



An Australian Government Initiative



Salt-tolerant Hybrid Eucalypts

**A report for the
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by Jen McComb

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Foreword

In order to extend the range of plants that can be grown on saline land and give a direct financial return, hybrids were produced between *Eucalyptus camaldulensis* lines that had been selected for salt tolerance, and *E. globulus* selected for high pulp quality and yield. The aim was to produce salt tolerant trees with high pulp quality.

This report describes the production, morphology, selection and seedling growth of hybrids of *E. globulus* x *camaldulensis*. It also describes the field performance of *E. globulus* x *camaldulensis* seedlings and clones, *E. camaldulensis* seedlings and clones, and *E. globulus* seedlings in trials on saline and non-saline sites. In the field, selected hybrids showed better survival than *E. globulus* on both saline and non saline sites. Further monitoring of growth and survival as the trees increase in biomass is necessary to determine the degree of superiority of the hybrids over the *E. globulus*. The clones giving the most vigorous growth were the same regardless of whether the site was saline or not over a range of rainfall from 432–948mm. Although the hybrids were originally selected for growth in saline areas, they have potential for growth elsewhere.

The results of this study suggest that selected eucalypt hybrids can have superior salt tolerance and vigour. Small numbers of clones are already commercially available for planting. These should be planted on a wider range of sites to evaluate climatic tolerance and growth. Further crossing to broaden the genetic base is also recommended.

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Executive Summary

What this report is about

This report is about selecting eucalypt hybrid material that can be grown for pulp production on saline lands. The report describes the production, morphology, selection and seedling growth of *E. globulus* x *camaldulensis* hybrids. It also describes the field performance of *E. globulus* x *camaldulensis* seedlings and clones, *E. camaldulensis* seedlings and clones, and *E. globulus* seedlings in trials on saline and non-saline sites. Preliminary assessment of 5-6 year old trees for fibre and sawlog properties was also undertaken.

Who is the report targeted at?

This report is for farm advisors, industry investors and researchers looking for appropriate genetic material to grow on saline and non-saline lands. The hybrids reported here have the potential for commercial returns while achieving land rehabilitation.

Background

There is increasing recognition that rising salinity is one of the biggest threats to productivity of agricultural land, and to infrastructure in both rural and urban areas. Existing and predicted damage to remnant native vegetation and wetlands is also significant. Tree planting has an important role for salinity control in both recharge and discharge areas.

This project initially concentrated on trees suitable for discharge areas, where both salt and waterlogging tolerance is important, and water uptake by the trees is predicted to slow or halt the rise in water table. Several species of trees and shrubs are suitable for planting on saline land, but at the time this project started, the species available for saline land produced materials such as fence posts, honey, firewood or provided shelter. None was appropriate for pulp production.

Aim

Our aim was to produce salt tolerant trees with high quality pulp, and for this purpose we developed hybrids between *Eucalyptus camaldulensis* x *E. globulus*. As the project progressed it became clear that 1) some highly productive hybrids were also suitable for plantations in non-saline areas, and 2) hybrids in both saline and non-saline areas could provide sawlogs. Therefore, preliminary assessment of fibre and sawlog wood properties was also undertaken.

Methods

The parents were selected for either their salt tolerance or pulp quality and yield. *E. camaldulensis* and *E. globulus* are not closely related but are placed in different sections of the sub genus *Symphomyrtus* of the genus *Eucalyptus*. As is usual with wide crosses in eucalypts, the progenies included large proportions of abnormal plants that died. Despite this there were some hybrids that showed excellent growth and form. It was clear that there was a wide range of compatibility between the parents, and that an initial survey of the compatibility (in terms of numbers of seeds per capsule, germination and proportion of abnormal plants) would be valuable before investing a large amount of time in extensive crossing. The superior clones from the present work are concentrated in five cross combinations.

The inheritance of morphological traits from the *E. globulus* parent made it easy to confirm that seedlings from crosses using *E. camaldulensis* as a female parent were in fact hybrid. The variation of mature leaf shape and size of the parent species and the hybrids made it impossible to distinguish the hybrid from the parents on the basis of leaf morphology, but the flowers and fruits of the hybrids were intermediate in shape and size.

Field trials were set up in 1995 at Mount Barker, Beverley and Pinjarra in Western Australia, and in 1997 in Narrikup and Boyup Brook, Western Australia; Coleambally, NSW and Cressy, Tasmania. All

sites were saline except for Pinjarra and Coleambally. Trees were assessed for survival, height, diameter and form.

A small sample of 5-6 year old trees was harvested and assessed for pulp yield, active alkali, basic density, milling and sawlog properties.

Results/Key findings

The hybrid families showed a level of tolerance to saline waterlogging intermediate between the parents in the glasshouse. In the field selected hybrids showed better survival than *E. globulus* on both saline and non saline sites. The best hybrids on all sites were equal to the surviving *E. globulus* in height, but for all hybrids combined, there was only a significant difference in the reduction in height due to soil salinity at the Mt Barker site (which was the most saline). On sites that were less suitable for *E. globulus*, several hybrids were significantly better than *E. globulus*. Further monitoring of growth and survival as the trees increase in biomass is necessary to determine the degree of superiority of the hybrids over *E. globulus* and to define areas in which it would be preferable to plant hybrids rather than *E. globulus*.

The clones giving the most vigorous growth were the same regardless of whether the site was saline or not, and over a rainfall range from 432–948mm. This may indicate that the selection has been for general vigour rather than for salt tolerance per se.

Although the hybrids were originally selected for growth in saline areas, they have potential for growth elsewhere. The hybrids grew well at Cressy, an area too cold for *E. globulus*, and at Coleambally and Beverley, which are too hot and dry for *E. globulus*. Use of hybrids could extend the regions that produce pulp wood further into drier regions than can be planted with *E. globulus*.

Timber from the hybrids was found to have potential for value-added purposes. Wood from 5-year-old trees had medium density and shrinkage greater than that of mature *E. camaldulensis* but less than that of 17–23 year old *E. globulus*.

Further crossing to extend the genetic base of the hybrids is desirable. The hybrids are reproductively fertile so backcrossing with *E. globulus* to improve wood properties is also possible.

Implications for stakeholders

These early results suggest that selected eucalypt hybrids can have superior salt tolerance and vigour to the parent stock. Farmers and industry investors can plant these hybrids for commercial products and rehabilitation of saline agricultural land. Small numbers of clones are already commercially available. Growth data should be collected from plantings on a wider range of sites, to evaluate climatic tolerance of the selected clones.

For researchers, this study provides clones suitable for testing on a range of sites to evaluate climatic tolerance and growth. Further crossing to broaden the genetic base is also recommended.

Recommendations for further research

1. Additional crosses should be undertaken to broaden the genetic base of the hybrids. Only crosses that give high numbers of seeds and a high proportion of normal progeny should be included in further screening trials and field trials. Crosses should also include backcrosses to *E. globulus* to improve the wood quality.
2. Plants from these new crosses should be grown to sufficient size for cuttings to be taken before a salt screening process that kills non-tolerant genotypes is imposed, as these may well have potential for use in non saline areas.
3. Further research to improve the efficiency of the micropropagation process should be undertaken. An IVS (in vitro soil) process that improves rooting and acclimatisation of plants of woody Australian

species grown in vitro has been developed by the Department of Agriculture WA (Newell *et al.* 2002). This should be tested using the hybrid lines.

4. Further field trials are needed to test:

- lines held in vitro that have not been included in any trials
- growth in areas too dry or too cold for *E. globulus* (both saline and non saline)
- growth in further sites in the Murray-Darling Basin using both non-saline and saline water for irrigation over the first few years
- water use patterns of the hybrids and selection of those with highest water use for planting in discharge areas.

5. Continued monitoring of existing trials is necessary to determine whether the trees, particularly the hybrids and *E. globulus* will continue to survive and grow to harvestable size.

6. Once trees in the Coleambally, Narrikup and Mount Barker sites reach appropriate size, further tests of pulp and timber properties should be done.

1 Development of the *Eucalyptus camaldulensis* x *globulus* hybrids

1.1 Crosses and analysis of seedling progeny

1.1.1 Introduction

Eucalyptus pulp plantations require land of reasonable quality and compete for land traditionally used for crops and pasture. This land use conflict, combined with the potential size of the industry, is making it increasingly attractive to use marginal land for plantations. For this to be successful breeding programmes are required to extend the environmental range of *Eucalyptus* species that have high growth rates and good fibre production. In Tasmania, such breeding programmes have concentrated on frost resistance, to allow plantations to be extended to cooler regions at higher altitudes (Tibbits 1986).

In the south west of Western Australia, the productivity of 440 000 ha of land has been partially or completely lost as a result of secondary salination and the area is increasing (Schofield 1992; Robertson 1996; Beresford *et al.* 2001). A breeding programme to extend the range of *Eucalyptus* pulp plantations to land marginalised by secondary salination would not only help alleviate the problem of land use conflict, but also assist in the rehabilitation of salt degraded land.

Eucalyptus species that have high pulp yields and growth rates, such as *E. globulus* Labill., tend to have a low tolerance to the combined stresses of salt and waterlogging (secondary salinised land is often seasonally waterlogged) (Marcar 1993). Attempts to increase their salt / waterlogging tolerance using natural intraspecific variation has been largely unsuccessful (Kabay and van der Moezel pers. comm.). Species that can tolerate salt / waterlogging conditions, such as *E. camaldulensis* Dehnh., have lower pulp quality and yields than *E. globulus* (Ona *et al.* 1996). However, *E. camaldulensis* is grown for paper pulp in a number of countries (Burma, USA, India, Morocco, Portugal and Spain) (Eldridge *et al.* 1993). One approach to breeding a salt / waterlogging tolerant *Eucalyptus* species that also has high pulp quality and yield is to hybridize *E. camaldulensis* with *E. globulus*.

The success of interspecific hybridisation in *Eucalyptus* is highly dependent on the extent of physiological, genetic and physical barriers between the species. Physiological and genetic barriers increase with taxonomic distance (Pryor 1951a,b; Pryor 1957; Griffin *et al.* 1988; Potts and Cauvin 1988; Ellis *et al.* 1991). Physical barriers are not influenced by taxonomic affinity, but relate to differences in flower size of the species to be crossed and are often unilateral (Pryor 1956; Potts and Cauvin 1988; Potts and Savva 1989; Gore *et al.* 1990). The proposed cross between *E. camaldulensis* and *E. globulus* subsp. *globulus* is potentially a difficult one as the species are in the same subgenus (*Symphomyrtus*) but in different sections (*E. camaldulensis*—*Exsertaria*; *E. globulus*—*Maidenaria*). They also have different sized flowers, with *E. camaldulensis* flowers several times smaller than *E. globulus* subsp. *globulus* flowers.

Crosses using *E. camaldulensis* as the male, and *E. globulus* subsp. *globulus* as the female parent have been unsuccessful (Potts *et al.* 1992). However, hybrid seedlings have been produced using *E. globulus* subsp. *maidenii* as the female parent (Pilipenko 1969). The difference in the success rate between the two subspecies is probably due to differences in their flower size. *E. globulus* subsp. *globulus* has much larger flowers than *E. globulus* subsp. *maidenii*.

