	0.1201	5	4	0.0927	0.4633	6	3	0.0516
E. sideroxylon	1	2	1	0	0	2	1.17%	0.0670	0.0079	5	6	0.0677	0.5572	F1	4	
E. socialis	38	3	0	0	38	3	10.80%	0.0867	0.1940	4	2	0.0635	0.1303	20	4	0.0544
E. transcontinentalis	35	3	10	0	25	3	8.37%	0.0789	0.1150	6	4	0.0126	0.2258	11	1	0.0684
									0.1235	5	5					
									0.1070	7	5					
									0.2438	17	1					
E. websteriana	1	2	1	0	0	2	2.17%	0.0665	0.0152	1	4	0.1219	0.6058	4	5	
E. youngiana	4	2	4	0	0	2	4.17%	0.0598	0.1392	4	4	0.1370	0.4745	1	2	0.0257
Total	314		111	0	203				0.0336	2	5					

[a] 'bunch' refers to a discrete cluster of similar individuals
[b] Mean of all MST values connecting female parent individuals
[c] MST link connecting female bunch to seedlings
[d] Seedlings (code number from dendrogram) linking to female (code number from dendrogram)
[e] Mean of all MST values connecting male parent individuals
[f] MST link connecting male group to seedlings
[g] Seedlings (code number from dendrogram) linking to male (code number from dendrogram)
[h] Mean of all MST values connecting seedlings

Table 8.6. Summary of measured variant (Y) and invariant (-) seedling characters (Table 8.2.) and significance (*) from correlation coefficient† (r^2) for *E. gillii*. [a]Characters significantly correlated (*) if greater than (r^2), calculated from PCC analysis, [b]characters invariant (-) if identical for all individuals in that cross.

E. gillii	r^{2a}	LSH	LST	LSB	LLE	LWI	LSW	LEA	LSM	LPS	LPL	LPO	LCO	SSH	STE	SSM	Total
E. caesia	0.65	Y*	-[b]	Y*	Y*	Y*	Y*	-	-	Y*	Y*	Y*	Y*	-	-	-	9/15
E. eremophila	0.60	Y*	Y*	Y*	Y*	Y*	-	Y*	Y*	Y*	Y*	Y*	Y*	Y*	Y*	Y*	14/15
E. gardneri	0.39	Y*	Y*	Y*	Y*	Y*	-	-	-	Y*	Y*	Y*	Y*	Y	Y	-	11/15
E. gillii intra	0.31	Y*	Y*	Y*	Y*	Y*	-	-	-	-	-	-	-	-	Y	-	6/15
E. kruseana	0.41	Y*	Y*	Y*	Y*	Y*	Y*	Y*	-	Y*	Y*	Y*	Y*	-	-	Y*	12/15
E. oldfieldii	0.36	Y*	Y*	Y*	Y*	Y*	-	-	-	Y*	Y*	Y*	Y*	Y*	Y*	Y*	12/15
E. orbifolia	0.59	Y*	Y*	Y*	Y*	Y*	-	-	-	Y*	Y*	-	Y*	-	Y*	Y*	10/15
E. polyanthemos	0.64	Y*	Y*	Y*	Y*	Y*	-	-	-	Y*	Y*	Y*	Y*	-	-	-	9/15
E. sideroxylon	0.76	Y*	Y*	Y*	Y*	Y*	-	Y*	-	Y*	Y*	Y*	Y*	Y*	Y*	-	12/15
E. socialis	0.39	Y*	Y*	Y*	Y*	Y*	-	-	-	-	-	Y*	Y*	Y*	Y*	-	9/15
E. transcontinentalis	0.39	Y*	Y*	Y*	Y*	Y*	-	-	-	-	-	-	Y*	Y*	-	-	7/15
E. websteriana	0.76	Y*	Y*	Y	Y*	Y*	-	Y*	-	Y*	Y*	Y*	Y*	Y*	Y*	-	12/15
E. youngiana	0.70	Y*	Y*	Y*	Y*	Y*	Y*	Y*	-	Y*	Y*	Y*	Y*	Y*	Y*	-	13/15

Correlation coefficient† : showing the fit (r^2) of the individual character vectors in the ordination space.

Table 8.7. Seedling morphological characters of seedlings from crosses between *E. gillii* and other species. Measurements in mm or presented as mean (range). [a]Characters unique or highly descriptive for that taxon according to PCC (Figs 8.28-8.40), [b]characters significantly correlated[†] (r^2) to individuals. Hybrid seedling crosses shaded.

	E. gillii	*E. gillii* x *E. caesia*	*E. caesia*	*E. gillii* x *E. eremophila*	*E. eremophila*	*E. gillii* x *E. gardneri*	*E. gardneri*
LSH	lanceolate or ovate, rarely linear [ab]	ovate	cordate [ab]	lanceolate or ovate	ovate or lanceolate [ab]	ovate or lanceolate	ovate or lanceolate [b]
LST	acute, rarely obtuse or acuminate [ab]	acute	acute	acute	obtuse [ab]	acute or mucronulate	acute, mucronulate or acuminate [b]
LSB	attenuate, obtuse or cordate [ab]	cordate	cordate, rarely obtuse [b]	obtuse or acuminate, rarely cordate	obtuse [b]	obtuse or attenuate	obtuse, oblique or attenuate [b]
LLE	27.4 (26.1-28.8) [ab]	29.0 (26.0-31.0)	46.6 (26.0-67.8) [ab]	30.2 (26.0-35.0)	39.6 (38.6-40.5) [ab]	29.3 (24.5-35.8)	53.1 (40.7-74.3) [ab]
LWI	16.0 (10.8-28.1) [ab]	19.2 (16.5-24.5)	52.1 (33.3-66.8) [ab]	15.3 (11.6-21.0)	23.7 (21.6-26.4) [ab]	16.3 (10.4-22.8)	26.8 (10.9-41.2) [ab]
LSW	dull or glaucous [a]	dull	shining [b]	dull	dull	dull	dull
LEA	concolorous	concolorous	concolorous	concolorous, rarely discolorous	concolorous [b]	concolorous	concolorous
LSM	smooth	smooth	smooth	smooth	not smooth	smooth	smooth
LPS	sessile	sessile	petiolate [ab]	sessile	petiolate [ab]	sessile	petiolate [ab]
LPL	0.0	0.0	11.9 (6.6-20.9) [ab]	0.0	10.0 (9.4-10.8) [ab]	0.0	8.4 (3.1-13.2) [ab]
LPO	opposite	opposite	alternate [ab]	opposite	alternate [ab]	opposite	alternate [ab]
LCO	green	green	dark green [ab]	green	grey green [ab]	blue green	blue green [b]
SSH	round, rarely angular	round	round	round	round	round	round
STE	branching	branching	branching	branching	branching	branching, rarely round	branching
SSM	smooth	smooth	smooth	smooth	not smooth	smooth	smooth

Continued

Table 8.7. Continued

	E. gillii x *E. gillii*	*E. gillii* x *E. kruseana*	*E. kruseana*	*E. gillii* x *E. oldfieldii*	*E. oldfieldii*	*E. gillii* x *E. orbifolia*	*E. orbifolia*
LSH	lanceolate or ovate	lanceolate or ovate	cordate or orbicular [b]	ovate or lanceolate	lanceolate [b]	ovate	orbicular, rarely cordate [ab]
LST	acute	acute, rarely obtuse	obtuse or mucronulate [ab]	acute or obtuse	mucronulate [ab]	acute, rarely obtuse	truncate or mucronulate [b]
LSB	attenuate, oblique, obtuse or cordate	attenuate, rarely acute, obtuse	obtuse or cordate [ab]	attenuate or obtuse	attenuate [b]	obtuse, cordate or attenuate	obtuse, cordate or attenuate [b]
LLE	28.0 (21.3-37.5)	29.6 (23.1-36.0)	23.7 (22.0-26.1) [b]	31.4 (25.1-37.8)	42.3 (38.0-45.1) [b]	30.1 (27.5-32.0)	23.9 (17.0-29.3) [b]
LWI	14.7 (9.0-20.5)	16.5 (9.4-20.8)	24.2 (22.1-26.8) [b]	17.1 (9.1-23.0)	16.4 (10.1-21.5) [b]	18.5 (13.5-23.0)	30.1 (19.7-41.8) [b]
LSW	dull	dull	dull or glaucous [ab]	dull	dull	dull	dull
LEA	concolorous	concolorous	discolorous [ab]	concolorous	concolorous	concolorous	concolorous
LSM	smooth	smooth	not smooth	smooth	smooth	smooth	smooth
LPS	sessile	sessile	petiolate [ab]	sessile	petiolate [ab]	sessile	petiolate [ab]
LPL	0.0	0.0	0.9 (0.7-1.5) [ab]	0.0	4.7 (2.5-6.1) [ab]	0.0	7.0 (2.9-10.8) [ab]
LPO	opposite	opposite	opposite [b]	opposite	alternate [ab]	opposite	opposite
LCO	green	blue green	blue green [b]	blue green, rarely grey green or green	grey green, blue green or green [b]	grey green, blue green or green	blue green [b]
SSH	round	round	angular	round	angular [ab]	angular	round
STE	branching	branching	branching	branching	branching [ab]	branching	single or branching [b]
SSM	smooth	smooth	not smooth [ab]	smooth	not smooth [ab]	smooth	not smooth [ab]

Continued

Table 8.7. Continued

	E. gillii x *E. polyanthemos*	*E. polyanthemos*	*E. gillii* x *E. sideroxylon*	*E. sideroxylon*	*E. gillii* x *E. socialis*	*E. socialis*
LSH	ovate or lanceolate	orbicular [ab]	lanceolate	lanceolate or ovate [b]	ovate or lanceolate	lanceolate, rarely ovate [b]
LST	acute	truncate or acute [ab]	acute	acute, sometimes obtuse [b]	acute, rarely obtuse or acuminate	acute [b]
LSB	obtuse	obtuse, cordate or acute [ab]	obtuse	attenuate, rarely obtuse [b]	attenuate, cordate rarely obtuse	attenuate or obtuse [b]
LLE	28.8 (27.0-29.5)	37.5 (31.0-47.1) [ab]	25.0	58.9 (43.6-76.9) [ab]	31.1 (12.1-25.9)	31.3 (20.3-38.8) [ab]
LWI	15.9 (13.5-19.5)	47.9 (31.3-68.6) [ab]	12.0	21.7 (9.9-33.3) [ab]	18.8 (12.1-25.9)	12.5 (5.9-30.4) [b]
LSW	dull	dull	dull	dull	dull	dull
LEA	concolorous	concolorous	concolorous	discolorous [ab]	concolorous	concolorous
LSM	smooth	smooth	smooth	smooth	smooth	smooth
LPS	sessile	petiolate [b]	sessile	petiolate [ab]	sessile	sessile
LPL	0.0	14.6 (9.0-20.0) [ab]	0.0	5.0 (2.0-8.7) [ab]	0.0	0.0
LPO	opposite	alternate [ab]	opposite	alternate [ab]	opposite	opposite or alternate [b]
LCO	blue green	blue green [ab]	green	grey green [ab]	blue green	blue green [ab]
SSH	round	round	round	angular [ab]	square, sometimes angular or round	square [b]
STE	branching	branching	branching	branching, rarely single [ab]	branching, rarely single	branching [b]
SSM	smooth	smooth	smooth	smooth	smooth	smooth

Continued

Table 8.7. Continued

	E. gillii x *E. transcontinentalis*	*E. transcontinentalis*	*E. gillii* x *E. websteriana*	*E. websteriana*	*E. gillii* x *E. youngiana*	*E. youngiana*
LSH	ovate, sometimes lanceolate, rarely linear or cordate	linear [b]	ovate	cordate or orbicular [ab]	ovate or lanceolate	ovate or cordate [b]
LST	obtuse, sometimes acute	obtuse [ab]	acute	truncate or obtuse [ab]	acute	mucronulate, acute or acuminate [ab]
LSB	attenuate or cordate, rarely obtuse	obtuse [b]	obtuse	obtuse, mucronulate or acuminate	obtuse	obtuse or attenuate [ab]
LLE	31.0 (20.7-39.8)	18.3 (16.6-21.2) [b]	27.5	22.2 (13.8-27.5) [ab]	31.5 (30.0-32.3)	59.9 (33.0-72.1) [b]
LWI	15.5 (6.8-25.3)	5.9 (5.2-7.1) [b]	16.5	25.0 (15.7-33.4) [ab]	15.2 (10.0-19.7)	30.0 (15.8-45.6) [b]
LSW	dull	dull	dull	dull	dull	dull or shining [ab]
LEA	concolorous	concolorous	concolorous	concolorous [b]	concolorous	concolorous [b]
LSM	smooth	smooth	smooth	smooth	smooth	smooth
LPS	sessile	sessile	sessile	petiolate [ab]	sessile	petiolate [b]
LPL	0.0	0.0	0.0	3.0 (1.2-4.7) [b]	0.0	9.9 (5.7-14.4) [b]
LPO	opposite	opposite	opposite	opposite or alternate [b]	opposite	alternate [b]
LCO	blue green, sometimes green, rarely green or dark green	blue green [ab]	light green	dark green, blue green, light green or green [ab]	blue green or grey green	dark green, rarely green [ab]
SSH	square, rarely angular or round	square [b]	round	round [b]	round	angular [b]
STE	branching	branching	branching	branching, rarely single [ab]	branching	single [b]
SSM	smooth	smooth	smooth	not smooth	smooth	smooth

8.4.2.2. *E. socialis* as the female parent

The results of the hierarchical and non-hierarchical analyses for each of the fifteen successful crosses with *E. socialis* as the female parent are summarised in Table 8.8. Numbers of seedlings measured for each cross ranged from two to eighteen, with most under ten (Table 8.4). The *E. socialis* x *E. socialis* cross produced ten seedlings. The cross with *E. pruinosa* did not produce seed. Seed from a commercial seed source were grown, in addition to seed from the female parent tree, as the parent seedling comparators, and the variation displayed reflects the naturally high levels of variability within this species.

Dendrograms from nine of the fifteen crosses divided the seedlings into two clusters: female parent with all seedlings, and all male individuals (Figures 8.27 to 8.35). Dendrograms from crosses between *E. socialis* and *E. gardneri* (Figures 8.36), *E. gillii* (Figure 8.37), *E. kruseana* (Figure 8.38), *E. steedmanii* (Figure 8.39), and *E. transcontinentalis* (Figure 8.40) were divided into three clusters, with around half the seedlings for each cross clustered separately to either parent, and the other half clustered with the female parent. The male parent seedlings were always clustered together. *E. socialis* x *E. socialis* seedlings were clustered with the female parent (Figure 8.41).

The MDS plots reflected the dendrogram groupings, with nine crosses showing two groups, one containing all female parent seedlings and seedlings, the other containing all male parent seedlings (Figures 8.42 to 8.50). The five interspecific crosses between *E. socialis* and *E. gardneri* (Figure 8.51), *E. gillii* (Figure 8.52), *E. kruseana* (Figure 8.53), *E. steedmanii* (Figure 8.54) and *E. transcontinentalis* (Figure 8.55), placed all or some of the seedlings between the parent groups. The plot of *E. socialis* x *E. socialis* (Figure 8.56), scattered the seedlings across the plot, with four groups apparent. The stress values for all SSH analyses were very low, between 3.66 and 7.30%.

The MST linkages gave finer resolution to the scatter plots. In the case of the seedlings of *E. socialis* x *E. caesia*, *E. socialis* x *E. kruseana*, *E. socialis* x *E. sideroxylon*, and *E. socialis* x *E. steedmanii*, the male parents were linked to *E. socialis* #1 (MST value range 0.3582 - 0.5043). For *E. socialis* x *E. transcontinentalis* (MST value 0.2418) and *E. socialis* x *E. youngiana* (MST value 0.4039), the male parent linked to *E. socialis* #2. These results indicate that the seedlings, at this stage, bear low resemblance to the male parent seedlings. The remaining crosses link between seedlings and male parent, with a high MST value (range 0.3771-0.5273). *E. socialis* x *E. gillii* (MST value 0.1976) shows the closest link between seedling #3 and *E. gillii*, indicating a relatively high similarity between them. The same data for the female parent group were used in each analysis, and the mean MST values between female individuals are between 0.0596 and 0.1831, indicating a moderate level of variability within the group. The mean MST value linking individuals for each male parent group is between 0.0125 and 0.0881, indicating that each group is very uniform. *E. youngiana* was the exception, with a value of 0.1502, indicating a moderate level of variability within the group. Principal canonical correlation analysis (Table 8.9) showed most characters to be significantly correlated to the MDS ordination coordinates (Appendix 2.2.: Figures 8.70 - 8.84).

Table 8.8. Summary data for *E. socialis*: UPGMA dendrogram, MDS plot and MST analysis
S = seedlings from cross; M = male; F = female

Cross	#S	# Dend clusters	# S in female cluster	# S in male cluster	# S in S cluster	# MDS bunch[a]	MDS SSH stress	Mean MST of female bunch[b]	MST female to S[c]	S link to female[d] S	F	Mean MST of male bunch[e]	MST male to S[f]	S link to male[g] S	M	Mean MST of S bunch[h]
E. caesia	6	2	6	0	0	2	4.66%	0.1567	0.1022	all S to F		0.0372	0.4875	F1	2	0.0622
E. eremophila	7	2	7	0	0	2	4.37%	0.1104	0.1303	all S to F		0.0125	0.5273	6	3	0.0459
E. gardneri	5	3	2	0	3	3	4.59%	0.1257	0.1679	all S to F		0.0579	0.3813	2	3	0.0910
E. gillii	5	3	1	0	4	3	6.42%	0.1375	0.1811	all S to F		0.0656	0.1976	3	3	0.3096
E. kruseana	8	3	5	0	3	3	5.65%	0.1831	0.1158	all S to F		0.0562	0.3582	F1	1	0.1095
E. macrocarpa	2	2	2	0	0	2	3.66%	0.1446	0.1431	2	4	0.0830	0.3994	2	1	0.1182
E. oldfieldii	10	2	10	0	0	2	5.22%	0.1457	0.0865	all S to F		0.0318	0.5498	5	5	0.1081
E. orbifolia	5	2	5	0	0	2	3.79%	0.1545	0.0888	all S to F		0.0500	0.3771	2	2	0.1643
E. polyanthemos	9	2	9	0	0	2	4.78%	0.0596	0.1233	all S to F		0.0611	0.4526	F1	3	0.0973
E. sideroxylon	3	2	3	0	0	2	5.90%	0.1314	0.2251	all S to F		0.0533	0.5043	F1	4	0.0
E. socialis (intra)	10	1	10	0	0	4	6.50%	0.0	0. 0	all S to F		0.0	0.0	0	0	0.0
E. steedmanii	9	3	5	0	4	3	6.08%	0.0863	0.1702	all S to F		0.0881	0.4108	F1	5	0.0946
E. transcontinentalis	12	3	3	0	9	3	6.77%	0.1507	0.3073	4	4	0.0488	0.2418	F2	1	0.0854
									0.1128	2	3					
E. websteriana	18	2	18	0	0	2	7.30%	0.0902	0.1526	5	5	0.0295	0.4150	9	5	0.0697
									0.0551	18	2					
E. youngiana	2	2	2	0	0	2	5.37%	0.1138	0.1787	1	3	0.1502	0.4039	F2	2	0.0
									0.1875	1	5					
Total	110		89	0	22											

[a] 'bunch' refers to a discrete cluster of similar individuals
[b] Mean of all MST values connecting female parent individuals
[c] MST link connecting female group to seedlings
[d] Seedlings (code number from dendrogram) linking to female (code number from dendrogram)
[e] Mean of all MST values connecting male parent individuals
[f] MST link connecting male group to seedlings
[g] Seedlings (code number from dendrogram) linking to male (code number from dendrogram)
[h] Mean of all MST values connecting seedlings

Table 8.9. Summary of measured variant (Y) and invariant (-) seedling characters (Table 8.2) and significance (*) from correlation coefficient† (r^2) for *E. socialis*. [a]Characters significantly correlated (*) if greater than (r^2), calculated from PCC analysis, [b]characters invariant (-) if identical for all individuals in that cross.

E. socialis	r^2	LSH	LST	LSB	LLE	LWI	LSW	LEA	LSM	LPS	LPL	LPO	LCO	SSH	STE	SSM	Total
E. caesia	0.66	Y*	Y*	Y*	Y*	Y*	Y*	-	-	Y*	Y*	Y*	Y*	Y*	Y*	-	12/15
E. eremophila	0.64	-	Y*	Y*	Y*	Y*	Y*	-	Y*	Y*	Y*	Y*	Y*	Y*	Y*	Y*	13/15
E. gardneri	0.67	-	Y*	Y*	Y*	Y*	Y*	-	-	Y*	Y*	Y*	Y	Y*	Y*	-	11/15
E. gillii	0.67	Y*	Y*	Y*	Y*	Y*	Y*	-	-	Y*	Y*	-	Y*	Y*	Y*	-	11/15
E. kruseana	0.63	Y*	Y*	Y	Y*	Y*	Y*	Y*	-	Y*	Y	Y*	Y*	Y*	Y*	Y*	14/15
E. macrocarpa	0.75	-	Y	Y*	Y*	Y*	Y*	-	-	Y*	Y*	Y*	Y*	Y*	Y*	Y*	13/15
E. oldfieldii	0.61	Y*	Y*	Y*	Y*	Y*	Y*	-	-	Y*	Y*	Y*	Y	Y*	Y*	Y*	13/15
E. orbifolia	0.69	Y*	Y*	Y*	Y*	Y*	Y	-	-	Y*	Y*	Y*	Y	Y*	Y*	Y*	13/15
E. polyanthemos	0.61	Y*	Y*	Y*	Y*	Y*	Y*	-	-	Y*	Y*	Y*	Y*	Y*	Y*	-	12/15
E. sideroxylon	0.72	Y*	Y*	Y*	Y*	Y*	Y*	Y*	-	Y*	Y*	Y*	Y*	Y*	Y*	-	13/15
E. socialis intra	0.59	Y*	Y*	Y*	Y*	Y*	Y*	-	-	Y*	Y*	Y*	Y*	Y*	Y*	-	12/15
E. steedmanii	0.62	Y*	Y*	Y*	Y*	Y*	Y*	-	Y	Y*	Y*	Y*	Y*	Y*	Y*	Y*	14/15
E. transcontinentalis	0.61	Y*	Y*	Y*	Y*	Y*	Y	-	-	Y*	Y*	Y*	Y*	Y*	Y*	-	12/15
E. websteriana	0.52	Y*	Y*	Y*	Y*	Y*	Y*	Y*	-	Y*	Y*	Y	Y*	Y*	Y*	Y*	14/15
E. youngiana	0.73	Y*	Y*	Y*	Y*	Y*	Y	-	-	Y*	Y*	Y*	Y*	Y*	Y	-	12/15

Correlation coefficient† : showing the fit (r^2) of the individual character vectors in the ordination space.

Table 8.10. Seedling morphological characters of seedlings from *E. socialis* and putative male parent species. Measurements in mm or presented as mean (range). [a]Characters unique or highly descriptive for that taxon according to PCC (Figures 8.71-8.85), [b]characters significantly correlated[†] (r^2) to individuals. Hybrid seedling crosses shaded.

	E. socialis	*E. socialis* x *E. caesia*	*E. caesia*	*E. socialis* x *E. eremophila*	E. *eremophila*	*E. socialis* x *E. gardneri*	*E. gardneri*	*E. socialis* x *E. socialis*
LSH	lanceolate, rarely ovate [ab]	ovate, rarely lanceolate	cordate [ab]	ovate	ovate or lanceolate	ovate	ovate or lanceolate	ovate, rarely lanceolate
LST	acute [ab]	acute, rarely obtuse	acute [b]	acute, mucronulate or obtuse	obtuse [b]	obtuse or acute	acute, mucronulate or acuminate [b]	obtuse, acute, rarely mucronulate
LSB	attenuate or obtuse [ab]	obtuse, rarely cordate	cordate, rarely obtuse [b]	obtuse, cordate or attenuate	obtuse [ab]	obtuse or cordate	obtuse, oblique or attenuate [b]	cordate, oblique, rarely attenuate
LLE	31.3 (20.3-38.8) [ab]	36.7 (25.5-49.0)	46.6 (26.0-67.8) [b]	36.1 (29.3-55.0)	39.6 (38.6-40.5) [ab]	37.9 (26.3-44.7)	53.1 (40.7-74.3) [ab]	36.3 (27.8-49.0)
LWI	12.5 (5.9-30.4) [ab]	23.8 (12.5-29.0)	52.1 (33.3-66.8) [ab]	25.6 (19.2-37.5)	23.7 (21.6-26.4) [ab]	24.9 (19.7-28.0)	26.8 (10.9-41.2) [b]	23.3 (14.6-31.5)
LSW	dull	dull	shining [b]	dull	dull [b]	dull	dull [b]	dull
LEA	concolorous	concolorous	concolorous	concolorous	concolorous	concolorous	concolorous	concolorous
LSM	smooth	smooth	smooth	smooth	not smooth [ab]	smooth	smooth	smooth
LPS	sessile [ab]	sessile	petiolate [b]	sessile	petiolate [b]	sessile	petiolate [ab]	sessile
LPL	0.0 [ab]	0.0	11.9 (6.6-20.9) [ab]	0.0	10.0 (9.4-10.8) [ab]	0.0	8.4 (3.1-13.2) [ab]	0.0
LPO	opposite, rarely alternate [ab]	opposite or alternate	alternate [ab]	opposite or alternate	alternate [b]	opposite	alternate [b]	opposite or alternate
LCO	blue green [ab]	grey green or blue green	dark green [ab]	green, grey green or blue green	grey green [b]	green, dark green or blue green	blue green	green, or rarely green
SSH	square [b]	square	round [ab]	square	round [ab]	square or angular	round [ab]	square orangular
STE	branching [b]	single	branching [b]	single or branching	branching [ab]	single or branching	branching [b]	single or branching
SSM	smooth	smooth	smooth	smooth	not smooth [ab]	smooth	smooth	smooth

Continued

Table 8.10 Continued

	E. socialis x *E. gillii*	*E. gillii*	*E. socialis* x *E. kruseana*	*E. kruseana*	*E. socialis* x *E. macrocarpa*	*E. macrocarpa*	*E. socialis* x *E. oldfieldii*	*E. oldfieldii*
LSH	ovate	lanceolate or ovate, rarely linear [b]	ovate or lanceolate	cordate or orbicular [ab]	ovate	ovate	ovate	lanceolate [b]
LST	acute or obtuse	acute, rarely obtuse or acuminate [ab]	mucronulate, acute, obtuse or acuminate	obtuse or mucronulate [ab]	acute, obtuse	acute or obtuse, rarely acuminate	acute or mucronulate	mucronulate [ab]
LSB	obtuse, cordate or attenuate	attenuate, obtuse or cordate [b]	cordate, obtuse or attenuate	obtuse or cordate	cordate	obtuse or attenuate [b]	obtuse, cordate or attenuate	attenuate [b]
LLE	32.2 (26.3-36.1)	27.4 (26.1-28.8) [b]	35.4 (16.7-47.4)	23.7 (22.0-26.1) [b]	40.3 (37.1-43.4)	50.7 (33.5-65.8) [ab]	32.3 (16.5-41.8)	42.3 (38.0-45.1) [b]
LWI	25.2 (9.7-32.9)	16.0 (10.8-28.1) [b]	23.6 (7.9-37.1)	24.2 (22.1-26.8) [b]	31.2 (28.1-34.2)	33.7 (19.3-45.3) [ab]	21.2 (11.1-29.4)	16.4 (10.1-21.5) [b]
LSW	dull	dull, sometimes glaucous [b]	dull	dull or glaucous [b]	glaucous	very glaucous [ab]	dull or glaucous	dull [b]
LEA	concolorous	concolorous	concolorous	discolorous [ab]	concolorous	concolorous or discolorous	concolorous	concolorous
LSM	smooth	smooth	smooth	not smooth	smooth	smooth	smooth	smooth
LPS	sessile or petiolate	sessile [b]	sessile	petiolate [ab]	sessile	sessile or petiolate [b]	sessile	petiolate [ab]
LPL	0.5 (0.0-2.0)	0.0 [b]	0.0	0.9 (0.7-1.5)	0.0	3.3 (0.0-6.1)	0.0	4.7 (2.5-6.1) [ab]
LPO	opposite or alternate	opposite	opposite or alternate	opposite [b]	opposite	opposite [b]	opposite or alternate	alternate [b]
LCO	green, grey green or blue green	green [b]	dark green, blue green or light green	blue green [b]	grey green	green or grey green [b]	green, grey green, blue green or light green	grey green, blue green or green [b]
SSH	round, square or angular	round, rarely angular [ab]	angular or square	angular [ab]	square	angular [ab]	square or angular	angular [ab]
STE	single or branching	branching [ab]	single or branching	branching [ab]	single	single [b]	single	branching [ab]
SSM	smooth	smooth [b]	smooth	not smooth [ab]	smooth	smooth [b]	smooth	not smooth [ab]

Continued

Table 8.10 Continued

	E. socialis x *E. orbifolia*	*E. orbifolia*	*E. socialis* x *E. polyanthemos*	*E. polyanthemos*	*E. socialis* x *E. sideroxylon*	*E. sideroxylon*	*E. socialis* x *E. steedmanii*	*E. steedmanii*
LSH	ovate	orbicular, rarely cordate [b]	ovate or orbicular	orbicular [b]	ovate	lanceolate or ovate [b]	orbicular or ovate	lanceolate or ovate [b]
LST	acute, mucronulate or obtuse	truncate or mucronulate	acute or obtuse	truncate or acute [b]	acute, mucronulate or obtuse	acute, sometimes obtuse [b]	acute, or mucronulate	acute, mucronulate or obtuse [b]
LSB	obtuse or cordate	obtuse, cordate or attenuate [b]	cordate, obtuse or attenuate	obtuse, cordate or acute [b]	attenuate or cordate	attenuate, rarely obtuse [b]	attenuate, obtuse or cordate	attenuate or obtuse [b]
LLE	35.2 (19.4-47.9)	23.9 (17.0-29.3) [b]	36.0 (19.7-46.0)	37.5 (31.0-47.1) [ab]	33.3 (22.3-42.0)	58.9 (43.6-76.9) [ab]	37.5 (23.6-45.0)	64.2 (38.6-81.3) [ab]
LWI	20.1 (13.1-31.6)	30.1 (19.7-41.8) [b]	22.3 (13.4-25.6)	47.9 (31.3-68.6) [ab]	25.1 (18.5-30.0)	21.7 (9.9-33.3) [ab]	31.5 (13.5-47.0)	14.5 (5.0-29.5) [b]
LSW	dull	dull	dull or glaucous	dull [b]	glaucous or dull	dull [b]	dull or glaucous	dull or shiny [b]
LEA	concolorous	concolorous	concolorous	concolorous	concolorous	discolorous [ab]	concolorous	concolorous
LSM	smooth	smooth	smooth	smooth	smooth	smooth	smooth	smooth or not smooth
LPS	sessile or petiolate	petiolate [ab]	sessile or petiolate	petiolate [ab]	sessile	petiolate [ab]	sessile or petiolate	petiolate [ab]
LPL	0.2 (0.0-1.0)	7.0 (2.9-10.8) [ab]	0.1 (0.0-0.5)	14.6 (9.0-20.0) [b]	0.0	5.0 (2.0-8.7) [ab]	2.33 (0.0-7.0)	5.4 (0.5-13.4) [ab]
LPO	opposite or alternate	opposite [b]	opposite	alternate [b]	opposite or alternate	alternate [b]	opposite or alternate	alternate [b]
LCO	blue green, dark green or green	blue green	blue green, light green or green	blue green [ab]	blue green	grey green [b]	blue green or grey green	blue green or green [b]
SSH	square	round [b]	square or angular	round [b]	square or angular	angular [ab]	round	round [ab]
STE	single	single or branching [ab]	single or branching	branching [ab]	single or branching	branching, rarely single [b]	branching	branching [ab]
SSM	smooth	not smooth [ab]	smooth	smooth	not smooth	smooth	smooth	not smooth [ab]

Continued

Table 8.10 Continued

	E. socialis x *E. transcontinentalis*	*E. transcontinentalis*	*E. socialis* x *E. websteriana*	*E. websteriana*	*E. socialis* x *E. youngiana*	*E. youngiana*
LSH	cordate, rarely ovate	linear [b]	ovate, rarely lanceolate	cordate or orbicular [b]	ovate	ovate or cordate [b]
LST	acute, rarely mucronulate or acuminate	obtuse [ab]	acuminate, acute or obtuse	truncate or obtuse [b]	obtuse or acute	mucronulate, acute or acuminate [b]
LSB	obtuse or cordate	obtuse [b]	obtuse or cordate	obtuse, mucronulate or acuminate [ab]	obtuse or cordate	obtuse or attenuate [b]
LLE	37.3 (23.7-50.6)	18.3 (16.6-21.2) [b]	33.3 (17.7-47.0)	22.2 (13.8-27.5) [ab]	34.4 (3.4-36.3)	59.9 (33.0-72.1) [ab]
LWI	26.0 (13.9-30.0)	5.9 (5.2-7.1) [b]	24.5 (10.5-36.4)	25.0 (15.7-33.4) [ab]	23.1 (22.7-23.5)	30.0 (15.8-45.6) [ab]
LSW	dull	dull	dull or glaucous	dull [ab]	dull or glaucous	dull or shining
LEA	concolorous	concolorous	concolorous	concolorous [b]	concolorous	concolorous
LSM	smooth	smooth	smooth	smooth	smooth	smooth
LPS	sessile or petiolate	sessile [b]	sessile	petiolate [b]	sessile	petiolate [b]
LPL	0.3 (0.0-2.1)	0.0 [b]	0.0	3.0 (1.2-4.7) [b]	0.0	9.9 (5.7-14.4) [ab]
LPO	alternate or opposite	opposite [b]	opposite	opposite, rarely alternate	opposite	alternate [ab]
LCO	blue green, green or light green	blue green [b]	light green, grey green or blue green	dark green, blue green, light green or green [b]	blue green	dark green, rarely green [ab]
SSH	angular or round	square [b]	angular or round	round [b]	angular	angular [ab]
STE	single or branching	branching [ab]	single or branching	branching, rarely single [b]	single or branching	single
SSM	smooth	smooth	smooth	not smooth [b]	smooth	smooth

Correlation coefficient† : showing the fit (r^2) of the individual character vectors in the ordination space.

8.4.2.3. Summary of crosses

The number of hybrids produced for each cross varied, from no hybrids from nine and eleven out of sixteen crosses for *E. gillii* and *E. socialis*, respectively, to 53 in the cross between *E. gillii* and *E. oldfieldii* (Table 8.3 and 8.4). The phylogenetic relationships between the species affected hybridisation success, with intraseries crosses producing more hybrid seedlings than interseries and intersectional crosses (Table 8.11). Observations of the morphology of these species indicated that a higher degree of hybridisation success was realised between species with similar flower sizes than between species with dissimilar flower sizes. Flowers from the female parents *E. gillii* and *E. socialis*, and pollen donors *E. kruseana*, *E. transcontinentalis*, *E. oldfieldii*, *E. gardneri*, *E. eremophila* and *E. orbifolia,* are between 9 and 18 mm long. Crosses between these species produced the most hybrids. The flowers of *E. polyanthemos, E. sideroxylon, E. pruinosa* and *E. websteriana* are less than 8 mm long, and only the *E. gillii* x *E. polyanthemos* cross produced hybrids. Flowers from *E. caesia* and *E. steedmanii* are between 19 and 25 mm long, flowers from *E. macrocarpa* and *E. youngiana* are greater than 25 mm long and only four hybrids were produced from these crosses, all from *E. socialis* x *E. steedmanii*.

Table 8.11. Number of hybrids produced in relation to phylogenetic placement by Pryor and Johnson (1971).

Female		Male		Number of hybrids	Total number of seedlings measured
Sect. *Bisectaria*		Sect. *Bisectaria*			
Ser. *Oleosae*	*E. gillii*	Ser. *Oleosae*	*E. gillii*	0	68
			E. transcontinentalis	25	35
			E. socialis	38	38
		Ser. *Macrocarpae*	*E. orbifolia*	1	10
			E. websteriana	0	1
			E. caesia	0	6
			E. macrocarpa	0	0
			E. oldfieldii	53	54
			E. youngiana	0	4
		Ser. *Kruseanae*	*E. kruseana*	38	38
		Ser. *Reduncae*	*E. gardnerii*	42	42
		Ser. *Occidentales*	*E. eremophila*	0	11
			E. steedmanii	0	0
Sect. *Adnataria*		*Pruinosae*	*E. pruinosa*	0	0
		Ser. *Polyanthemae*	*E. polyanthemos*	6	6
		Ser. *Melliodorae*	*E. sideroxylon*	0	1
Sect. *Bisectaria*		Sect. *Bisectaria*			
Ser. *Oleosae*	*E. socialis*	Ser. *Oleosae*	*E. socialis*	0	10
			E. transcontinentalis	9	12
			E. gillii	4	5
		Ser. *Macrocarpae*	*E. orbifolia*	0	5
			E. websteriana	0	18
			E. caesia	0	6
			E. macrocarpa	0	2
			E. oldfieldii	0	10
			E. youngiana	0	2
		Ser. *Kruseanae*	*E. kruseana*	3	8
		Ser. *Reduncae*	*E. gardnerii*	3	5
		Ser. *Occidentales*	*E. eremophila*	0	7
			E. steedmanii	4	9
Sect. *Adnataria*		Ser. *Pruinosae*	*E. pruinosa*	0	0
		Ser. *Polyanthemae*	*E. polyanthemos*	0	9
		Ser. *Melliodorae*	*E. sideroxylon*	0	3

8.5. Discussion

Naturally occurring hybrids involving the species used in this study were reviewed by Griffin *et al.* (1988), with twenty-five records listed. These consisted of one intersectional cross between *E. kruseana* (sect. *Bisectaria* ser. *Kruseana*) and *E. ovularis* (sect. *Dumosae* ser. *Dundasianae*), thirteen interseries crosses (twelve from sect. *Adnataria*) and eleven intraseries crosses, from ser. *Macrocarpae* and ser. *Oleosae* (sect. *Bisectaria*), and ser. *Melliodorae* and ser. *Polyanthemae* (sect. *Adnataria*). The listings for recorded manipulated or spontaneous crosses are fewer (six), with five between species from different sections e.g. *E. caesia* (sect. *Bisectaria* ser. *Macrocarpae*) and *E. pulverulenta* (sect. *Maidenaria* ser. *Viminales*), and one between species from different series in the same section.

In this study, the flowers were emasculated but not bagged following pollination, as bagging resulted in high levels of drop of the small flowers. Some intraspecific seedlings were produced amongst the hybrid seedlings following interspecific hybridisation, either through faulty emasculation or via insect visits. The substantial numbers of hybrid seedlings produced indicates either that contamination was low, or that these species have a high propensity for interspecific hybridisation.

Three different trees were used as female parents for each of *E. gillii* and *E. socialis*, and over 3000 flowers pollinated, however, only one tree from each species produced viable seed. This may have been due to the age of the trees: all of the *E. gillii* trees and two of the *E. socialis* trees were under six years old at the time of pollination, and although each produced large numbers of flowers, most dropped after pollination. The trees may not have been mature enough physiologically to carry high seed loads, although they carried a small number of capsules from the previous year. The *E. gillii* tree that produced seed was under six years old, but was much larger than the other two used. The *E. socialis* tree that produced seed was 22

years old, a large, mature specimen. The high seedling mortality observed in seedlings from crosses with *E. gillii* as the female parent may have been caused by lethal gene combinations or incompatible crosses, resulting in low seedling vigour and higher disease susceptibility.