Hybrids between *E. globulus* and *E. camaldulensis* using *E. camaldulensis* as the female parent have been produced but details have not been published (Hayat Mesbah, National Centre for Forest Tree Breeding, Morocco, pers. comm.).

Previous work using *Eucalyptus camaldulensis* intraspecific crosses showed that pollination three days after emasculation, when styles had just turned red and stigmas were enlarged, yellow and sticky gave

maximum seed set (45–55 seeds per capsule and 95–100% capsule set). The period of stigma receptivity varied between cultivars. Reasonably high levels of seed (> 25 seed per capsule and >65 % capsule set) could also be produced when flowers were pollinated at the time of emasculation opening up the possibility of ‘one-stop pollination’ (Oddie and McComb 1998).

This chapter describes the hybridisation between salt / waterlogging tolerant *E. camaldulensis* clones and *E. globulus* lines selected for pulp quality and yield, and the performance of the seedlings. This work was completed before the commencement of the JVAP funding but it is included here to describe the lines used for this project.

1.1.2 Materials and methods

Controlled pollinations using clones of salt tolerant *E. camaldulensis* (van der Moezel and Bell 1990; Bell *et al.* 1994) as the female parent were conducted at Kwinana, 50 Km south of Perth, WA in December 1992 – January 1993 and December 1993 (Table 1.1).

Four *E. globulus* pollen parents were used in the interspecific crosses. They originated from King Island, Tas. (K), Otways, Vic. (O), Taranna, Tas. (T) and Flinders Island, Tas. (F). Pollen supplied by North Forests Eucalypt Research Centre (formerly APPM) was from families with high pulp quality and yield, K (KI 5 R), O (O 4 E) and T (T 1 S). The F pollen was a mixture of pollen collected from a natural stand of *E. globulus* trees by Kylisa Seeds Pty Ltd. Pollen was freeze dried and stored at –20°C or at 4°C ± 2°C.

Table 1.1 *E. camaldulensis* clones used in controlled pollination experiments

Clone	No. of ramets	Provenance
42	4	Capelis, WA
83	2	Mt. Fouracre, WA
84	2	Mt. Fouracre, WA
85	3	Broken Hill, NSW
87	4	Erudina, SA
52 ^a	1	Silverton, NSW

^a Clone 52 was only used as a pollen parent in intraspecific crosses

Seventeen intraspecific outcrosses, five selfs and eighteen *E. camaldulensis* x *globulus* crosses were produced using five *E. camaldulensis* clones as females. In the interspecific crosses all *E. camaldulensis* clonal lines were mated with four *E. globulus* families excluding the crosses 84 x K and 84 x O. The controlled pollination technique used in this study was as described by Oddie and McComb (1998).

Between 24–81 flowers were pollinated for each intraspecific outcross, 78–106 flowers for each self and 36–164 flowers for each interspecific cross. At least 20 flowers per clone were emasculated and bagged but not pollinated to act as controls. Open pollinated capsules were collected from the same mother trees.

E. camaldulensis capsules were harvested in March – April. At this time at least 20 open pollinated capsules were also collected from each clone. Each capsule was placed in a vial and stored over silica gel to dry. The percent capsule set was calculated and the number of seeds in each capsule was counted. One hundred seeds from each family (less if < 100 seeds were produced) were weighed to calculate the mean seed weight.

For all selfs and interspecific crosses and in a selection of intraspecific outcrosses 5–10 styles at 2, 4, 6 and 8 days after pollination were fixed to view the pollen tubes (Oddie and McComb 1998).

A few controlled pollinations using *E. globulus* as the female parent were conducted at Manjimup, WA in September 1993. Three *E. globulus* x *camaldulensis* crosses (76 flowers) and two intraspecific (28 flowers) crosses were attempted. Flowers were pollinated 6–7 days after emasculation. Six or thirteen days after pollination 6 styles were harvested and fixed from each cross. In November 1993 the fruit set was assessed.

Seedling growth

The *E. camaldulensis* x *camaldulensis* and *E. camaldulensis* x *globulus* seed produced from controlled crosses in December 1992, open pollinated *E. camaldulensis*, *E. globulus* seed from open pollinated trees of K, T and O and seeds from Flinders Island (but not the same tree as the F pollen parent) were sown in a glasshouse in August 1993 in 64 cell trays containing 3 l of peat:perlite (2:1) and a nutrient mix (Oddie 1996). Germination was recorded 1, 3 and 7 weeks after sowing.

Six months after the seeds were sown, 20 *E. camaldulensis* x *globulus*, 10 *E. camaldulensis* (open pollinated and intraspecific crosses) and 10 *E. globulus* seedlings from each family were repotted into 200 mm black plastic pots. Abnormal seedlings were excluded from selection. The repotted plants were randomly placed on weed mat in a paddock at Murdoch University and fertilised once a fortnight with a general liquid fertiliser (Wuxal®).

The number and type of abnormalities present in seedlings was recorded when they were 3, 11 and 22 months old. The number of 'normal' seedlings produced from each pollinated flower was calculated using data on capsule set, the number of seeds per capsule and the % of abnormal seedlings produced after 22 months.

Statistical analysis

Analyses were carried out using SPSS (Anon. 1988). Prior to analysis all data were screened for assumptions of normality, homogeneity of variance and non-correlation between means and variances. Percentage capsule set and percentage germination were arcsine transformed to convert them to a normal distribution (Tabachnick and Fidell 1996). The number of normal seedlings per flower and mean seed weights were log transformed to correct for unequal variances. Log transformation of the seeds per capsule data could not correct for unequal variances so untransformed data were used, but the 0.01 significance level was used rather than the 0.05 level to protect against type 1 errors (Tabachnick and Fidell 1996). One way analysis of variance across all types of crosses was carried out on the number of seeds per capsule. Contrast analysis was then used to test the *a priori* hypotheses that:

1. intraspecific crosses differ from interspecific hybrids
2. intraspecific crosses differ from selfs
3. intraspecific crosses differ from open pollinated progenies
4. selfs differ from interspecific hybrids
5. selfs differ from open pollinated progenies
6. interspecific hybrids differ from open pollinated progenies.

LSD tests were used to determine statistical differences between families within each cross type.

One way analyses of variance and LSD tests were performed to determine statistical differences in % capsule set, mean seed weight, % seed germination and the number of normal seedlings produced per flower between the different types of crosses (*E. camaldulensis* open pollinated, *E. camaldulensis* selfs, *E. camaldulensis* outcrosses and the *E. camaldulensis* x *globulus* crosses).

A log-linear analysis was carried out on the *E. camaldulensis* x *globulus* crosses using the design variables of cross and time since germination, and the response variable of normality of the seedlings. This determined if the proportion of normal seedlings observed was related to the cross involved or the time since germination.

1.1.3 Results

Pollen tube growth

The germination of pollen grains and growth of pollen tubes was too prolific to quantify in all the intraspecific outcrosses, selfed and interspecific crosses examined. Pollen tube abnormalities such as bulbous swellings and forked growths were observed in the styles of all types of crosses with no obvious differences in the nature and number of abnormalities between intraspecific and interspecific crosses. In the *E. globulus* x *globulus* crosses pollen tubes had grown beyond the base of the style 13 days after pollination. In all *E. globulus* x *camaldulensis* crosses pollen tubes had grown at most 6.5–7.5 mm down the styles (which had a mean length of 8.5 mm) by day 6 and grew no further by day 13.

Capsule and seed set

All *E. camaldulensis* intraspecific and interspecific crosses produced some capsules, each producing at least one seed. The mean number of seeds produced per capsule was significantly greater ($F_{(3, 1322)}=290.27(p<0.001)$) for intraspecific outcrosses than for selfs and interspecific crosses (Table 1.2). The intraspecific cross 85 x 87 and the reciprocal were an exception, producing only two seeds per capsule; the lowest of all crosses including interspecific crosses (Table 1.2; Fig. 1.1). Only three other intraspecific outcrosses 83 x 42, 83 x 87 and 42 x 87 produced less than a mean of 35 seeds per capsule. The only intraprovenance cross included 83 x 84 (Mt. Fouracre, WA) displayed high capsule and seed set (Table 1.2).

In all types of crosses (*E. camaldulensis* selfs, *E. camaldulensis* outcrosses and the *E. camaldulensis* x *globulus* crosses) there was a significant difference in the percentage capsule set and the number of seeds produced per capsule ($p < 0.01$) between the different male / female combinations (Table 1.2). Crosses involving clones 83 and 87 as the female parent had the highest percent capsule set, while crosses with clone 42 as the female parent produced the least (Table 1.2). Clone 84 produced the most seeds per capsule for the two intraspecific outcrosses attempted.

The mean number of seeds per capsule produced by selfed *E. camaldulensis* clones ranged from two in clone 83 to eleven in clone 42 (Table 1.2). There was also variation in the capsule set of the selfed clones. Clone 42 producing the highest selfed capsule set at 81 % and clone 87 producing the lowest at 8 %. Selfed capsule set was below 50 % for the other clones.

The mean number of seeds produced per capsule was significantly ($F_{(1, 1058)}=26.57 (p < 0.001)$) greater for interspecific crosses than for selfs (Table 1.2). Only two crosses, 83 x O and 84 x F produced more than an average of 17 seeds per capsule (Table 1.2). In clones 85 and 87 crosses with King Island pollen produced the highest number of seeds per capsule followed by Otways, Flinders and Taranna. In all clones the number of seeds per capsule was lowest (mean of 3.5–4.5) when Taranna was used as the pollen parent.

Mean seed weight of *E. camaldulensis* outcrosses was less than the *E. camaldulensis* open pollinated, selfs and *E. camaldulensis* x *globulus* hybrids. However the difference was not statistically significant ($F_{(13,41)}=1.54(p=0.22)$) (Table 1.2). Mean weights ranged from 0.114 mg (83 x O) to 0.416 mg (85 x T). Families with clone 85 and 87 as the female parent produced the heaviest seeds (overall mean of 0.26). As the number of seeds produced per capsule increased the weight of seeds tended to decrease ($r_{(18)}=-0.52 (p < 0.02)$).

Table 1.2 Capsule set, mean number of seeds per capsule and seed weight of *E. camaldulensis* open pollinated, selfed, intraspecific, and interspecific crosses using *E. globulus* as the male parent

Cross	Year of crossing	Number of flowers pollinated ¹	% capsule set ¹	Mean no. seeds per capsule (se)	Mean seed weight (mg)
<i>E. camaldulensis</i> —Open pollinated					
42				11.3 (1.12) ^b	0.148
83				5.3 (1.24) ^a	0.180
84				19.9 (1.36) ^b	0.138
85				4.3 (0.85) ^a	0.311
87				5.6 (0.87) ^a	0.321
Mean (se)				6.6 (0.57) ^A	0.22 (0.04) ^A
<i>E. camaldulensis</i> —Self pollinated					
42	1993	16	81.3	10.6 (1.68) ^d	0.152
83	1993	61	45.9	1.9 (0.27) ^a	0.182
84	1992	41	41.5	6.9 (0.51) ^c	0.200
85	1992 & 1993	95	32.6	3.1 (0.34) ^b	0.314
87	1992	51	45.1	6.8 (1.07) ^{cd}	0.279
Mean (se)			49.3 (8.35) ^A	5.86 (0.77) ^A	0.23 (0.03) ^A
<i>E. camaldulensis</i> x <i>E. camaldulensis</i>					
42 x 83	1993	24	70.8	36.2 (6.21) ^{bcd}	0.140
42 x 85	1993	14	64.3	64.1 (5.93) ^f	0.142
42 x 87	1992	43	34.9	27.3 (3.82) ^{bc}	0.149
83 x 42	1993	23	78.3	18.3 (1.81) ^b	0.192
83 x 84	1993	10	100.0	51.4 (3.69) ^{ef}	0.142
83 x 85	1992 & 1993	52	90.4	47.5 (1.75) ^e	0.122
83 x 87	1993	30	70.0	32.7 (2.46) ^{cde}	0.174
84 x 85	1992	38	34.2	73.9 (5.06) ^f	0.139
84 x 87	1992	21	71.4	81.6 (6.08) ^f	0.132
85 x 42	1993	22	72.7	44.8 (3.69) ^{de}	0.163
85 x 52	1992	46	58.7	55.0 (2.81) ^{ef}	0.192
85 x 84	1993	16	93.8	37.2 (3.17) ^{cde}	0.140
85 x 87	1992 & 1993	69	34.8	2.0 (0.16) ^a	0.279
87 x 42	1992 & 1993	75	82.7	42.6 (2.34) ^{de}	0.173
87 x 83	1993	19	94.7	38.7 (2.50) ^{de}	0.190
87 x 84	1993	28	100.0	45.1 (2.52) ^{de}	0.211
87 x 85	1992 & 1993	174	34.5	2.3 (0.18) ^a	0.321
Mean (se)			69.8 (5.69) ^B	41.2 (1.24) ^C	0.17 (0.01) ^A
<i>E. camaldulensis</i> x <i>globulus</i> —Interspecific hybrids					
42 x K	1993	47	10.6	8.6 (3.47) ^{abc}	0.195
83 x K	1993	24	58.3	7.1 (1.43) ^c	0.148
85 x K	1992	87	56.3	16.5 (1.18) ^d	0.245
87 x K	1992	70	85.7	14.1 (0.52) ^d	0.224
42 x O	1993	39	43.6	6.7 (0.87) ^c	0.130
83 x O	1993	37	51.4	25.8 (2.16) ^e	0.114

Cross	Year of crossing	Number of flowers pollinated ¹	% capsule set ¹	Mean no. seeds per capsule (se)	Mean seed weight (mg)
85 x O	1992	61	41.0	8.7 (0.87) ^c	0.215
87 x O	1992	76	85.5	8.5 (0.42) ^c	0.286
42 x F	1992	69	34.8	9.6 (2.05) ^c	0.196
83 x F	1992	65	47.7	4.6 (0.56) ^b	0.126
84 x F	1992	51	43.1	23.0 (2.54) ^e	0.136
85 x F	1992	110	35.5	5.4 (0.42) ^b	0.323
87 x F	1992	124	64.5	5.5 (0.36) ^{ab}	0.296
42 x T	1993	11	18.2	4.3 (0.82) ^{abc}	0.238
83 x T	1993	16	50.0	3.5 (1.12) ^{ab}	0.208
84 x T	1992	13	23.5	4.5 (1.50) ^{abc}	0.222
85 x T	1992	94	62.8	3.7 (0.25) ^a	0.416
87 x T	1992	70	85.7	4.5 (0.25) ^{ab}	0.339
Mean (se)			49.9 (5.17) ^A	9.1 (1.55) ^B	0.23 (0.02) ^A

Note: Numbers refer to *E. camaldulensis* clones and letters to *E. globulus*. Results were bulked where there were no significant differences ($p < 0.01$) in the number of seeds per capsule for the same cross made in 1992 & 1993.

¹ Total numbers of flowers pollinated. For %capsule set, those capsules lost because of parrot damage or broken branches were excluded from the data.

^{AB} Different upper case letters after means and standard errors refer to significant differences ($p < 0.01$) between overall means for each type of cross for each column.

^{abcdef} Different lower case letters after means and standard errors for data on mean number of seeds per capsule refer to significant differences ($p < 0.01$) between means within a particular type of cross.

No capsules were produced in the controls (emasculated and bagged but not pollinated) of clones 42, 83, 85 and 87. In clone 84 one capsule set one seed from a control emasculated in 1992. No seeds resulted from the intraspecific or interspecific *E. globulus* crosses and few open pollinated capsules were seen.

Seed germination

E. globulus seed germinated over several weeks and at the time of the last germination count (7 weeks) some seeds had just emerged. In contrast *E. camaldulensis* and *E. camaldulensis* x *globulus* seed germinated within three weeks.

There was no significant difference ($F_{(3,20)} = 2.157$ ($p > 0.12$)) in the percentage of seeds that germinated between the various cross types (Table 1.3) with the percentage germination of hybrid families being greater than or comparable to the parental families (Table 1.3). Seed germination was consistently poor in both intra and interspecific families with *E. camaldulensis* clone 84 as the female parent (Table 1.3).

Seedling abnormalities

The incidence of abnormal seedlings in the *E. camaldulensis* open pollinated, *E. globulus* open pollinated and *E. camaldulensis* x *camaldulensis* crosses was very low, never exceeding 2% after 22 months. These abnormalities took the form of severely twisted stems, divided leaves or twisted leaves. However *E. camaldulensis* x *globulus* hybrids produced up to 97% abnormal seedlings after 22 months. A log-linear analysis for the *E. camaldulensis* x *globulus* crosses using the design variables of cross and time since germination and the response variable of normality of the seedlings showed a significant 3-way interaction ($\chi^2_{59} = 2037$, $p < 0.001$). While all crosses showed an increase in the proportion of abnormal seedlings with time, some families showed little abnormality at 3 and 11 months before increasing at 22 months (83 x K, 84 x K), some showed moderate levels of

abnormalities at 3 months and this increased steadily through 11 and 22 months (85 x K, 85 x O, 85 x F, 85 x T, 87 x O, 87 x F, 87 x T) while one cross showed only a slight increase over time after the initial 3 months (87 x K). Hybrid families with *E. globulus* K as the male parent produced the least abnormalities while those with *E. globulus* T produced the most (Table 1.3). There was a significant negative correlation ($r_{(18)} = -0.57, p < 0.01$) between the number of seeds produced per capsule and the percentage of plants that had developed abnormalities by 22 months.

Some types of abnormalities were rare. They included the production of only one cotyledon (three seedlings), fused cotyledons (one seedling) and the production of three leaves at each node (one seedling). The more common abnormalities took one of two forms. In the first type, seedlings developed normally until the first or second pair of seedling leaves were produced. At this stage growth stopped and the cotyledons and leaves became variegated red / brown and green. Seedlings in this state remained alive for several months without further shoot growth and all eventually died. In eight of the ten hybrid families (all with *E. camaldulensis* clones 85 or 87 as the female parent) between 38 and 50 % of seedlings were affected by this type of abnormality (Table 1.3). In the remaining two hybrid families (83 x K and 84 x F) only 1 % of seedlings were affected.

The second type of abnormality developed in apparently 'normal' seedlings from the time they were about six months old. Affected seedlings stopped growing, leaves became increasingly variegated, thick and wrinkled, usually curving downwards and veins became thick and woody in appearance (Fig. 1.1). In some cases new shoots were produced from axillary buds low on the stem. Initially these shoots appeared 'healthy' and grew rapidly, but eventually they developed the abnormalities (Fig. 1.1c). The majority of abnormal seedlings died within twelve months.

The number of 'normal' seedlings produced per pollinated flower was significantly greater ($F(1,14)=37.28$ ($p < 0.001$)) in interspecific compared to intraspecific crosses (Table 1.3). *E. camaldulensis* x *globulus* families with *E. globulus* K as the male parent produced the most 'normal' seedlings per pollination (2.7–4.7) while families with *E. globulus* T as the male parent produced the least 'normal' seedlings (0.1) (Table 1.3).

Table 1.3 The percentage germination and development of abnormalities in *E. camaldulensis*, *E. globulus* and *E. camaldulensis* x *globulus* seedling families

Family	% germination	% abnormal seedlings at months ¹	% abnormal seedlings at 11 months ²	% abnormal seedlings at 22 months ²	No. 'normal' seedlings / flower ³
<i>E. camaldulensis</i> —Open pollinated					
83	81.3	0	0	0	
84	56.3	0	0	0	
85	64.1	0	0	0	
87	76.6	2	2	2	
Mean (se)	69.6 (5.72) ^A	0.5 (0.5)	0.5 (0.5)	0.5 (0.5)	
<i>E. camaldulensis</i> x <i>E. camaldulensis</i>					
42 x 87	65.0	0	0	0	6.2
83 x 85	72.0	0	0	0	30.9
84 x 85	21.0	0	0	0	5.3
84 x 87	59.0	1.7	1.7	1.7	33.8
85 x 52	69.0	1.4	1.4	1.4	15.4
87 x 42	78.0	0	0	0	27.5
Mean (se)	60.7 (7.63) ^A	0.4 (0.29)	0.4 (0.29)	0.4 (0.29)	19.9 (5.14) ^A
<i>E. camaldulensis</i> x <i>globulus</i> —Interspecific hybrids					
83 x K	67.0	0.8	5.8	50.4	2.8
85 x K	78.5	38.2	50.6	62.9	2.7
87 x K	91.0	43.1	48.8	57.3	4.7
85 x O	50.0	46.0	67.6	75.7	0.4
87 x O	87.0	46.0	56.8	73.0	1.7
84 x F	50.0	1.0	10.9	63.6	1.8
85 x F	80.0	43.8	49.4	71.9	0.4
87 x F	76.0	50.0	55.0	73.7	0.7
85 x T	78.5	45.9	59.4	97.0	0.1
87 x T	74.5	47.7	60.8	97.1	0.1
Mean (se)	73.3 (4.39) ^A	36.3 (5.97)	46.5 (6.62)	72.3 (4.84)	1.5 (0.47) ^B
<i>E. globulus</i> —Open pollinated					
K	55.0	0	0	0	
O	55.0	0	0	0	
F	30.0	0	0	0	
T	67.0	0	0	0	
Mean (se)	51.8 (7.78) ^A	0	0	0	0

Note: One hundred *E. camaldulensis* x *camaldulensis* and *E. globulus* seeds and two hundred *E. camaldulensis* x *globulus* seeds were sown to obtain the germination data.

¹ % abnormal seedlings from the number of germinated seeds.

² Cumulative % abnormal seedlings calculated from a sample of 10 (parental species) or 18–20 (interspecific) 'normal' seedlings from plants that were potted out when they were six months old.