The program produced over 400 seedlings from the two female parent species crossed with the sixteen male parent species. Hierarchical and non-hierarchical analysis of all seedlings, based on seedling morphology, placed approximately 50% intermediately between their parents on both the dendrograms and the ordination plots. The remaining seedlings were positioned with the female parents and considered to be the product of self pollination. Crosses between species from the same series, *E. gillii*, *E. socialis* and *E. transcontinentalis* (Ser. *Oleosae*), produced a high proportion of hybrid seedlings, while crosses between *E. gillii* and *E. socialis* and species from the next closest series, ser. *Macrocarpae*, produced few. Most species selected from ser. *Macrocarpae* had large flowers, and this may have contributed to the low success rate, with the exception of *E. gillii* x *E. oldfieldii*, where the flowers were similar in size. Crosses between *E. gillii* and *E. kruseana*, from distantly related series, produced a high proportion of hybrids, as did the crosses *E. gillii* x *E. gardneri*, *E. socialis* x *E. kruseana* and *E. socialis* x *E. gardneri*, again this may be due to similar flower sizes. Six hybrid seedlings were produced from the intersectional cross between *E. gillii* (sect. *Bisectaria*) and *E. polyanthemos* (sect. *Adnataria*), however the majority of intersectional pollinations failed, possibly due to the relativley large genetic distance between the species. The relatively low dissimilarities between the female parent and the seedlings, shown on the dendrograms, reflects probable short term inheritance of maternal characters. As the seedlings mature and exhibit adult morphology, further clarification of hybrid status will be possible.

8.6. Conclusion

Of the 246 seedlings resulting from the interspecific controlled pollinations with *E. gillii* as the female parent, 43 seedlings clustered with *E. gillii*, and there is a high probability they are the result of self pollination. The remaining 203 seedlings, from crosses between *E. gillii* and *E. gardneri*, *E. kruseana*, *E. oldfieldii*, *E. orbifolia*, *E. polyanthemos*, *E. socialis* and *E. transcontinentalis*, clustered between the female and male parent clusters, which indicated that the seedlings are morphologically intermediate, and thus suggests that the seedlings are F1 hybrids.

Of the 111 seedlings resulting from the interspecific controlled pollinations with *E. socialis* as the female parent, 88 seedlings clustered with the female parent and there is a high probability they are the result of self pollination. The remaining 23 seedlings, from crosses between *E. socialis* and *E. gardneri, E gillii, E. kruseana, E. steedmanii* and *E. transcontinentalis,* clustered with neither the male nor female parent, which indicated that the seedlings are morphologically intermediate, and thus suggests that the seedlings are F1 hybrids.

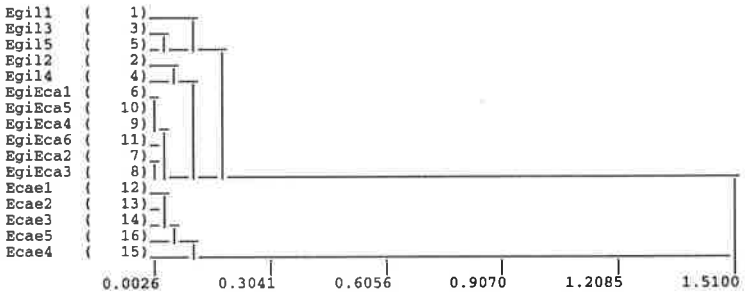


Figure 8.1. Dendrogram of *E. gillii* x *E. caesia* seedlings and parent seedlings; *E.* gillii (Egil), *E. caesia* (Ecae) and *E. gillii* x *E. caesia* (EgiEca). Scale represents standardised Manhattan distance (Gower metric) based on variant seedling characters (Table 8.6) for dendrograms 8.1 to 8.13.

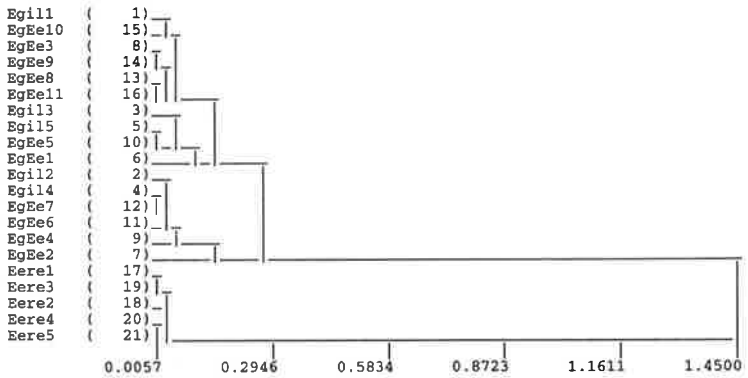


Figure 8.2. Dendrogram of *E. gillii* x *E. eremophila* seedlings and parent seedlings; *E. gillii* (Egil), *E. eremophila* (Ecae) and *E. gillii* x *E. eremophila* (EgEe).

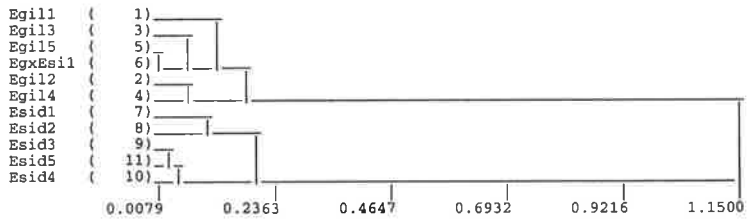


Figure 8.3. Dendrogram of *E. gillii* x *E. sideroxylon* seedlings and parent seedlings; *E. gillii* (Egil), *E. sideroxylon* (Esid) and *E. gillii* x *E. sideroxylon* (EgxEsi).

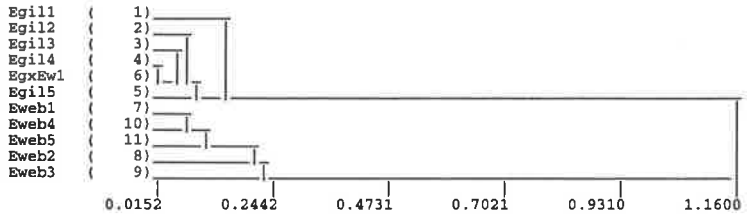


Figure 8.4. Dendrogram of *E. gillii* x *E. websteriana* seedlings and parent seedlings; *E. gillii* (Egil), *E. websteriana* (Eweb) and *E. gillii* x *E. websteriana* (EgxEw).

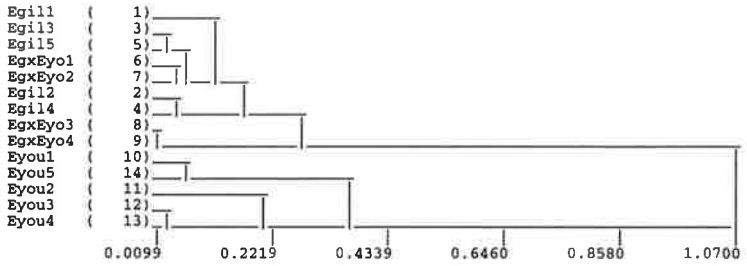


Figure 8.5. Dendrogram of *E. gillii* x *E. youngiana* seedlings and parent seedlings; *E. gillii* (Egil), *E. youngiana* (Eyou) and *E. gillii* x *E. youngiana* (EgxEyo).

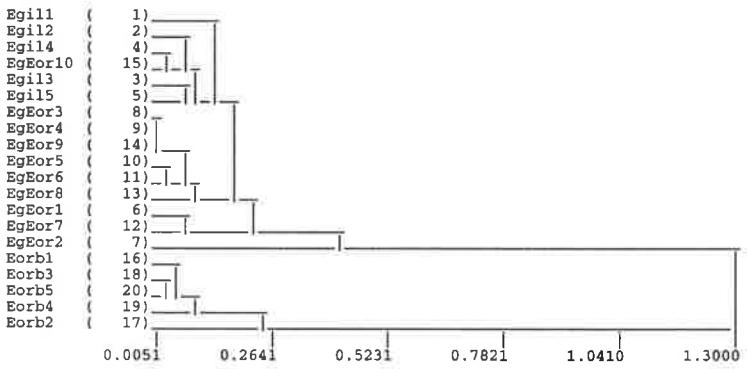


Figure 8.6. Dendrogram of *E. gillii* x *E. orbifolia* seedlings and parent seedlings; *E. gillii* (Egil), *E. orbifolia* (Eorb) and *E. gillii* x *E. orbifolia* (EgEor).

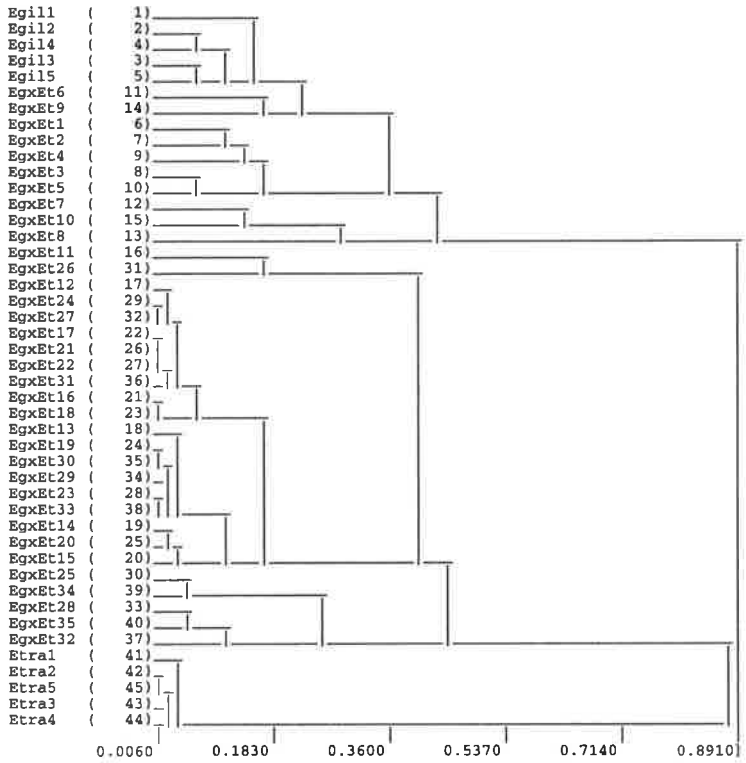


Figure 8.7. Dendrogram of *E. gillii* x *E. transcontinentalis* seedlings and parent seedlings; *E. gillii* (Egil), *E. transcontinentalis* (Etra) and *E. gillii* x *E. transcontinentalis* (EgxEt).

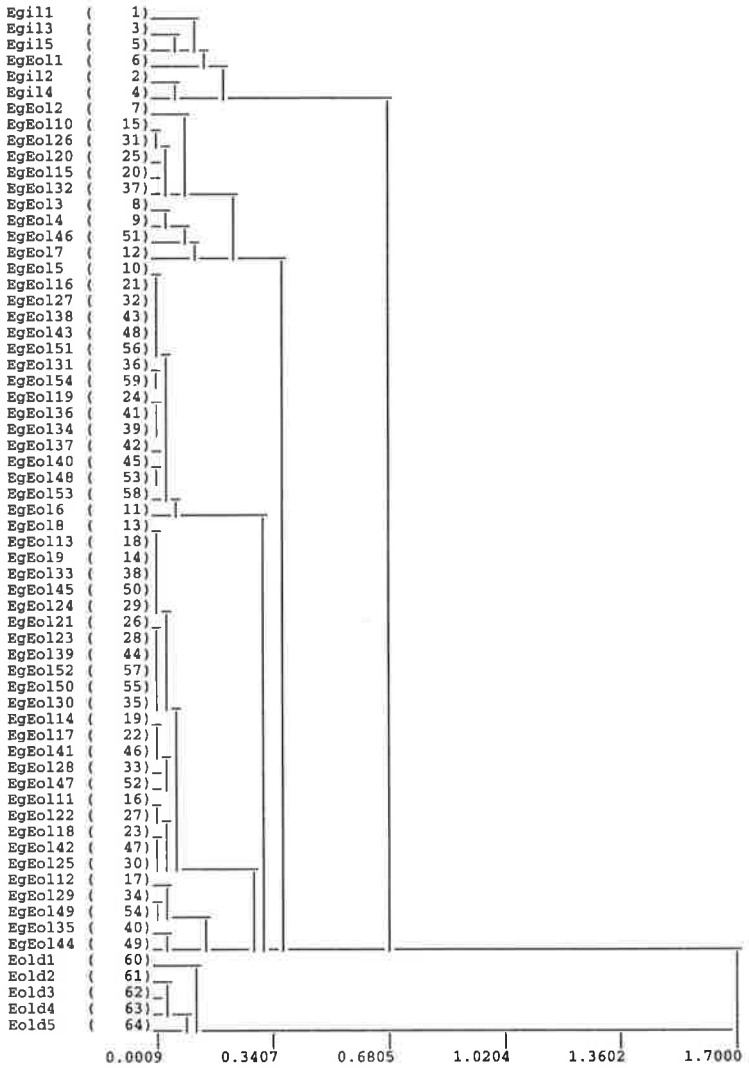


Figure 8.8. Dendrogram of *E. gillii* x *E. oldfieldii* seedlings and parent seedlings; *E. gillii* (Egil), *E. oldfieldii* (Eold) and *E. gillii* x *E. oldfieldii* (EgEol).

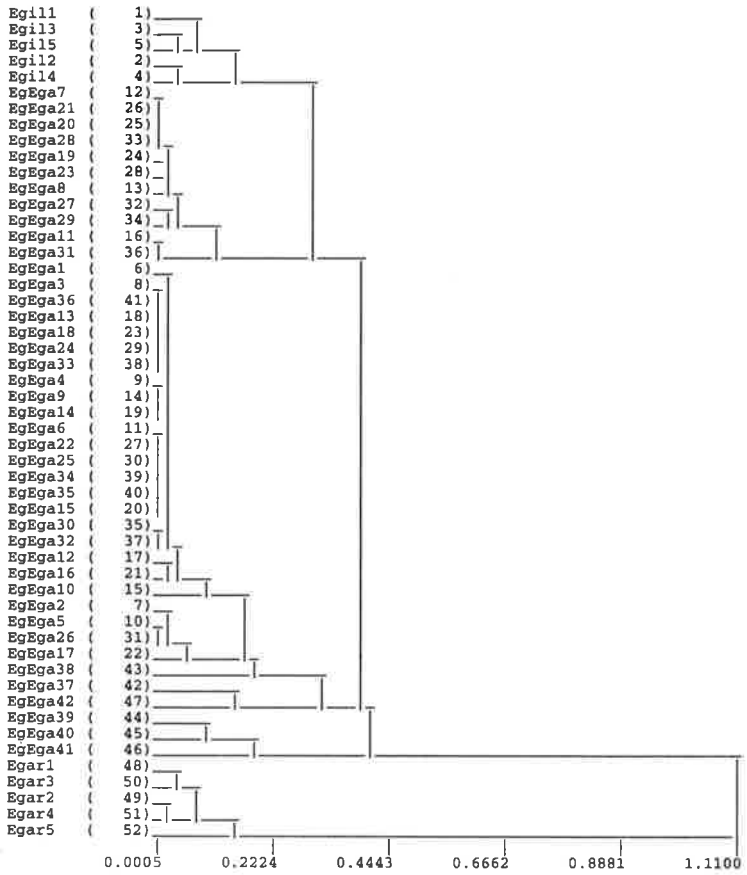


Figure 8.9. Dendrogram of *E. gillii* x *E. gardneri* seedlings and parent seedlings; *E. gillii* (Egil), *E. gardneri* (Egar) and *E. gillii* x *E. gardneri* (EgEga).

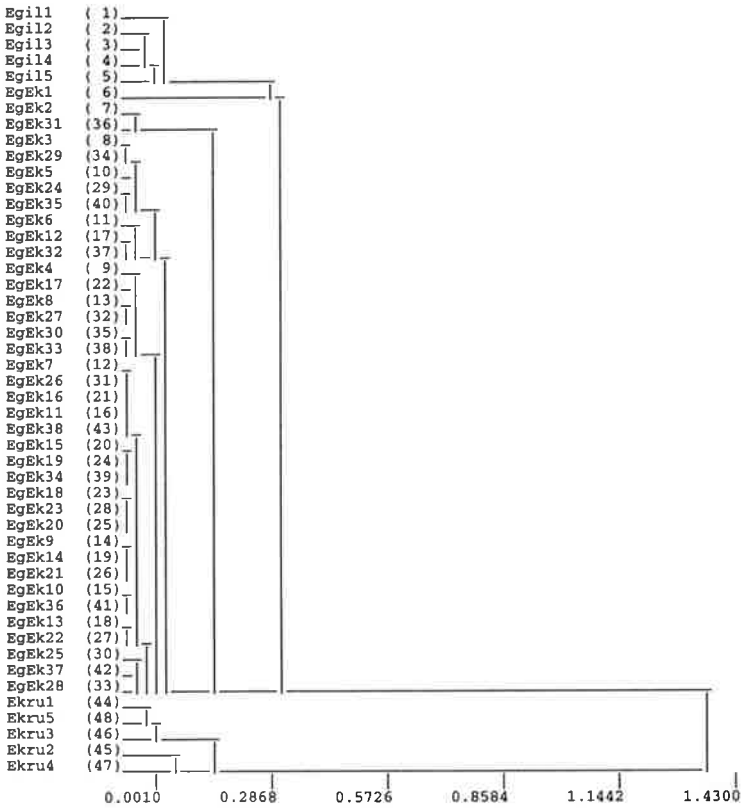


Figure 8.10. Dendrogram of *E. gillii* x *E. kruseana* seedlings and parent seedlings; *E. gillii* (Egil), *E. kruseana* (Ekru) and *E. gillii* x *E. kruseana* (EgEk).

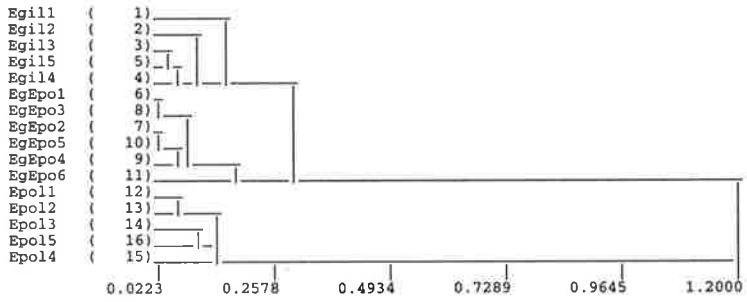


Figure 8.11. Dendrogram of *E. gillii* x *E. polyanthemos* seedlings and parent seedlings; *E. gillii* (Egil), *E. polyanthemos* (Epol) and *E. gillii* x *E. polyanthemos* (EgEpo).

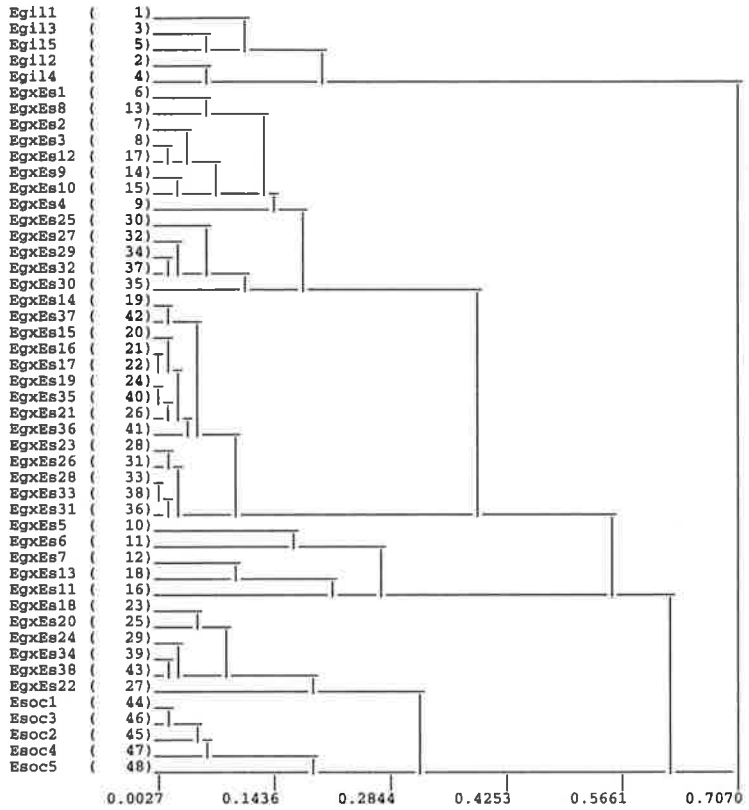


Figure 8.12. Dendrogram of *E. gillii* x *E. socialis* seedlings and parent seedlings; *E. gillii* (Egil), *E. socialis* (Esoc) and *E. gillii* x *E. socialis* (EgxEs).

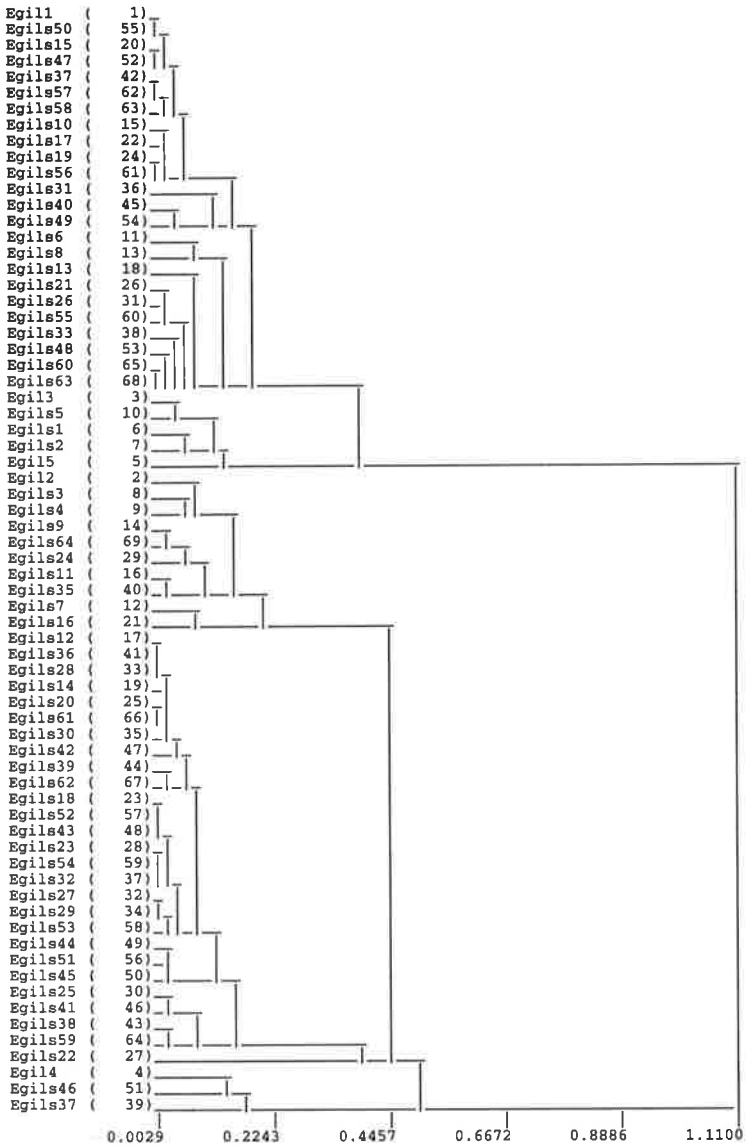


Figure 8.13. Dendrogram of *E. gillii* x *E. gillii* seedlings and parent seedlings; *E. gillii* (Egil) and *E. gillii* x *E. gillii* (Egils).

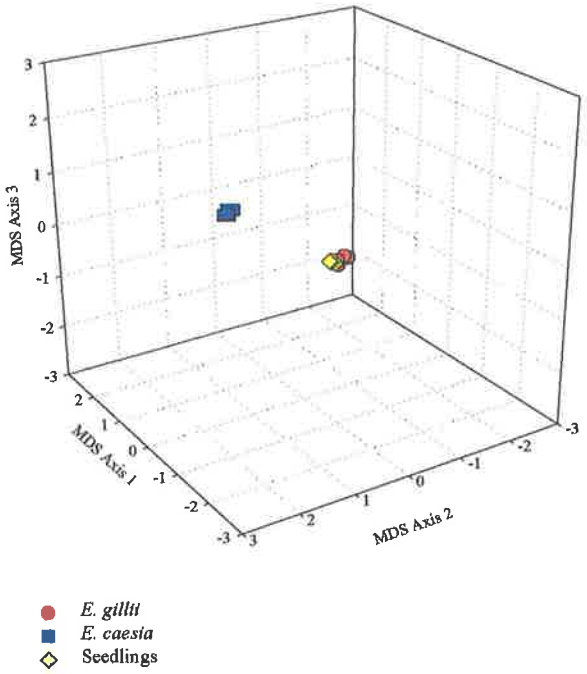


Figure 8.14. Multidimensional ordination of *E. gillii* x *E. caesia* seedlings and parents.

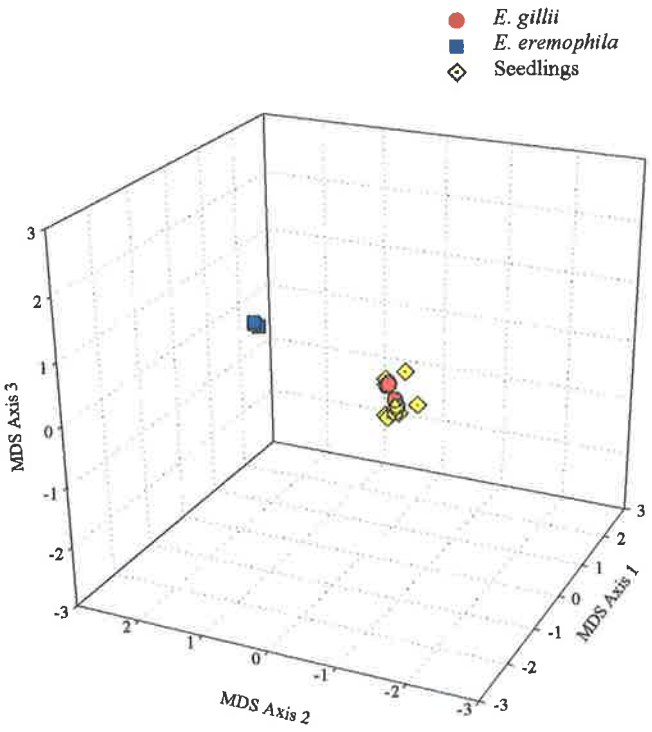


Figure 8.15. Multidimensional ordination of *E. gillii* x *E. eremophila* seedlings and parents.

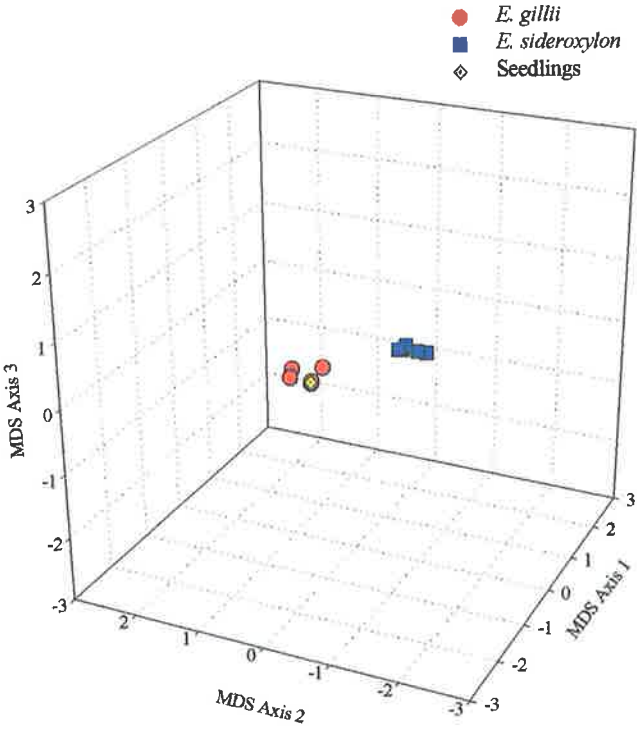


Figure 8.16. Multidimensional ordination of *E. gillii* x *E. sideroxylon* seedlings and parents.

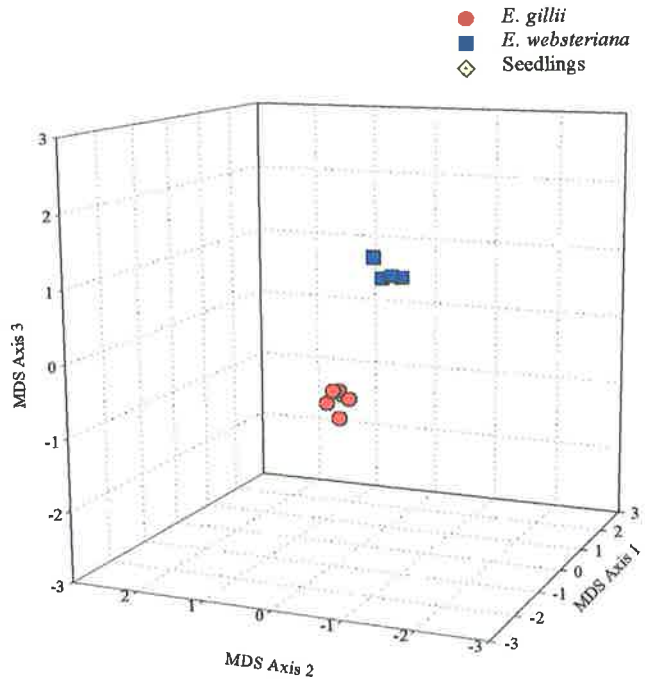


Figure 8.17. Multidimensional ordination of *E. gillii* x *E. websteriana* seedlings and parents.

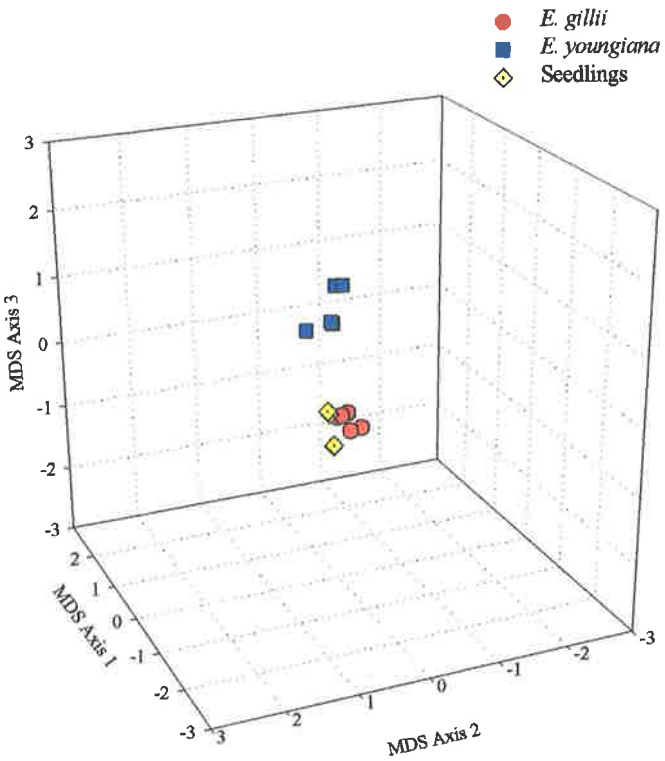


Figure 8.18. Multidimensional ordination of *E. gillii* x *E. youngiana* seedlings and parents.

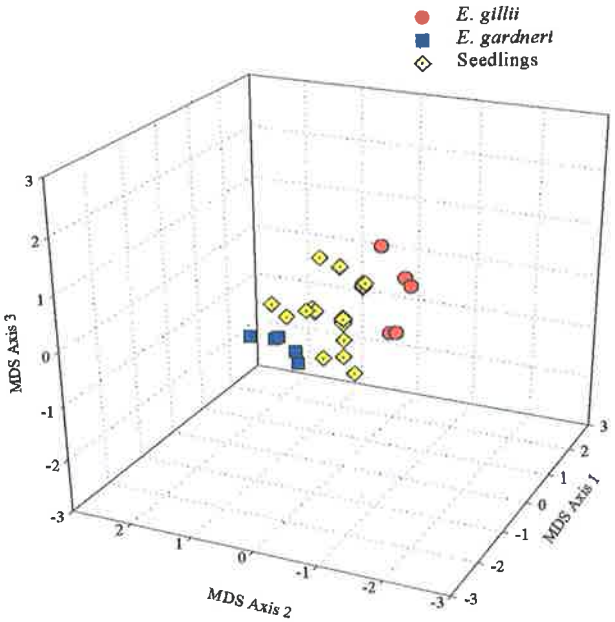


Figure 8.19. Multidimensional ordination of *E. gillii* x *E. gardneri* seedlings and parents.

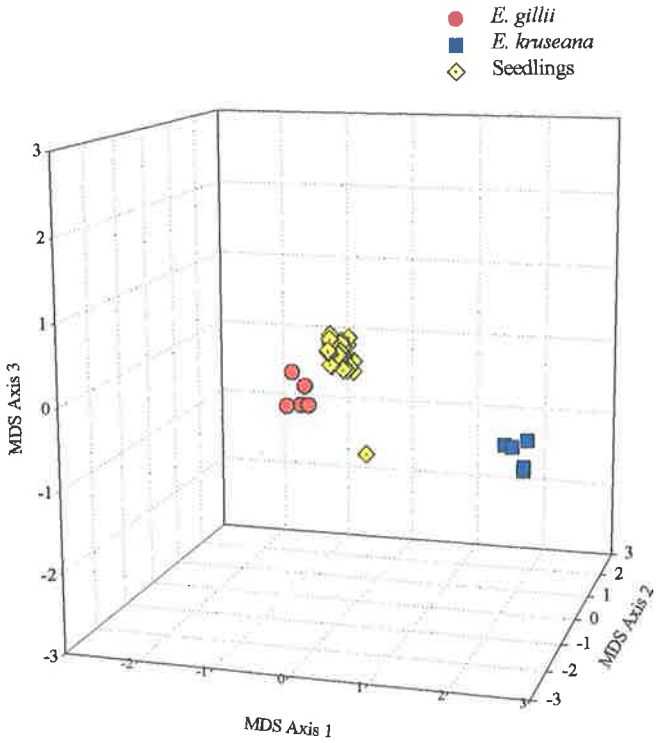


Figure 8.20. Multidimensional ordination of *E. gillii* x *E. kruseana* seedlings and parents.

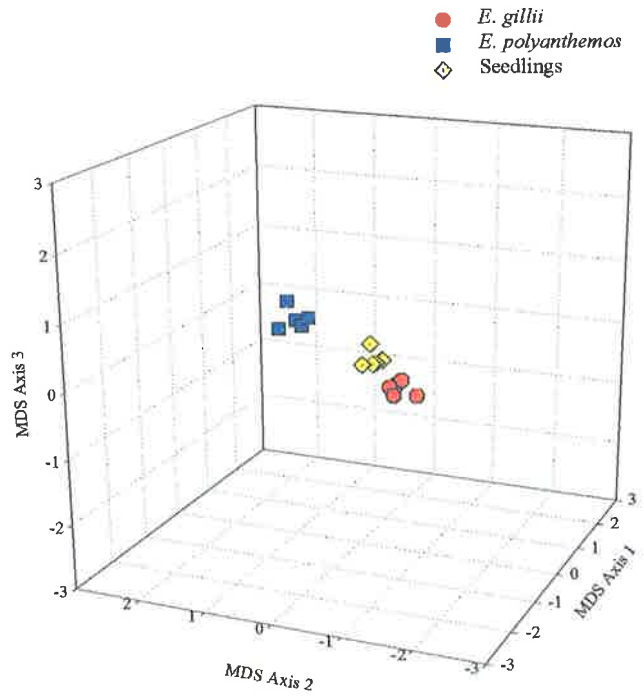


Figure 8.21. Multidimensional ordination of *E. gillii* x *E. polyanthemos* seedlings and parents.

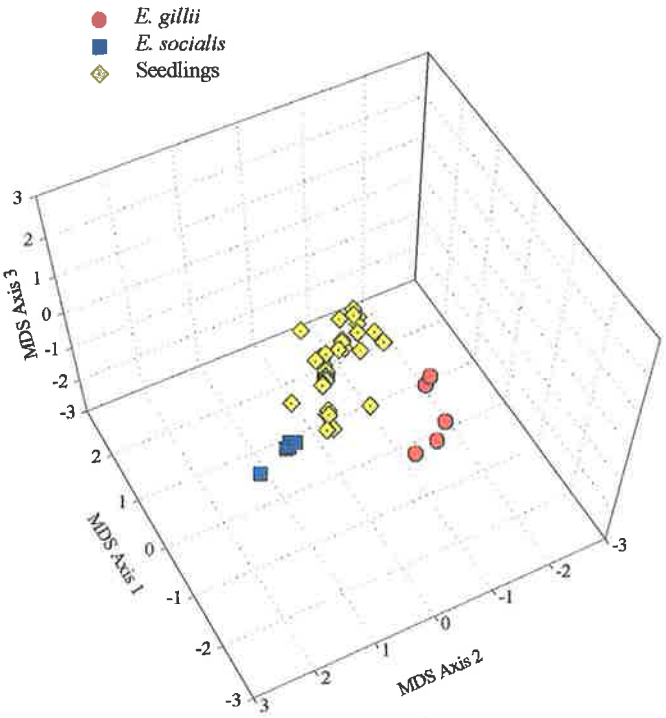


Figure 8.22. Multidimensional ordination of *E. gillii* x *E. socialis* seedlings and parents.

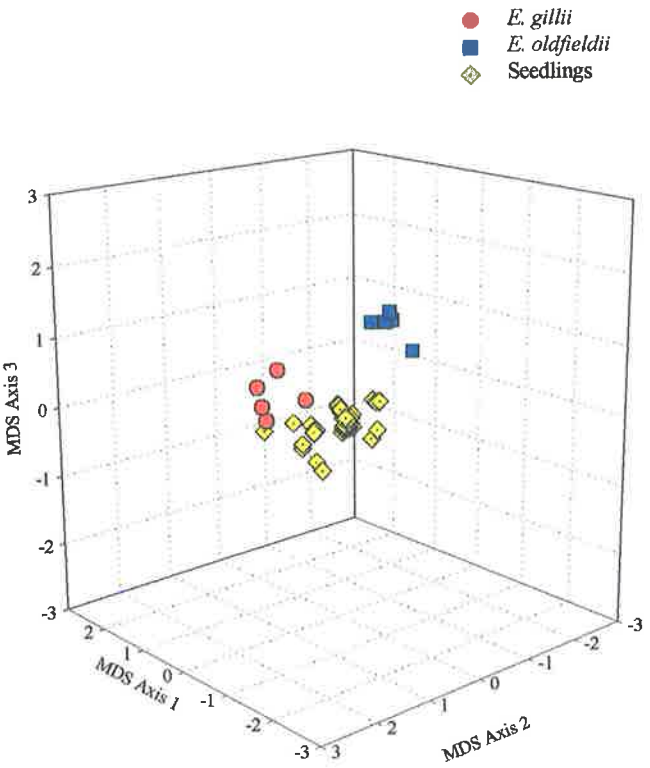


Figure 8.23. Multidimensional ordination of *E. gillii* x *E. oldfieldii* seedlings and parents.

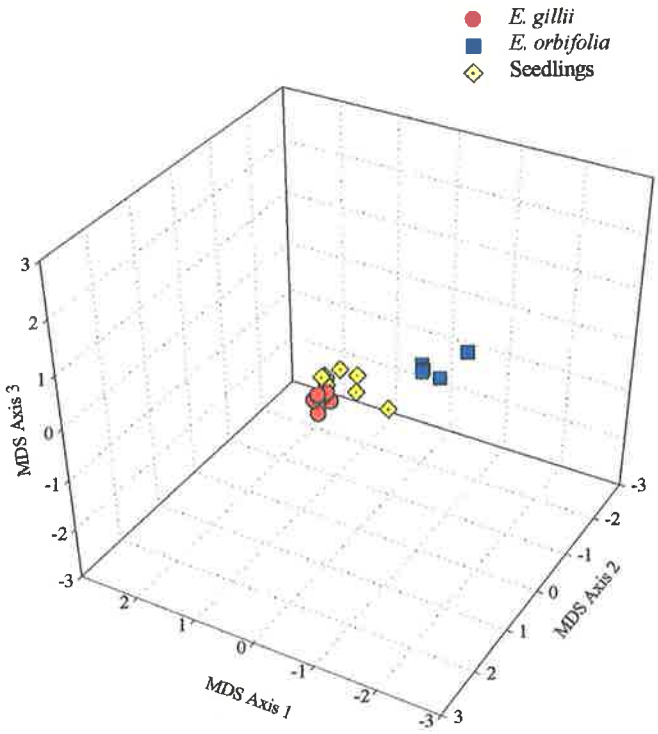


Figure 8.24. Multidimensional ordination of *E. gillii* x *E. orbifolia* seedlings and parents.