³ Number of normal seedlings per flower calculated from germination and abnormality data (this Table) and seeds per capsule and capsule set (Table 1.2).

^{A,B} Different upper case letters after means and standard errors refer to significant differences ($p < 0.01$) between means in each column.



Figure 1.1 Normal and abnormal growth of *E. camaldulensis* x *globulus* hybrids

A. 9-month-old 'normal' *E. camaldulensis* x *globulus* seedling (87 x K), B. 10-month-old *E. camaldulensis* x *globulus* seedling (87 x O) displaying abnormal growth. Note leaves curving downwards, C. 10-month-old *E. camaldulensis* x *globulus* seedling (87 x T). Note wrinkled leaves with prominent veins, downward curve of leaves and new, initially normal growth from low axillary buds. Pots have 200 mm diameters.

1.1.4 Discussion

In *E. camaldulensis* there are significant barriers to selfing and interspecific hybridisation with *E. globulus*. The production of the hybrids was impeded by a 28 % reduction in percentage capsule set, a 78 % reduction in the number of seeds produced per capsule and a 99 % increase in the number of seedlings displaying detrimental abnormalities (by 22 months) compared to outcrossed

E. camaldulensis (Tables 1.2, 1.3). These substantial barriers to the hybridisation of *E. camaldulensis* and *E. globulus* reflect their taxonomic distance.

Although all hybrid families showed reduced seed production the extent of this varied significantly between families. *E. globulus* pollen from Taranna was the least compatible (as assessed by seed production) with all the *E. camaldulensis* clones (Table 1.2). Compatibility of the other *E. globulus* families varied with the different *E. camaldulensis* clones, each had relatively high seed set with at least one clonal mother. It is interesting that such variation, caused by comparatively small genetic differences between families is discernible over the substantial barriers that exist when making an intersectional cross.

Reduced seed production in the *E. camaldulensis* x *globulus* hybrids was not due to abnormal pollen growth in the style. In other interspecific crosses of *Eucalyptus* evidence of incompatibility has been observed in the form of pollen tube abnormalities in the style and a reduction in the number of pollen tubes penetrating ovules compared to intraspecific outcrosses (Ellis *et al.* 1991). It is possible the barriers responsible for the reduced seed production between *E. camaldulensis* and *E. globulus* are at the stage of pollen tube penetration of the ovule, or during embryo/endosperm development.

All crosses using *E. globulus* subsp. *globulus* (intra and interspecific) as the female parent failed. A high proportion of open pollinated buds also aborted suggesting the failure of the crosses was not due to controlled pollination techniques. In all *E. globulus* x *camaldulensis* crosses pollen tubes appeared normal but failed to grow the full length of the style. Although it was only possible to test one *E. globulus* genotype it appears that there is unilateral incompatibility between *E. globulus* subsp. *globulus* and *E. camaldulensis*. Unilateral incompatibility has been reported between a number of species with differing style lengths; pollen of the small flowered species being unable to grow the entire length of the style of the large flowered species (Pryor 1956; Williams and Rouse 1988; Potts and Savva 1989; Gore *et al.* 1990). This may be due to the pollen tubes exhausting reserves in the pollen grain or as a result of genetic pre programming (Williams and Rouse 1988). It has recently been shown that a cut style technique allows ‘one stop pollination’ of large style eucalypt flowers (Harbard *et al.* 1999; Williams *et al.* 1999). It is possible that this technique might allow use of pollen of small flowered eucalypts such as *E. camaldulensis* on the cut styles of the large flowered *E. globulus*.

Barriers to crossing were also observed in one of the *E. camaldulensis* outcrosses. When clones 85 and 87 were crossed there was a 95% reduction in the number of seeds produced per capsule compared to the other intraspecific crosses. Capsule set was also low with overall seed production lower than that obtained in the selfs. Reciprocal crosses between these two clones were made over two years with the same result. The success of other crosses using 85 or 87 as the male parent and *in vivo* pollen germination tests ruled out pollen viability as an explanation for the low seed yield. *In vivo* pollen tests also determined the partial incompatibility between the two clones was not manifested in the stigma or style. Analyses of the DNA from clones 85 and 87 showed them to be different ruling out mistakes in “labelling” the clones (R. Teasdale pers com.). Low seed production has been reported when crossing closely related trees of *E. gunnii* (Potts *et al.* 1987). However clones 85 and 87 originated from different provenances Broken Hill, NSW and Erudina, SA. Partial cross incompatibility in an interprovenance cross has also been reported in *E. nitens* in the form of abnormally slow seedling growth (Tibbits 1988). Further research on controlled crosses between a broad range of provenances and individuals is required to determine the frequency of partial cross incompatibility in *E. camaldulensis*. Although no other *E. camaldulensis* clone combination displayed such strong barriers to crossing there was some variation in the combining ability of the clones (Tables 1.2, 1.3) and this may be significant enough to have implications for the management of seed orchards.

Partial self incompatibility in *E. camaldulensis* was observed as a 29 % reduction in capsule set and an 86 % reduction in the number of seeds produced per capsule compared to outcrossed *E. camaldulensis*. Germination and subsequent seedling growth were not investigated in this study but reduced survival and vigour is common after selfing in *Eucalyptus* species (Hodgson 1976; Venkatesh and Vakshasya 1977; Van Wyk 1980; Eldridge and Griffin 1983; Potts *et al.* 1987).

The germination of *E. camaldulensis* x *globulus* seeds was not significantly different from the parental species. Tibbits (1988) has also reported high germination in hybrids between *Eucalyptus* species of the *Viminalis* series while Griffin *et al.* (2000) found a reduction in germination for hybrids of *E. globulus* and *E. nitens*, *E. urophylla*, *E. dunnii* or *E. grandis*.

The production of *E. camaldulensis* x *globulus* hybrids was impeded by the high percentages of seedlings that developed abnormalities. All hybrid families had some individuals with abnormalities by three months of age with 36% of hybrid individuals affected. By the time the seedlings were twenty two months old 72% of individuals were affected.

High levels of abnormalities have also been reported for other taxonomically wide crosses between *Eucalyptus* species suggesting a degree of genetic incompatibility (Pryor 1957; Pilipenko 1969; Potts *et al.* 1992; Griffin *et al.* 2000). The abnormalities are often described as 'dwarfs' and display high rates of mortality. In a cross between *E. globulus* subsp. *maidenii* and *E. camaldulensis* Pilipenko (1969) reported extremely high rates of abnormalities with only one seedling developing normally. The only abnormal seedling that survived developed into a normal tree after remaining in a 'dwarf' state for several years.

In contrast, barriers to hybridisation are much lower in taxonomically closer crosses. Potts *et al.* (1992) found seventy seven percent of families in the intraseries cross *E. nitens* x *globulus* developed abnormalities after one year's plantation growth, with twenty two percent of hybrid individuals affected. In a study involving a range of intraseries crosses Tibbits (1988) found between 0 and 20% of hybrid individuals with abnormalities.

There was a wide range in the percentages of seedlings that developed abnormalities in the various *E. camaldulensis* x *globulus* families (50–97 %) (Table 1.3). As was observed for seed production the two families with *E. globulus* T as the male parent were the least compatible while families with K as the male parent performed relatively well. Griffin *et al.* (2000) also reported significant variation between parental genotypes in ability to produce viable offspring in a number of eucalypt crosses.

The number of normal seedlings produced per pollinated flower gives an indication of the viability of the various crosses. The *E. camaldulensis* intraspecific families produced from 5 to 34 'normal' seedlings per pollinated flower. Overall the *E. camaldulensis* x *globulus* hybrids produced 92% fewer normal seedlings per pollinated flower than the *E. camaldulensis* intraspecific crosses. Families with *E. globulus* T as the male parent produced 0.1 'normal' seedlings per flower (Table 1.3). In contrast the families with K as the male parent produced between 2.7 and 4.7 'normal' seedlings per flower. These results again suggest differential compatibility occurs between the various genotype combinations of *E. camaldulensis* and *E. globulus*. Differential compatibility has also been reported in a cross between *E. nitens* and *E. globulus* (Volker 1995). It is particularly interesting to note that the parent combinations that displayed poor compatibility also involved *E. globulus* males derived from Taranna.

Although barriers to the crossing of *E. camaldulensis* and *E. globulus* exist, hybrid seed can be produced when *E. camaldulensis* is the female parent and seedlings can be successfully raised. The variation in compatibility between the various genotypes indicate that further research on the combining ability of different *E. camaldulensis* and *E. globulus* families may increase potential seed production and seedling vigour.

1.1.5 Summary

Controlled pollination techniques were used to produce hybrids between *E. camaldulensis* and *E. globulus* and comparisons made with outcrosses and selfs of *E. camaldulensis*. When *E. globulus* was used as the female parent *E. camaldulensis* pollen tubes failed to grow the entire length of *E. globulus* styles. When *E. camaldulensis* was used as the female parent, interspecific crosses were successful, although there were substantial barriers to hybridisation. In the *E. camaldulensis* x *globulus* hybrids there was a 28 % reduction in percentage capsule set, a 78 % reduction in the number of seeds

produced per capsule and a 99 % increase in the number of lethal abnormalities amongst seedlings (by 22 months of age) compared to outcrossed *E. camaldulensis*. Overall this equated to a 92 % reduction in the number of normal seedlings produced per pollinated flower in the *E. camaldulensis* x *globulus* compared to the *E. camaldulensis* outcrosses. The intensity of these barriers varied with the different hybrid families. Families with *E. globulus* originating from Taranna as the male parent produced less than 0.1 normal seedlings per pollinated flower. In contrast families with *E. globulus* originating from King Island as the male parent produced means of 2.7–4.7 normal seedlings per flower. *E. camaldulensis* is partially self incompatible. This was manifested as a 29 % reduction in the percentage capsule set and an 86 % reduction in the number of seeds produced per capsule compared to outcrosses. One reciprocal combination of *E. camaldulensis* clones originating from Erudina and Broken Hill gave very low seed set.

1.2 Selection of salt–waterlogging tolerant genotypes

1.2.1 Introduction

The *E. camaldulensis* x *globulus* hybrids produced in the above breeding programme, and seedlings of the parent species and the *E. camaldulensis* x *camaldulensis* crosses were raised to seven months old, then normal plants were selected to be screened for salt waterlogging tolerance. Whilst there is a number of tree species suitable for upslope plantings of saline areas, few of commercial use are suitable for planting in discharge areas where both salinity and waterlogging is a problem. Winter waterlogging can reduce the ability of plants to tolerate salinity (Marcar 1993). *Eucalyptus camaldulensis* Dehnh. is moderately tolerant of salinity and highly tolerant of waterlogging (Bell 1999, Niknam and McComb 2000) and the aim was to select those hybrid genotypes that showed this maternal characteristic. This work was completed before the commencement of the JVAP funding and has been published as Meddings *et al.* (2001). A brief account is included here to describe the selection screen to which the plants were exposed.

1.2.2 Materials and methods

Micropropagated clones of *E. camaldulensis*, seedlings of *E. camaldulensis* x *E. camaldulensis*, *E. globulus* and hybrid families were grown to 6 months old then repotted. 4 plants per pot, in 255 mm plastic pots of fine white sand. After a further 4 weeks growth they were subjected to waterlogging with 1/5 Hoagland nutrient solution. After a week NaCl was added to the tanks to achieve a concentration of 50mmol/L. Salinity was increased by this amount each week until 350 mmol/L was reached and held for 3 weeks. Survival and health of the plants were recorded each week. Each seedling line, clone or hybrid family had 8 replicate pots of 4 plants.

1.2.3 Results

Survival of the *E. camaldulensis* clonal lines was comparable to outcrossed *E. camaldulensis* x *camaldulensis* seedlings for the first 8 weeks of the experiment, at which time NaCl concentrations had reached 350 mmol/L (Fig. 1.2). After this time the *E. camaldulensis* seedlings died more quickly than the clones. Three *E. camaldulensis* clonal lines (42, 83, 85) and one outcrossed *E. camaldulensis* (83 x 85) family showed over 80% survival at the end of the trial (Table 1.4). Final survival of *E. camaldulensis* x *E. camaldulensis* families was usually intermediate or comparable to the lowest clonal parent (Table 1.4). The mean final survival of all the *E. camaldulensis* seedlings was 54% compared to 72% in the *E. camaldulensis* clonal lines. The onset of seedling death in *E. globulus* and the *E. camaldulensis* x *globulus* hybrids was earlier than for *E. camaldulensis* (Fig. 1.2). Survival in *E. globulus* was very poor with only 1 seedling alive at the end of the trial while the final mean survival of hybrid families ranged from 16–40%, intermediate between the *E. camaldulensis* and *E. globulus* parents (Fig. 1.2).

In *E. globulus* salt waterlogging damage included chlorosis of older leaves then death of the growing tip. *E. camaldulensis* plants died more slowly than the *E. globulus* showing leaf chlorosis but then

abscission of older leaves. The growing tip was the last part to die. Amongst the hybrids the more sensitive individuals showed damage similar to *E. globulus* while the more tolerant ones were similar to *E. camaldulensis*.

1.2.4 Discussion

Marcar (1993) found unselected *E. camaldulensis* seedlings were more tolerant of saline waterlogged conditions than *E. globulus* seedlings and in this trial clonal *E. camaldulensis* which had been previously selected for salt waterlogging tolerance showed better survival than seedlings of *E. globulus*, *E. camaldulensis* x *camaldulensis* or *E. camaldulensis* x *globulus* parents. Survival of the interspecific hybrid families was intermediate between *E. camaldulensis* and *E. globulus*. Within hybrid families there was a large range of salt-waterlogging tolerance enabling selection of genotypes that would possibly approach the tolerance levels of the parental *E. camaldulensis* clones. The *E. camaldulensis* x *globulus* families, 85 x K and 87 x K displayed relatively higher survival under saline waterlogged conditions than other hybrid families. These two hybrid families show particular promise as they also produced the highest number of 'normal' seedlings per pollinated flower compared to other hybrid families (Table 1.3).

The glasshouse trial indicated that selected genotypes from the *E. camaldulensis* x *globulus* hybrids will survive and grow under higher salinity than is possible for pure lines of *E. globulus*. The best of the *E. camaldulensis* x *globulus* seedlings that survived the salt-waterlogging trial were selected for micropropagation (Chapter 2).

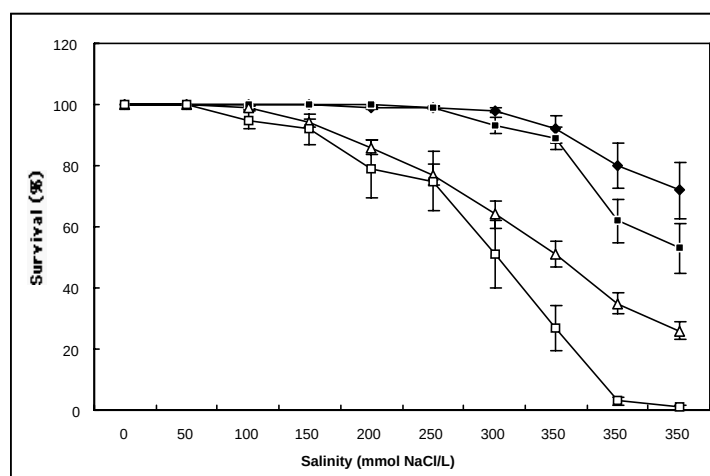


Figure 1.2 Mean survival percentages of clones of *E. camaldulensis*, seedlings of *E. camaldulensis* x *camaldulensis*, *E. camaldulensis* x *globulus* and *E. globulus*

Note: *E. camaldulensis* (♦), seedlings of *E. camaldulensis* x *camaldulensis* (■), *E. camaldulensis* x *globulus* (Δ) and *E. globulus* (○). Bars represent standard errors of the means. (Reproduced with permission from the Australian Journal of Experimental Agriculture 41, 788).

Table 1.4 Percentage survival of *E. camaldulensis* clones, seedlings of *E. camaldulensis* x *camaldulensis*, *E. camaldulensis* x *globulus* and *E. globulus* families

	No. of weeks of flooding									
	1	2	3	4	5	6	7	8	9	10
	Salinity mmol NaCl/L									
	0	50	100	150	200	250	300	350	350	350
<i>E. camaldulensis</i> clones										
42	100	100	100	100	97	97	97	94	94	87
83	100	100	100	100	100	100	97	94	91	91
84	100	100	100	100	100	97	97	75	56	52
85	100	100	100	100	100	100	100	100	91	81
87	100	100	100	100	100	100	100	97	69	47
Mean	100	100	100	100	99 (0.6)	99 (0.7)	98 (0.7)	92 (4.4)	80 (7.5)	72 (9.2)
<i>E. camaldulensis</i> x <i>camaldulensis</i> seedlings										
42 x 87	100	100	100	100	100	100	94	91	53	41
83 x 85	100	100	100	100	100	100	100	97	88	81
84 x 87	100	100	100	100	100	100	95	95	55	45
85 x 52	100	100	100	100	100	97	94	84	50	37
87 x 42	100	100	100	100	100	100	84	78	66	62
Mean	100	100	100	100	100	99 (0.6)	93 (2.6)	89 (3.5)	62 (7.0)	53 (8.2)
<i>E. camaldulensis</i> x <i>globulus</i>										
83 x K	100	100	100	97	90	69	63	47	40	31
84 x F	100	100	94	91	78	72	38	28	19	12
85 x K	100	100	100	94	81	75	72	68	53	34
85 x O	100	100	95	90	87	87	65	60	50	40
85 x F	100	100	100	94	87	75	57	47	31	25
85 x T	100	100	100	97	97	94	66	47	28	22
87 x K	100	100	100	97	91	81	81	75	47	34
87 x O	100	100	100	94	75	62	56	47	19	16
87 x F	100	100	100	87	84	66	56	44	25	22
87 x T	100	100	100	100	94	91	88	50	41	28
Mean	100	100	99 (0.7)	94 (1.2)	86 (2.2)	77 (3.4)	64 (4.5)	51 (4.2)	35 (3.4)	26 (2.8)
<i>E. globulus</i>										
K	100	100	100	97	94	94	78	47	3	0
O	100	100	87	78	54	50	34	25	6	3
F	100	100	94	91	75	69	31	12	0	0
T	100	100	100	100	94	87	59	22	3	0
Mean	100	100	95 (3.1)	92 (4.9)	79 (9.5)	75 (9.9)	51 (11.1)	27 (7.4)	3 (1.2)	1 (0.8)

Note: There were 32 replicates for each line, except 84 x 87 and 85 x O for which there were 20. (S.E. of means are shown in parenthesis. Reproduced with permission from the Australian Journal of Experimental Agriculture **41**, 789).

1.2.5 Summary

The salt-waterlogging tolerance of *E. camaldulensis* x *globulus* hybrids, clones of *E. camaldulensis*, open pollinated seedlings of *E. globulus* and *E. camaldulensis* x *E. camaldulensis* seedlings was assessed. Potted plants of seven-month-old plants in a glasshouse were waterlogged with increasing concentrations of salt to 350 mmol/L NaCl over a period of 10 weeks.

E. globulus seedlings were less tolerant of the salt waterlogging conditions than *E. camaldulensis* clones. The hybrid *E. camaldulensis* x *globulus* families were intermediate between the two parental lines as was the performance of progeny from crosses between salt tolerant *E. camaldulensis* lines. Some hybrid individuals performed as well as *E. camaldulensis*. The most tolerant plants were selected for micropropagation for field trials on saline agricultural land (Chapters 2–5).

2 Clonal propagation of *Eucalyptus camaldulensis* x *globulus* for field trials

2.1 Introduction

Hybrids are not true breeding so that if hybrid plants with desirable traits are to be propagated the procedure must be clonal, not seed based. Possibilities include use of micropropagation or cuttings. This chapter reports on three aspects of clonal propagation; media additives to improve growth in vitro, long-term storage protocols and success of propagation through cuttings.

2.2 Codes used to identify lines

The female parent of a cross is always given first. The code numbers given to the selected lines are as follows. The first number is the number assigned to a particular cross. The second number is the number of the selected genotype from that cross. An example of a code is (87 x K 7.1). This identifies a hybrid from the cross *E. camaldulensis* 87 x *E. globulus* King Island (all hybrids from that cross are given the number 7). The number 7.1 indicates ramets from seedling number one; this was one of the plants that survived the salt waterlogging screening. A full list of clone numbers is seen in Table 2.1.

2.3 In vitro growth

Micropropagation is most successful if juvenile plants, or at least juvenile shoots, are used as a source of explants. The *E. camaldulensis* x *globulus* hybrids were approximately 9 months old at the end of the salt waterlogging trial. Genotypes selected as highly tolerant had the salt washed from their roots, and were repotted in fresh soil. Attempts were made to clone a total of 51 hybrid genotypes and some of the progeny from the *E. camaldulensis* x *camaldulensis* crosses. Some plants died before resprouting and plants recovering from stress were particularly susceptible to fungal diseases. There were considerable problems in obtaining cultures free of contaminants. There were 41 successful culture lines established at Murdoch using the techniques described by McComb *et al.* (1996) (Table 2.1).

E. camaldulensis grows well in tissue culture where as *E. globulus* is difficult and slow unless cultures are initiated from newly germinated seedlings (Bennett *et al.* 1994). Cultures of the *E. camaldulensis* x *globulus* hybrids from the 9-month old salt tolerant plants showed variable in vitro performance, but were generally closer to *E. camaldulensis* than *E. globulus* (Table 2.2). Two media additives were tested in attempt to improve the shoot growth and rooting of hybrid lines. Culture media were supplemented with a surfactant: Pluronic F-68 or the demethylating agent: 5-azacytidine. The non-ionic surfactant Pluronic F-68 has been found to promote growth of cultured plants. Surfactants may stimulate growth by increasing permeability of cell membranes, therefore increasing uptake of nutrients and growth regulators (Khatun *et al.*, 1993; Khehra *et al.*, 1995). The methylation status of plants in tissue culture has been investigated and results demonstrate frequent methylation change after tissue culture (Brown, 1989). The inactivation of genes through methylation can be reversed through the application of 5-azacytidine (Lambe *et al.*, 1995).