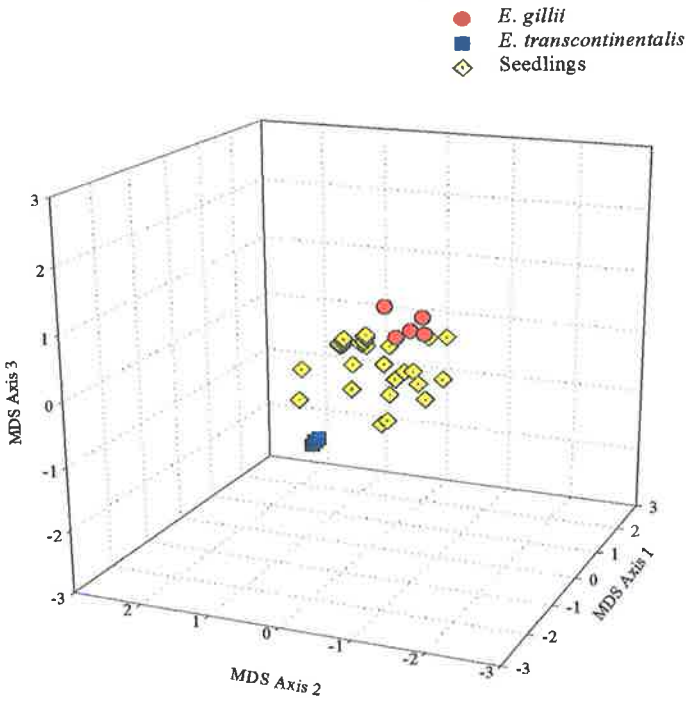


Figure 8.25. Multidimensional ordination of *E. gillii* x *E. transcontinentalis* seedlings and parents.

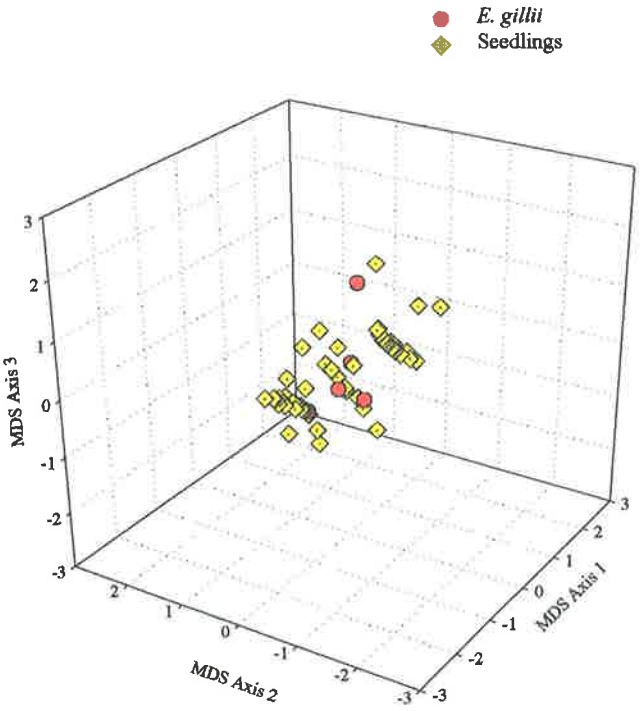


Figure 8.26. Multidimensional ordination of *E. gillii* x *E. gillii* seedlings and parents.

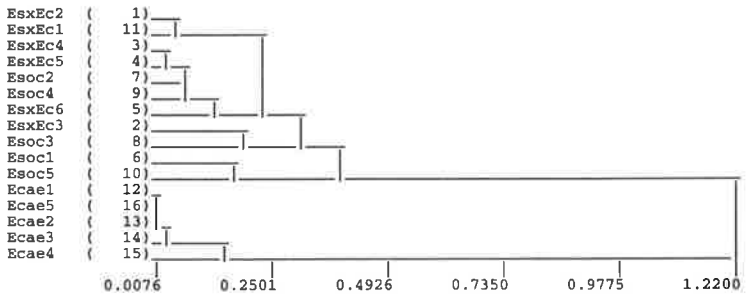


Figure 8.27. Dendrogram of *E. socialis* x *E. caesia* seedlings and parent seedlings; *E. socialis* (Esoc), *E. caesia* (Ecae) and *E. socialis* x *E. caesia* (EsxEc). Scale represents standardised Manhattan distance (Gower metric) based on variant seedling characters (Table 8.9) for dendrograms 8.27 to 8.41.

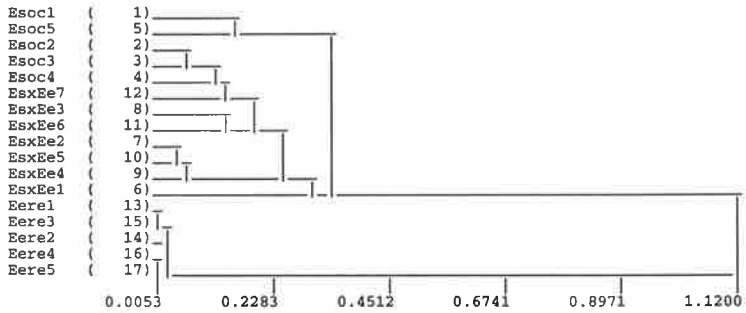


Figure 8.28. Dendrogram of *E. socialis* x *E. eremophila* seedlings and parent seedlings; *E. socialis* (Esoc), *E. eremophila* (Eere) and *E. socialis* x *E. eremophila* (EsxEe).

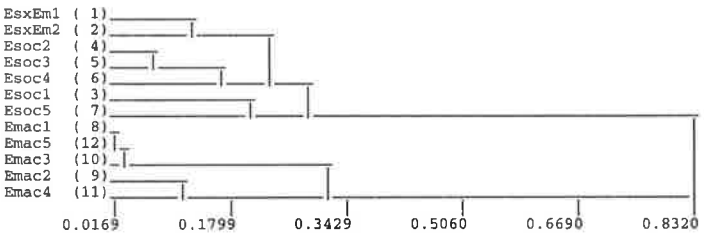


Figure 8.29. Dendrogram of *E. socialis* x *E. macrocarpa* seedlings and parent seedlings; *E. socialis* (Esoc), *E. macrocarpa* (Emac) and *E. socialis* x *E. macrocarpa* (EsxEm).

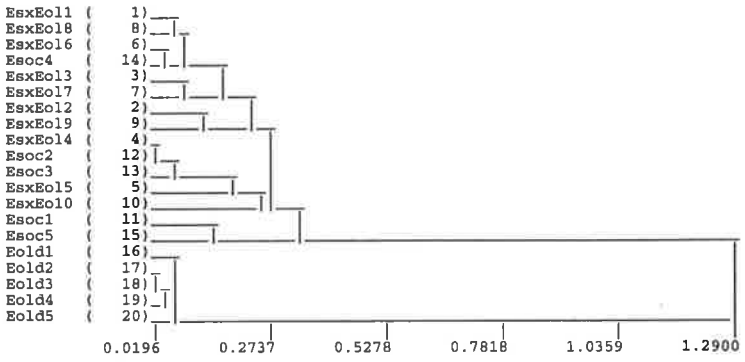


Figure 8.30. Dendrogram of *E. socialis* x *E. oldfieldii* seedlings and parent seedlings; *E. socialis* (Esoc), *E. oldfieldii* (Eold) and *E. socialis* x *E. oldfieldii* (EsxEol).

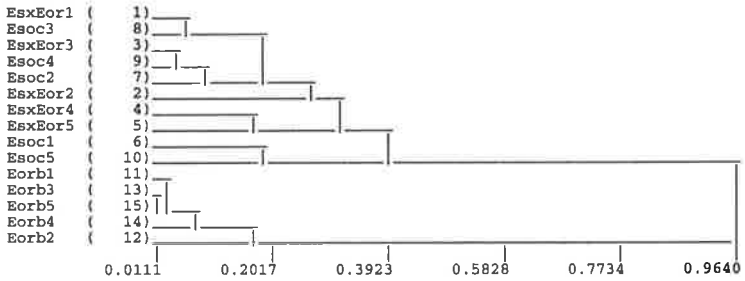


Figure 8.31. Dendrogram of *E. socialis* x *E. orbifolia* seedlings and parent seedlings; *E. socialis* (Esoc), *E. orbifolia* (Eorb) and *E. socialis* x *E. orbifolia* (EsxEor).

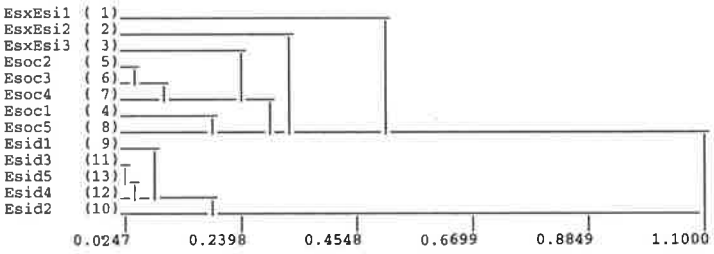


Figure 8.32. Dendrogram of *E. socialis* x *E. sideroxylon* seedlings and parent seedlings; *E. socialis* (Esoc), *E. sideroxylon* (Esid) and *E. socialis* x *E. sideroxylon* (EsxEsi).

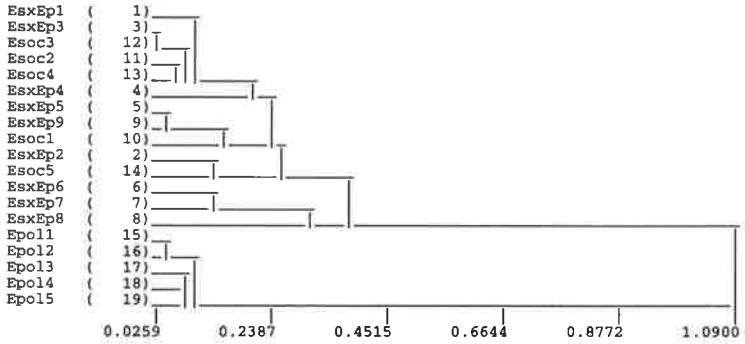


Figure 8.33. Dendrogram of *E. socialis* x *E. polyanthemos* seedlings and parent seedlings; *E. socialis* (Esoc), *E. polyanthemos* (Epol) and *E. socialis* x *E. polyanthemos* (EsxEp).

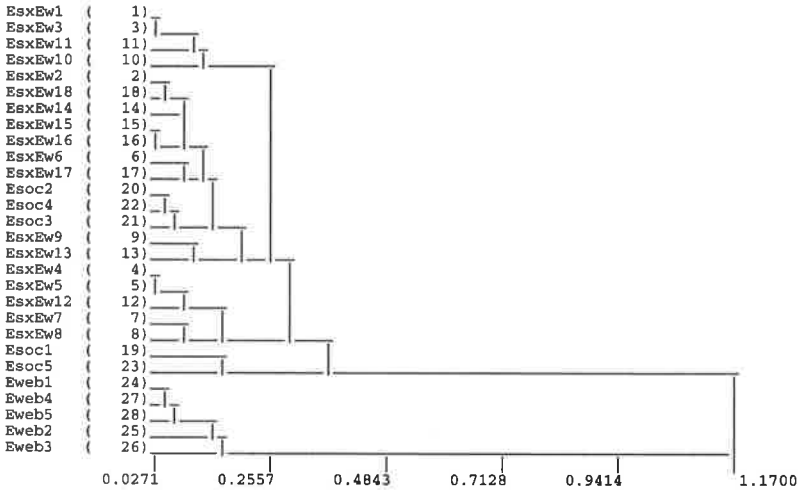


Figure 8.34. Dendrogram of *E. socialis* x *E. websteriana* seedlings and parent seedlings; *E. socialis* (Esoc), *E. websteriana* (Eweb) and *E. socialis* x *E. websteriana* (EsxEw).

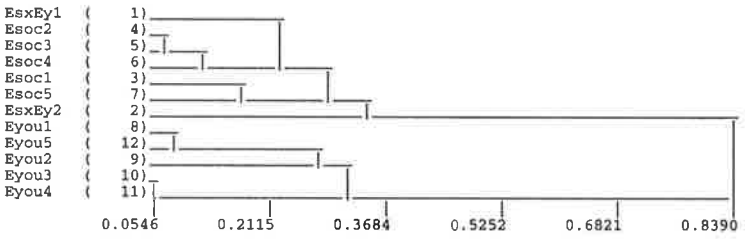


Figure 8.35. Dendrogram of *E. socialis* x *E. youngiana* seedlings and parent seedlings; *E. socialis* (Esoc), *E. youngiana* (Eyou) and *E. socialis* x *E. youngiana* (EsxEy).

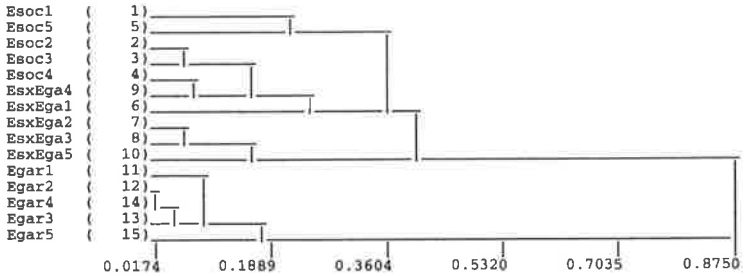


Figure 8.36. Dendrogram of *E. socialis* x *E. gardneri* seedlings and parent seedlings; *E. socialis* (Esoc), *E. gardneri* (Egar) and *E. socialis* x *E. gardneri* (EsxEga).

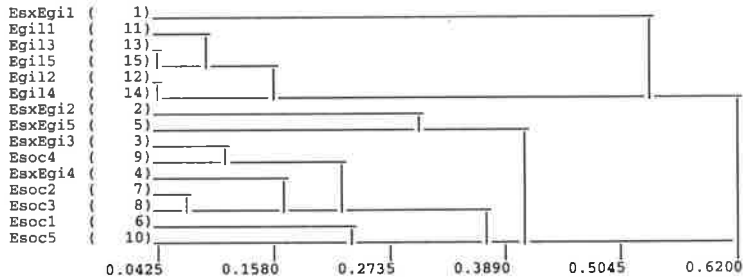


Figure 8.37. Dendrogram of *E. socialis* x *E. gillii* seedlings and parent seedlings; *E. socialis* (Esoc), *E. gillii* (Egil) and *E. socialis* x *E. gillii* (EsxEgi).

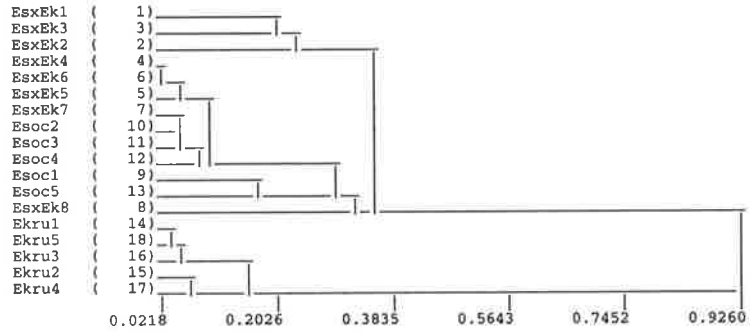


Figure 8.38. Dendrogram of *E. socialis* x *E. kruseana* seedlings and parent seedlings; *E. socialis* (Esoc), *E. kruseana* (Ekru) and *E. socialis* x *E. kruseana* (EsxEk).

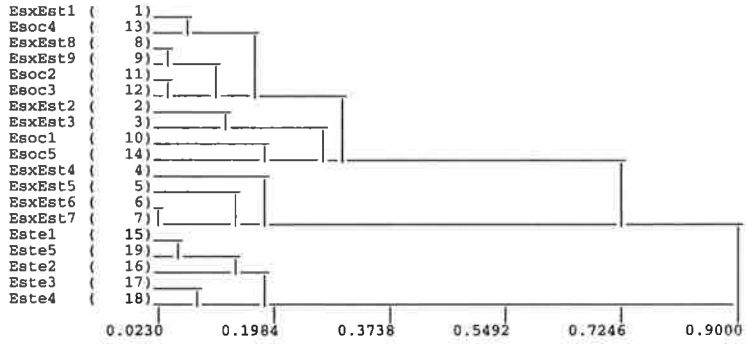


Figure 8.39. Dendrogram of *E. socialis* x *E. steedmanii* seedlings and parent seedlings; *E. socialis* (Esoc), *E. steedmanii* (Este) and *E. socialis* x *E. steedmanii* (EsxEst).

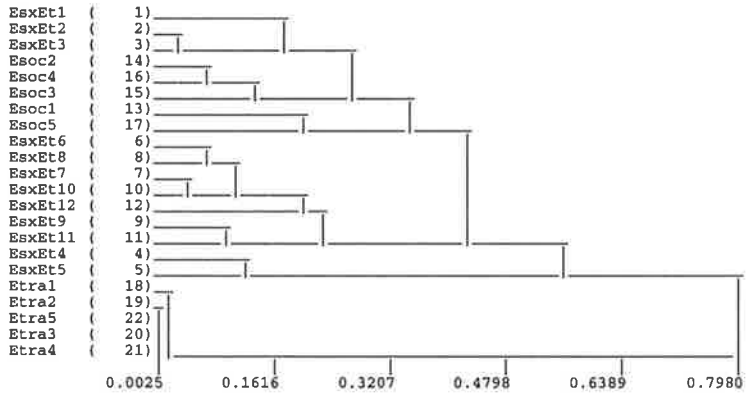


Figure 8.40. Dendrogram of *E. socialis* x *E. transcontinentalis* seedlings and parent seedlings; *E. socialis* (Esoc), *E. transcontinentalis* (Etra) and *E. socialis* x *E. transcontinentalis* (EsxEt).

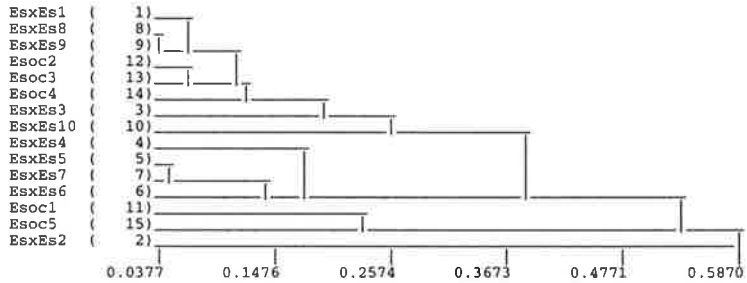


Figure 8.41. Dendrogram of *E. socialis* x *E. socialis* seedlings and parent seedlings; *E. socialis* (Esoc), and *E. socialis* x *E. socialis* (EsxEs).

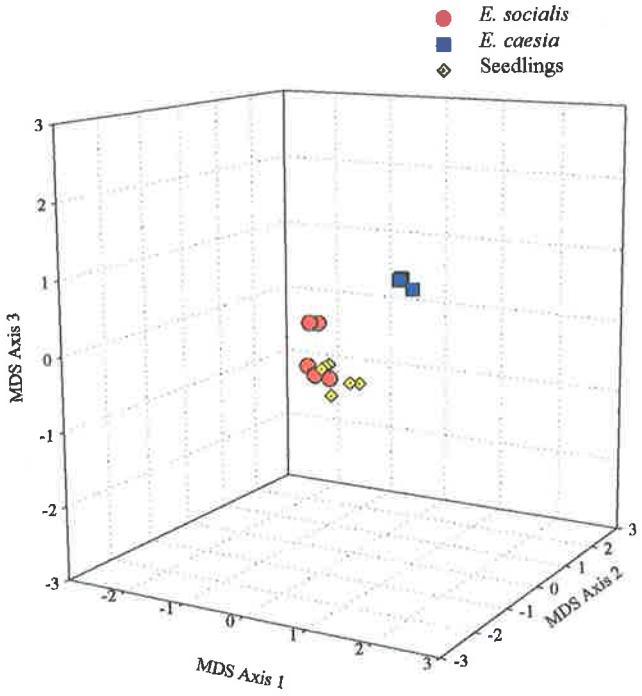


Figure 8.42. Multidimensional ordination of *E. socialis* x *E. caesia* seedlings and parents.

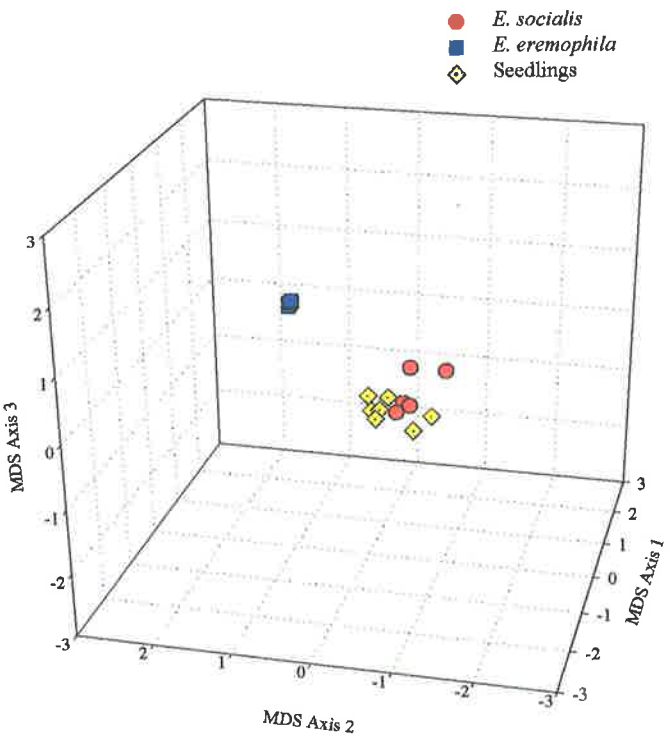


Figure 8.43. Multidimensional ordination of *E. socialis* x *E. eremophila* seedlings and parents.

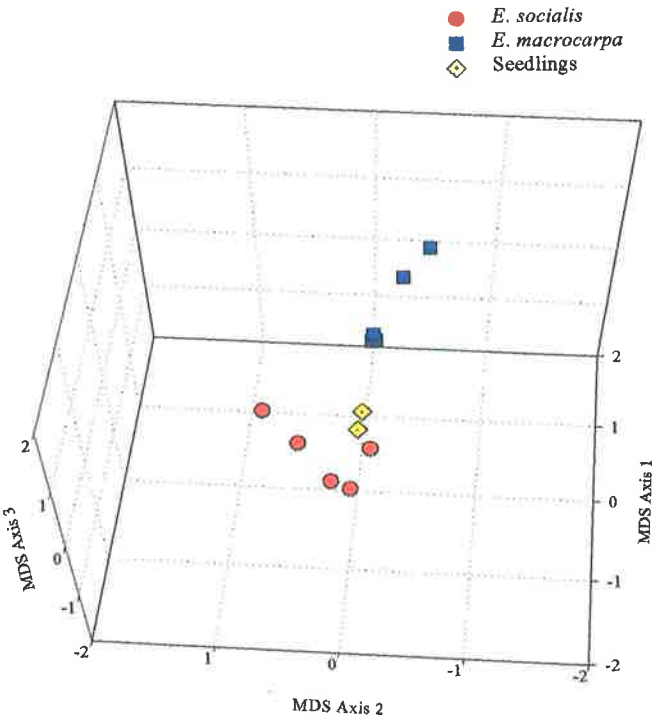


Figure 8.44. Multidimensional ordination of *E. socialis* x *E. macrocarpa* seedlings and parents.

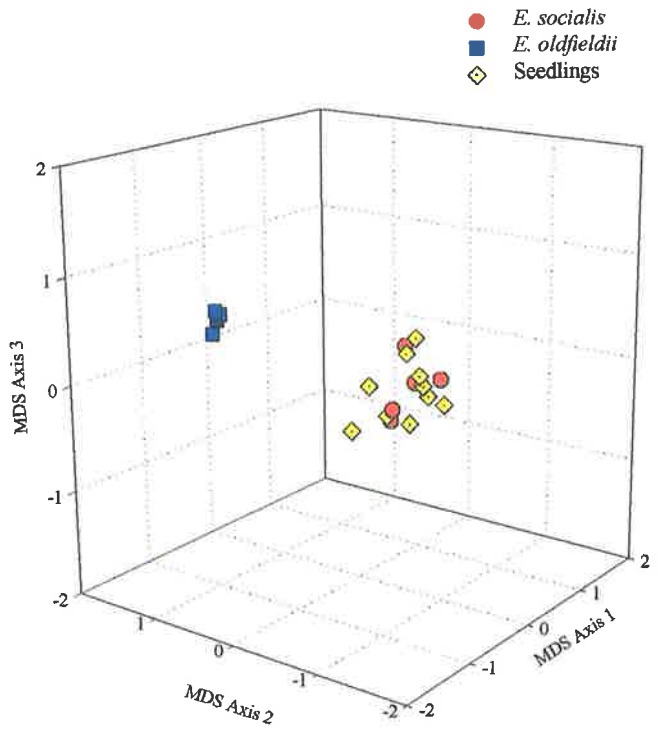


Figure 8.45. Multidimensional ordination of *E. socialis* x *E. oldfieldii* seedlings and parents.

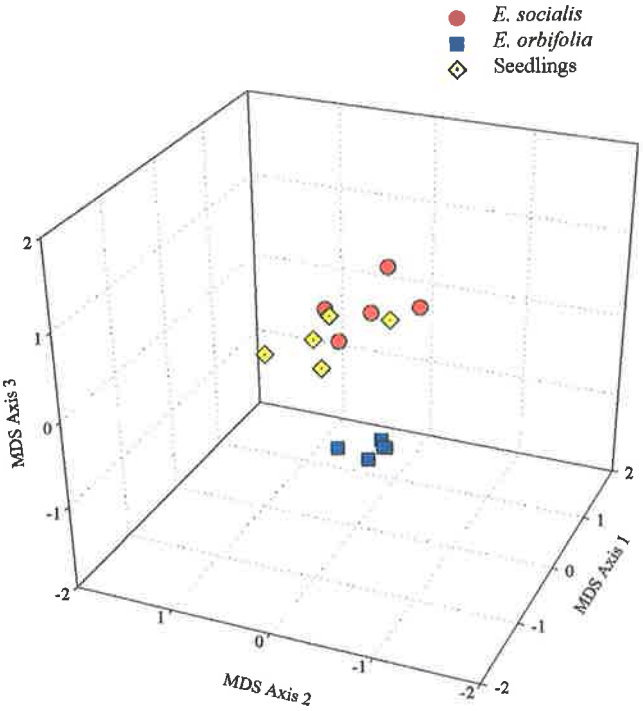


Figure 8.46. Multidimensional ordination of *E. socialis* x *E. orbifolia* seedlings and parents.

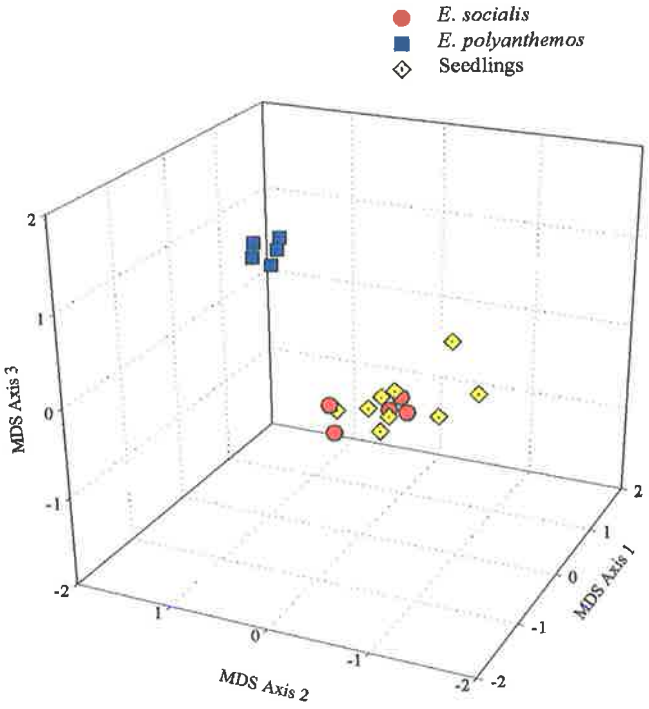


Figure 8.47. Multidimensional ordination of *E. socialis* x *E. polyanthemos* seedlings and parents.

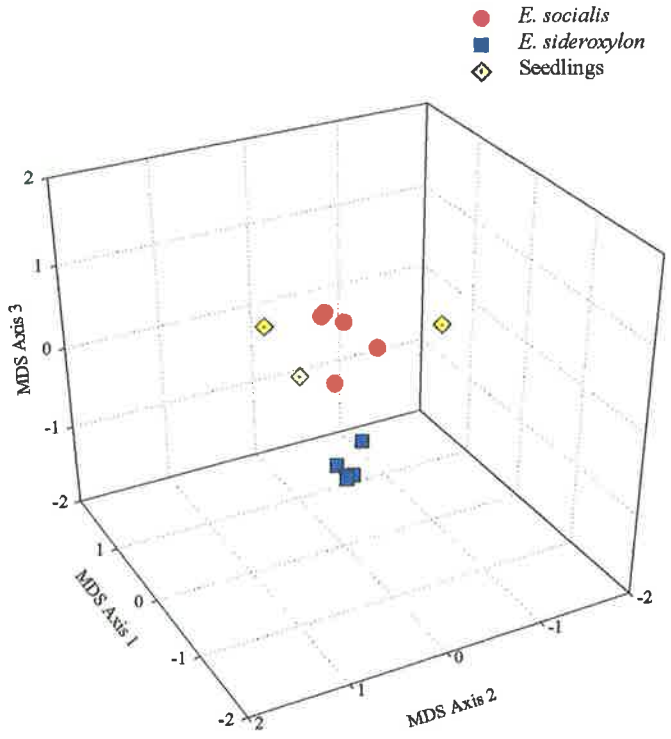


Figure 8.48. Multidimensional ordination of *E. socialis* x *E. sideroxylon* seedlings and parents.

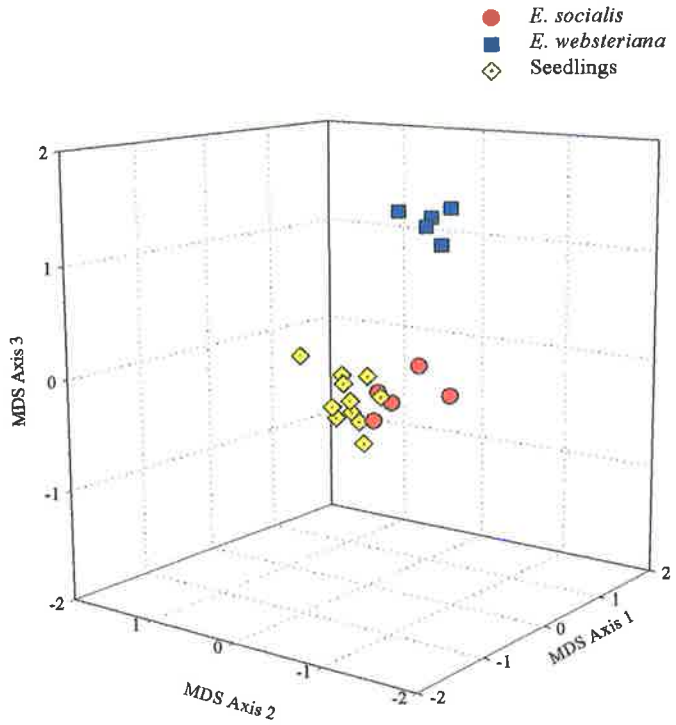


Figure 8.49. Multidimensional ordination of *E. socialis* x *E. websteriana* seedlings and parents.

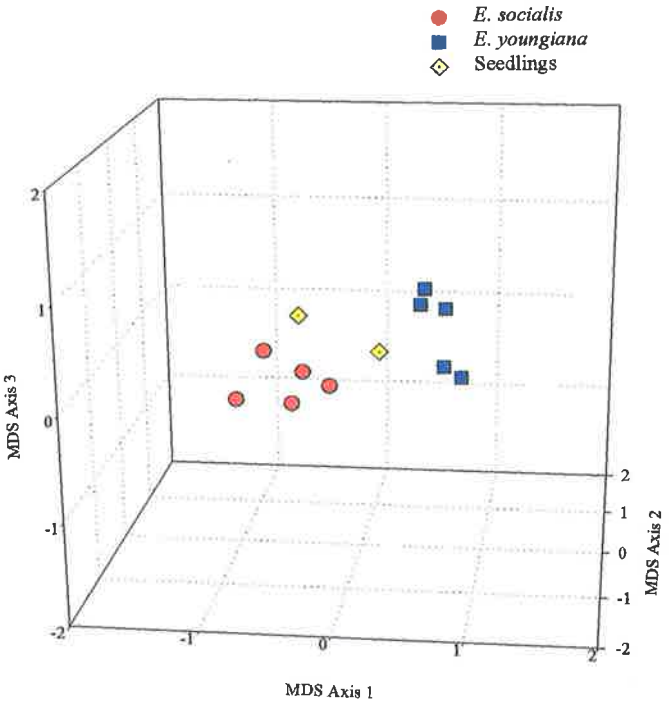


Figure 8.50. Multidimensional ordination of *E. socialis* x *E. youngiana* seedlings and parents.

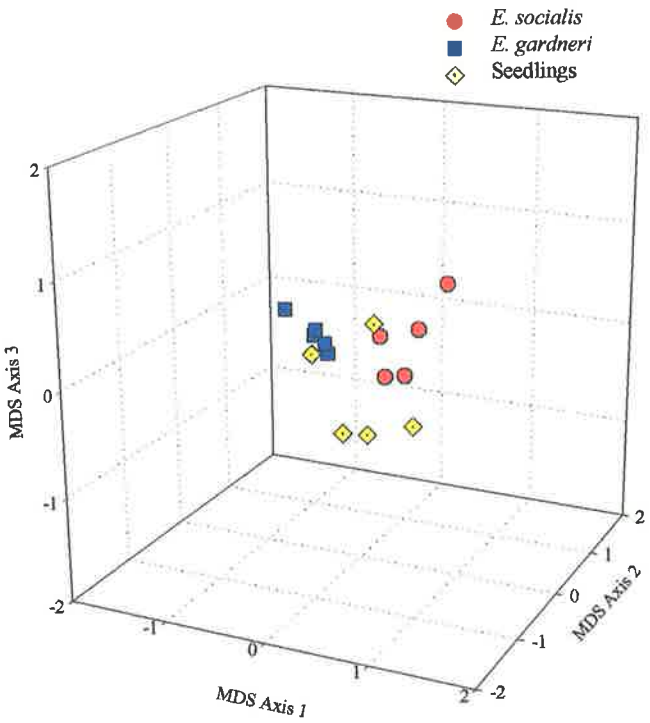


Figure 8.51. Multidimensional ordination of *E. socialis* x *E. gardneri* seedlings and parents.

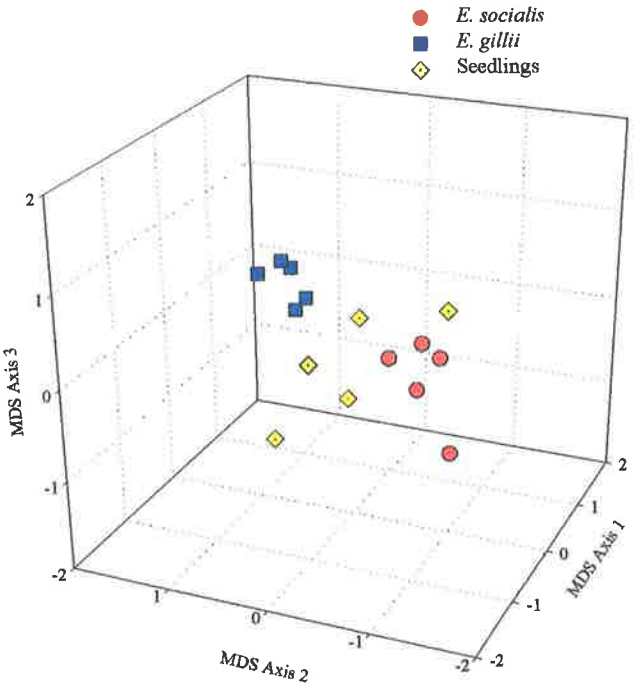


Figure 8.52. Multidimensional ordination of *E. socialis* x *E. gillii* seedlings and parents.

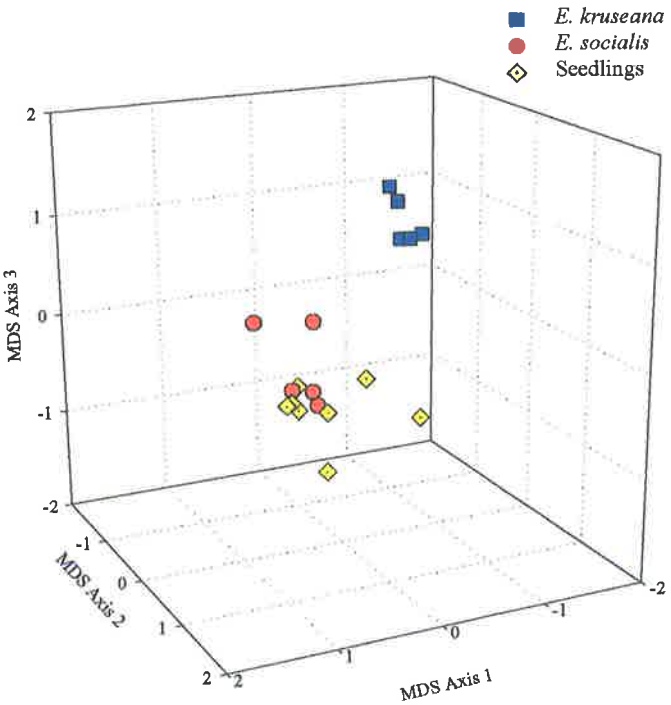


Figure 8.53. Multidimensional ordination of *E. socialis* x *E. kruseana* seedlings and parents.

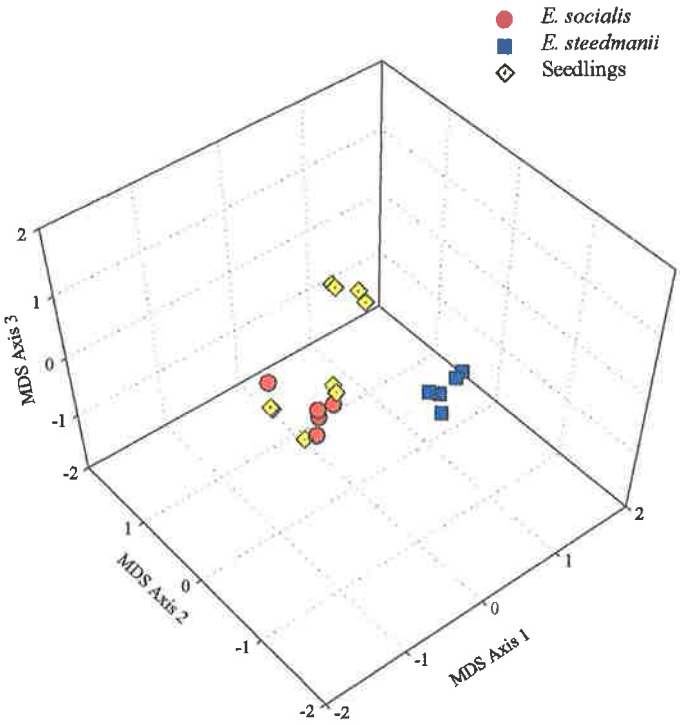


Figure 8.54. Multidimensional ordination of *E. socialis* x *E. steedmanii* seedlings and parents.