Table 2.1 Clonal lines established in vitro

Cross	Line	Established in culture at Murdoch	Multiplied by NET	Maintained in culture	Discarded due to poor field performance
87 x F	1.2	*	*	*	
	1.3	*	*	*	
	1.8	*	*	*	
84 x F	3.1	*			
	3.2	*			
83 x K	5.1	*			
	5.2	*	*	*	
85 x K	6.1	*	*	*	
	6.2	*	*	*	
	6.3	*	*	*	
	6.4	*	*	*	
	6.5	*	*	*	
	6.6	*	*	*	
	6.7	*			
	6.8	*	*	*	
	6.9	*			
	6.10	*	*	*	
	6.11	*	*	*	
	6.12	*	*	*	
	7.1	*	*	*	
	7.2	*	*	*	marginal
87 x K	7.3	*	*	*	marginal
	7.4	*	*	*	
	7.6	*	*	*	
	7.7	*	*		*
	7.9	*	*	*	
	7.11	*	*	*	
	7.12	*	*	*	
	8.1	*	*	*	
	8.2	*			
	9.1	*			
85 x O	9.2	*	*	*	
	9.4	*	*	*	
	9.6	*	*	*	
87 x T	10.3	*	*		*
	10.4	*	*		*
	10.5	*	*		*
	10.6	*	*	*	
	10.7	*	*	*	
85 x T	11.1	*			
	11.2	*	*		*
<i>E. camaldulensis</i>	83	*	*		**
	85	*	*		**
	42	*	*		**

Clonal lines established in vitro (*). Those presently maintained and those discarded because of consistently poor field performance are also shown. ** discarded as a non commercial species.

Table 2.2 Comparison of the mean shoot multiplication and rooting in vitro of *E. camaldulensis*, *E. globulus* and the hybrids

Species	In vitro	
	multiplication	% rooting
<i>E. globulus</i> *	1.7	81
<i>E. camaldulensis</i> **	2.5	85
<i>E. camaldulensis</i> x <i>globulus</i> ***	2.4	71

*data from Bennett *et al.* (1994)

** lines 42, 83 and 85

*** clones 5.2, 6.1, 6.2, 7.1, 7.3, 7.4, 10.3, 11.1, 11.2

5-azacytidine

Two clonal lines (7.3, 11.2) were grown on media containing (0, 1, 5, 10 μ M) 5-azacytidine for four weeks. Two tubs of each concentration were set up, each tub containing five shoot clusters. The height and weight of each shoot cluster were measured at time 0 and after 4 weeks of growth on the treatment media.

After 4 weeks of growth on the medium containing 5-azacytidine the shoots were transferred to root initiation media. From each tub the ten best single shoots were chosen and placed on standard hybrid root initiation medium and the number of shoots which grew roots was scored after 4 weeks.

Treatment with 5-azacytidine led to a small increase in height in both the clonal lines (Fig. 2.1) and in the weight of clone 11.2. The number of shoots producing roots was increased in clonal line 7.3 after treatment with 5-azacytidine (Fig. 2.2). The improvement in growth of clonal lines was not significant when a greater range of clonal lines was tested. It is now apparent that clone 11.2 was an unfortunate choice to test as it had abnormal plagiotrophic growth in the field (Chapter 4).

Pluronic F-68

Two clonal lines (9.4, 10.7) were treated with Pluronic F-68. The plants were grown on media containing (0, 0.25, 0.5, 1%) Pluronic F-68 for four weeks. Two tubs of each concentration were set up, each containing five shoot clusters. The height and weight of each shoot cluster were measured at weeks 0 and 4. After 4 weeks' growth on media containing Pluronic F-68 the shoots were transferred to root initiation media. From each tub the ten best single shoots were chosen and placed on standard hybrid root initiation media and the number of shoots which grew roots was scored after four weeks.

The Pluronic F-68 either had no effect or suppressed growth (in both height and weight), with increase in concentration (data not shown). In both clonal lines, Pluronic F-68 reduced root production with an increase in concentration.

2.4 Long-term storage of cultures in vitro

One disadvantage of micropropagation is that cultures are routinely subcultured at intervals of 3–4 weeks. This results in considerable costs during non-production periods. In addition as a number of years of growth in the field must pass before tree quality can be assessed, cultures must be maintained over this period before final selections can be made. Consequently experiments were undertaken to develop long-term storage protocols for the cultures to reduce cost of monthly subculturing. The effect was examined of the inclusion of 2mM choline chloride into the medium before storage at 5° or 25°C. This compound has been shown to reduce the level of stress in plants in vitro as assessed by the proline content of shoots (K. Dixon, Kings Park and Botanic Gardens, pers. com). Shoots were placed 5 per container in glass jars each with 30ml of medium. Harvests of shoots at 2, 4 and 6 months without subculture showed that the addition of choline chloride did not improve survival or affect the level of proline in the shoots. Surprisingly cultures could be stored successfully at either 5° or 25°C

without subculture but recovery after storage was better for material stored at 25°C. Thus cultures that are not required for current production purposes can be subcultured at longer intervals. The culture room where such material is stored must be immaculate and routinely sprayed for mites as infestation of stored cultures is a constant danger.

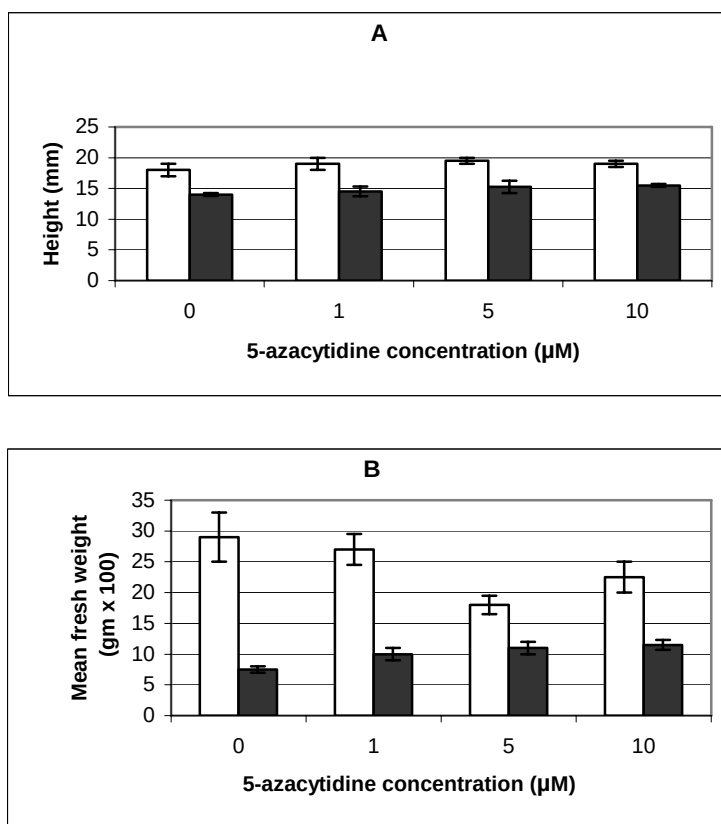


Figure 2.1 The effect of 5-azacytidine on shoot growth in vitro

A. height, B. fresh weight. Note: Clear bars clone 7.3, black bars clone 11.2.

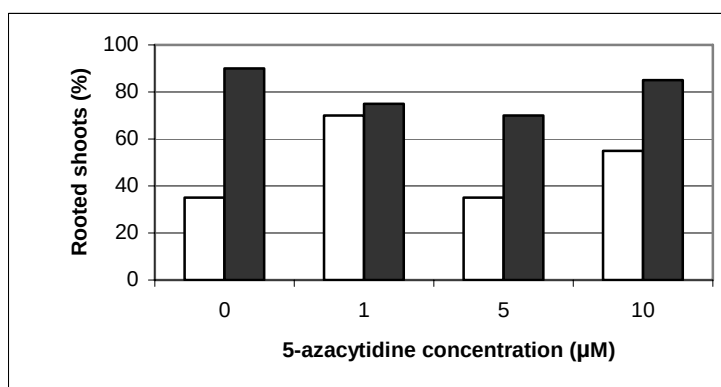


Figure 2.2 The effect of 5-azacytidine on rooting of shoots in vitro

Note: Clear bars clone 7.3, black bars clone 11.2.

2.5 Propagation of hybrids and *E. camaldulensis* from cuttings

Hybrids and the *E. camaldulensis* parents were tested for ability to root as cuttings. Similar to their performance in vitro, the *E. camaldulensis* cuttings root easily whilst for *E. globulus* a very large number of genotypes must be screened to identify ones that will give a commercially acceptable level of rooting of cuttings. To obtain maximum success from cuttings, hybrid and parent plants raised from tissue culture were grown in pots at North Eucalypt Technologies then hedged to yield vigorous juvenile shoots. Cuttings were hormone treated and rooted under high humidity following a proprietary protocol. All genotypes of *E. camaldulensis* gave a high percentage of rooting. Rooting was more variable amongst hybrid genotypes with some lines being as poor as *E. globulus* and others showing 100% rooting. The mean rooting of the hybrid lines was closer to the *E. camaldulensis* parent than the *E. globulus* (Table 2.3).

Table 2.3 Comparison of the rooting ability of cuttings from hedged mother plants of *E. camaldulensis* and the hybrids

Species	% Rooting of cuttings	
	Mean	Range
<i>E. camaldulensis</i> **	97	94–99
<i>E. camaldulensis</i> x <i>globulus</i> ***	67	4–100

** lines 42, 83 and 85

*** clones 5.2, 6.1, 6.2, 7.1, 7.3, 7.4, 10.3, 11.1, 11.2

2.6 Maintenance of the clones

The clones were initiated at Murdoch and some plants for field trials were produced. Clones were then transferred to North Eucalypt Technologies, Burnie Tasmania, and most of the clonal material for the trials was produced from their tissue culture laboratory. Later, when North Eucalypt Technologies was taken over by Gunns Pty Ltd the maintenance of the clones was subcontracted to Plant Biotechnologies Tasmania (also in Burnie).

2.7 Summary

Hybrids selected as superior from the salt waterlogging trial were micropropagated. Addition of the surfactant Pluronic F-68, or the demethylating agent 5-azacytidine did not enhance growth in vitro. Cultures could be stored at 25°C without subculture for up to 6 months. For production of large numbers of plants for field trials, in vitro culture was used to produce mother plants from which cuttings were taken and rooted. The performance of the hybrids both in vitro and as cuttings was closer to the *E. camaldulensis* parent than the *E. globulus*. Most of the clonal plants for the field trials reported in later chapters were raised by North Eucalypt Technologies (Burnie Tasmania).