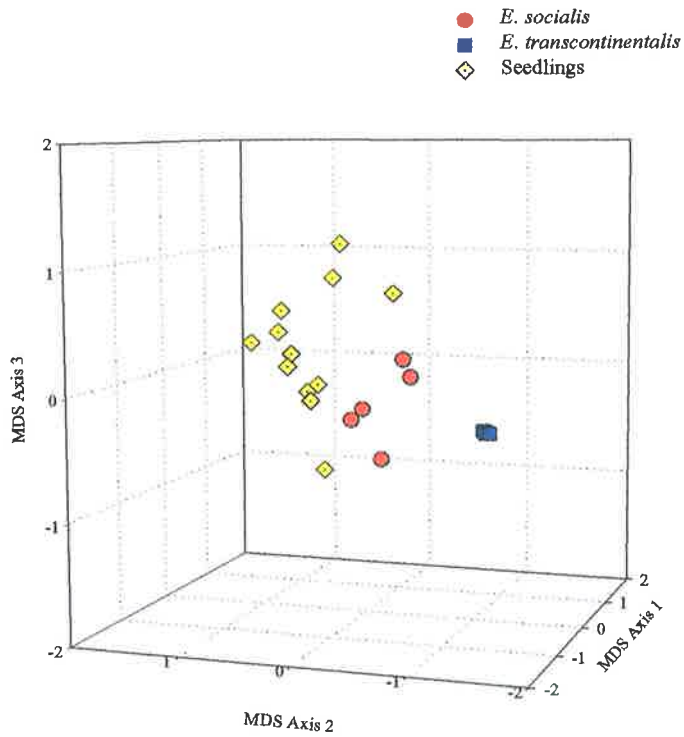


Figure 8.55. Multidimensional ordination of *E. socialis* x *E. transcontinentalis* seedlings and parents.

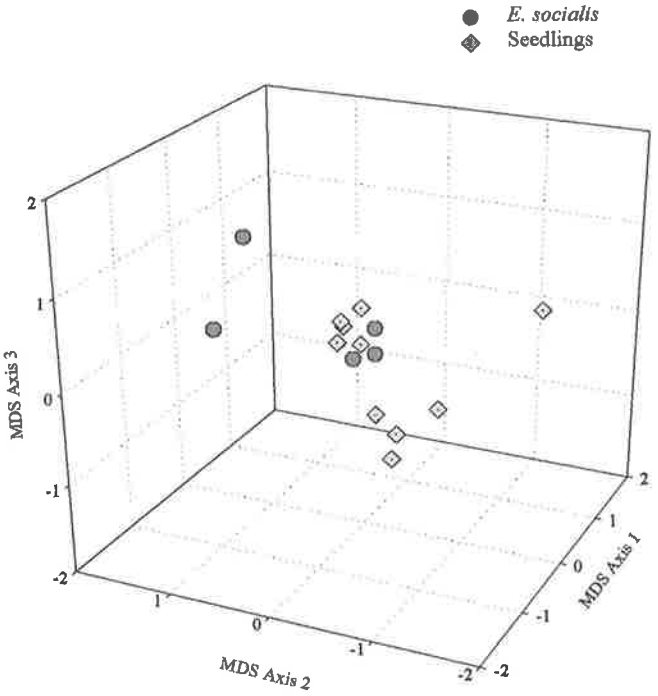
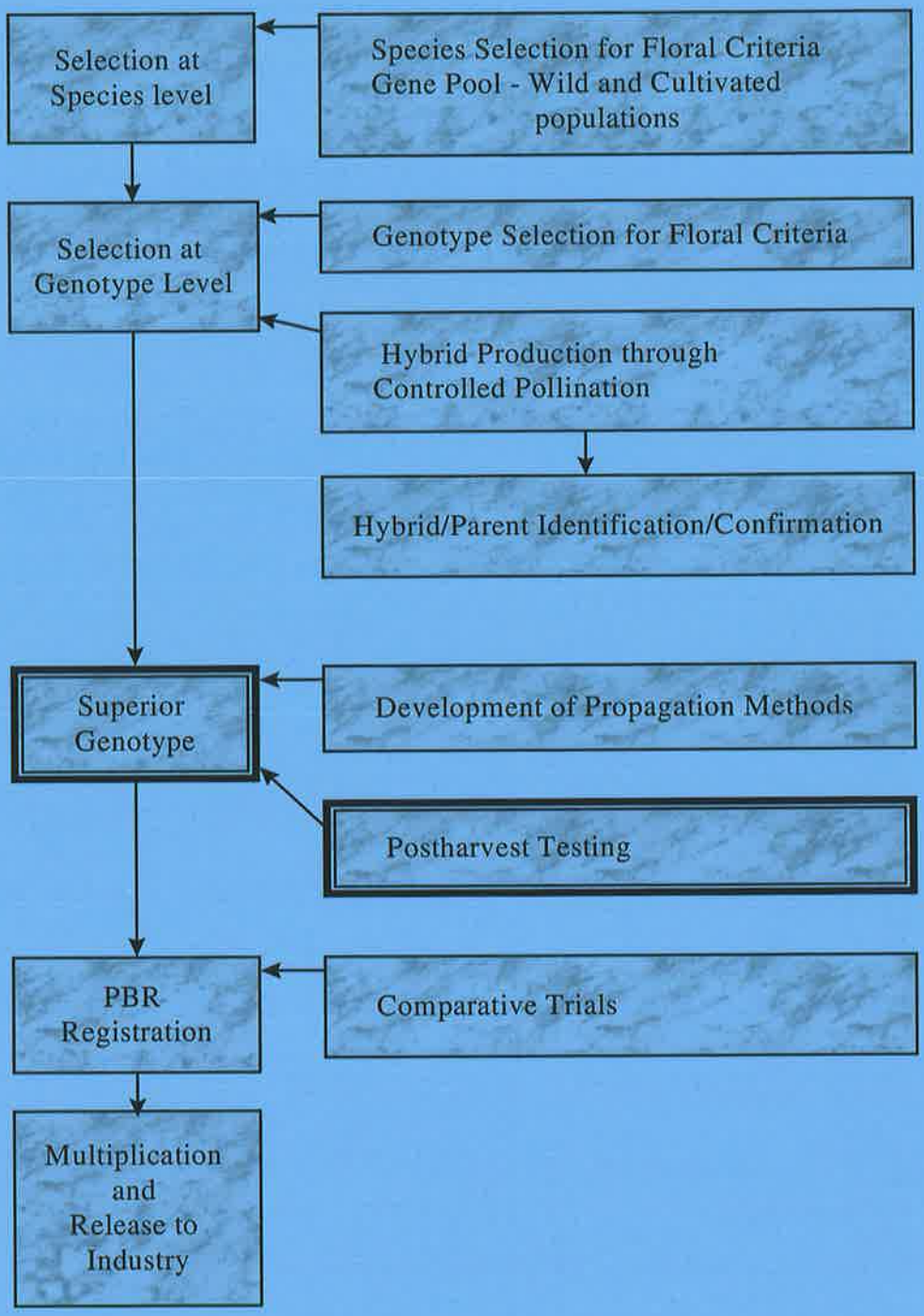


Figure 8.56. Multidimensional ordination of *E. socialis* x *E. socialis* seedlings and parents.



Ornamental eucalypt breeding cycle flow chart.

Areas covered by this chapter are highlighted.

Chapter Nine

Postharvest vase life of four flowering *Eucalyptus* species

9.1. Abstract

The effects of sucrose, citric acid and cold storage were assessed on flowering stems of *Eucalyptus tetragona*, *E. youngiana, E. forrestiana* subsp. *forrestiana* and *E. stoatei*. Continuous application of 0.5 to 5.0% sucrose in the vase solution reduced vase life of *E. tetragona* flowers, from >13 days for the reverse osmosis water control to <10 days; vase life of *E. youngiana* was not affected by sucrose. Pulsing with 0.5 to 10% sucrose, in conjunction with cold dry storage at 3°C for one to two weeks, had no effect on vase life of *E. tetragona* flowers, which had a subsequent vase life of 11-12 days. Preliminary trials with *E. forrestiana* subsp. *forrestiana* and *E. stoatei* indicate that pulsing with sucrose at 2.0 to 5.0% may be beneficial to vase life. Sucrose, at any concentration, pulsed or in holding solution, did not increase flower opening after harvest of *E. tetragona* or *E. youngiana*. Citric acid had no effect on vase life of any species tested. Significant differences in vase life were found between plants within a species; the implications of this for future testing, selection, and breeding programs are discussed.

9.2. Introduction

Some species from the genus *Eucalyptus* are recognised on world floriculture markets as foliage filler crops, but little attention has been paid to the flowers and buds of eucalypts, which have potential as features in floral arrangements (Sedgley, 1998). There are a number of species that may prove suitable, and research is required into production, postharvest handling and varietal selection.

The eucalypt flower is distinctive, as it has no visible sepals and petals when open. The stamens, often brightly coloured, are a significant component of the showy display of the flower, while the sepals and petals have evolved to become fused to form the cap, or operculum, which covers the flower buds. The cap is shed at anthesis, enabling the inflexed filaments to unfold radially and the anthers to dehisce, releasing pollen (Eldridge *et al.*, 1993). In some species the operculum may be coloured and attractive, as may the hypanthium. Inflorescences are either axillary or terminal, with one to many flowers. The lack of sepals and petals, the clustering of flowers in an inflorescence, and the progressive opening of flowers from the base to the apex of the shoot, will influence the response of the stem to postharvest treatments.

Studies to date have focussed on the production and postharvest care of eucalypt foliage, indicating low levels of sucrose may be beneficial to vase life (Wirthensohn *et al.*, 1996; Jones *et al.*, 1994), although levels of 10% caused leaf browning and damage (Jones *et al.*, 1994; Jones and Sedgley, 1993). Dry storage at cool temperatures (1-5°C) is possible for up to 30 days with no reduction in vase life, however, higher storage temperatures reduce vase life significantly (Wirthensohn *et al.*, 1996; Jones *et al.*, 1994; Jones *et al.*, 1993; Forrest, 1991). Antimicrobial treatments have an effect on vase life, depending on the substance and plant material. Jones *et al.* (1993) concluded that vase life of *E. gunnii* was not increased by

five different antimicrobial compounds, whereas vase life of *E. crenulata* increased with the addition of 5 mg/L BCDMH (1-bromo-3-chloro-5,5-dimethylhydrantoin, 10 mg/L available chlorine) with no positive effect by 50 mg/L sodium dichloroisocyanuric acid (DICA), 250 mg/L benzalkonium chloride, 250 mg Hydroxy-quinoline-citrate (8HQC/L) or 50 mg/L *n*-alkyl dimethyl ethylbenzyl ammonium chloride (*Physan*-20) with water as the control. Sucrose pulsing (between 0.5% and 10% sucrose) for 24 hours has no significant effect on the vase life of a range of eucalypt foliage species (Wirthensohn *et al.*, 1996; Jones *et al.*, 1993; Jones *et al.* 1994).

Research on flowers of similar Australian native species, such as *Grevillea* and *Acacia*, indicate that a 3% sucrose holding solution may increase vase life of some *Grevillea* genotypes, but higher sucrose concentrations damage leaves (Ligawa *et al.*, 1997). *Acacia* cut flower stems require a flower opening solution immediately after harvest, containing Agral, aluminum sulphate and 1% sucrose, supplied as a cool pulse to enhance flower opening (Jones and Horlock, 1997). The pH of the holding solution should be low, about 3 to 4, and may reduce microbial activity (Halevy and Mayak, 1981). The pH is generally lowered by the addition of citric acid, which significantly increased the vase life of *Acacia amoena* flowering stems (Williamson and Milburn, 1995). The inclusion of citric acid in rehydration solutions for flowers such as *Acacia, Protea* and *Helicona*, amongst others, is recommended (Sacalis, 1993).

There is considerable variation across species and genotypes, and the potential of hybrids and superior genotypes to significantly increase vase life is apparent (Wirthensohn *et al.*, 1996), highlighting the importance of selection and rigorous standardised testing of all suitable species. Selection of genotypes can be made on the basis of postharvest performance. Anecdotal evidence suggests that the flowers of eucalypts have physical characteristics that

relate to different methods of pollination. One example is the secretion of nectar during anthesis, to encourage nectar feeding birds to the flower, which may not be desirable in a cut flower. The recording and study of such characteristics is important, and should be considered during selection at both species and genotype level.

In contrast to eucalypt foliage production, where plants are kept juvenile to produce suitable material (Wirthensohn *et al.*, 1996), the production of *Eucalyptus* buds and flowers requires the plants reach adulthood as early as possible. Buds and flowers should also be terminal, with little or no shoot growth above the buds, and flowers should continue to open after harvest.

A selection and crossing program was commenced in 1991 at the University of Adelaide (Ellis *et al.*, 1991; Wirthensohn *et al.*, 1999) and continues to identify and develop *Eucalyptus* species for cut flower and bud production, in addition to cut foliage. One species identified with potential for cut flower and bud production is *E. tetragona* (Plate 9.1A). *E. tetragona* is a medium shrub with opposite grey leaves and square stems, covered in a thick layer of wax (Wirthensohn and Sedgley, 1996). The seed capsules, produced after flowering, are also well accepted on world cut flower markets. Flower buds appear approximately 6 weeks before the flower season (November to January). Buds are small and white, in umbels of 3 in every leaf axil, opening to a small cream flower (Brooker and Kleinig, 1990). The white stems and flowers of *E. tetragona*, in combination with the time of flowering, indicate the potential of this species for the Christmas market, and interest by the industry is apparent (Jones and Sedgley, 1993). Other species identified with potential for the cut flower market are *E. youngiana* (Plate 9.1B), *E. forrestiana* subsp. *forrestiana* (Plate 9.1C), *E. forrestiana* subsp. *dolichorhyncha* Brooker, and *E. stoatei* (Plate 9.1D). *E. youngiana* has very large flowers (from yellow to deep red) during Spring and Summer, while *E. forrestiana* subsp. *forrestiana*,

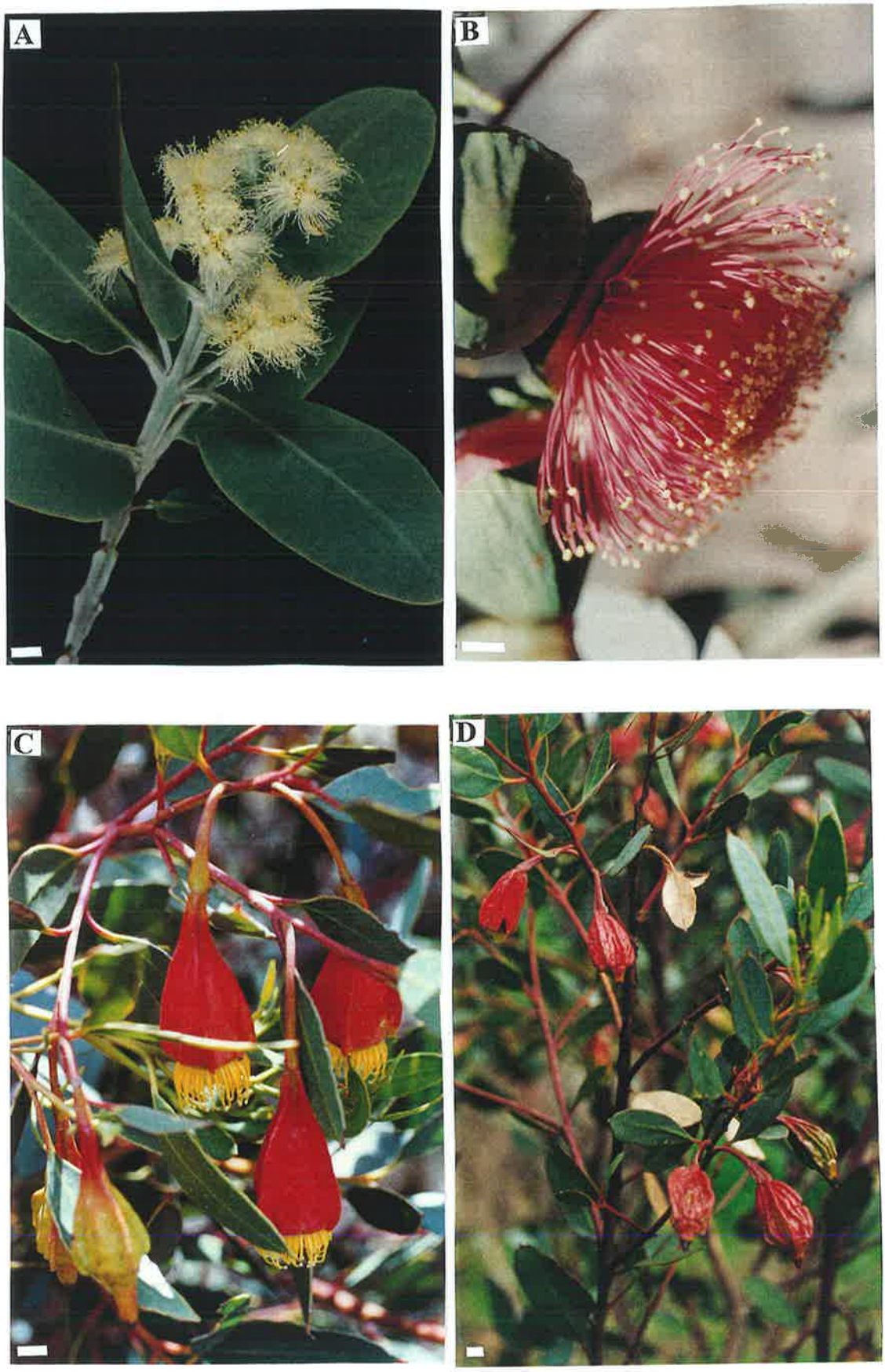


Plate 9.1.
Species used in postharvest trials. A: *E. tetragona;* B: *E. youngiana;* C: *E. forrestiana* subsp. *forrestiana;* D: *E. stoatei* . Bar = 1cm.

E. forrestiana subsp. *dolichorhyncha* and *E. stoatei* have bright red, pendulous buds throughout the year.

We conducted our study to establish the optimal postharvest holding conditions of *E. tetragona*, investigating continual holding in sucrose solutions, sucrose pulsing and cold storage to simulate transport to distant markets and their effect on flower opening and vase life. *E. youngiana,* was trialed with continual sucrose, while *E. stoatei* and *E. forrestiana* were also tested on a preliminary basis.

9.3. Materials and Methods

9.3.1. Plant material

Eucalyptus tetragona material was harvested from six trees between 2.5 and 4.5 years of age in the Laidlaw Plantation. Plants were grown from seed of unknown provenance, purchased from Nindethana Seed Company, W.A. Plants were grown under conditions of summer irrigation and quarterly fertiliser applications. Material from *E. youngiana* (six trees), *E. forrestiana* subsp. *forrestiana* (one tree) and *E. stoatei* (two trees) was harvested from 20-25 years old trees in the Monarto Woodland. Trees were grown from seed of unknown provenance, under natural conditions with no supplementary irrigation or fertilisers.

9.3.2. Harvest

Stems, between 15-40 cm, were harvested from all species when the first 1-2 flowers had opened. They were cut from the tree, left dry for up to two hours, and brought to the laboratory as quickly as possible. The lower leaves were removed, the stems recut (diagonal cut removing lower 10-20 mm of stem) and placed in RO water until allocation to treatments.

9.3.3. Vase life of flowers and leaves

The number of flowers on each stem was recorded at harvest, after pulsing and then daily as one of 4 stages: 1 - not open, 2 - operculum lift, 3 - flower fully open, 4 - flower wilt or stamen drop. Vase life of flowers was considered terminated when more than 50% of the flowers were at stage 4. The 'percentage of flowers open' for each stem was calculated as the maximum number of flowers at stage 2 and 3 throughout the assessment period, divided by the total number of flowers on that stem. The general appearance and colour of the leaves was recorded daily, and vase life of leaves was considered terminated when more than 50% of leaves showed desiccation and browning. Fresh weight and water uptake of each stem were measured daily, allowing the calculation of the first day of negative weight gain, indicating the onset of vascular blockage. Solutions were renewed daily as no biocide was used.

9.3.4. Experimental Treatments

9.3.4.1. Continual sucrose

Stems of *E. tetragona* and *E. youngiana* were placed in solutions containing Reverse Osmosis (RO) water (pH 6 to 7), RO water + 0.05 g/L citric acid (CA) (AnalaR, BDH Chemicals, Australia) (pH 3.5), 0.5% sucrose (AR Bulk, AJAX Chemicals, Australia)+ RO water + CA (pH 3.5), 1.0% sucrose + RO water + CA (pH 3.5), 2.0% sucrose + RO water + CA (pH 3.5) or 5.0% sucrose + RO water + CA (pH 3.5). Vase life was assessed at 22°C in a controlled temperature environment, with 12 hour day/night cycles under standard fluorescent lights (5.5 $\mu mol.m^{-2}.s^{-1}$).

9.3.4.2. Sucrose pulsing and cold dry storage

Stems of *E. tetragona, E. forrestiana* subsp. *forrestiana* and *E. stoatei* were pulsed in solutions containing RO water (pH 6 to 7), RO water + citric acid (CA) (pH 3.5), 0.5% sucrose + RO water + CA (pH 3.5), 2.0% sucrose + RO water + CA (pH 3.5) or 10.0%

sucrose + RO water + CA (pH 3.5) for 24 hours at 22°C. After pulsing, the stems were placed in cardboard boxes that were lined with a layer of newspaper then a layer of plastic sheet (100 μm), and placed in cold, dry storage at 3°C for 0, 7 or 14 days. After pulsing and storage, the stems were recut (diagonal cut to remove bottom 10-20 mm), placed in RO water and kept at 22°C in a controlled temperature environment, with 12 hours day/night cycles under standard fluorescent lights (5.5 $\mu mol.m^{-2}.s^{-1}$), for vase life measurement.

9.3.5. Statistical Analysis

The statistical designs (Randomised Complete Block Design) were: 1a) continual sucrose *(E. tetragona)* - five plants (as replicates) with six sucrose treatments and three stems per treatment [5 x 6 x 3 = 90 stems] with stems per plant randomly assigned to sucrose treatments; 1b) continual sucrose *(E. youngiana)* - six plants (as replicates) with five sucrose treatments and three stems per treatment [6 x5 x 3 = 90 stems] with stems per plant randomly assigned to sucrose treatments; and 2) sucrose pulsing and cold storage *(E. tetragona)* - three storage periods for six plants (as replicates) with five sucrose treatments and three stems per treatment [3 x 6 x 5 x 3 = 270 stems] with stems per plant randomly allocated to pulses and storage periods. Trials with *E. forrestiana* and *E. stoatei* were set up as design 2) with three storage treatments, five sucrose treatments and three stems per treatments, but only one and two plants per species were available. General Analysis of Variance (ANOVA) was used to determine the effect of the treatments on the criteria measured. Where no significant effect of treatment was found, analysis for the effect of plants was undertaken (One-way ANOVA). The data was tested using Genstat 5 Release 4.1. (PC/Windows NT, 1997, Lawes Agricultural Trust, Rothamsted Experimental Station) with L.S.D. and Power Analysis used where appropriate (Appendix 1.3.).

9.4. Results

9.4.1. Continual Sucrose

Maximum vase life of *E. tetragona* flowers and leaves was 13 days and 17 days respectively, with no effect of citric acid (Table 9.1). Vase life was reduced with continual sucrose exposure but the effect was largely independent of concentration (Figure 9.1). Continual high sucrose (5.0%) also induced leaf margin browning, and hastened desiccation. No difference due to sucrose was evident in the percentage flowers opening after harvest (Figure 9.2) or time to weight loss after harvest (Figure 9.3). Differences between plants were evident (Table 9.2), with plant 2 exhibiting the longest vase life of flowers and leaves, 12 and 16 days respectively (Figure 9.4). The most flowers opened after harvest on plant 2 (Figure 9.5). Time to weight change is not a good indicator to predict life of stems from different plants, as plant 2 showed negative weight gain after three days, while Plant 3, with the next longest vase life, showed negative weight gain after five days (Fig 9.6). Visual observations of the stem did not reveal any detrimental features of the species, with little nectar production, insignificant pollen release and limited stamen drop.

The maximum vase life of flowers and leaves of *E. youngiana* was 11.1 and 13.5 days, respectively, with no effect of sucrose treatment or acidification of vase solution (Table 9.1., Figure 9.7). There was no effect of sucrose on the percentage flowers opening after harvest (Figure 9.8) or day of weight change after harvest (Figure 9.9). Further analysis indicated that significant differences between plants were evident (Table 9.2), with plant 2 having the longest vase life (Figure 9.10) and longest time to negative weight change (Figure 9.11), but least percent flowers opening after harvest (Figure 9.13). Plants 3, 4 and 5 had the shortest vase life and fastest day to weight change, but the highest percentage of flowers opening after harvest. Plant 6 showed similar vase life and 56.7% flowers open after harvest. Undesirable features recorded for *E. youngiana* included the secretion of considerable quantities of nectar

from each flower during the first three days after opening, and the dropping of large quantities of pollen during the same period.

Table 9.1. Effect of continual sucrose on vase life of flowers and leaves, flowers opening after harvest and time to negative weight change for *Eucalyptus tetragona* and *E. youngiana* stems.

	Vase life (days)[y]		Flowers open after harvest	Time to negative weight change
	Flowers	Leaves	(%)	(days)
E. tetragona				
control (pH 6-7)	13.3[a]	17.5[a]	79.7[a]	3.2[a]
control + CA[z] (pH 3.5)	11.7[ab]	16.3[a]	80.7[a]	4.1[a]
0.5% sucrose + CA (pH 3.5)	10.2[bc]	13.1[b]	73.1[a]	3.9[a]
1.0% sucrose + CA (pH 3.5)	9.5[bc]	11.1[bc]	66.3[a]	4.0[a]
2.0% sucrose + CA (pH 3.5)	8.1[c]	10.2[c]	54.0[a]	3.3[a]
5.0% sucrose + CA (pH 3.5)	8.8[c]	10.1[c]	62.3[a]	3.9[a]
E. youngiana				
control (pH 6-7)	8.1[a]	12.7[a]	68.2[a]	2.7[a]
control + CA[z] (pH 3.5)	8.3[a]	12.4[a]	48.4[a]	3.3[a]
0.5% sucrose + CA (pH 3.5)	9.4[a]	12.2[a]	77.9[a]	4.0[a]
1.0% sucrose + CA (pH 3.5)	11.1[a]	13.5[a]	53.9[a]	3.3[a]
2.0% sucrose + CA (pH 3.5)	10.7[a]	12.7[a]	56.4[a]	3.8[a]

[y] Data are averages of three stems from each of five plants (as replicates) with six treatments for *E. tetragona*, or averages of three stems each of six plants (as replicates) with five treatments for *E. youngiana*. Different superscripts indicate significant differences in a column at $P<0.05$.
[z] CA = citric acid

Anova tables Appendix 1.3

Table 9.2. Effect of plant on vase life of flowers and leaves, flowers opening after harvest and time to negative weight change in continual sucrose trials for *E. tetragona* and *E. youngiana* stems.

	Vase life (days)[y]		Flowers open after harvest	Time to negative weight change
	Flowers	Leaves	(%)	(days)
E. tetragona				
Plant 1	8.7^{c}	11.9^{bc}	40.9^{c}	2.9^{b}
Plant 2	12.3^{a}	15.7^{a}	92.0^{a}	3.4^{b}
Plant 3	11.9^{ab}	14.6^{a}	68.3^{b}	5.4^{a}
Plant 4	10.1^{bc}	13.6^{ab}	90.0^{a}	4.6^{a}
Plant 5	8.4^{c}	9.6^{c}	54.4^{bc}	2.3^{c}
E. youngiana				
Plant 1	8.5^{bc}	15.5^{a}	41.5^{bc}	2.7^{b}
Plant 2	11.4^{a}	15.3^{a}	35.2^{bc}	5.7^{a}
Plant 3	7.4^{c}	10.3^{c}	70.0^{ab}	2.7^{b}
Plant 4	9.4^{abc}	10.8^{c}	82.5^{a}	2.8^{b}
Plant 5	9.2^{abc}	10.9^{c}	79.8^{a}	2.9^{b}
Plant 6	11.2^{ab}	13.4^{b}	56.7^{abc}	3.7^{b}

[y] Data are averages of 18 stems from each of five plants (as replicates) for *E. tetragona*, or averages of 18 stems each of six plants (as replicates) for *E. youngiana*. Different superscripts indicate significant differences in a column at $P<0.05$.

Anova tables Appendix 1.3

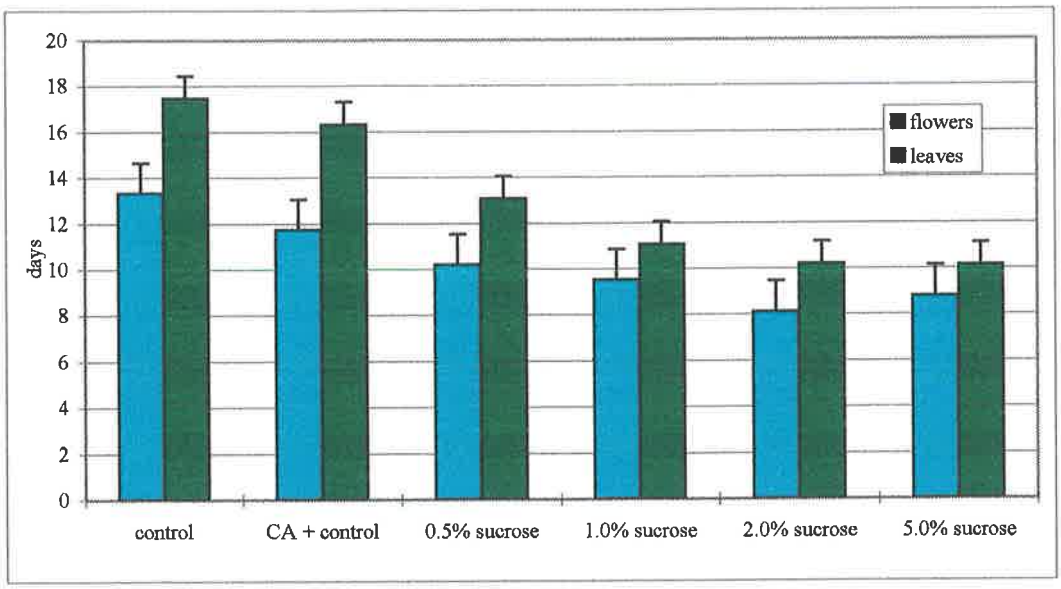


Figure 9.1. Vase life (days) of *E. tetragona* with sucrose

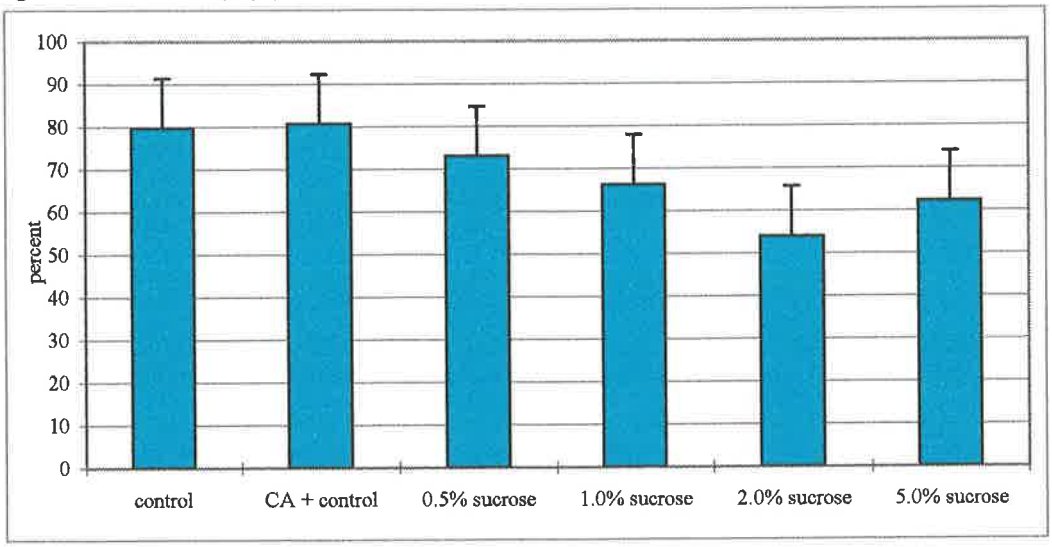


Figure 9.2. Percent flowers opening of *E. tetragona* with sucrose

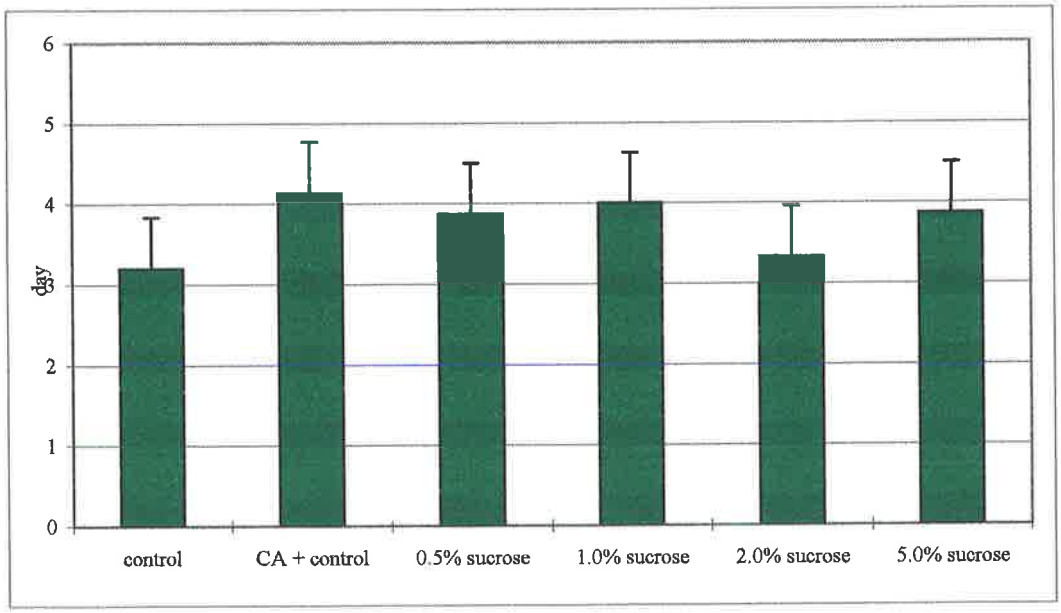


Figure 9.3. Time to negative weight change of *E. tetragona* with sucrose

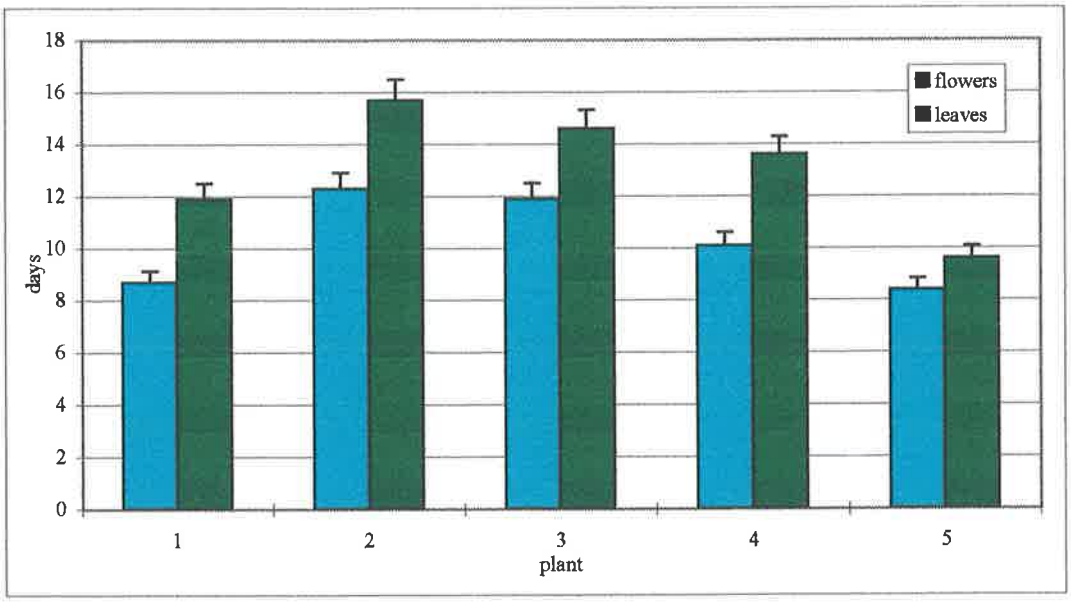


Figure 9.4. Vase life (days) of *E. tetragona* plants with sucrose

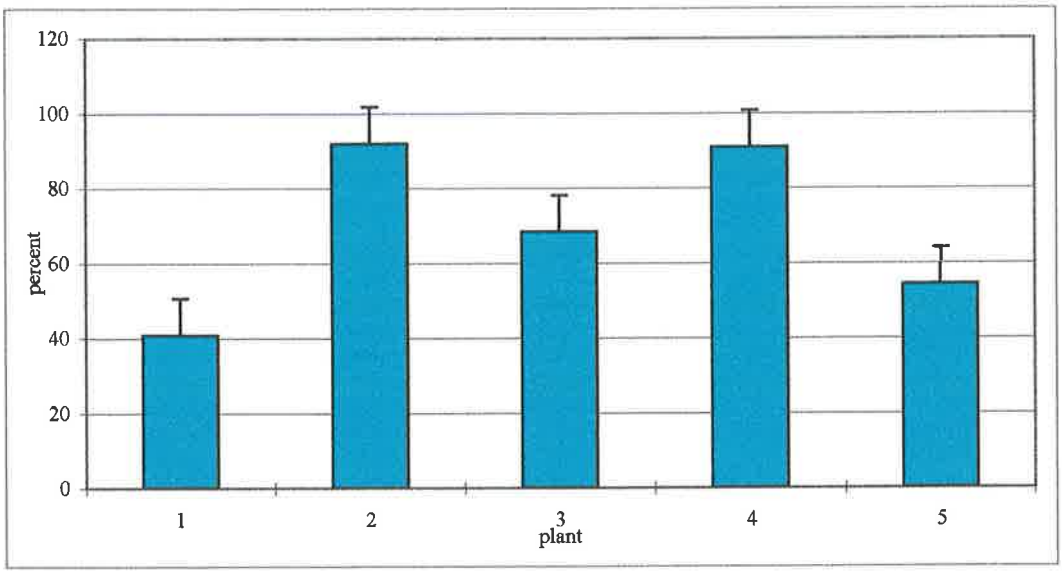


Figure 9.5. Percent flowers openingof *E. tetragona* plants with sucrose

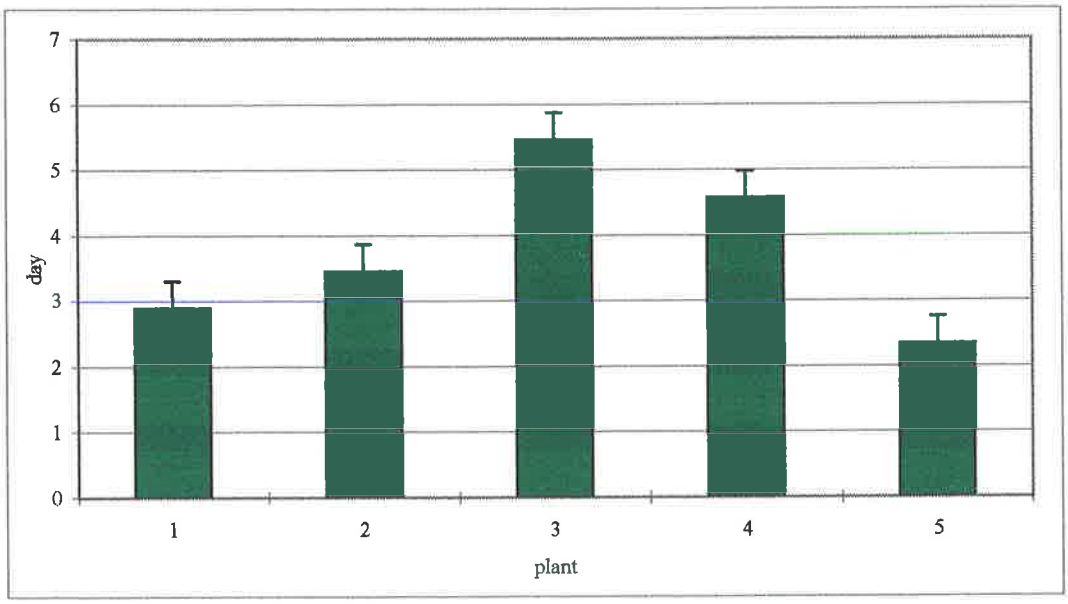


Figure 9.6. Time to negative weight change of *E. tetragona* plants with sucrose

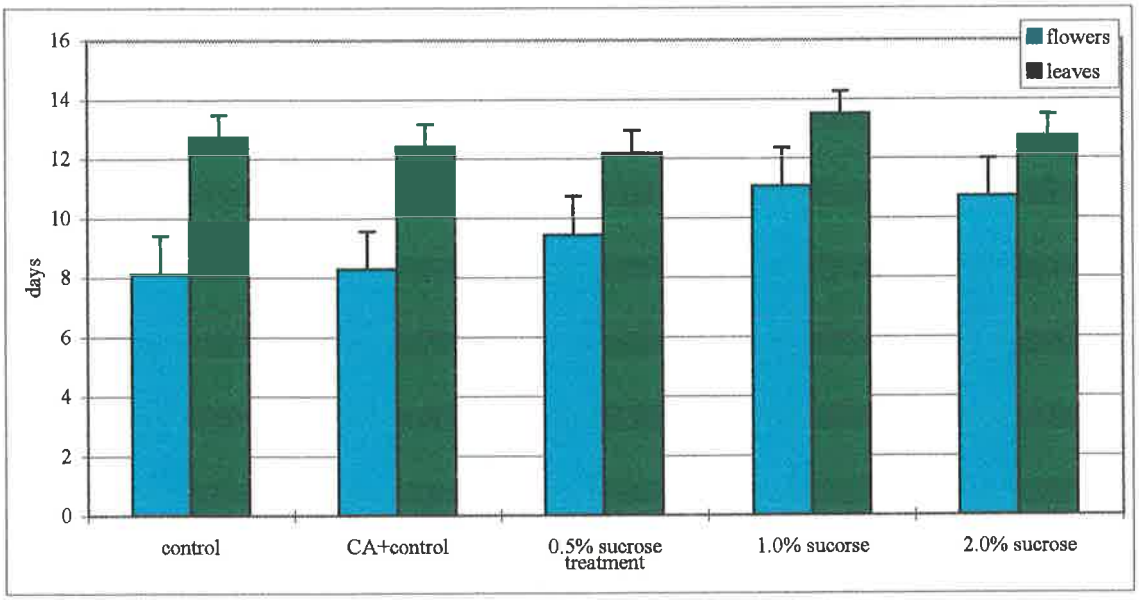


Figure 9.7. Vase life (days) of *E. youngiana* with sucrose

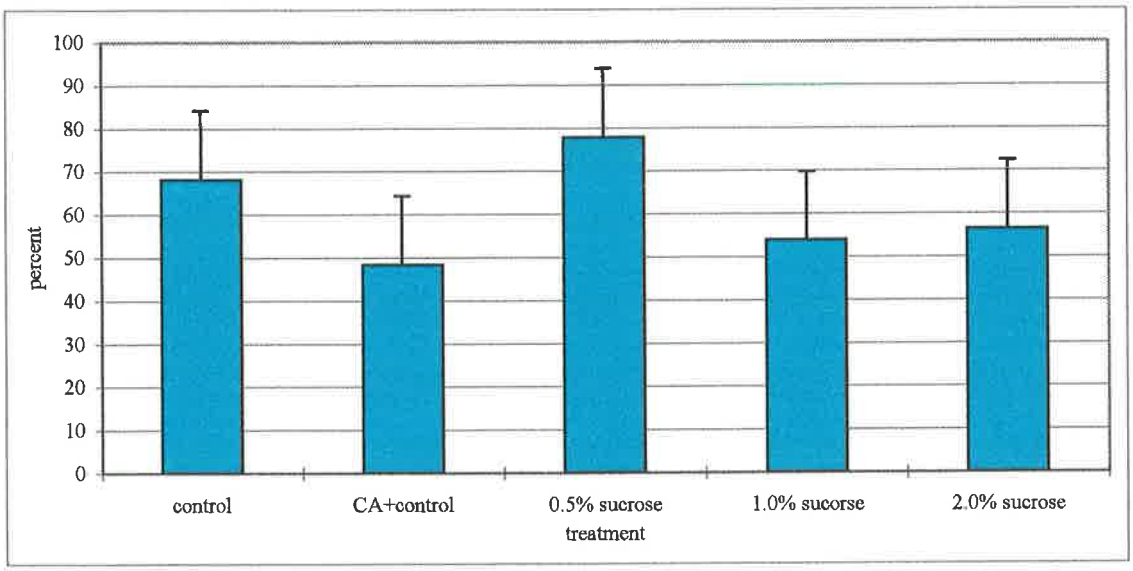


Figure. 9.8. Percent flowers opening of *E. youngiana* with sucrose

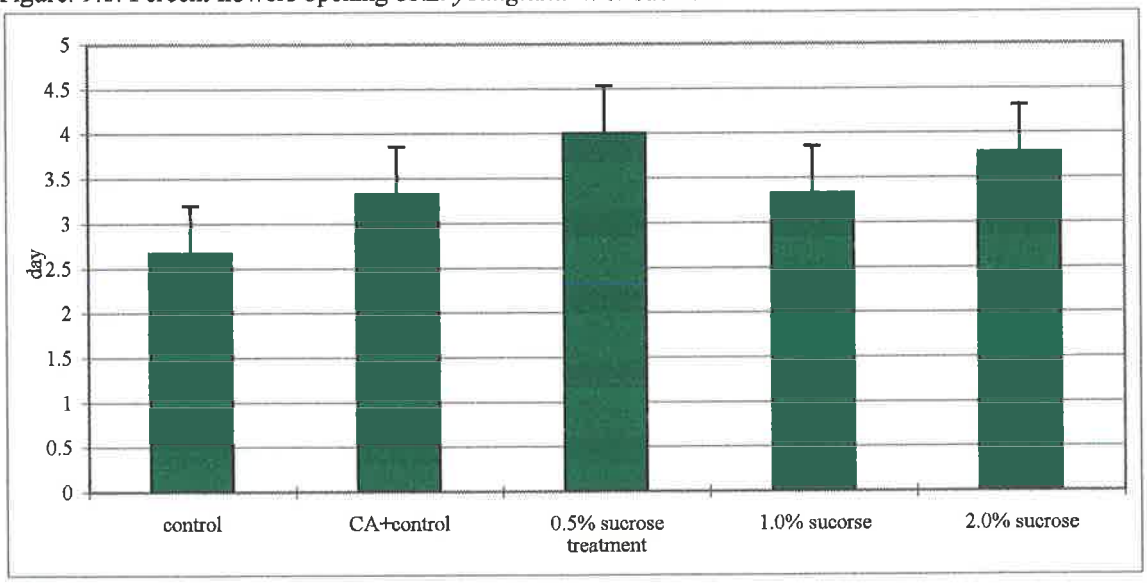


Figure 9.9. Time to negative weight change of *E. youngiana* with sucrose

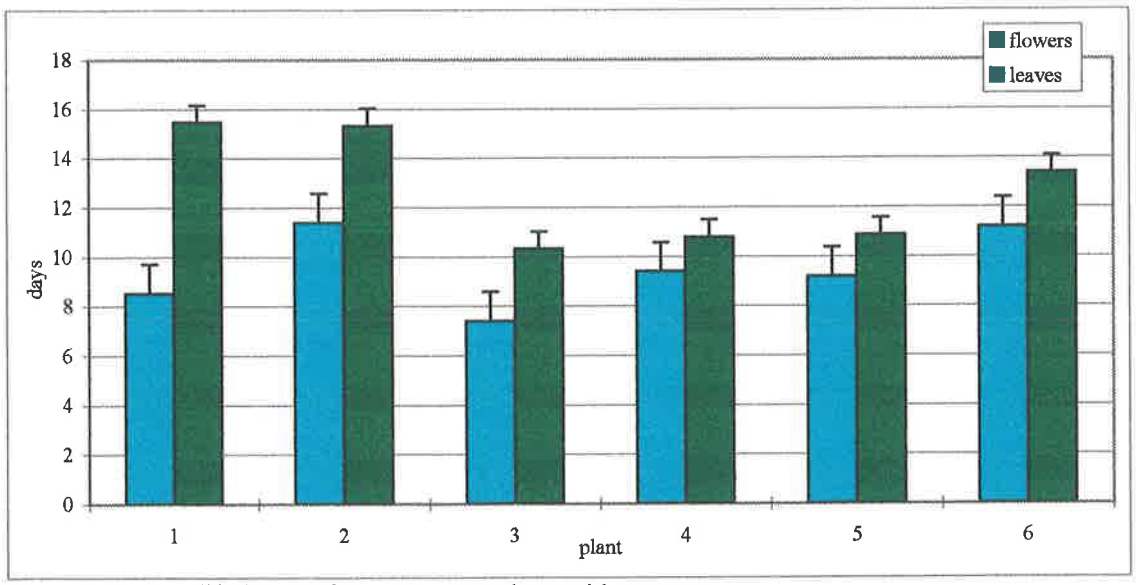


Figure 9.10. Vase life (days) of *E. youngiana* plants with sucrose

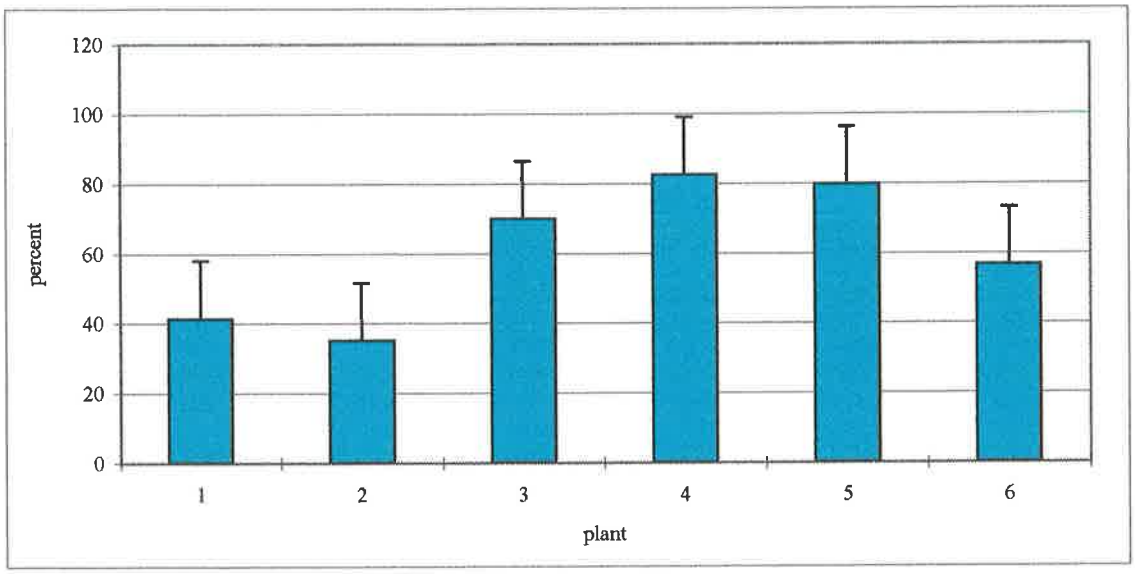


Figure 9.11. Percent flowers open of *E. youngiana* plants withsucrose

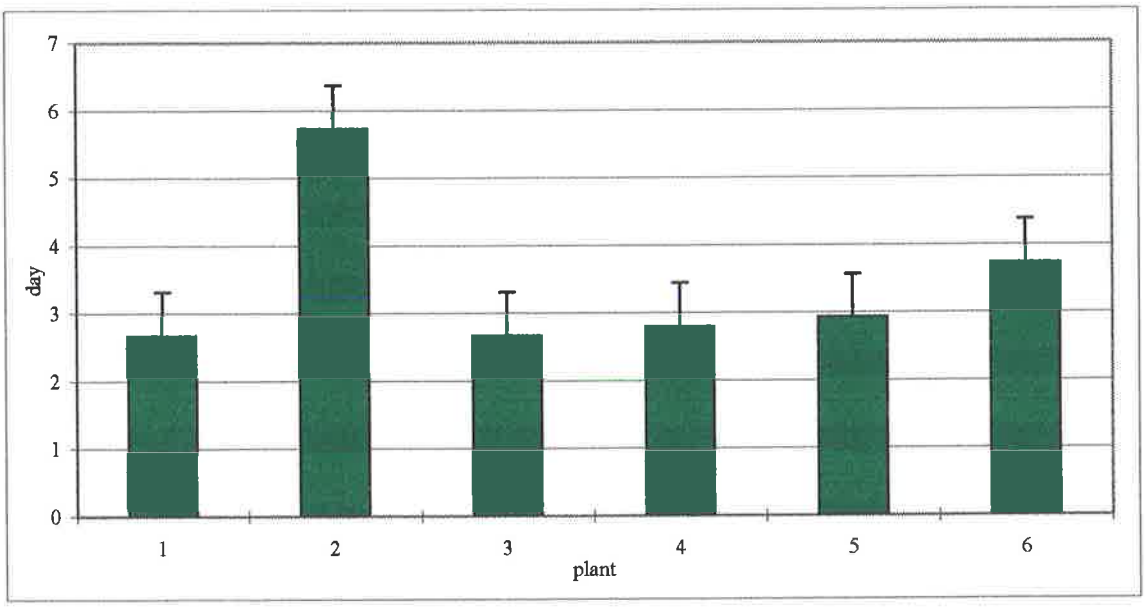


Figure 9.12. Time to negative weight change of *E. youngiana* plants with sucrose

9.4.2. Sucrose pulsing and cold storage

There was no effect of citric acid or sucrose pulsing on any of the criteria measured on stems of *E. tetragona* that were subsequently held in water (Table 9.3). Vase life ranged from 10.5 to 11.6 days for flowers and 15.8 to 17.2 days for leaves (Figure 9.13). Percentage of flowers opening after harvest was between 83 and 89% (Figure 9.14), and time to negative weight change was 9.9 to 10.3 days (Figure 9.15). There was no significant interaction between sucrose pulsing and cold storage on any of the criteria measured. Cold storage for 7 days extended the vase life of *E. tetragona* leaves adding 1.3 days, induced 7.2% more flowers to open after harvest and extended time to negative weight change by 1.4 days. Cold storage for 14 days, when compared to no storage, added 0.6 days vase life, induced 4.4% more flowers to open and extended time to negative weight change by 1.6 days. Further analysis determined there to be differences between plants of *E. tetragona* in all criteria measured (Table 9.4). Plant 2 had the longest vase life of flowers and leaves (Figure 9.16), 14 and 20 days respectively, and longest time to negative weight change, 11 days (Figure 9.17), but less than 78% flowers opening after harvest (Figure 9.18). The other plants were variable in their response, but worthy of note are plants 6 and 8, each with over 96% flowers opening after harvest, but with less than eleven days vase life of flowers. Pulsing with sucrose at 10% induced marginal blueing on the leaves; however, this disappeared within 24 hours of the stem being placed in RO water.

Table 9.3. Effect of sucrose pulsing and cold storage on vase life of flowers and leaves, flowers opening after harvest and time to negative weight change for *Eucalyptus tetragona* stems.

	Vase life (days)[y]		Flowers open after harvest	Time to negative weight change
	Flowers	Leaves	(%)	(days)
sucrose				
control (pH 6-7)	10.5[a]	15.8[a]	87.7[a]	9.9[a]
control + CA[z] (pH 3.5)	11.6[a]	17.1[a]	89.3[a]	10.3[a]
0.5% sucrose + CA (pH 3.5)	11.6[a]	16.6[a]	83.0[a]	10.0[a]
2.0% sucrose + CA (pH 3.5)	11.0[a]	17.2[a]	87.0[a]	10.1[a]
10.0% sucrose + CA (pH 3.5)	11.4[a]	16.6[a]	83.8[a]	10.2[a]
cold storage				
0 days at 3°C	11.4[a]	16.0[a]	82.3[b]	9.1[b]
7 days at 3°C	11.4[a]	17.3[b]	89.5[a]	10.5[a]
14 days at 3°C	10.9[a]	16.6[ab]	86.7[ab]	10.7[a]

[y] Data are averages of three stems each of six plants (as replications) with five sucrose x three cold treatments. Different superscripts indicate significant differences in a column at $P<0.05$.
[z] CA = citric acid

Anova tables Appendix 1.3

Table 9.4. Effect of plant on vase life of flowers and leaves, flowers opening after harvest and time to negative weight change in sucrose pulsing trial for *Eucalyptus tetragona* stems.

	Vase life (days)[z]		Flowers open after harvest	Time to negative weight change
	Flowers	Leaves	%	(days)
Plant 2	14.0[a]	20.0[a]	79.7[b]	11.0[a]
Plant 5	11.3[b]	15.7[c]	79.3[b]	10.0[bc]
Plant 6	9.0[c]	13.5[d]	96.9[a]	9.7[bc]
Plant 7	11.3[b]	16.6[c]	79.3[b]	9.9[bc]
Plant 8	10.4[b]	18.2[b]	97.9[a]	10.6[ab]
Plant 9	11.4[b]	15.8[c]	83.9[b]	9.2[c]

[z] Data are averages of 18 stems each of six plants (as replications).
Different superscripts indicate significant differences in a column at $P<0.05$.

Anova tables Appendix 1.3

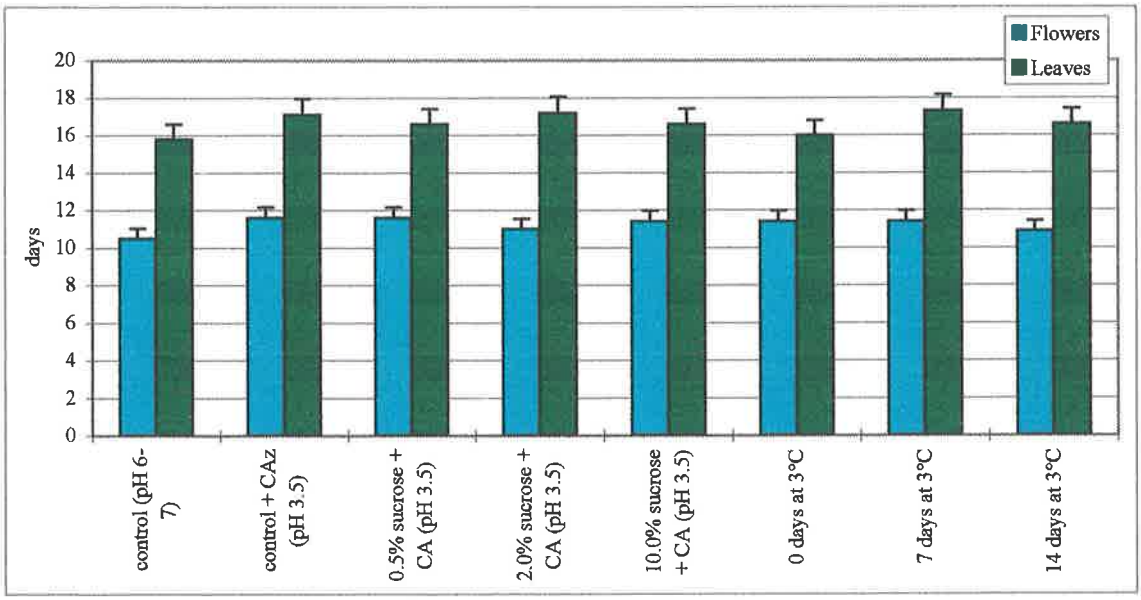


Figure 9.13. Vase life (days) of *E. tetragona* with sucrose and cold storage

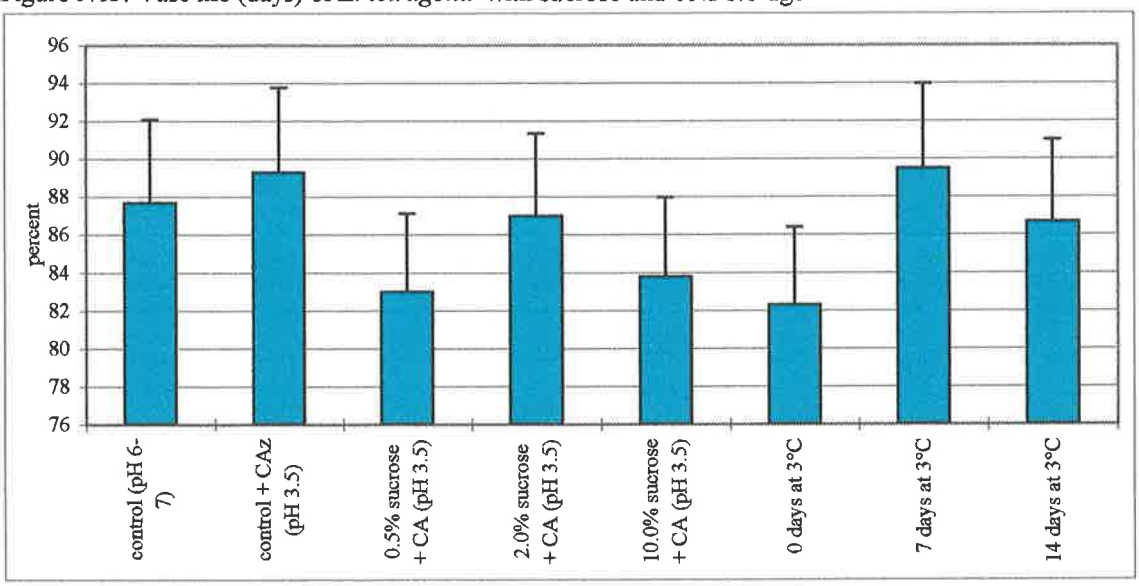


Figure 9.14. Percent flowers open of *E. tetragona* with sucrose and cold storage

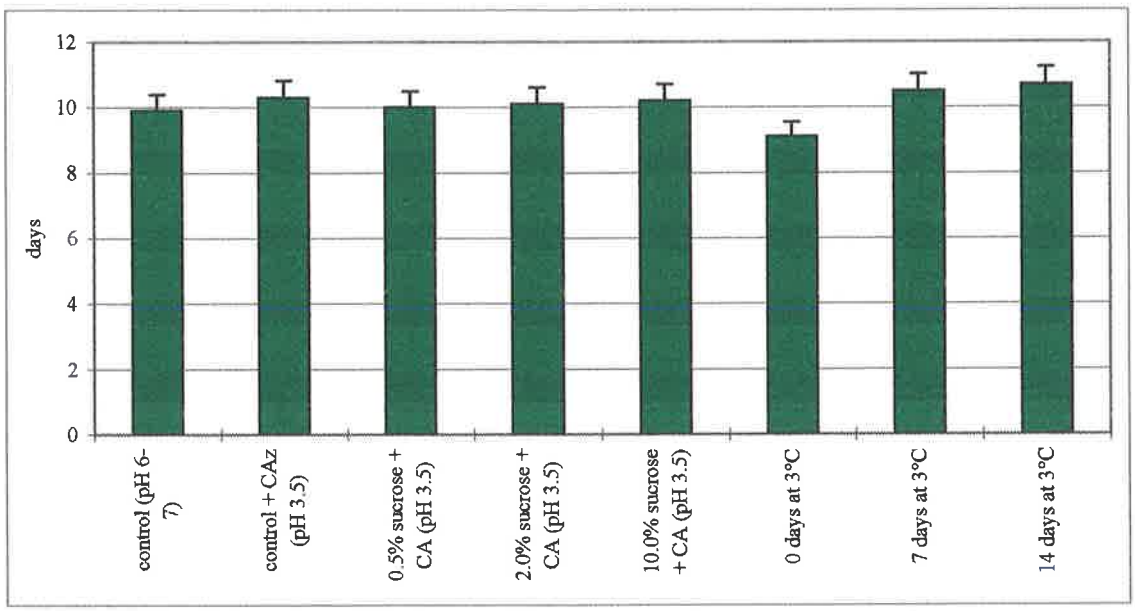


Figure 9.15. Time to negative weight change of *E. tetragona* with sucrose and cold storage

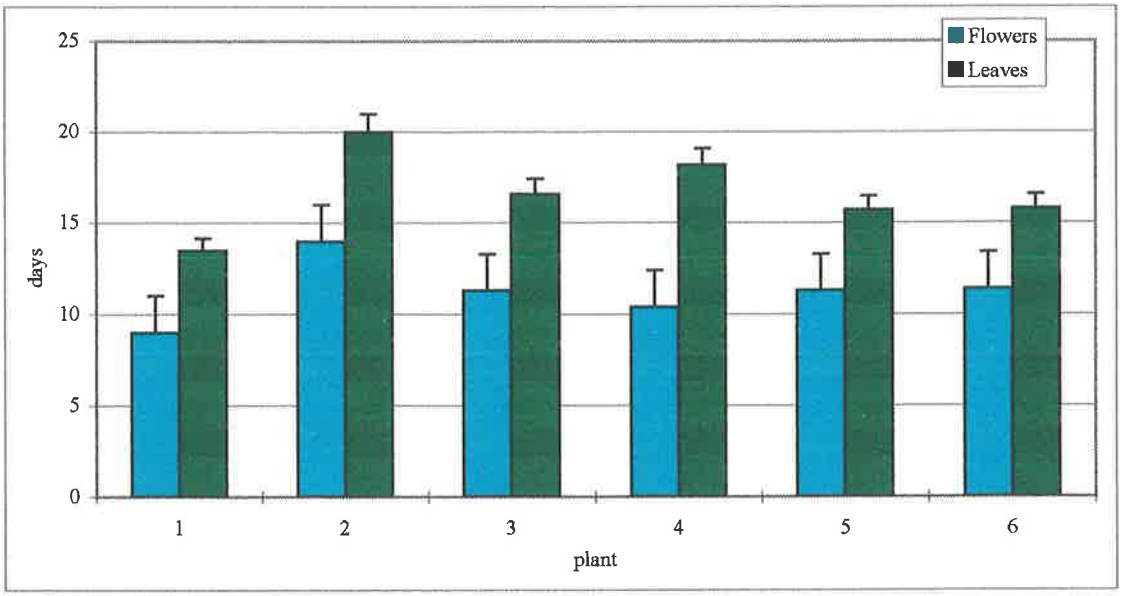


Figure 9.16. Vase life (days) of *E. tetragona* plants with sucrose pulsing and cold storage

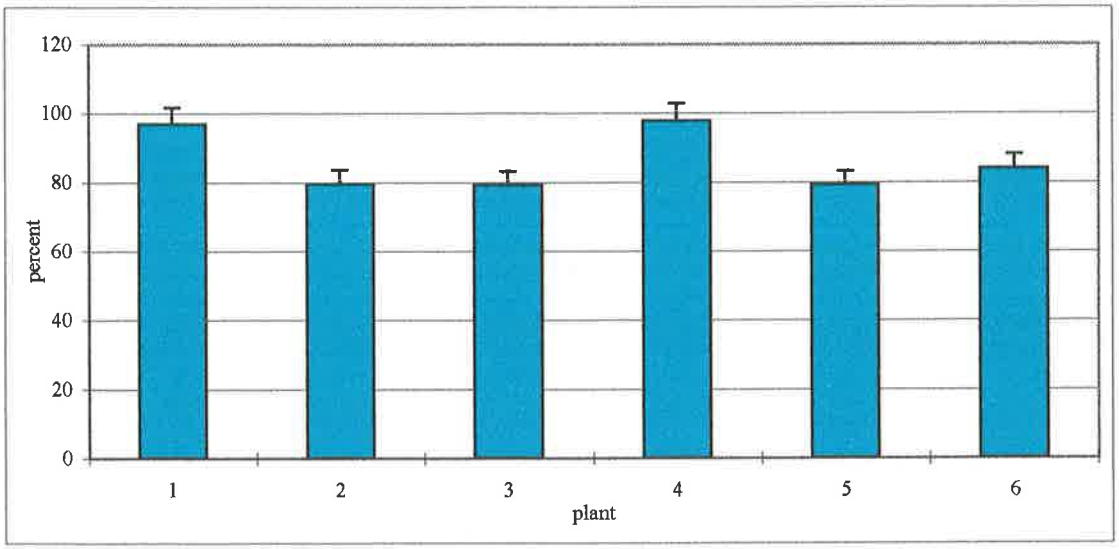


Figure 9.17. Percent flowers open of *E. tetragona* plants with sucrose pulsing and cold storage

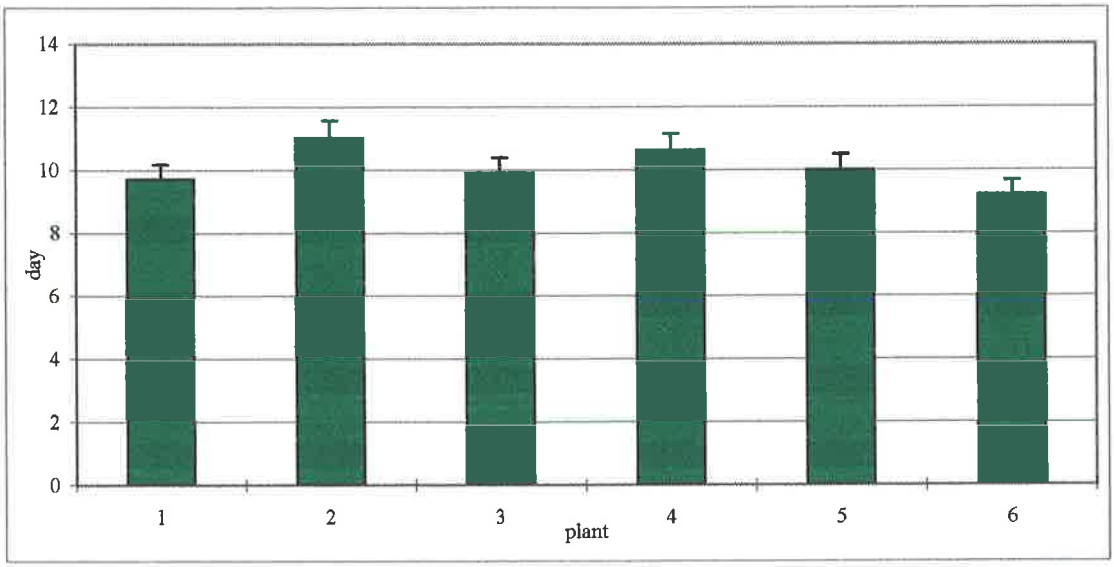


Figure 9.18. Time to negative weight change of *E. tetragona* plants with sucrose pulsing and cold storage

9.4.3. Observations of other species

As only one plant was available of *E. forrestiana* subsp. *forrestiana*, and only two plants for *E. stoatei*, these results can be used only to indicate potential effects of sucrose pulsing and cold storage on vase life. Sucrose pulsing with 2.0 % and 5.0% extended vase life to 12.8 and 12.1 days, respectively, compared to 10.3 days in RO water. No interaction between pulsing and cold dry storage was observed, and the latter did not affect vase life for *E. stoatei,* but there was a possible effect of sucrose pulsing on vase life, with a vase life of 18.6 days for those stems pulsed with 5% sucrose compared to 12.9 days in RO water and 13.3 days in RO water + citric acid. Cold storage for 7 days increased vase life by three days. Overall, these two species show acceptable vase life characters, and warrant further testing.

9.5. Discussion

The four flowering species studied varied in their response to postharvest treatments. Continual sucrose reduced vase life of *E. tetragona* by three to five days, compared to the RO water control, but this was not concentration dependent. The exogenously supplied sucrose had deleterious effects, such as browning and desiccation of leaves, at levels of 5.0%. These results are in contrast to the pulsing trials, where sucrose pulsing, with concentrations as high as 10%, had no effect on vase life and did not induce leaf browning. This difference could be due to the constant uptake of sucrose in the continual sucrose, resulting in a constantly high level within the plant, compared to the pulse, where a high concentration is taken up once only and thereafter only water is taken up. The high concentration, as a continuous supply, may do more damage on a cellular level, similar to the effect on rose leaves (Markart and Harper, 1995) than the single uptake of a high concentration. Neither continual sucrose nor sucrose pulsing increased the percentage of flowers open after harvest or cold storage, and had no effect on time to weight change. These results were reflected in the continual

sucrose trials with *E. youngiana.* Trials with *E. forrestiana* subsp. *forrestiana* and *E. stoatei* indicate that there may be a positive effect of sucrose pulsing in prolonging vase life, but these results are only preliminary and further testing is required.

Sucrose in solution, at varying concentrations, is used to prolong vase life and enhance flower opening of some flowering species. The presence of sucrose enables the plant to replace depleted endogenous carbohydrates, utilised during and after storage (Halevy and Mayak, 1981). It improves the water balance of the stem by triggering the closure of stomata resulting in a reduction of water loss; the sugar also accumulates in the flowers, increasing their osmotic concentration, and improving their ability to absorb water and maintain turgidity (Halevy and Mayak, 1981). However, sucrose does not benefit all species, in some cases the presence of sucrose is deleterious to the quality of the stem and overall vase life. The flower is not the only part of the flower to be considered the leaves are just as important in assessing vase life. In many cases the concentration of sugars and other substances used in solutions for 'pulsing' and 'bud opening' of flowers is determined by the sensitivity of the foliage to these ingredients and not by the optimal effect on flower development and longevity (Halevy and Mayak 1979). Markart and Harper (1995) found that the amount and speed of crisping of leaves of roses was positively related to the amount of sucrose in the preservative solution. Jones *et al.* (1993) found that sucrose increased the vase life of immature cut foliage stems of *Eucalyptus* species, and commented that exogenous sucrose may have caused an improvement in the development of immature leaf buds and maintenance of turgor in leaves.

Solution uptake rates were not affected by sucrose concentration (data not shown). There is considerable difference in time to weight change between the two trials, for *E. tetragona* between three and four days for continual sucrose and nine and ten days for sucrose pulsing. This cannot be attributed to sucrose treatment, as each trial included controls of RO water and

RO water with citric acid, both of which reflected the time to weight change of the other treatments. Both trials were conducted on material collected in mid December of consecutive years, negating the possible effect of time of year. It is possible that the preharvest condition of the plants affected this, and raises questions regarding the influence of production methods on vase life.

The addition of citric acid, or similar acid, to the holding solutions may increase longevity by reducing microbial population levels, or by increasing the flow of water through the stem (Halevy and Mayak, 1981). In these trials, acidification of the water by addition of citric acid had no discernible effect on vase life. Macroscopic observation of the solution did not reveal any evidence of increased microbial activity in the RO water (pH 6-7) compared to the acidified solutions. Water was replaced daily to reduce microbial activity, however microbial growth was apparent on the stems of *E. youngiana*, *E. forrestiana* subsp. *forrestiana* and *E. stoatei*, with no correlation to sucrose treatment or solution pH level. There is no obvious stem wax on any of these three species. At no stage, even at the end of the trial, was there evidence of microbial growth on the stem of *E. tetragona*. This may be caused by the high levels of wax on the surface of the stem. The precise physiological significance of stem wax is not known, but could be similar to that of epicuticular wax, which has been reported to act as a water repellent, in plant pathogen resistance and as an insect deterrent (Wirthensohn and Sedgley, 1996).

The vase life for *E. tetragona* flowers stored at 3°C for 7 or 14 days was the same as that for flowers with no storage at all, indicating that this length of cold storage is not detrimental to vase life. Although statistically significant differences were recorded for each of the cold storage periods for the criteria of vase life of leaves, percent flowers opening after harvest and time to negative weight change, the limiting factor was the life of the flowers. The

preliminary trials for *E. forrestiana* subsp. *forrestiana* and *E. stoatei* indicated some effect of sucrose pulsing and cold storage on vase life, with higher concentrations of sucrose extending vase life by several says, and 7 day cold dry storage also extending vase life by several days.

Cold temperatures slow the metabolic processes of the stem, enabling flowering material of some plants to be stored for some time with no reduction in vase life. The stem should be harvested at an optimal stage for storage - one that will allow flowers to continue to develop afterwards. Some flowers, such as rose, gladioli and iris, are picked at the bud stage, while others, such as carnation, chrysanthemum, and gerbera, do not develop once picked, and thus are picked at a later stage (Goszczyñska & Rudnicki 1988). Observations of the four species studied in these trials indicated that the flowers will continue to open up to seven days after harvest or storage, but only if they were sufficiently developed. The majority of *E. forrestiana* subsp. *forrestiana* and *E. stoatei* flowers did not open after harvest or storage.

Both *E. tetragona* and *E. youngiana* exhibited considerable differences between plants. For example, *E. tetragona* plant 2 was identical in both trials and showed the longest vase life in both of them. Plant 5 was identical in both trials, and in both trials showed medium vase life of flowers. Visual observations of these plants showed very similar morphology, with minor variation in flower size and number of flowers per stem.

The six plants tested for *E. youngiana* showed variation in vase life of flowers, from seven to eleven days. The most noticeable variation was shown in the percentage of flowers opening after harvest, where values ranged from 35 to 82%. With the exception of plant 1, those plants with the longest vase life had the lowest number of flowers opening after harvest, and the greatest time to negative weight change, indicating a possible positive correlation between flower opening and rate of senescence. The flowers of *E. youngiana* are very large, possibly

exacting a heavy toll on the physiological reserves of the cut stem when open and releasing nectar, resulting in faster senescence, as opposed to those stems whose flowers stay closed and static. It was determined that while pre-harvest health of the plant and time of harvest may have an effect on subsequent vase life, genotype may have a greater effect. Improvement of postharvest longevity is possible through testing a range of genotypes and selection of those with a long vase life for further development and breeding. It is likely that vase life is influenced by a combination of a number of heritable components, as was found in gerbera (Wernett *et al.*, 1996). Eucalypt breeding and selection to date has focussed on phenotypic quantitative and qualitative traits associated with forestry production, but the technique could be used to breed and select for genotypes with superior floral attributes and postharvest life.

The reproductive cycle of eucalypts is common to all species, but varies in length and time of year, between and within species. The cycle consists of six stages: inflorescence formation, formation of inflorescence initials (macroscopic appearance), development of flower buds from pin to cylindrical to plump, operculum shed and flowering, capsule development and seed dissemination. This cycle may start when the tree is one year old, but commonly commences in the third or fourth year after germination (Eldridge *et al.*, 1993). The cycle may take less than 12 months, from emergence of inflorescence initials to maturation of seed, to five years, depending on species, location and season (Florence, 1996). Trees may have buds, flowers and capsules at all stages on the tree at one time, so a knowledge of this cycle is important in production systems to ensure a harvestable crop each year.

Inflorescences consist of a number of buds grouped in umbels, with the number of buds in an umbel varying from 1, 3, 5, 7 or 11, depending on species. The opening, or operculum shedding, of each individual flower in an umbel may take over seven days, and the spread of flowering on a tree may take from one to ten months. Once the operculum is shed and the

stamens fully reflexed, it may take between two and fourteen days for the stigma to become receptive to pollen, so in most cases an individual flower may be in 'bloom' for over two weeks. There have been no published reports specifically relating to the effect of pollination on flower life of eucalypts, although a study by Savva *et al.* (1988) recognised that treatments preventing pollination appear to delay anther senescence for *E. urnigera*. In contrast, Sturt's Desert Pea, once pollinated, begins to senesce, and is completely perished in three days. This has necessitated extreme care in postharvest handling procedures and the possible development of pollen sterile varieties (Kirby, 1996). The lack of this kind of information for eucalypts highlights the need for a better understanding of the reproductive biology and its effects on postharvest vase life, handling and treatments.