3 Morphometrics of *Eucalyptus camaldulensis* x *globulus* hybrids

3.1 Introduction

In Chapter 1 the crosses between *E. camaldulensis* x *globulus* were described and the variation in frequency of abnormal plants shown to depend largely on the provenance of the *E. globulus* male parent. Here the vegetative and floral morphology of the normal seedlings and mature trees is described.

Natural interspecific hybridisation is well documented within most subgenera of *Eucalyptus* (Ashton and Sandiford 1988; Clifford and Binet 1954; Drake 1980; Hopper *et al.* 1978; Maiden 1904; Potts and Reid 1983, 1985; Pryor 1951b; Pryor 1976; Pryor and Johnson 1971). Although frequency of hybridisation generally increases with the closeness of the taxonomic affinity of the parental species, natural hybridisation is a relatively rare occurrence (Griffin *et al.* 1988). Interspecific hybrids are usually fertile in *Eucalyptus* (Sedgley and Griffin 1989; Venkatesh and Sharma 1976) and in some circumstances, usually in disturbed habitats, barriers to interspecific hybridisation break down and hybrids, backcrosses and later generations form a hybrid swarm (Ashton and Sandiford 1988; Clifford and Binet 1954; Drake 1980; Potts and Reid 1985; Pryor and Johnson 1971). The F2 generations display large phenotypic variations so hybrids used commercially are usually vegetatively propagated.

The production of intersubgeneric hybrids has been reported in *Eucalyptus* but the evidence is weak. In all but one case the resultant seedlings died or showed very poor vigour, or the performance of the seedlings was not reported (Murphy 1976; Pilipenko 1969). In the exceptional case a cross between *Symphyomyrtus* (*E. globulus* subsp. *maidenii*) and *Monocalyptus* (*E. fastigata*) yielded 'hybrid' seedlings but their morphology was very similar to *E. globulus* subsp. *maidenii*, leaving the possibility open that the seedlings were selfs (Pilipenko 1969).

Generally, the wider crosses between sections within subgenera have a high proportion of seedlings with low vigour and severe abnormalities. However there are exceptional highly vigorous intersectional hybrids including the spontaneous *E. grandis* x *urophylla* hybrid used commercially in Brazil (Brandao *et al.* 1984; Campinhos and Ikemori 1989; Potts and Cauvin 1988).

3.2 Materials and methods

3.2.1 Plant material

E. camaldulensis x *camaldulensis*, *E. globulus* and *E. camaldulensis* x *globulus* seedlings six months old were potted into 200 mm pots. Plants were placed in a random design on weed mat in a paddock at Murdoch University. They were fertilised once a fortnight with Wuxal® a general liquid fertiliser.

After 22 months in pots the plants, together with additional hybrid and parental seedlings 7 months old and 6–7 month old clonal *E. camaldulensis* were planted at Pinjarra (lat.32° 53', long.115° 50' S) South of Perth Western Australia in 1995 (Chapter 4–6). The soil was sand over clay and old agricultural land on which an *E. globulus* plantation had been largely unsuccessful, suffering high mortality of 4 year old trees over the summer of 1993/4. Each plant was fertilised with two 50g DAP fertilizer tablets (Bailey's) at the time of planting. Plants were 3m apart in rows 4m apart.

3.2.2 Assessment of traits

When seedlings were at the 2 leaf stage, the length, width and depth of emargination in cotyledons was measured in 10 seedlings from each family. As the size of cotyledons is at least partly dependent on

seed size, the morphology of the cotyledons was assessed by the length/width ratio and the depth of the notch expressed as a proportion of cotyledon width.

When plants were 7–8 months old several morphological characters were measured at the fifth node: petiole length, lamina length, lamina width at the widest point, the vertical distance between the axillary buds, and leaf angle (angle between the main stem and the midvein of the lamina). Abnormal plants were excluded from selection.

The morphometric data on juvenile plants and the operculum diameter and petiole length of adult plants were log transformed and proportional data arc sine transformed before one-way analyses of variance and Tukey's B test were performed to determine statistical differences between hybrid and parental families using Statistical Package for Social Sciences x (Anon., 1988). For morphological characters in which one of the parental species had no variance (*E. globulus*—petiole length, distance between axillary buds and flower bud number, *E. camaldulensis*—cotyledon notch depth) one-way analyses of variance were performed between the hybrid and remaining parent only.

Material for assessment of vegetative and floral morphology of field-grown trees was collected mainly in the flowering season of 1998/9 with some additional material from the 1999/2000 season. Only healthy trees of *E. globulus* were included. (All but 3 of the 80 of the *E. globulus* planted at Pinjarra had died by July 2000, after reaching heights of up to 9m.) The *E. globulus* at Pinjarra did not flower and flowers and fruits from the trees of the same provenance growing in Tasmania were used in the analysis.

Flowering time—Trees were assessed for flowers at anthesis every 2 weeks from the beginning of September to the end of December 1998.

Vegetative morphology—From each tree 10–15 fully expanded mature leaves were collected. The length, breadth, distance from the widest part of the leaf to the petiole, and the petiole length were measured.

Floral morphology—From each tree the number of buds in a panicle was recorded from 10 flowers. On each of 10 mature flower buds the operculum height and diameter was measured, and on 10 flowers at anthesis, the style and anther length were recorded. Anther length was estimated from 10 randomly selected anthers from each flower. On 10 fruits from each tree the height and diameter of the capsule and the number of valves were recorded. Pollen viability was measured by scoring the number of full and shrunken grains amongst a sample of 200 grains from pollen mounted in 2% aceto-orcein.

3.3 Results

3.3.1 Morphology—germination to 5 months old

E. camaldulensis and *E. globulus* seedlings are easily distinguished by the shape of the cotyledons. *E. camaldulensis* cotyledons are entire and orbicular, while *E. globulus* cotyledons are several times larger and reniform with a deep notch (Figure 3.1). The shape of the hybrid cotyledons was intermediate between the parents but closer to *E. globulus* than *E. camaldulensis*. All hybrids had emarginate cotyledons. However the depth of emargination was not as great as in *E. globulus* (Table 3.1). The ratio of cotyledon length over width was not significantly different in *E. globulus* and *E. camaldulensis* x *globulus* hybrid seedlings (Table 3.1).



Figure 3.1 Cotyledons from *E. globulus*, *E. camaldulensis* x *globulus* and *E. camaldulensis*

Note: Cotyledons from *E. globulus* (F) LHS, *E. camaldulensis* x *globulus* (84 x F) centre and *E. camaldulensis* (84 x 85) RHS. Bar is 1cm.

There was very little difference between the leaf lamina length, width, or length/width ratio of the hybrid and parental families (Table 3.1).

E. camaldulensis and *E. globulus* juvenile leaves are sessile and opposite. The switch to alternate, petiolate intermediate leaves happens gradually in both species but starts lower on *E. camaldulensis* than in *E. globulus*. In the hybrid, petiole length and distance between axillary buds at node 5, were intermediate between the two parental species but closer to *E. globulus* than *E. camaldulensis* (Table 3.1). The logarithmic mean of the distance between the axillary buds at a node in the hybrids was equivalent to the combined logarithmic mean of the parental families. Leaf orientation (angle) was the only morphological character measured in which *E. camaldulensis* characteristics predominated in the hybrid seedlings (Table 3.1).

3.3.2 Morphology—five-year-old trees

The leaf morphology of the parents and the mature hybrids was variable and although the hybrids had statistically longer leaves it was impossible to identify trees on this basis in the field (Table 3.2). Hybrids and the *E. camaldulensis* overlapped in flowering time, with the hybrids being earlier in the year (September to December) than the *E. camaldulensis* (November to January). (*E. globulus* did not flower in the first 5 years).

The flowers of *E. camaldulensis* x *globulus* were in pedicellate clusters of 3–6 flowers, more similar to the *E. camaldulensis* parent than the single sessile flowers seen in the *E. globulus* parent (Table 3.2). The flowers were intermediate in all other characteristics measured: operculum height and diameter, style and anther length (Table 3.2, Fig. 3.2). The fruit of *E. camaldulensis* is smooth and rounded while that of *E. globulus* is ridged and warty. The hybrids were intermediate in size between the parents and had variable development of slight ridging and a warty surface. Fruit valves of *E. camaldulensis* are strongly exerted while those of *E. globulus* are more or less flat—the hybrid had exerted valves. Fruit diameter, fruit height and operculum diameter were the most important traits in canonical analysis (Table 3.3, Fig. 3.4).

Pollen fertility of the hybrids and *E. camaldulensis* parent was within the same range (70.5–78.5% for the hybrids and 70.1–82.2% for the *E. camaldulensis*).

Table 3.1 The means (se) of untransformed data for morphological characters of seedlings

Morphological character	<i>E. camaldulensis</i> x <i>camaldulensis</i>	<i>E. camaldulensis</i> x <i>globulus</i>	<i>E. globulus</i>
Cotyledon length / cotyledon width	0.44 (0.01) ^a	0.35 (0.01) ^b	0.37 (0.01) ^b
Depth of emargination / cotyledon width	0 (0)	0.30 (0.02) ^a	0.61 (0.03) ^b
Petiole length	4.08 (0.15) ^a	1.20 (0.07) ^b	0 (0)
Distance between buds at node 5	5.5 (0.33) ^a	1.5 (0.15) ^b	0 (0)
Lamina length	49.0 (1.17) ^a	51.2 (0.53) ^a	51.7 (1.42) ^a
Lamina width	18.3 (0.51) ^a	21.9 (0.27) ^a	20.9 (0.70) ^a
lamina length / lamina width	2.76 (0.08) ^a	2.35 (0.02) ^a	2.54 (0.08) ^a
Leaf angle (°)	48.96 (0.82) ^a	48.87 (0.69) ^a	21.84 (2.00) ^b

Cotyledons were measured when plants were at the two seedling leaf stage, and other traits at the fifth node on 7–8 months old seedlings.

60 *E. camaldulensis* x *camaldulensis*, 40 *E. globulus* and 200 *E. camaldulensis* x *globulus* seedlings were assessed. All linear measures in mm.

^{a,b,c} — different letters refer to statistical differences ($P < 0.05$) between the different types of seedlings.

Table 3.2 The means (se) of untransformed data for vegetative morphological characters of 4–5-year-old trees

Line	Leaf		Dist Widest ¹	Petiole length
	Length	Width		
<i>E. camaldulensis</i>				
42	175	28.1	32.6	23.8
83	178	32.0	38.2	23.0
84	168	32.6	38.9	26.2
85	198	27.8	36.2	23.3
87	183	26.8	35.8	21.3
Mean	180.4	29.5	36.3	23.5
(SE)	(4.93) ^c	(1.18) ^b	(1.10) ^c	(0.79) ^b
<i>E. camaldulensis</i> x <i>globulus</i>				
42xF	237	45.2	44.4	27.0
83xF	203	36.7	41.4	25.5
83xK	213	36.0	43.8	27.5
83xO	220	37.1	42.6	28.2
84xF	197	35.3	37.5	31.6
85xF	207	32.2	40.3	26.2
85xK	222	37.5	42.3	26.8
85xO	205	32.7	41.1	24.7
87xF	193	31.0	36.6	25.0
87xK	217	35.9	40.9	25.6
87xO	219	32.4	40.6	28.5
Mean	212.2	35.6	41.0	27.0
(SE)	(3.79) ^a	(1.17) ^a	(0.71) ^a	(0.60) ^a
<i>E. globulus</i>				
F	183	31.8	36.2	25.9
K	204	38.5	39.8	30.0
O	190	31.1	34.1	27.3
T	205	36.0	38.3	26.8
Mean	195.3	34.4	37.1	27.5
(SE)	(5.44) ^b	(1.77) ^a	(1.25) ^b	(0.89) ^a

Note: All linear measures in mm.