9.6. Conclusion

The results for *E. tetragona* indicate enormous potential for this species as a cut bud and flower crop. The species has a long vase life, and even after cold storage, the flowers continue to open and develop normally, with no detrimental features such as copious nectar production or stamen drop. There is variation within the species, allowing for selection and breeding of superior genotypes to improve on the current vase life, as well as for selection of different leaf and flower sizes. Trials with *E. youngiana* indicate a suitable vase life of flowers, but problems such as nectar production and stamen drop detract from the commercial potential of the species. There is variation within the species, and this should be explored to select those with smaller flowers, less nectar production and a longer vase life. Preliminary data for *E. forrestiana* subsp. *forrestiana* and *E. stoatei* indicate that both cold storage and sucrose pulsing may increase vase life. However, the percentage of flowers opening after harvest was very low in both cases, indicating that more studies on timing of harvest and optimal bud opening treatments are required to assess the optimal postharvest vase life and

requirements of these species.

Chapter 10
General Discussion

The project aimed to improve species from the *Eucalyptus* genus for floriculture and for garden or pot plants, by addressing three aspects of plant improvement: species and genotype selection; interspecific hybridisation and hybrid identification; and post harvest physiology and treatments.

Within most eucalypt species, plants exhibit variation in morphology; with differences in the size, shape and colour of buds (operculum and hypanthium), flowers (stamens), leaves, stems and fruit. Such variability is an asset to breeders, as there is an increased chance for improvement, but is not desirable in commercial plantations, as it leads to lack of consistent supply.

Eucalyptus plants suitable for ornamental horticulture (floriculture and amenity planting) have certain morphological features, principally large, colourful, ornate buds and flowers. Leaves, stems and fruit are of secondary importance, but must complement the flowers and buds. Plants from selected species are assessed in trials, to facilitate comparisons of growth rate, flowering and pest resistance. From these trials, plants exhibiting fast growth, exceptional flowers and high levels of disease resistance, are considered superior and suitable for further assessment. Further assessment requires quantification of the response of each plant to cultivation techniques such as pruning, postharvest treatments and clonal propagation. Plants identified with desired characters can be used in breeding programs.

Of the thirty *Eucalyptus* species studied in this work, 17 have potential for development, primarily for floriculture. These are *E. caesia, E. erythrocorys, E. erythronema, E. forrestiana, E. gillii, E. kruseana, E. lesouefii, E. macrocarpa, E. orbifolia, E. oldfieldii, E. pruinosa, E. pyriformis, E. stricklandii, E. stoatei, E. tetragona, E. websteriana* and *E. youngiana.* An additional 23 species with potential for development were identified but not studied. These are *E. burracoppinensis, E. campaspe, E. clelandii, E. cocinna, E. coronata, E. corrugata, E. crucis, E. drummondii, E. eudesmoides, E. ewartina, E. kingsmillii, E. lane-poolei, E. leptopoda, E. megacarpa, E. nutans, E. oxymitra, E. pachyphylla, E. pimpiniana, E. preissiana, E. rameliana, E. rhodantha, E. synandra,* and *E. tetraptera.* All species listed have medium to large ornate buds and colourful or contrasting flowers.

Selection at genotype level facilitates the identification of plants exhibiting superior characters, in the areas of flowering, plant form and plant health. Twenty plants from nine species were subjected to selection at this level through growth trials, where rates of growth and development were determined and compared, both within and between species. Seventeen plants from *E. forrestiana* subsp. *dolichorhyncha*, *E. lesouefii* and *E. tetragona* were selected for further assessment for plant response to cultivation, clonal propagation and postharvest testing. The majority of remaining genotypes have some desirable features and should be included in intra or interspecific breeding programs.

The use of manipulated hybridisation to generate hybrids with superior characters to either parent is a common and reliable technique in ornamental plant breeding, as well as in eucalypt breeding for forestry. Interspecific hybridisation has been adapted as the method of choice for ornamental eucalypts, as the majority of major cut flower lines have hybrid pedigree, one of the most spectacular examples is the vast range of new cultivars which resulted from new methods to hybridise orchids. Hybrid plants, generated through controlled pollination, can be

identified quickly and parental status confirmed through measurement and analysis of seedling morphological characters and DNA fingerprints. Those plants with intermediate characters can be identified as early as three months after germination, enabling the culling of those with limited hybrid value. The possibility exists that those plants identified as hybrids will exhibit superior floral characters to either parent under cultivation conditions. This opens the way to quick assessment of the hybrids for floral characters that will lead on to development as ornamentals. The use of DNA fingerprinting to clarify parentage, as demonstrated in chapter 5 and noted in chapter 7, will enable each unique hybrid to be defined, opening the way to registration as a cultivar. The study of the putative natural hybrid, *E.* 'Urrbrae Gem', used adult and seedling morphology, as well as DNA fingerprinting, to clarify parentage, with all three methods identifying the same species as the male parent.

The ability to generate hybrids through controlled pollination was investigated in three studies involving a number of different *Eucalyptus* species. The first study aimed to reproduce *E.* 'Urrbrae Gem' by crossing the known female parent species with both possible male parents; the second crossed three closely related species with similar morphology; while the third crossed 16 species from different series within the genus, with differing floral morphology. The results of these trials suggested that while taxonomic relatedness was an important consideration, as some species were simply too distant to hybridise successfully, a further factor to be taken into account when developing pollination matrices was the comparative size of the flowers of both male and female species. Species with similar sized flowers hybridised more successfully than those which differed. This problem has been noted in other studies with *Eucalyptus*, however, it may be overcome with alterations to the pollination technique, including physical manipulation of the style to reduce style length.

While *E.* 'Urrbrae Gem' could be commercialised now with the development of appropriate clonal propagation techniques, the seedlings generated in this study must be grown to the flowering stage for evaluation. In the future, the development of molecular markers, for characters such as flower colour, disease tolerance or waxiness, could accelerate the selection process by identification of potential superior genotypes at the seedling stage. This could be achieved by either bulked segregant analysis (Michelmore, *et al.*, 1991) or by mapping of the genome. Maps of some eucalypts species have been developed already (Brondani and Grattapaglia, 1999; Bundock and Vaillancourt, 1999; Shepherd *et al.*, 1999), and it may be possible to adapt these maps for use with ornamental species.

Once a superior genotype or hybrid has been identified, determination of the response of the genotype to postharvest treatments, cultivation techniques, such as pruning, and to clonal propagation must be undertaken.

Observations of stems and flowers after harvest, and their response to various treatments, will facilitate the development of optimal treatments for maximum vase life and postharvest flower opening. The addition of sucrose to vase solutions and cold storage were treatments that extend vase life of a number of different plants, however, their effect on *Eucalyptus* buds and flowers was not known. By assessing the response of flowering stems of different plants to a variety of sucrose concentrations, the role of such additives to postharvest vase life can be determined. This study found that sucrose is not necessary to prolong vase life of some species, nor to enhance flower opening after harvest, while for others it may have a slightly beneficial effect. Cold storage does not have a detrimental effect on vase life, and may in fact enhance flower opening with some species. The undertaking of this trial over two consecutive years on some of the same plants from a species has shown that the pre-harvest condition and genotype of the plant may have a greater effect on vase life than any

postharvest treatment. Investigation of a greater number of species is necessary prior to development of a standard procedure for all flowering eucalypt stems. Trail consignments of stems to distant markets will test the postharvest procedures and also consumer acceptability of new lines.

Various cultivation practices are used in production of cut flower stems of eucalypts, and depend on the climate of the growing area, with practices such as spacing, supplementary irrigation and weed control varying with location. The effect of pruning and harvesting on the future cropping potential of plants is unknown and requires investigation.

Eucalyptus material available for commercial plantations is grown from seed sourced from natural and cultivated populations, with no control over the parentage. The outcrossing nature of the genus means that seedlings from such populations are variable, often lacking the superior qualities of the source tree. The variability leads to problems with management of commercial plantations, and ultimately to problems with the continual supply of quality product. The supply of quality product is foremost in the minds of producers, and species or cultivars that produce poorly will be quickly discarded. The solution to the problem of variability is the development of viable methods of clonal propagation.

Clonal propagation is widely used for ornamental plants, and successful protocols have been established for some *Eucalyptus* species desired for forestry. The exploration of these protocols for use on ornamental eucalypts, and the development of quick, cost-effective methods for their propagation, is vital to the ongoing task of producing ornamental eucalypts.

The careful selection and thorough testing of both natural genotypes and artificially generated hybrids is vital for the development of *Eucalyptus* as ornamentals. If these points are

addressed conscientiously, only superior cultivars will be released to the industry. The future lies in the production of hybrids, the selection of cultivars and the development of universal protocols for optimal postharvest treatments, cultivation techniques and clonal propagation.

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Appendix 1.1

Anova tables for section 4.4.1. (Table 4.1.).

Final tree height

General Analysis of variance - Variate: final height

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	4	3276279	819070	3.85	
block.*Units* stratum					
species	9	48382609	5375845	25.26	<0.001
Residual	170(16)	36172871	212782		
Total	183(16)	85507764			

Tree height growth rate

General Analysis of variance - Variate: growth rate

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	4	3311.8	827.9	3.65	
block.*Units* stratum					
species	9	40787.7	4532.0	19.99	<0.001
Residual	170(16)	38547.1	226.7		
Total	183(16)	80814.8			

Final tree trunk diameter

General Analysis of variance - Variate: final diameter

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	4	1343.7	335.9	2.55	
block.*Units* stratum					
species	9	10009.0	112.1	8.46	<0.001
Residual	170(16)	22357.4	131.5		
Total	183(16)	33173.8			

Tree trunk growth rate

General Analysis of variance - Variate: growth rate

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	4	1.3098	0.3275	2.27	
block.*Units* stratum					
species	9	10.5888	1.1765	8.16	<0.001
Residual	170(16)	24.5178	0.1442		
Total	183(16)	35.8191			

Note: Each of the analyses showed minimal differences between plants within species, and high species level differences meant that no further information could be gathered by including another level in the analysis.

Appendix 1.2

Anova tables for section 7.3.3 (Tables 7.4, 7.5, 7.6 and 7.7).

All species cannot be analysed due to insufficient replication for all crosses (only one female for *E. macrocarpa*).

E. macrocarpa as female parent species – only one female plant so no analysis possible

E. pyriformis as female parent species

Mean number of seeds per flower pollinated

General Analysis of variance - Variate: mean number of seeds

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
female stratum	4	786.12	196.53	7.80	
female.*Units* stratum					
male	3	383.76	127.92	5.07	0.017
Residual	12	302.54	25.21		
Total	19	1472.42			

Mean weight of seeds per flower pollinated

General Analysis of variance - Variate: mean seed weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
female stratum	4	0.353E-05	0.883E-06	7.71	
female.*Units* stratum					
male	3	0.700E-07	0.233E-07	0.20	0.892
Residual	12	0.138E-05	0.115E-06		
Total	19	0.498E-05			

Percent seeds germinated

General Analysis of variance - Variate: percent seed germinated

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
female stratum	4	3386.3	846.6	2.60	
female.*Units* stratum					
male	3	892.9	297.6	0.91	0.463
Residual	12	3908.4	325.7		
Total	19	8187.6			

E. youngiana as female parent species

Mean number of seeds per flower pollinated

General Analysis of variance - Variate: mean number of seeds

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
female stratum	3	871.2	290.4	1.03	
female.*Units* stratum					
male	3	2633.3	877.8	3.10	0.082
Residual	9	2548.8	283.2		
Total	15	6053.3			

Mean weight of seeds per flower pollinated

General Analysis of variance - Variate: mean seed weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
male stratum	3	0.608E-07	0.203E-06	0.09	
male.*Units* stratum					
Female	3	0.608E-07	0.162E-05	7.57	0.008
Residual	9	0.193E-07	0.215E-07		
Total	15	0.687E-05			

Percent seeds germinated

General Analysis of variance - Variate: percent seed germinated

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
male stratum	3	3285.6	1095.2	2.07	
male.*Units* stratum					
female	3	4553.1	1517.7	2.87	0.096
Residual	9	4766.4	529.6		
Total	15	12605.1			

Appendix 1.3

Anova tables for section 9.4. (Tables 9.1, 9.2, 9.3 and 9.4).

E. youngiana : effect of treatment on

Flower vase life

General Analysis of variance - Variate: days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plant stratum	5	179.122	35.824	2.40	
Plant.sucrose stratum					
sucrose	4	132.067	33.017	2.21	0.104
Residual	20	298.600	14.930	1.97	
Plant.sucrose.stem1 stratum					
	60	454.667	7.578		
Total	89	1064.456			

Leaf vase life

General Analysis of variance - Variate: days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plant stratum	5	414.767	82.953	15.99	
Plant.sucrose stratum					
sucrose	4	18.400	4.600	0.89	0.490
Residual	20	103.733	5.187	1.67	
Plant.sucrose.stem1 stratum					
	60	186.000	3.100		
Total	89	722.900			

Percent flowers open after harvest

General Analysis of variance - Variate: days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plant stratum	5	29417	5883	2.57	
Plant.sucrose stratum					
sucrose	4	10236	2559	1.12	0.377
Residual	20	45868	2293	1.18	
Plant.sucrose.stem1 stratum					
	60	116137	1936		
Total	89	201658			

Time to negative weight change

General Analysis of variance - Variate: days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plant stratum	5	108.89	21.618	8.47	
Plant.sucrose stratum					
sucrose	4	18.844	4.711	1.85	0.160
Residual	20	51.022	2.551	0.80	
Plant.sucrose.stem1 stratum					
	60	192.000	3.200		
Total	89	369.956			

E. tetragona : effect of treatment on

Flower vase life

General Analysis of variance - Variate: days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plant stratum	5	108.89	21.618	8.47	
Plant.sucrose stratum					
sucrose	4	18.844	4.711	1.85	0.160
Residual	20	51.022	2.551	0.80	
Plant.sucrose.stem1 stratum					
	60	192.000	3.200		
Total	89	369.956			

Leaf vase life

General Analysis of variance - Variate: days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plant stratum	5	414.767	82.953	15.99	
Plant.sucrose stratum					
sucrose	4	18.400	4.600	0.89	0.490
Residual	20	103.733	5.187	1.67	
Plant.sucrose.stem1 stratum					
	60	186.000	3.100		
Total	89	722.900			

Percent flowers open after harvest

General Analysis of variance - Variate: days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plant stratum	5	29417	5883	2.57	
Plant.sucrose stratum					
sucrose	4	10236	2559	1.12	0.377
Residual	20	45868	2293	1.18	
Plant.sucrose.stem1 stratum					
	60	116137	1936		
Total	89	201658			

Time to negative weight change

General Analysis of variance - Variate: days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plant stratum	5	108.89	21.618	8.47	
Plant.sucrose stratum					
sucrose	4	18.844	4.711	1.85	0.160
Residual	20	51.022	2.551	0.80	
Plant.sucrose.stem1 stratum					
	60	192.000	3.200		
Total	89	369.956			

E. tetragona : effect of plant on

Flower vase life

One-Way Analysis of variance - Variate: days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plant	4	234.71	58.68	5.61	<0.001
Residual	85	889.78	10.47		
Total	89	1124.49			

Leaf vase life

One-Way Analysis of variance - Variate: days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plant	5	412.71	103.18	7.17	<0.001
Residual	85	1223.11	14.39		
Total	89	1635.82			

Percent flowers open after harvest

One-Way Analysis of variance - Variate: days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plant	5	36209.5	9052.4	10.64	<0.001
Residual	85	72296.8	850.6		
Total	89	108506.4			

Time to negative weight change

One-Way Analysis of variance - Variate: days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plant	5	114.489	28.622	18.01	<0.001
Residual	85	135.111	1.509		
Total	89	249.600			

E. youngiana : effect of plant on

Flower vase life

One-Way Analysis of variance - Variate: days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plant	5	179.12	35.82	3.40	0.008
Residual	84	885.33	10.54		
Total	89	1064.46			

Leaf vase life

One-Way Analysis of variance - Variate: days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plant	5	414.767	82.953	22.61	<0.001
Residual	84	308.133	3.668		
Total	89	722.900			

Percent flowers open after harvest

One-Way Analysis of variance - Variate: days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plant	5	29417	5883	2.87	0.019
Residual	84	172241	2050		
Total	89	201658			

Time to negative weight change

One-Way Analysis of variance - Variate: days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plant	5	108.089	21.618	6.93	<0.001
Residual	84	261.867	3.117		
Total	89	369.956			

E. tetragona : effect of treatment on

Flower vase life

General Analysis of variance - Variate: days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plant stratum	5	597.707	119.541	12.40	
Plant.*Units* stratum					
Sucrose	4	42.126	10.531	1.09	0.361
Cold	2	17.474	8.737	0.91	0.405
Sucrose.Cold	8	34.452	4.306	0.45	0.892
Residual	250	2409.459	9.638		
Total	269	3101.219			

Leaf vase life

General Analysis of variance - Variate: days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plant stratum	5	1123.896	224.779	24.48	
Plant.*Units* stratum					
Sucrose	4	70.237	17.559	1.91	0.109
Cold	2	74.007	37.004	4.03	0.019
Sucrose.Cold	8	44.030	5.504	0.60	0.778
Residual	250	2295.104	9.180		
Total	269	3607.274			

Percent flowers open after harvest

General Analysis of variance - Variate: days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plant stratum	5	17715.2	3543.0	13.84	
Plant.*Units* stratum					
Sucrose	4	1538.4	384.6	1.50	0.202
Cold	2	2363.6	1181.8	4.62	0.011
Sucrose.Cold	8	2553.9	319.2	1.25	0.272
Residual	250	63978.4	255.9		
Total	269	88149.6			

Time to negative weight change after harvest

General Analysis of variance - Variate: days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plant stratum	5	90.033	18.007	3.91	
Plant.*Units* stratum					
Sucrose	4	6.681	1.670	0.36	0.835
Cold	2	134.467	67.233	14.59	<0.001
Sucrose.Cold	8	23.830	2.979	0.65	0.738
Residual	250	1152.356	4.609		
Total	269	1407.367			

E. tetragona : effect of plant on

Flower vase life

One-Way Analysis of variance - Variate: days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plant	5	597.707	119.541	12.61	<0.001
Residual	264	2503.511	9.483		
Total	269	3101.219			

Leaf vase life

One-Way Analysis of variance - Variate: days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plant	5	1123.896	224.779	23.90	<0.001
Residual	264	2483.378	9.407		
Total	269	3607.274			

Percent flowers open after harvest

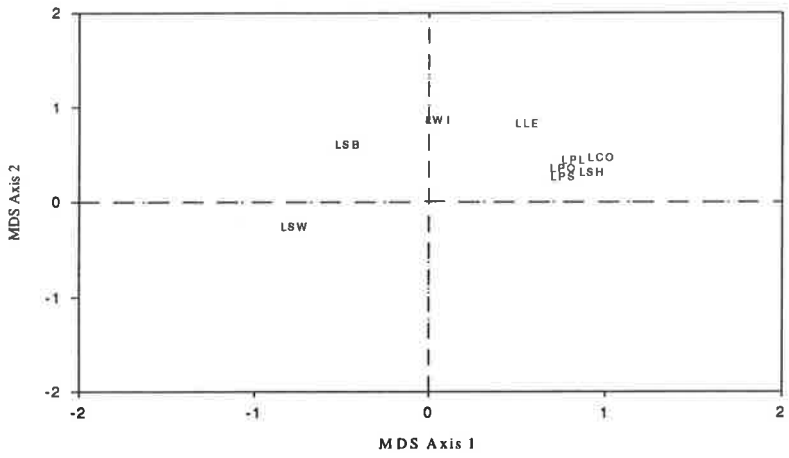
One-Way Analysis of variance - Variate: days

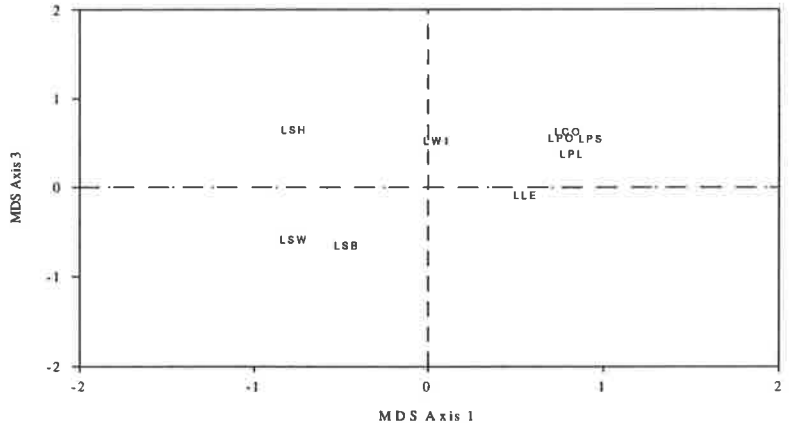
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plant	5	17715.2	3543.0	13.28	<0.001
Residual	264	70434.4	266.8		
Total	269	88149.6			

Time to negative weight change

One-Way Analysis of variance - Variate: days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plant	5	90.033	18.007	3.61	0.004
Residual	264	1317.333	4.990		
Total	269	1407.367			





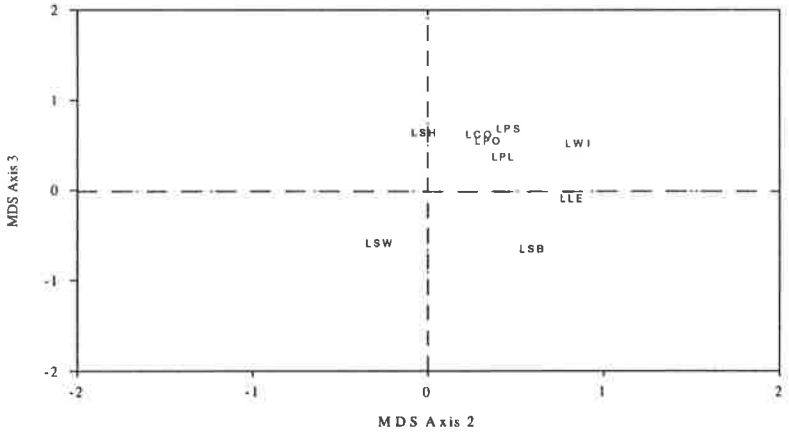
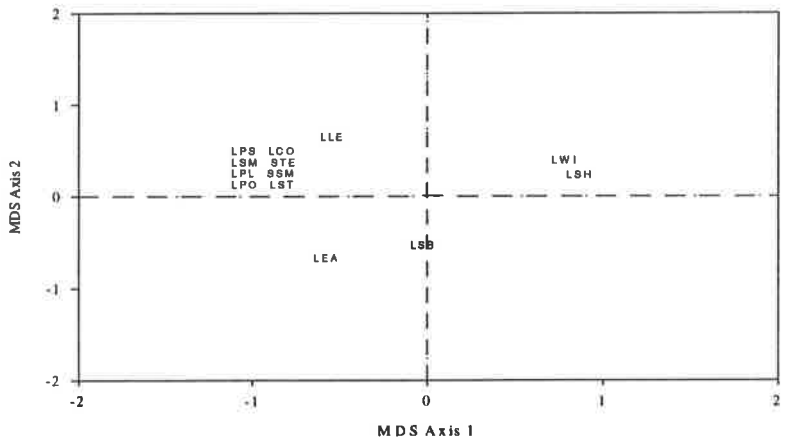
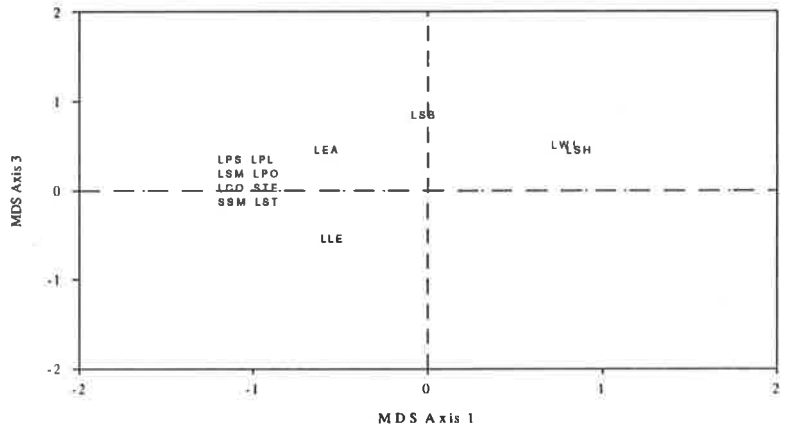


Figure 8.57. Principal canonical correlation graphs of *E. gillii* x *E. caesia* seedlings and parents. Characters in italics and underlined not significantly correlated to MDS ordinations (Table 8.7).





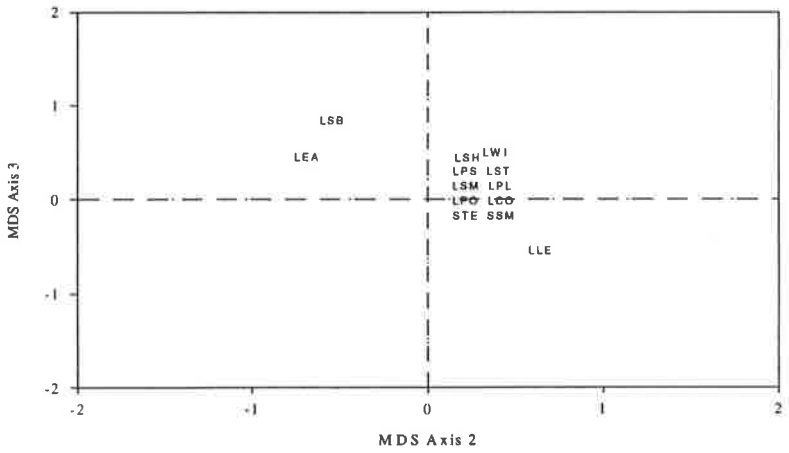
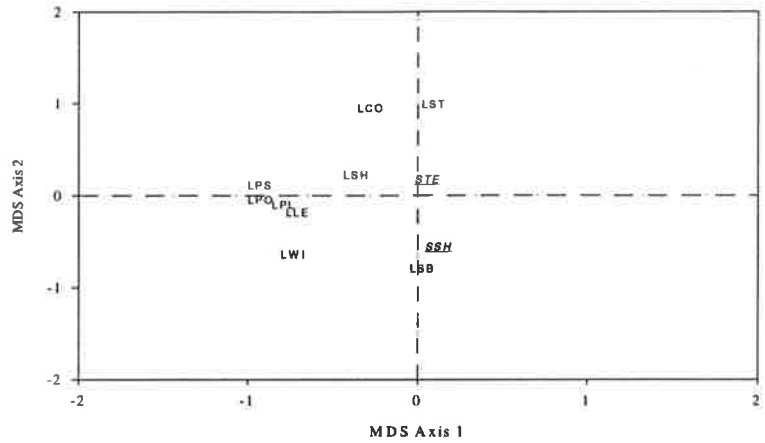
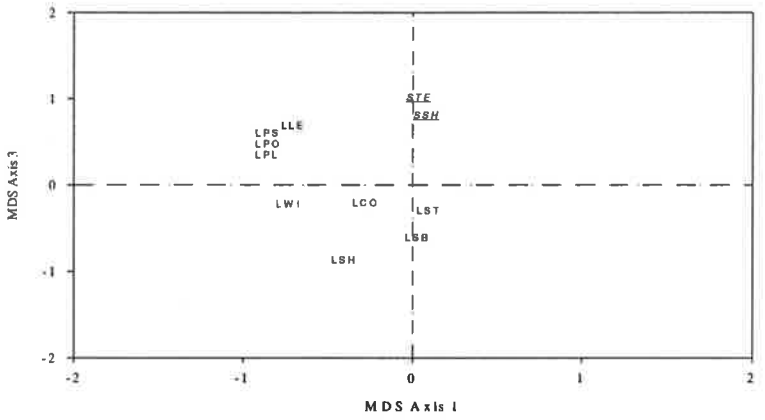


Figure 8.58. Principal canonical correlation graphs of *E. gillii* x *E. eremophila* seedlings and parents.





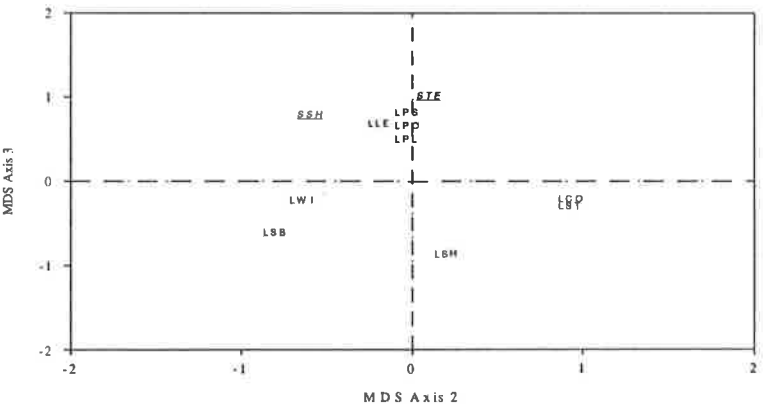
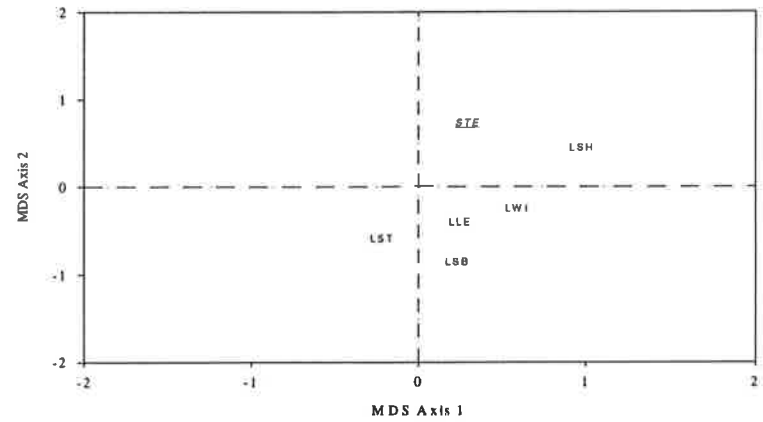
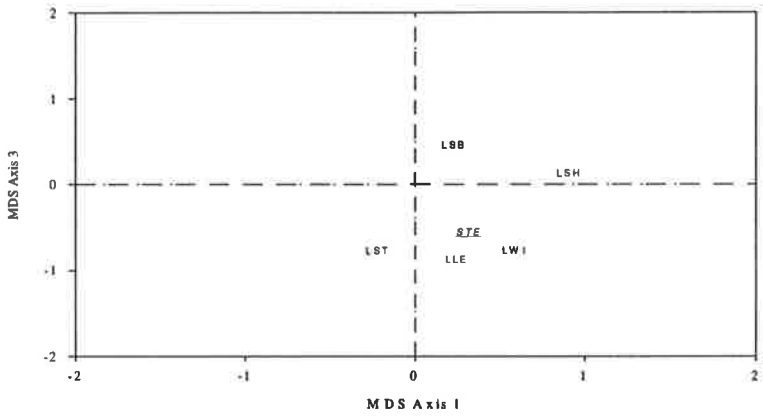


Figure 8.59. Principal canonical correlation graphs of *E. gillii* x *E. gardneri* seedlings and parents. Characters in italics and underlined not significantly correlated to MDS ordinations (Table 8.7).





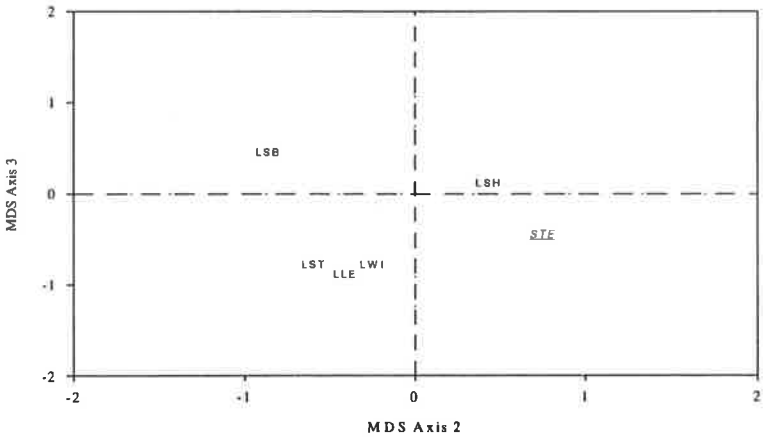
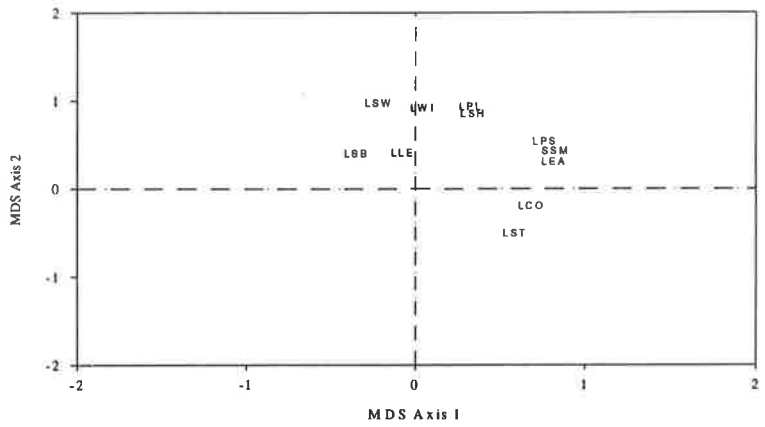
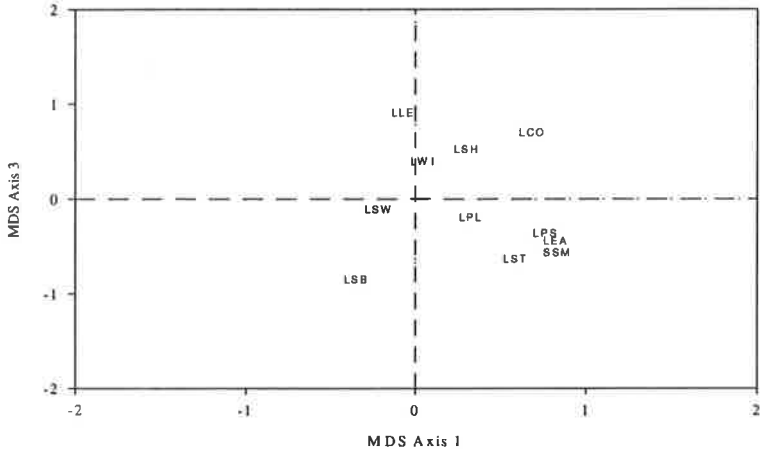


Figure 8.60. Principal canonical correlation graphs of *E. gillii* x *E. gillii* seedlings and parents. Characters in italics and underlined not significantly correlated to MDS ordinations (Table 8.7).





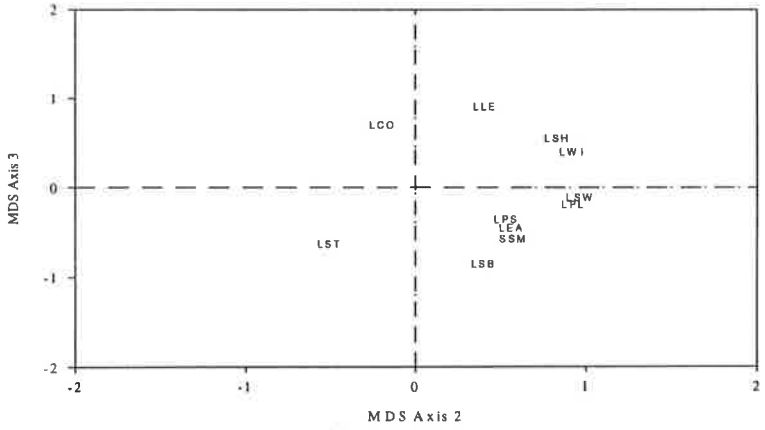


Figure 8.61. Principal canonical correlation graphs of *E. gillii* x *E. kruseana* seedlings and parents.

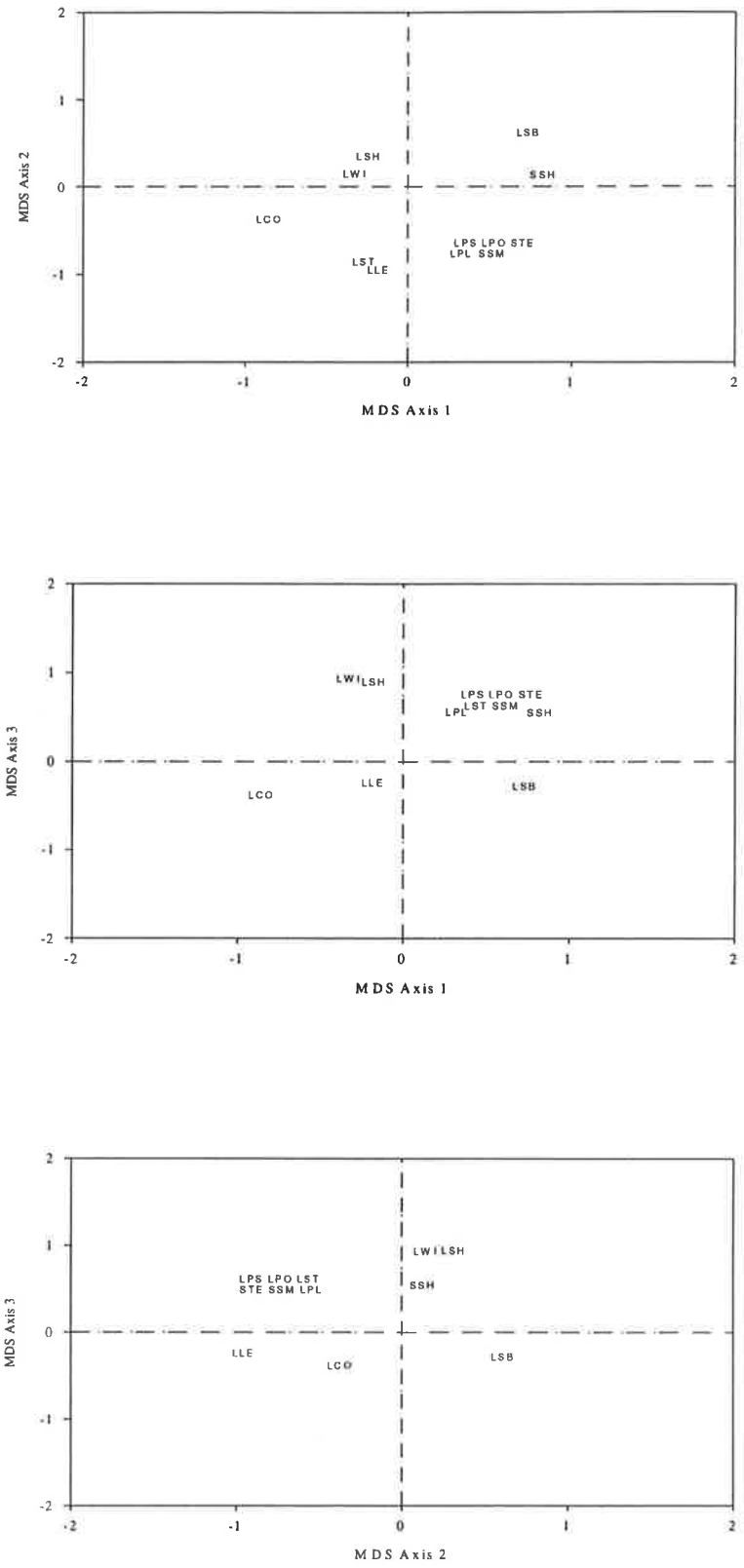
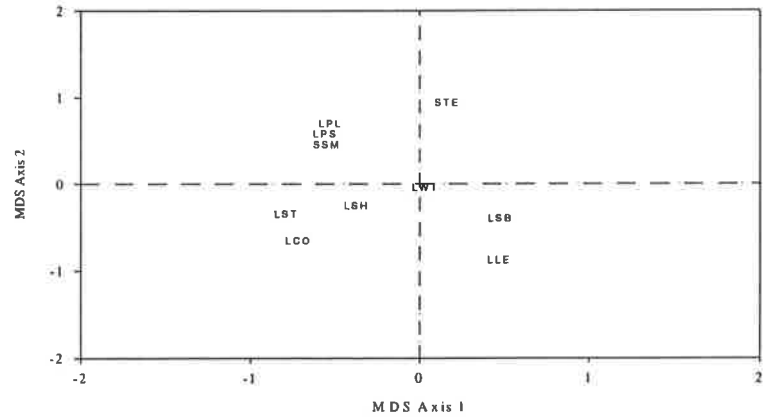
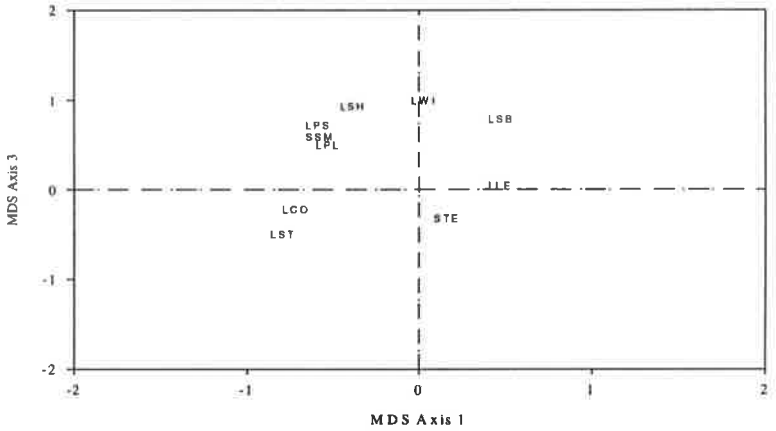


Figure 8.62. Principal canonical correlation graphs of *E. gillii* x *E. oldfieldii* seedlings and parents.





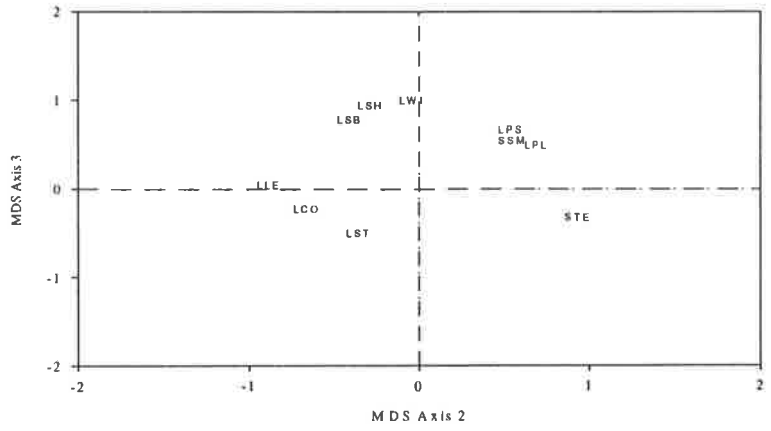
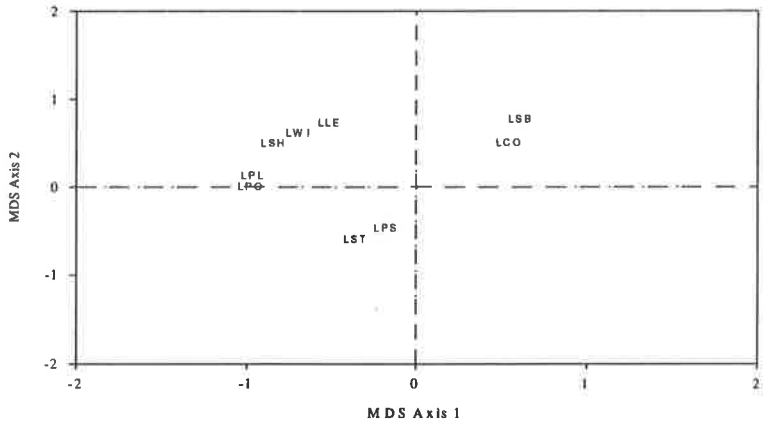
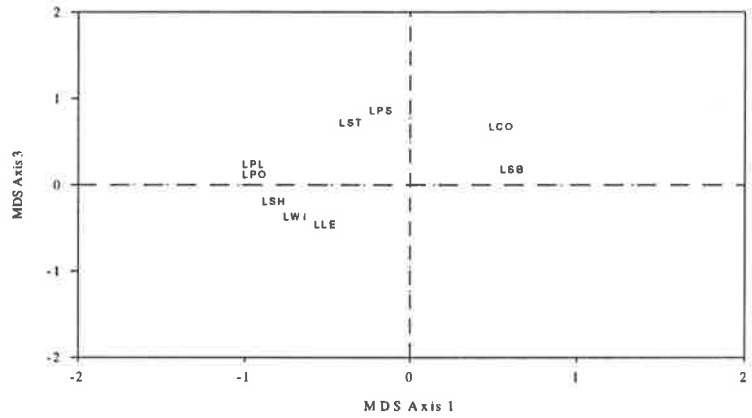


Figure 8.63. Principal canonical correlation graphs of *E. gillii* x *E. orbifolia* seedlings and parents.





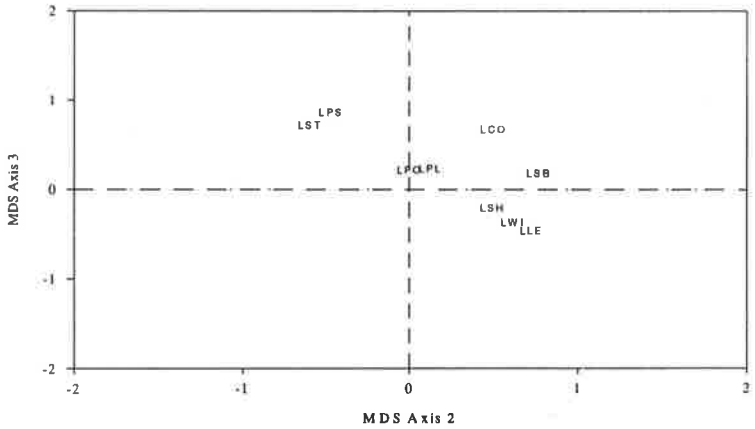
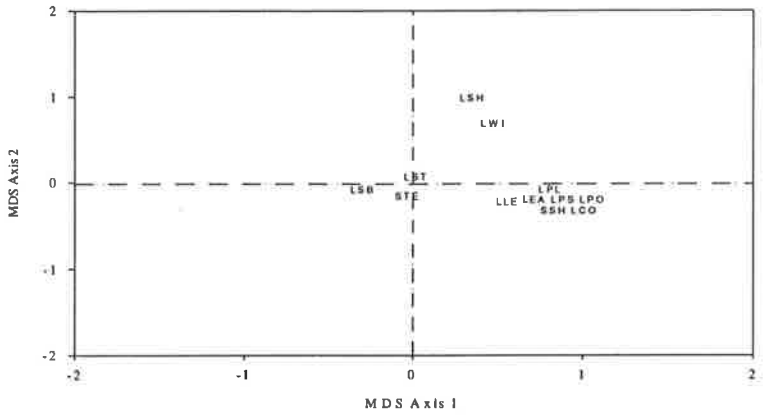
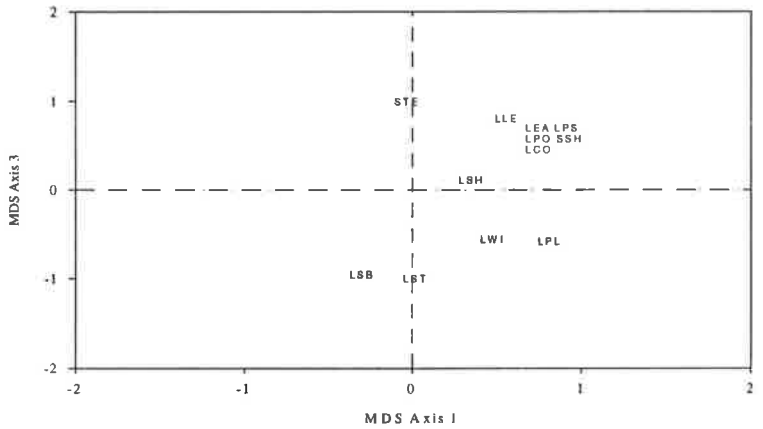


Figure 8.64. Principal canonical correlation graphs of *E. gillii* x *E. polyanthemos* seedlings and parents.





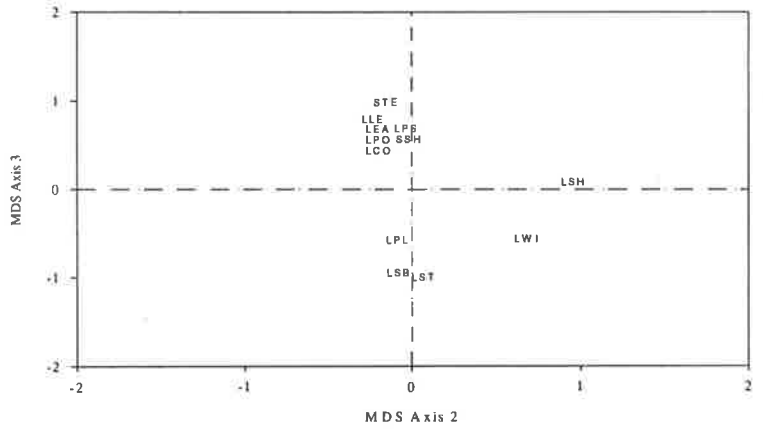
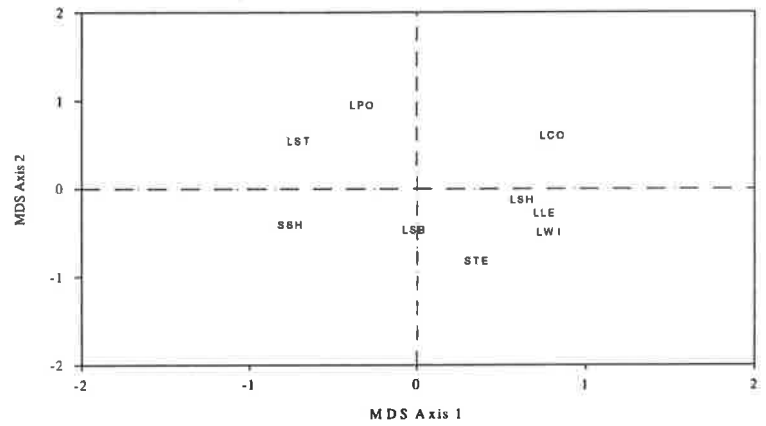
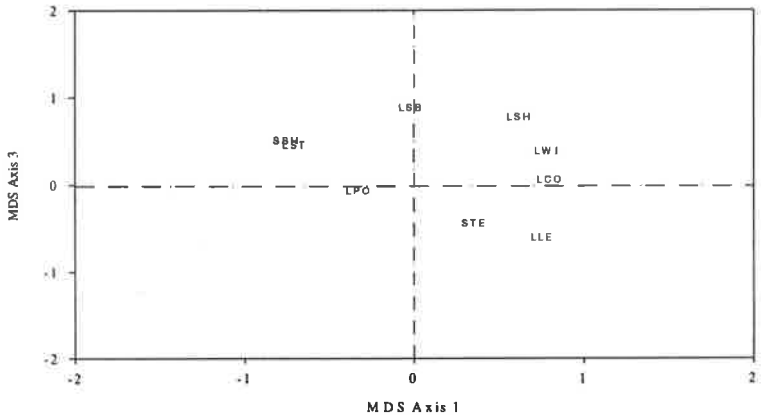


Figure 8.65. Principal canonical correlation graphs of *E. gillii* x *E. sideroxylon* seedlings and parents.





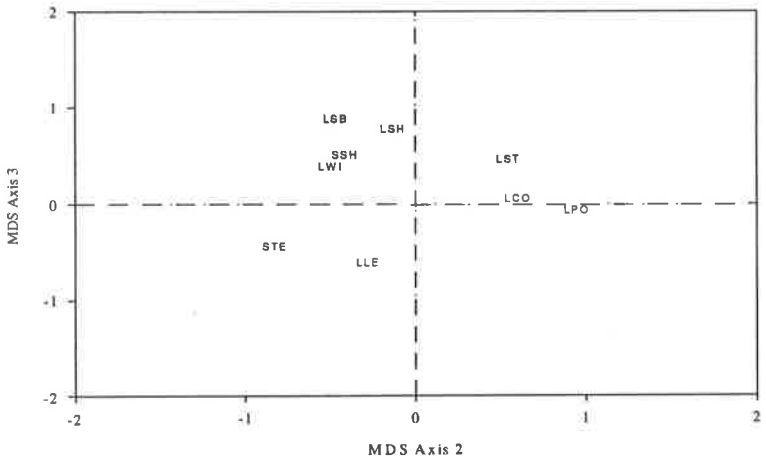
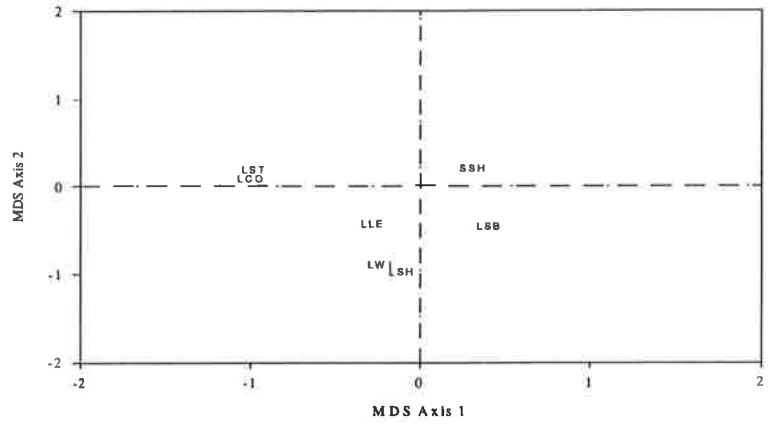
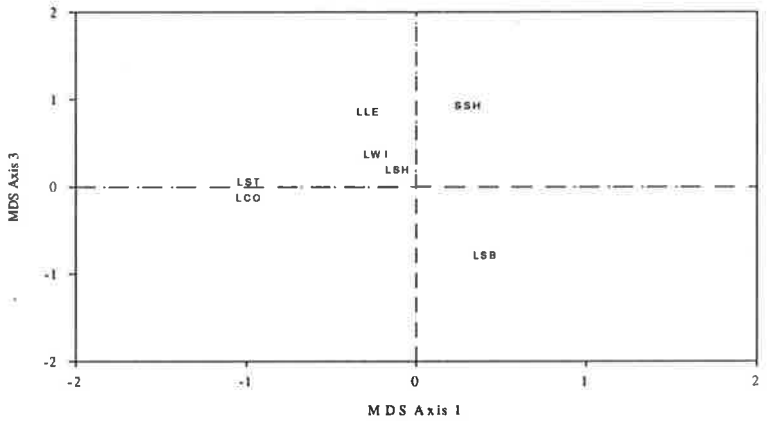


Figure 8.66. Principal canonical correlation graphs of *E. gillii* x *E. socialis* seedlings and parents.





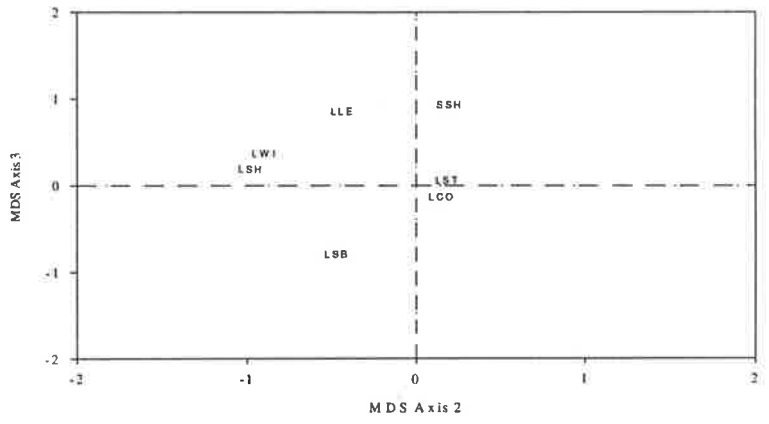
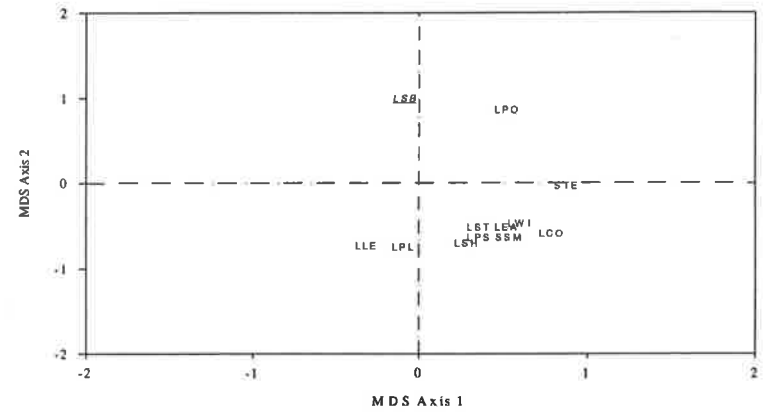
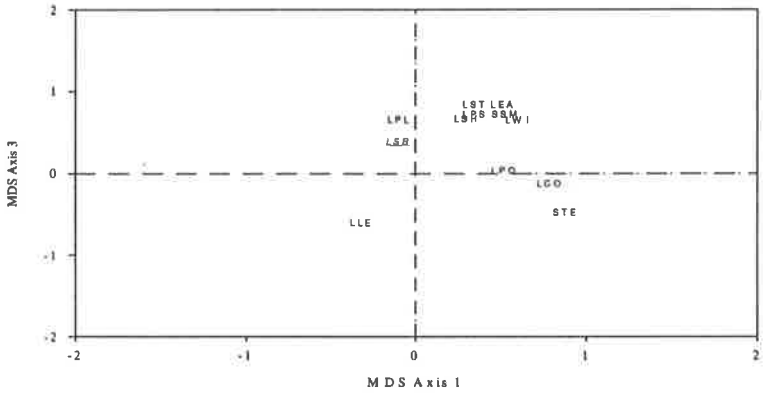


Figure 8.67. Principal canonical correlation graphs of *E. gillii* x *E. transcontinentalis* seedlings and parents.





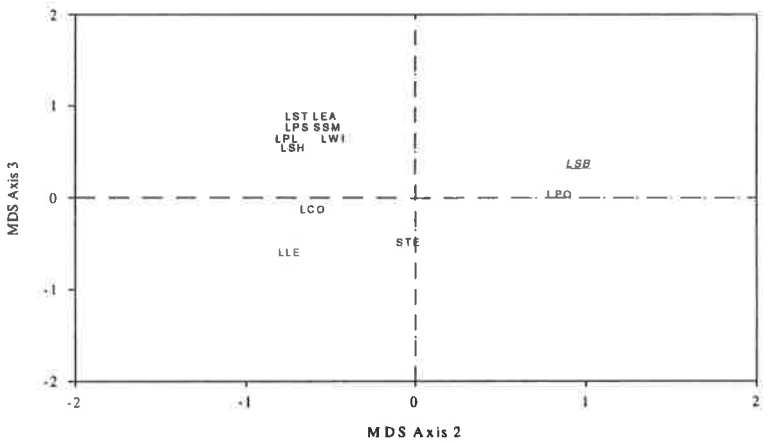
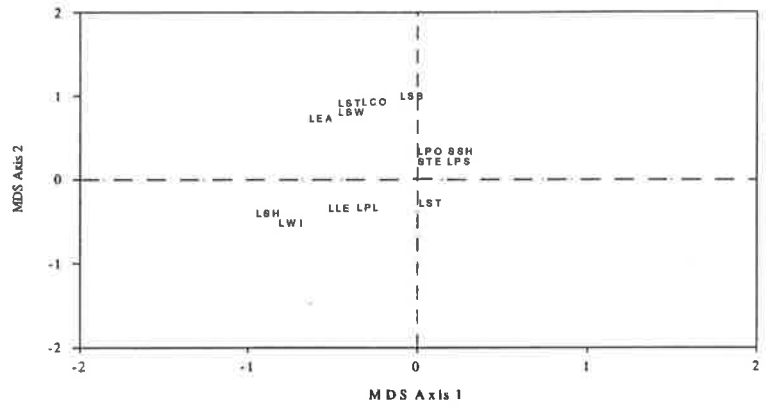
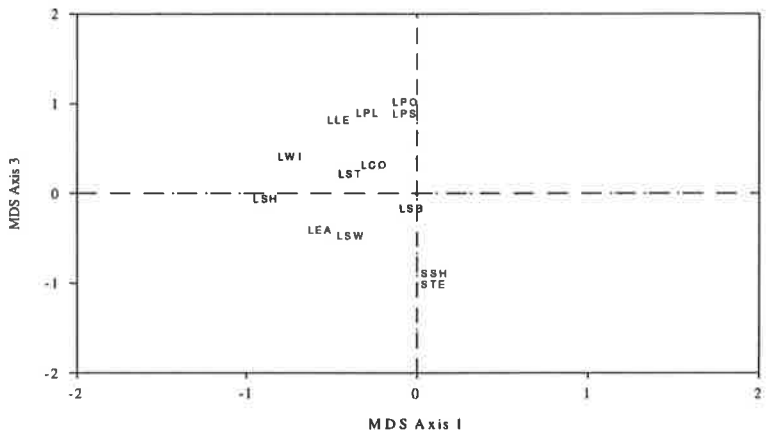


Figure 8.68. Principal canonical correlation graphs of *E. gillii* x *E. websteriana* seedlings and parents. Characters in italics and underlined not significantly correlated to MDS ordinations (Table 8.7).





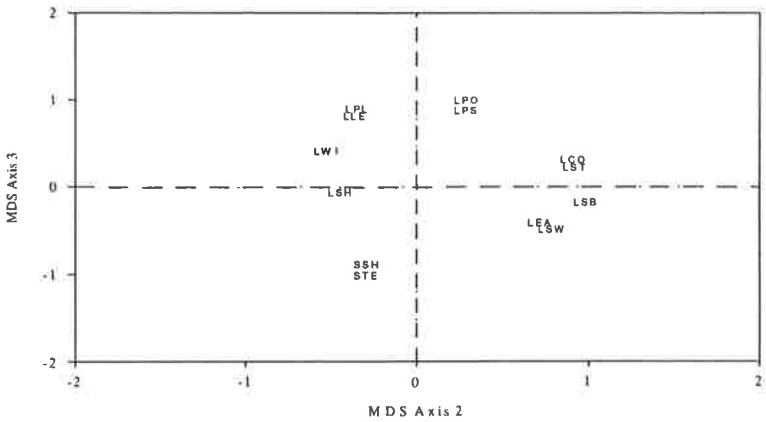


Figure 8.69. Principal canonical correlation graphs of *E. gillii* x *E. youngiana* seedlings and parents.

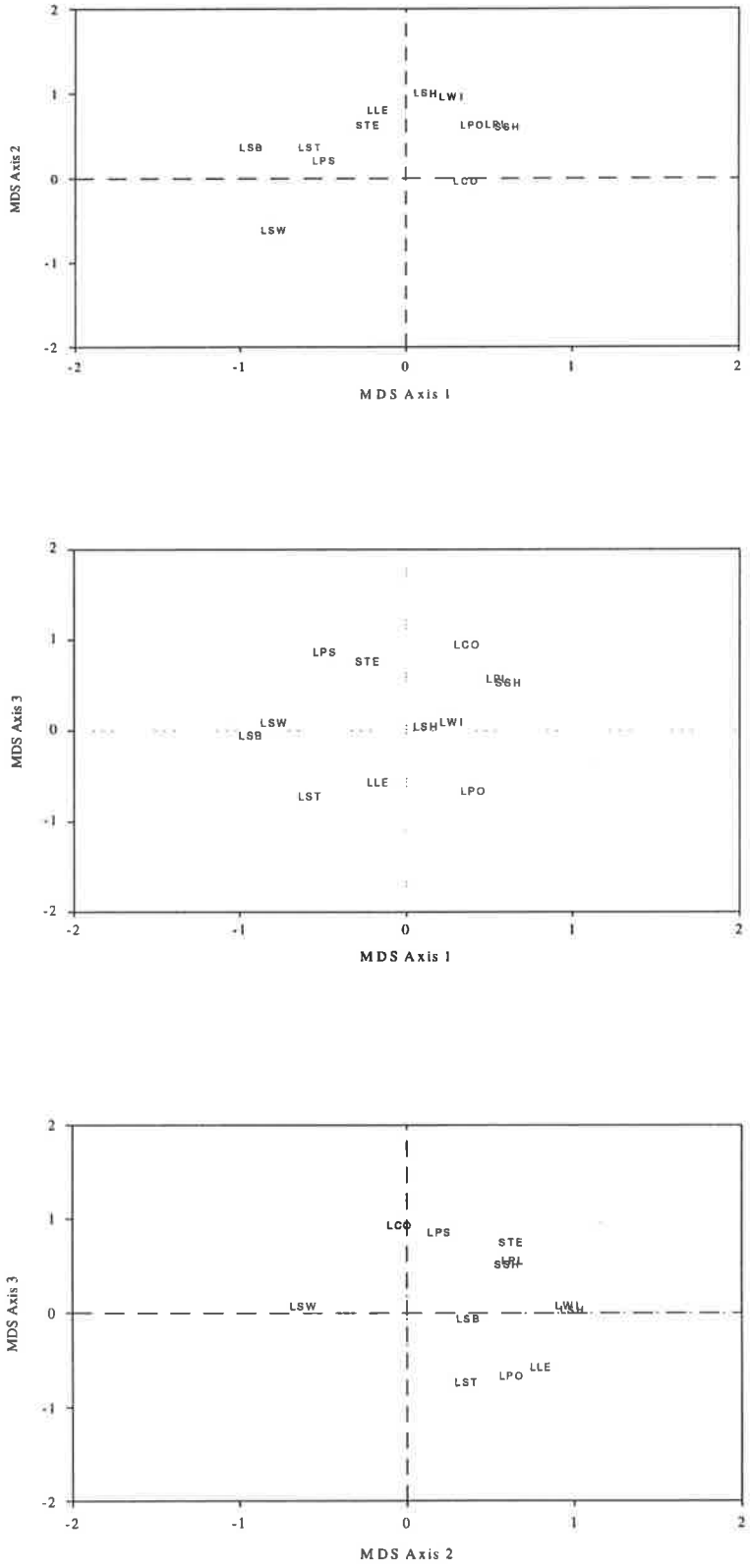


Figure 8.70. Principal canonical correlation graphs of *E. socialis* x *E. caesia* seedlings and parents.

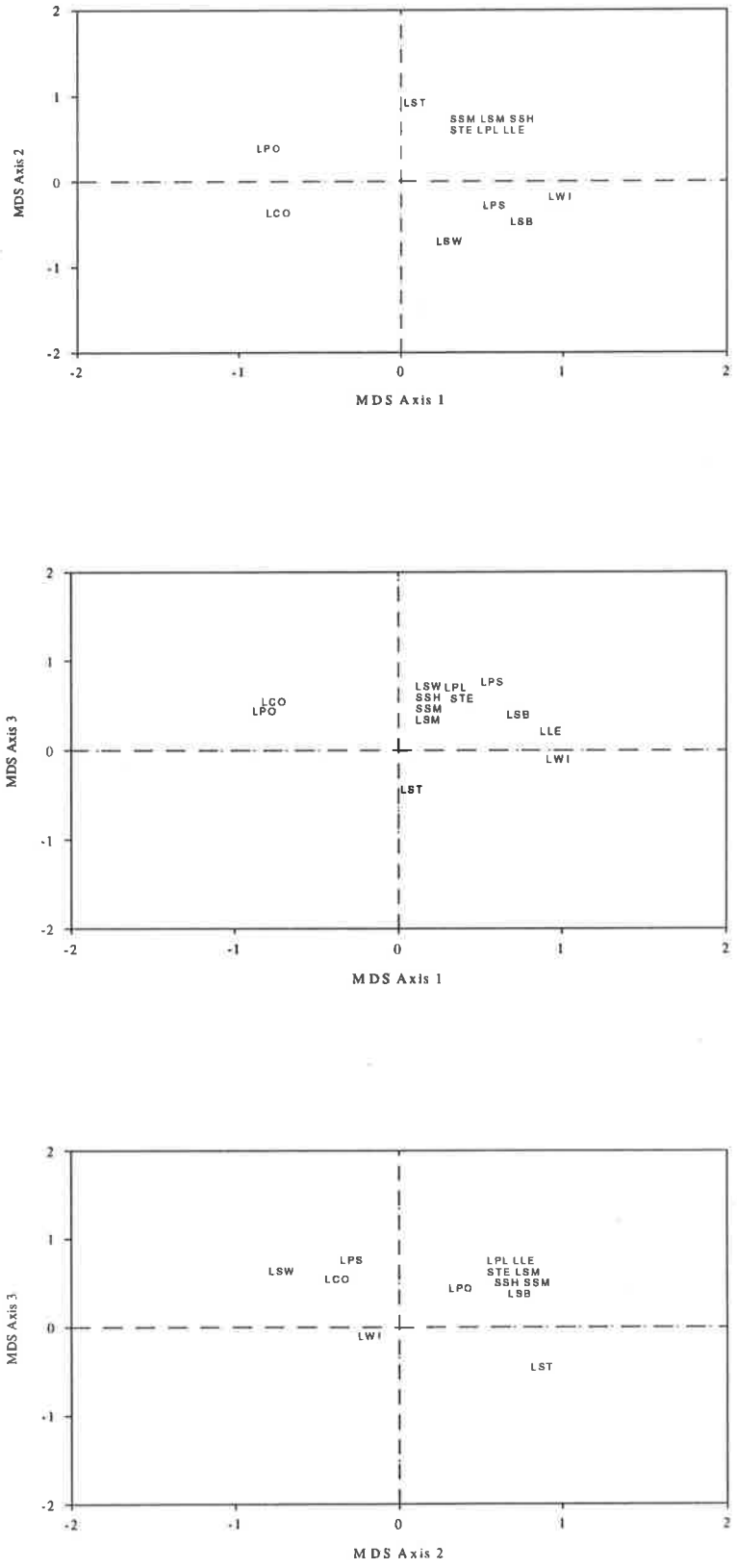


Figure 8.71. Principal canonical correlation graphs of *E. socialis* x *E. eremophila* seedlings and parents.

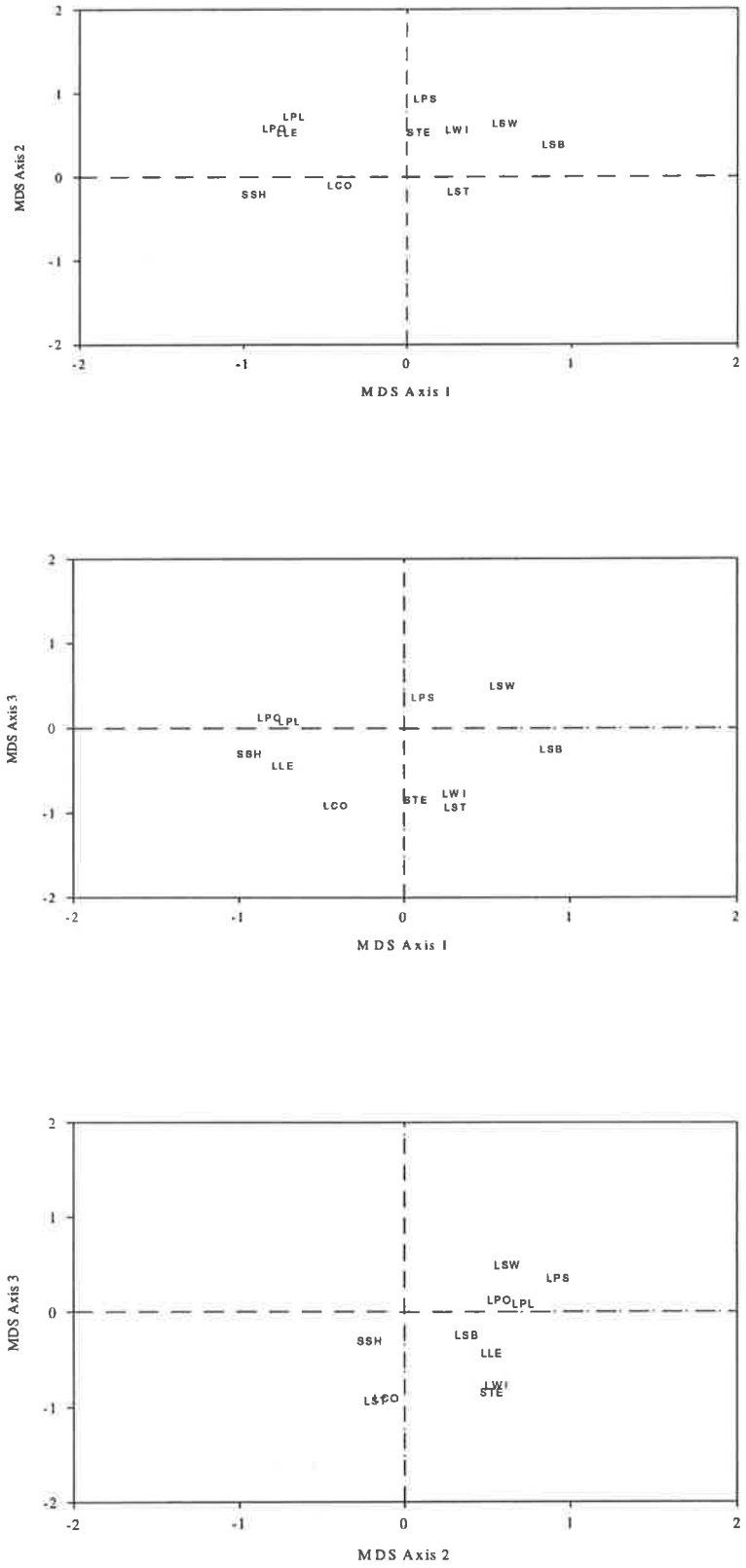
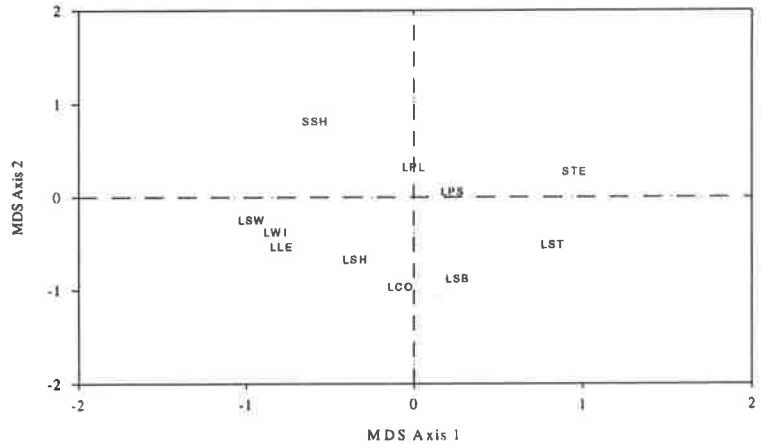
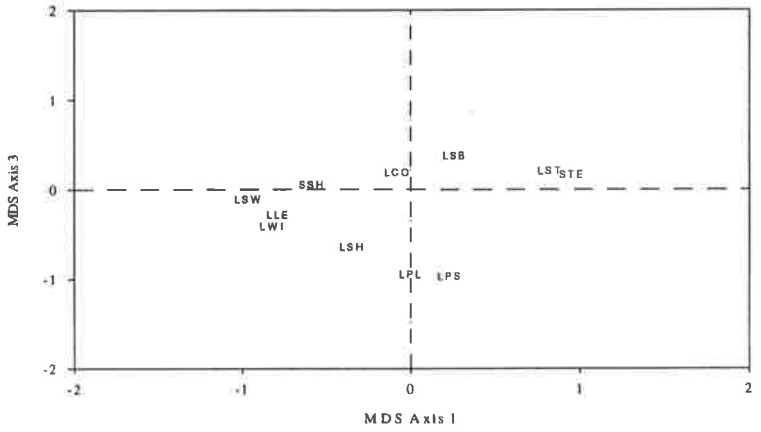


Figure 8.72. Principal canonical correlation graphs of *E. socialis* x *E. gardneri* seedlings and parents.





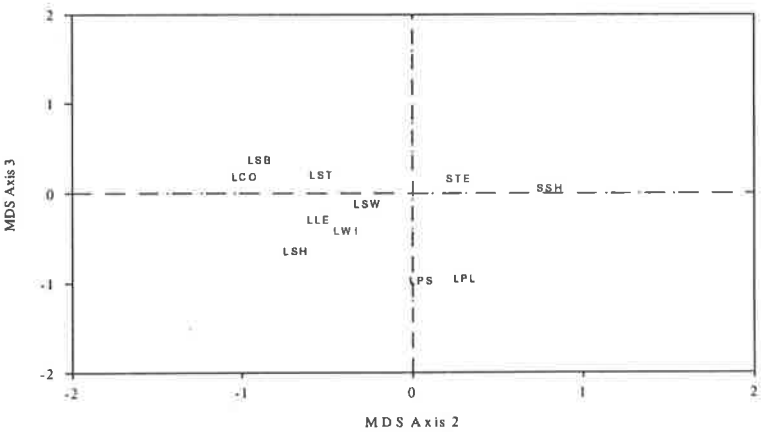


Figure 8.73. Principal canonical correlation graphs of *E. socialis* x *E. gillii* seedlings and parents.

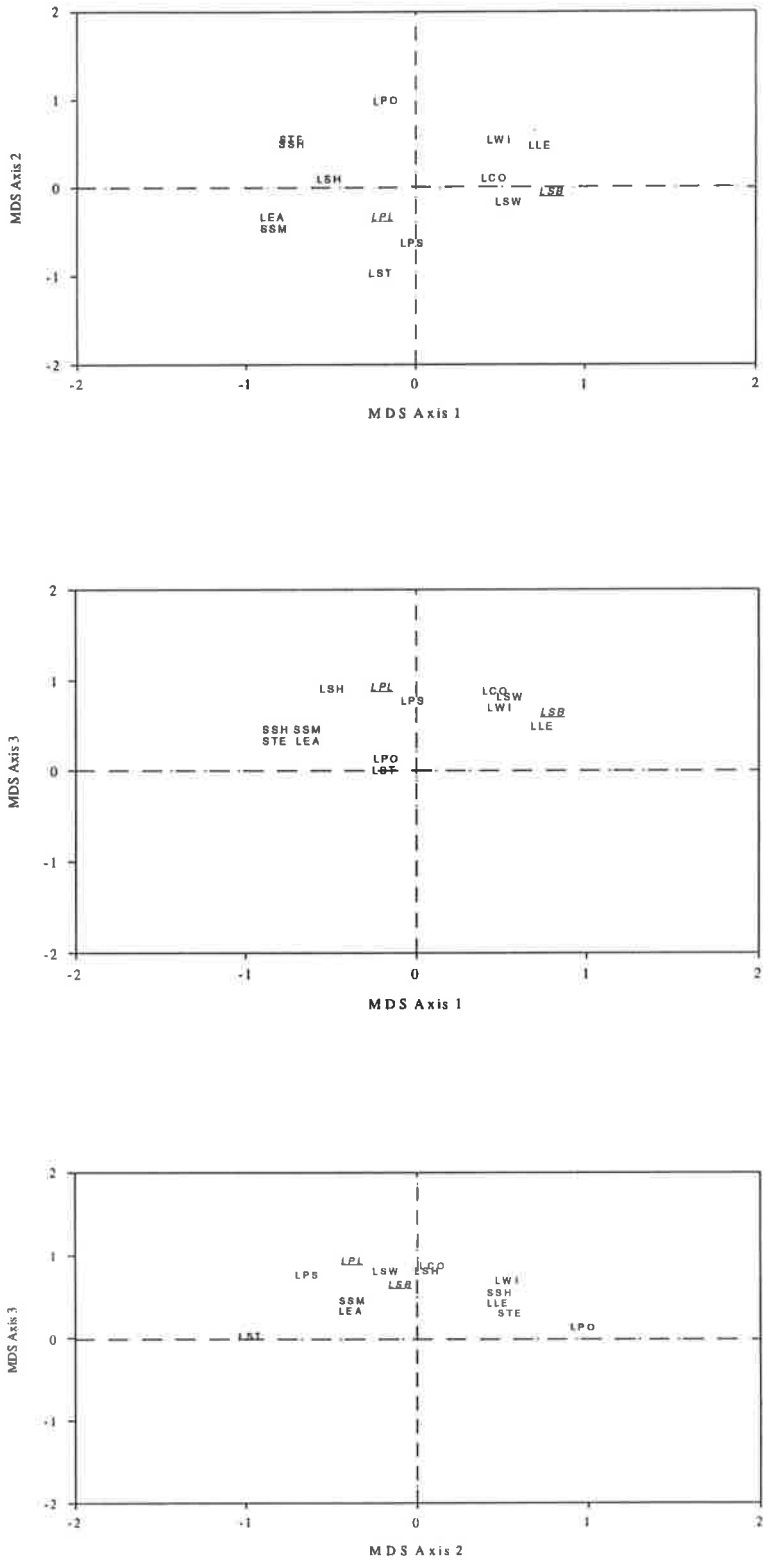
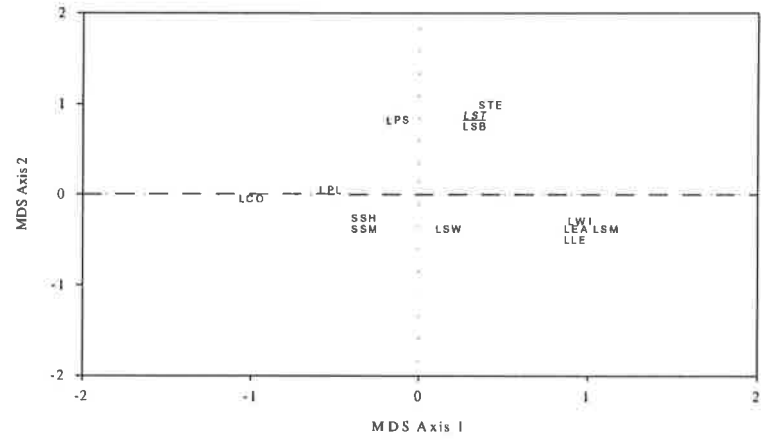
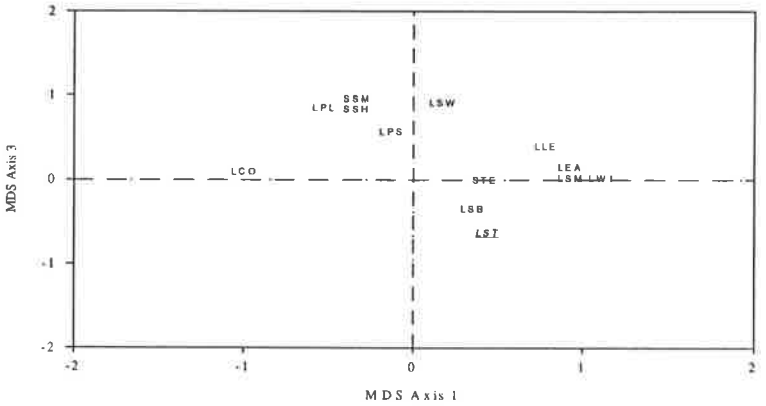


Figure 8.74. Principal canonical correlation graphs of *E. socialis* x *E. kruseana* seedlings and parents. Characters in italics and underlined are not significantly correlated to MDS ordinations (Table 8.10).





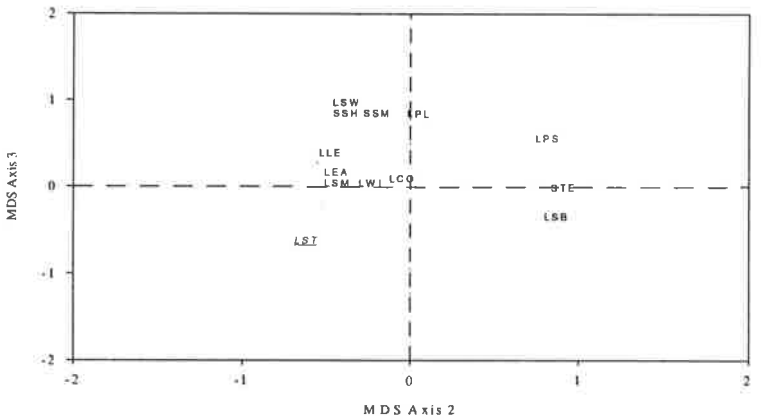
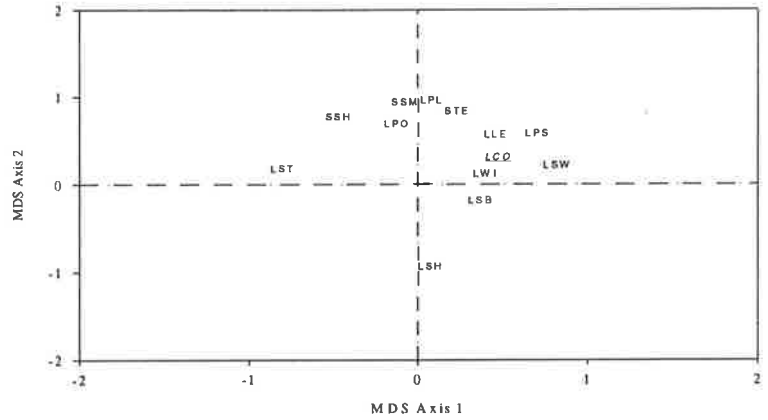
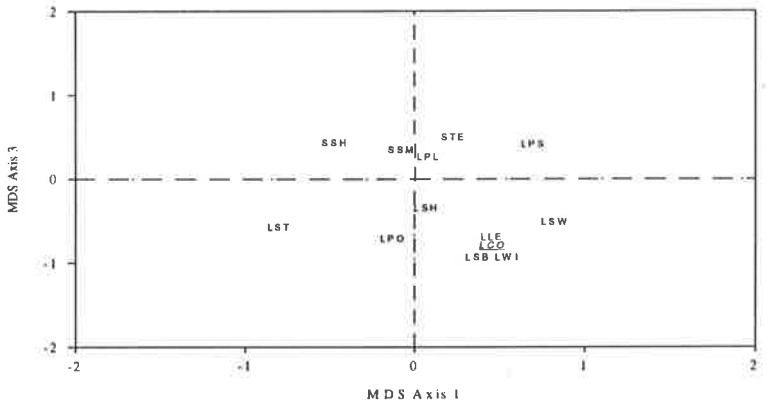


Figure 8.75. Principal canonical correlation graphs of *E. socialis* x *E. macrocarpa* seedlings and parents. Characters in italics and underlined are not significantly correlated to MDS ordinations (Table 8.7).





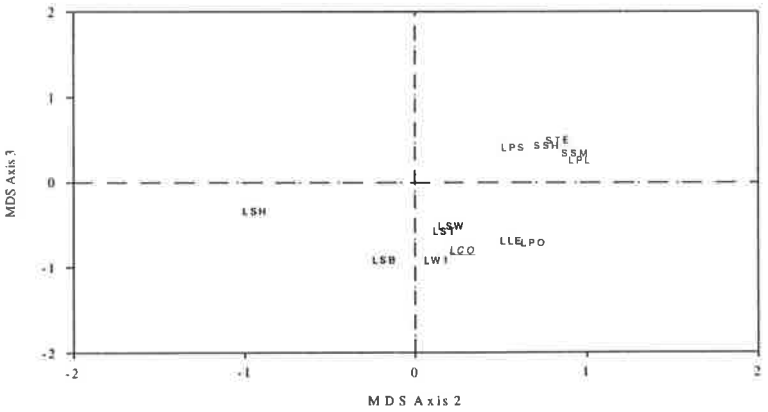


Figure 8.76. Principal canonical correlation graphs of *E. socialis* x *E. oldfieldii* seedlings and parents. Characters in italics and underlined are not significantly correlated to MDS ordinations (Table 8.10).

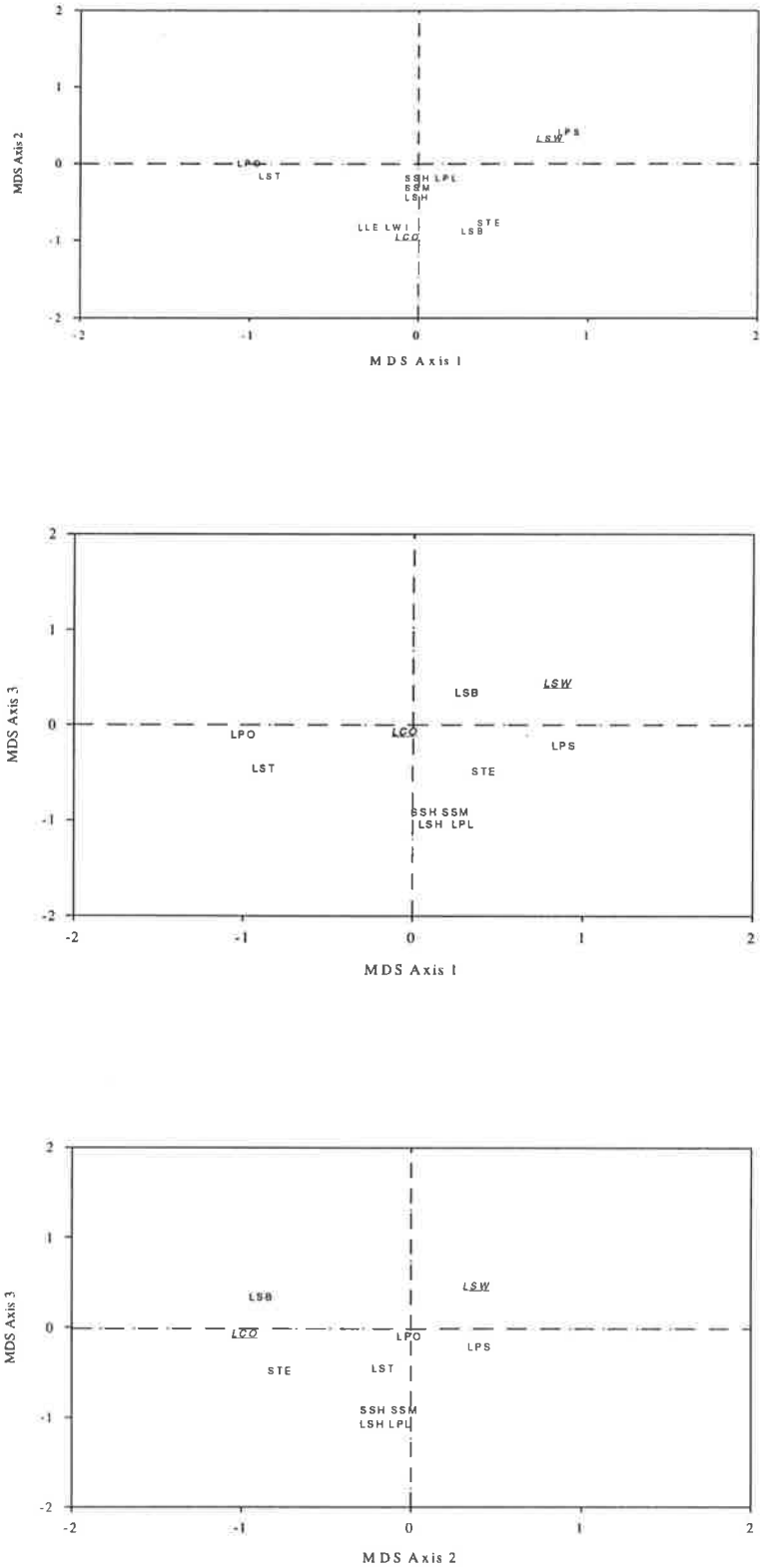


Figure 8.77. Principal canonical correlation graphs of *E. socialis* x *E. orbifolia* seedlings and parents. Characters in italics and underlined are not significantly correlated to MDS ordinations (Table 8.10).

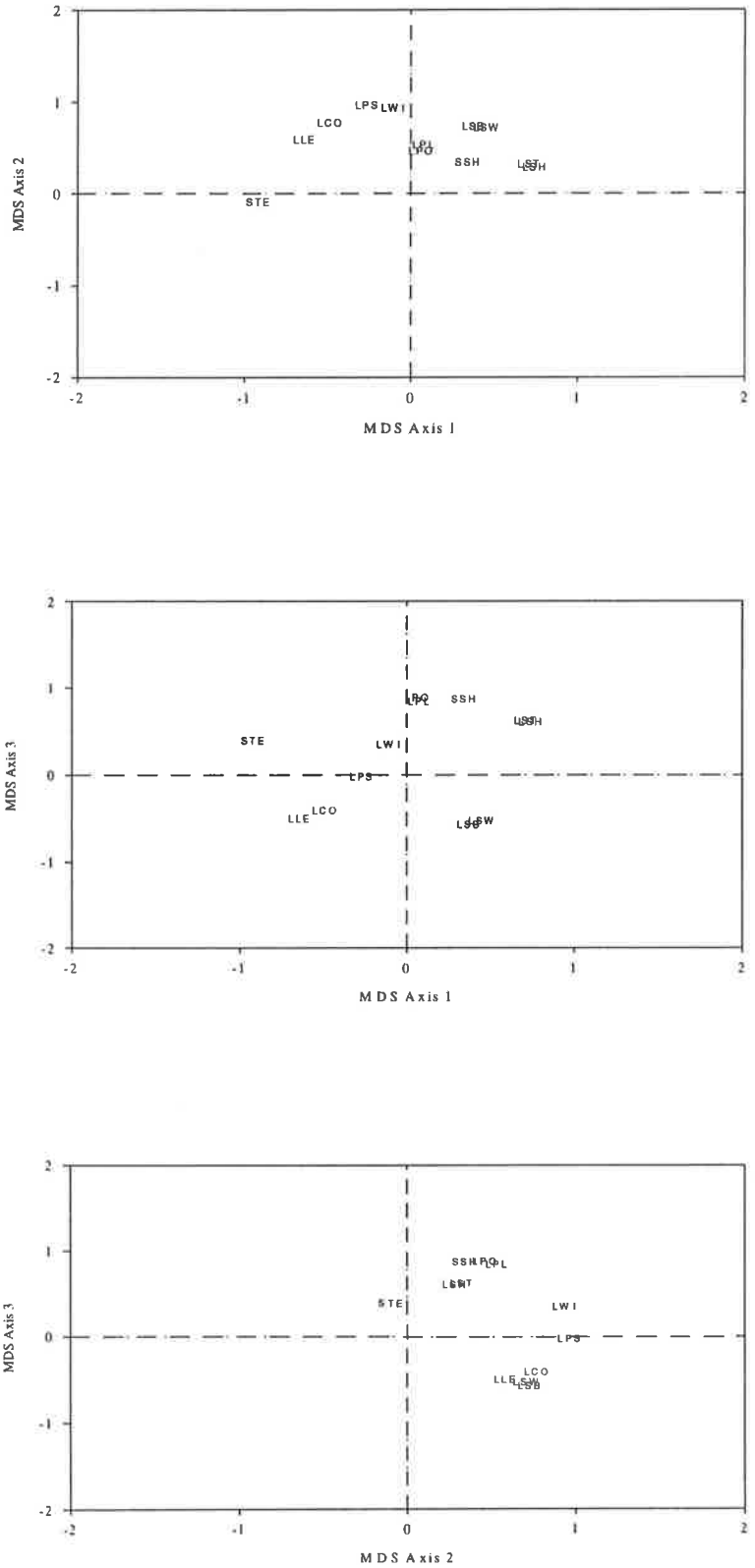
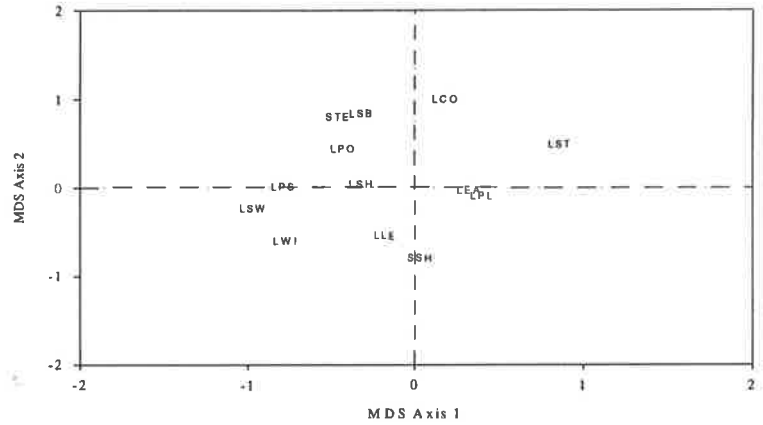
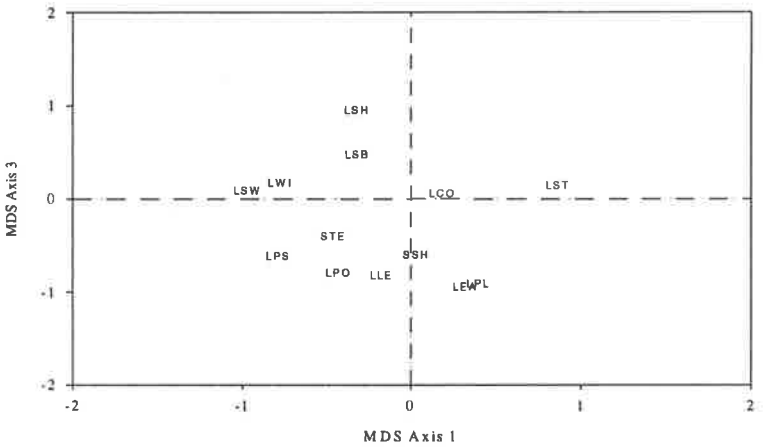


Figure 8.78. Principal canonical correlation graphs of *E. socialis* x *E. polyanthemos* seedlings and parents.





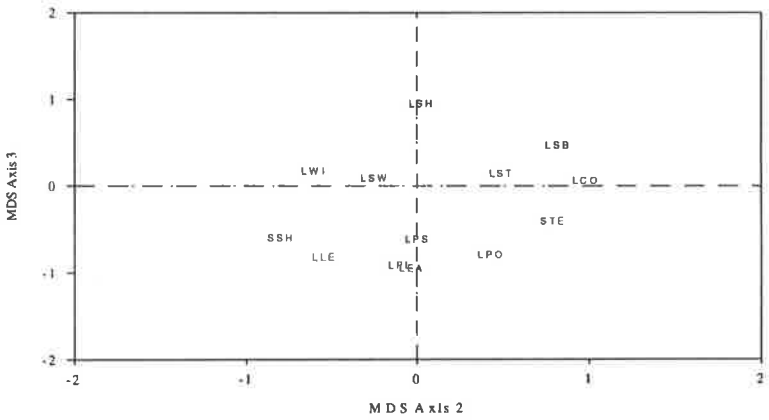
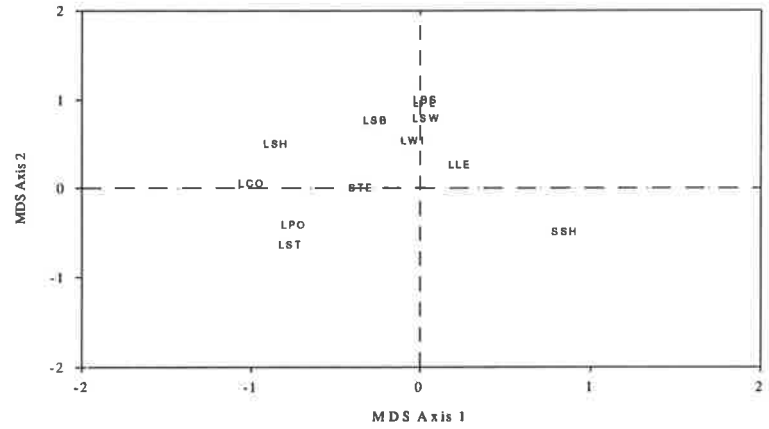
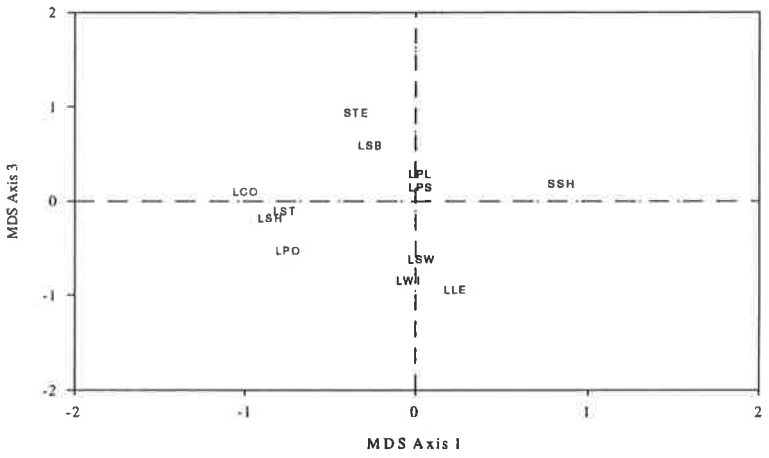


Figure 8.79. Principal canonical correlation graphs of *E. socialis* x *E. sideroxylon* seedlings and parents.





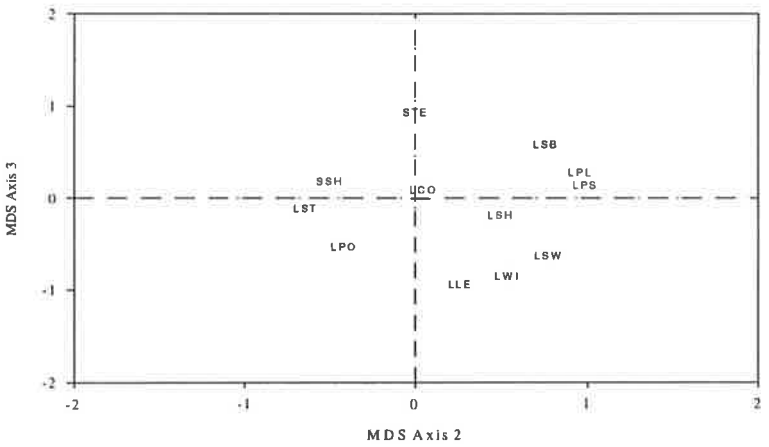


Figure 8.80. Principal canonical correlation graphs of *E. socialis* x *E. socialis* seedlings and parents.

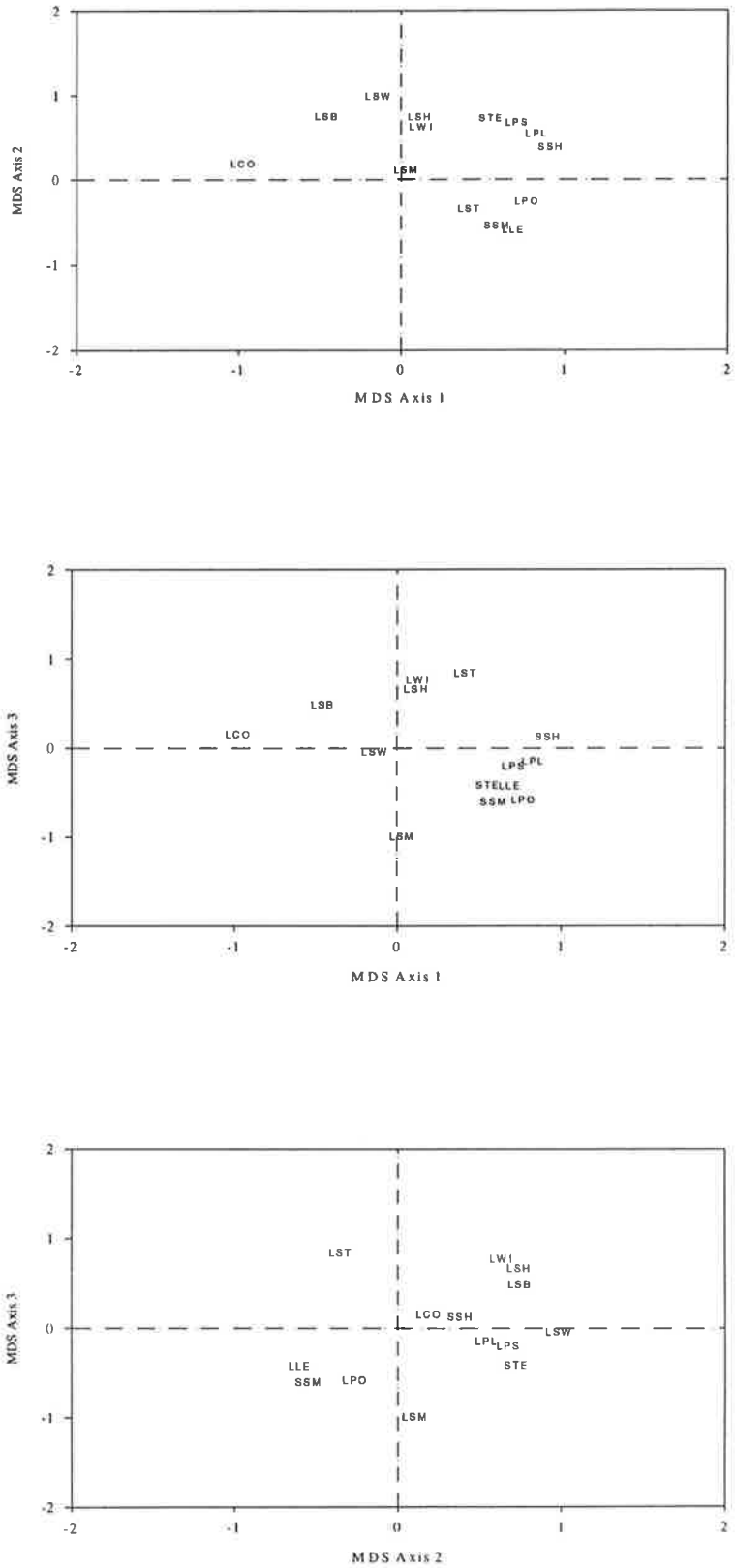
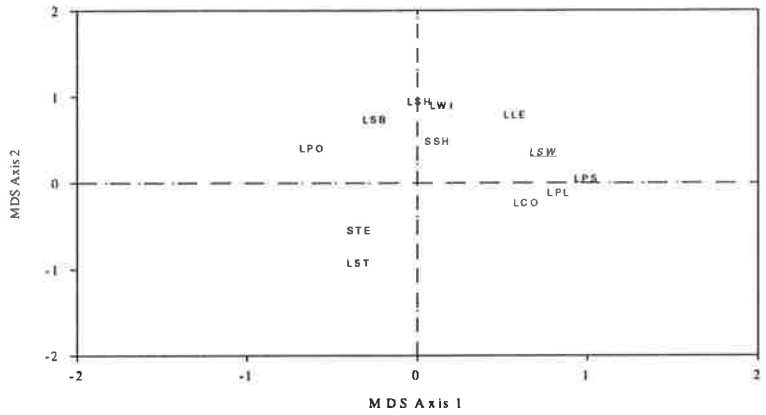
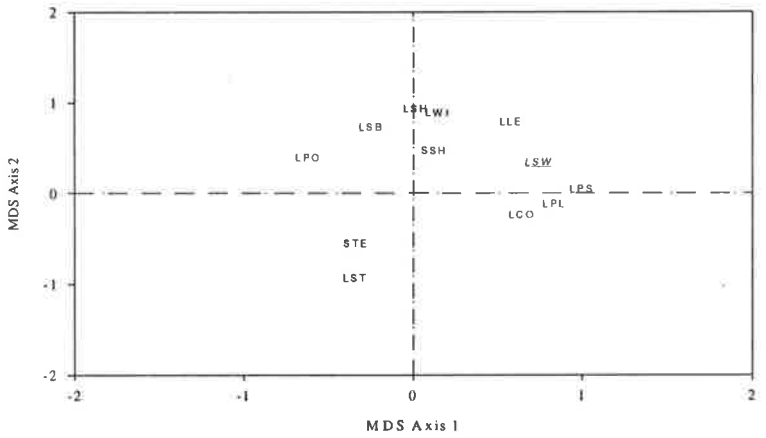


Figure 8.81. Principal canonical correlation graphs of *E. socialis* x *E. steedmanii* seedlings and parents.





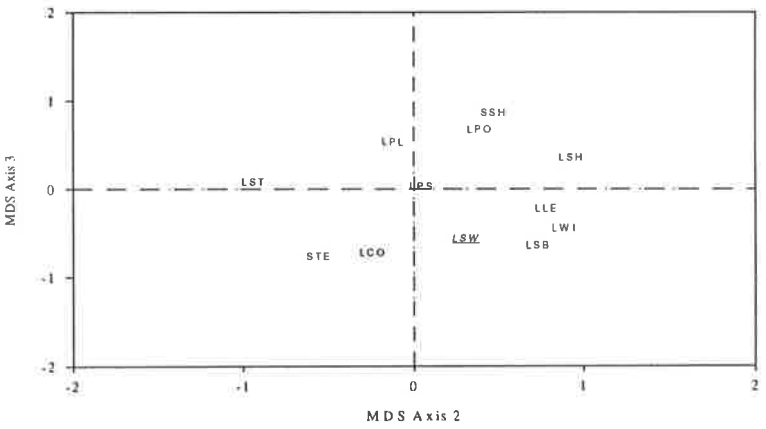
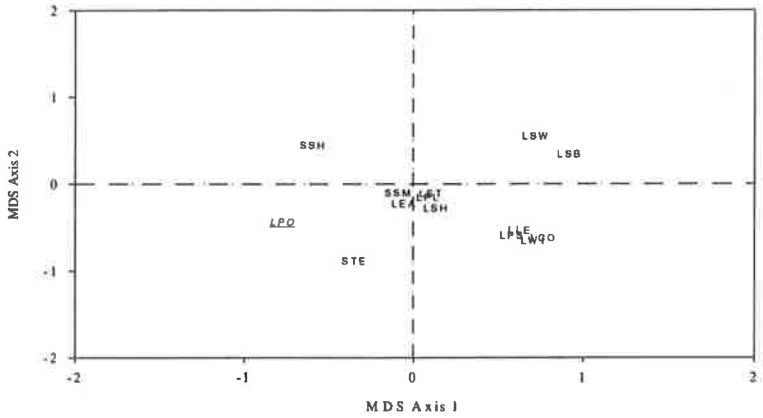
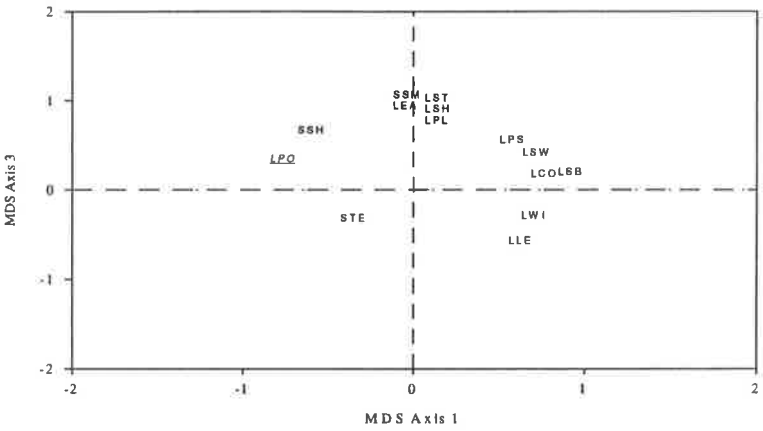


Figure 8.82. Principal canonical correlation graphs of *E. socialis* x *E. transcontinentalis* seedlings and parents. Characters in italics and underlined are not significantly correlated to MDS ordinations (Table 8.10).





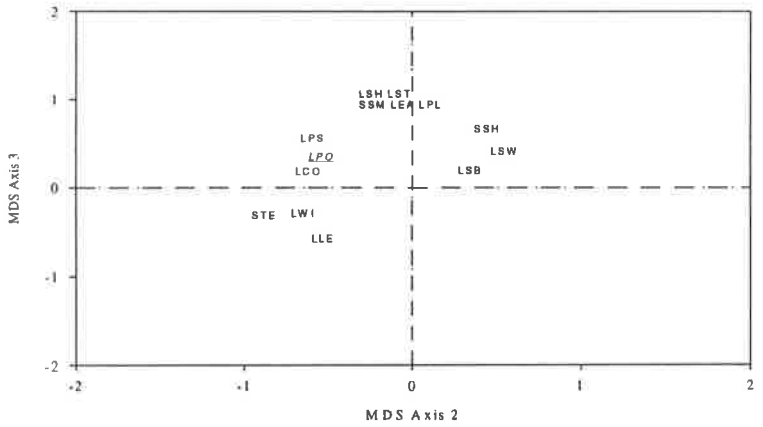
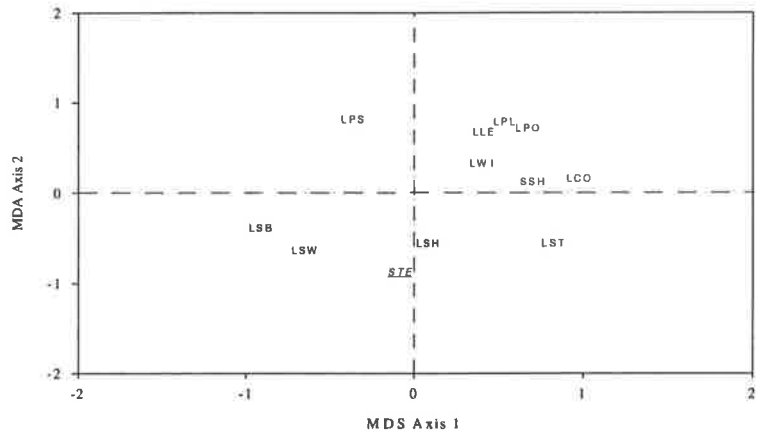
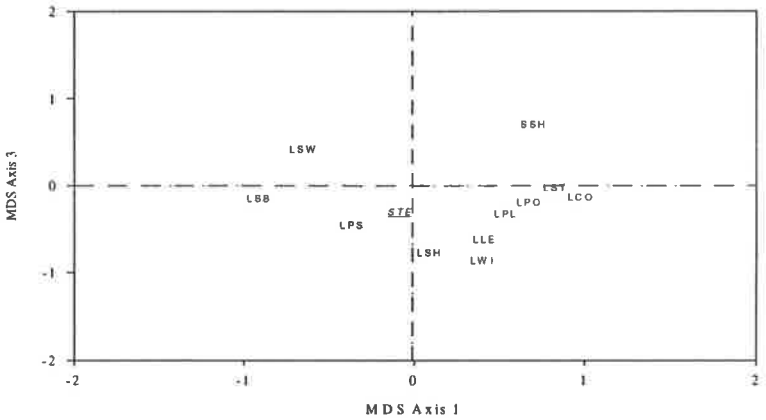


Figure 8.83. Principal canonical correlation graphs of *E. socialis* x *E. websteriana* seedlings and parents. Characters in italics and underlined are not significantly correlated to MDS ordinations (Table 8.10).





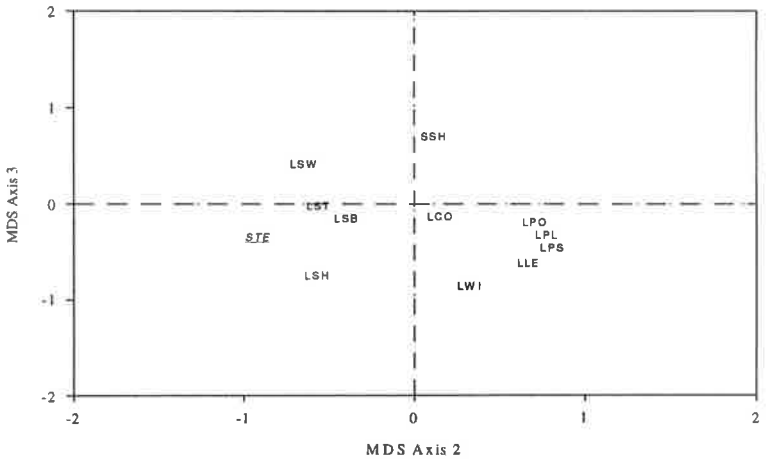


Figure 8.84. Principal canonical correlation graphs of *E. socialis* x *E. youngiana* seedlings and parents. Characters in italics and underlined are not significantly correlated to MDS ordinations (Table 8.10).

Appendix 3.1 - Presentations

17th October, 1997	Research update to Scholarship donors and interested parties Urrbrae House, Waite Agricultural Research Institute "Improvement of Ornamental Eucalypts"
11th February, 1999	Summary of research for Playford Memorial Trust Scholarship Horticulture donors and other interested parties Plant Research Centre Auditorium, Waite Agricultural Research Institute "Improvement of Ornamental Eucalypts"
16th April, 1999	5th Australian Wildflower Conference Melbourne, 14th to 17th April, 1999 "Development of Ornamental Eucalypts"
21st April, 1999	Adelaide Botanic Gardens Guides Adelaide Botanic Gardens "Improvement of Ornamental Eucalypts"
2nd June, 1999	Departmental Seminar Plant Research Centre Auditorium, Waite Agricultural Research Institute "Improvement of Ornamental Eucalypts"
2nd June, 1999	Interdepartmental Seminar Department of Environmental Biology, North Terrace "Improvement of Ornamental Eucalypts"

Appendix 3.2. - Posters

Delaporte, K.L., Collins, G., Conran, J., Klieber, A. and Sedgley, M. (1999). Improvement of Ornamental Eucalypts. 14th National AFPGA Conference, Hahndorf, South Australia, 20-22 August, 1999.

Delaporte, K.L., Conran, J. and Sedgley, M. (2000). Hybridisation in *Eucalyptus* ser. *Curviptera*: a morphological study. Flowers 2000: The first Australian Flower Conference, Tumbi-Umbi, New South Wales, 2-6 August, 2000.

Appendix 3.3. - Papers

Delaporte, K.L., Klieber, A. and Sedgley, M. (2000). Postharvest vase life of two flowering *Eucalyptus* species. *Postharvest Biology and Technology* 19, 181-186.

Delaporte, K.L., Conran, J. and Sedgley, M. (2000). Hybridisation in *Eucalyptus* ser. *Curviptera*: a morphological study. In: *Flowers 2000: The first Australian Flower Conference* (Ed: R. Worrall), Tumbi-Umbi, New South Wales, 2-6 August, 2000.

Delaporte, K.L., Collins, G., Conran, J. and Sedgley, M. (2000). Molecular analysis of an interspecific ornamental Eucalypt hybrid for parental identification. *Euphytica* (in press).

Delaporte, K.L., Conran, J. and Sedgley, M. (2000). Morphological analysis to identify the pollen parent of an ornamental interspecific hybrid *Eucalyptus*. *Scientia Horticulturae* (in press).

Delaporte, K.L., and Sedgley, M. (2000). *Ornamental Eucalypts:* Selection within species for cut bud and flower production. *Australian Plants* (in press).

Delaporte, K.L., Collins, G., Conran, J., Klieber, A. and Sedgley, M. (1999). *Development of Ornamental Eucalypts.* Proceedings of the 5th Australian Wildflower Conference, Melbourne, 14th to 17th April, 1999. pp:51-52.

Delaporte, K.L., Conran, J. and Sedgley, M. (2000). Interspecific hybridisation between three closely related *Eucalyptus* species: *E. macrocarpa*, *E. youngiana* and *E. pyriformis. (In preparation)*

Delaporte, K.L., Conran, J. and Sedgley, M. (2000). Interspecific hybridisation within *Eucalyptus (Myrtaceae):* subgenus *Symphyomyrtus,* sections *Bisectaria* and *Adnataria. (In preparation).*