^{a,b,c} — different letters refer to statistical differences ($P < 0.05$) between the different types of trees.

¹ — distance from the petiole to the widest part of the leaf lamina

Table 3.3 The means (se) of untransformed data for floral morphological characters of 4–5-year-old trees

Line	No. buds ¹	Operculum		Style length	Anther length	Fruit		No. valves
		Diameter	Height			Height	Diameter	
<i>E. camaldulensis</i>								
42	5.3	4.8	5.0	3.2	5.3	5.4	6.4	4.0
83	5.6					6.0	6.3	4.0
84	5.2	4.5	4.2	2.7	4.0	7.2	9.1	4.0
85	6.0	4.5	4.9	3.6	5.5	6.0	6.2	4.1
87	5.4	4.2	5.0	3.6	5.1	4.9	6.0	4.1
Mean	5.5	4.6	4.8	3.3	5.0	5.9	6.8	4.0
(SE)	(0.02) ^a	(0.07) ^c	(0.19) ^b	(0.22) ^b	(0.34) ^c	(0.39) ^c	(0.58) ^c	(0.02) ^a
<i>E. camaldulensis</i> x <i>globulus</i>								
42xF								
83xF	2.8	5.0	4.9			8.8	10.6	4.0
83xK	6.2							
83xO								
84xF	2.8	7.6	7.9	4.4	8.2	9.5	10.1	4.7
85xF	5.8					5.9	7.0	4.1
85xK	3.5	7.2	7.0	4.0	8.3	5.1	8.7	4.1
85xO	4.2	6.7	6.8	4.2	8.0	9.8	10.0	4.1
87xF	3.2					11.8	8.4	4.0
87xK	4.4	7.4	7.8	4.3	9.0	7.8	7.9	4.3
87xO						11.5	7.6	4.0
Mean	4.1	6.8	6.9	4.2	8.4	8.8	8.8	4.2
(SE)	(0.47) ^b	(0.47) ^b	(0.54) ^a	(0.09) ^b	(0.02) ^b	(0.86) ^b	(0.46) ^b	(0.08) ^a
<i>E. globulus</i>								
F	1.0							
K	1.0	12.8	6.2			16.5	18.9	4.2
O	1.0	11.9	6.5	7.3	10.3	14.9	21.1	4.0
T	1.0	12.0	8.8			20.7	22.0	4.0
Mean	1.0	12.2	7.2	7.3	10.3	17.4	20.7	4.1
(SE)	(0)	(0.28) ^a	(0.82) ^a	(-) ^a	(-) ^a	(1.73) ^a	(0.92) ^a	(0.07) ^a

All linear measures in mm.

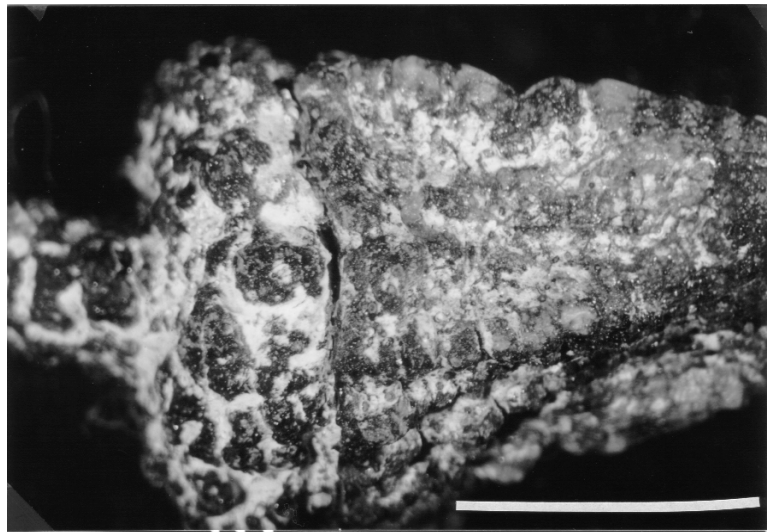
^{a,b,c} — different letters refer to statistical differences ($P < 0.05$) between the different types of trees.

¹ — number of flower buds in a panicle

A



B



C



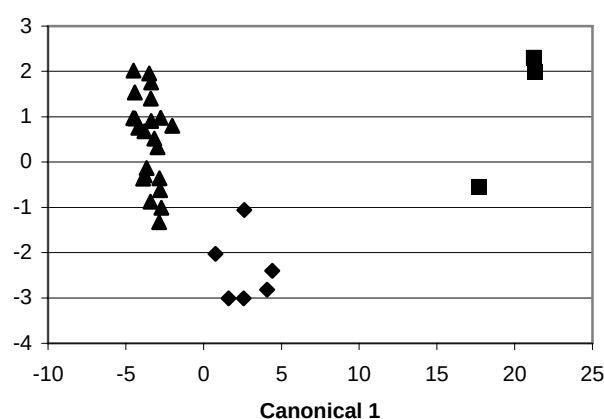
Figure 3.2 A. Flower buds of *E. camaldulensis* 85 and *E. camaldulensis* x *globulus* (85 x O). B. Flower bud of *E. globulus* K. C. Fruit of *E. camaldulensis* 85 and *E. camaldulensis* x *globulus* (85 x K)

A. Flower buds of *E. camaldulensis* 85 (LHS) and *E. camaldulensis* x *globulus* (85 x O) (RHS). B. Flower bud of *E. globulus* K. C. Fruit of *E. camaldulensis* 85 (LHS) *E. camaldulensis* x *globulus* (85 x K) (RHS). Bar is 5 mm.

Table 3.4 Structure of canonical variables using nine traits

Trait	Canonical 1	Canonical 2
Fruit diameter	-0.992513	0.032669
Fruit height	-0.915004	0.013801
Log operculum diameter	-0.895887	0.409824
Log Petiole length	-0.567099	0.428056
Operculum height	-0.533809	0.771415
Leaf width	-0.503352	0.094947
Leaf length	-0.397650	0.304640
Widest distance ¹	-0.190363	0.398437
Number of buds	0.858112	-0.368688

¹ distance from the petiole to the widest part of the leaf lamina

**Figure 3.3** Plot of discriminant canonical functions incorporating fruit diameter, fruit height and log operculum diameter

▲ *E. camaldulensis*, ◆ *E. camaldulensis* x *globulus*, ■ *E. globulus*

3.4 Discussion

3.4.1 Seedling morphology

The morphology of *E. camaldulensis* x *globulus* seedlings was distinct from both parental species. In the majority of seedling characters measured the hybrids had values that were equivalent to *E. globulus* or closer to *E. globulus* than *E. camaldulensis* (Table 3.1). There was thus a clear distinction between hybrids and seedlings resulting from the rare accidental self pollination with *E. camaldulensis*. It is common for *Eucalyptus* hybrids to have intermediate characters that are closer to one parent than the other, with the logarithmic mean of hybrids often equivalent to the combined logarithmic mean of the parents (Pilipenko, 1969; Pryor, 1976; Tibbits, 1988). In most previous studies the parental species closest to the hybrid varied with different morphological characters, with the overall morphology of hybrids intermediate between both parents (Pryor, 1951a; Pryor, 1954; Pryor, 1976; Tibbits, 1988). The apparent predominance of *E. globulus* characters in the morphology of the *E. camaldulensis* x *globulus* seedlings may be biased by the number and type of morphological characters that were measured. However Pilipenko (1969) has reported the predominance of one parent in some *Eucalyptus* hybrids, including a cross between *E. globulus* subsp. *maidenii* and *E. camaldulensis* in which the hybrids appeared to be closer in morphology to *E. globulus* subsp.

maidenii than *E. camaldulensis*. Thus it appears the dominance of *E. globulus* traits is seen whether the species is used as the male or female parent.

The distance between axillary buds at node 5 in the hybrid seedlings (a measure of whether leaves are alternate or opposite) was intermediate between the parental species, but closer to *E. globulus* than *E. camaldulensis*. The results of other crosses indicate that opposite leaves dominate over alternate leaves (Pilipenko, 1969; Pryor, 1954). In these studies the character was assessed subjectively. In the present study the distance measured between buds at node 5 was greater in the hybrids than in *E. globulus* but it was so small that the arrangement of hybrid leaves could still be visually classified as opposite.

3.4.2 Morphology of mature trees

While it would be difficult to discriminate between trees of the *E. camaldulensis* x *globulus* hybrids and the parent species on the basis of leaf morphology, the floral and capsule features were similar to *E. camaldulensis* (flowers with pedicels, number of flowers in a cluster and valve exsertion) or intermediate between the parents (operculum height and diameter, stamen and style length, ribbing and warts on capsule) (Table 3.2). Hybrid pollen fertility was comparable to that of *E. camaldulensis*. In a backcross of hybrid 87xK with *E. globulus* pollen there was an average of 17 seeds set per capsule compared with the mean of 14 obtained in the original cross (J. McComb, unpubl.).

The disparity in flower size between the parent species meant that using conventional pollination techniques we were able to make the cross only in one direction using the smaller flowered *E. camaldulensis* as a female parent. It is possible that the intermediate sized hybrid flower might be pollinated by either *E. camaldulensis* or *E. globulus* but at present we have only shown the latter to be so. The technique of cutting the style and embedding the pollen in the bleeding sap has proved successful for ‘one-stop’ controlled pollination of *E. globulus* (Harbard *et al.* 1999; Williams *et al.* 1999) and may allow use of *E. camaldulensis* as a male parent to create hybrids with the *E. globulus* cytoplasm.

The overlap of flowering time of the hybrid with the *E. camaldulensis* means that natural back crossing would be possible when hybrids are grown near natural stands of the parental species. The development of hybrid swarms in eucalypts depends on habitat availability with hybrids often being confined to disturbed areas, or out-competing the parent species on marginal but not favourable sites (Chaperon 1984). Thus while plantations of the *E. camaldulensis* x *globulus* hybrids near the parental species could result in further introgression and it is possible that these hybrids could compete with *E. camaldulensis* on disturbed river flats.

3.5 Summary

Crosses between *Eucalyptus camaldulensis* and *E. globulus* have the potential to produce hybrids with both salt-waterlogging tolerance and good wood pulp properties. Here we describe the morphology of the hybrids with reference to the parental species. Cotyledon shape of the *E. camaldulensis* x *globulus* hybrids was intermediate between the two parents and an excellent way of checking that a seedling was a hybrid and not an accidental self. Shape and size of juvenile leaves did not distinguish the hybrids, but hybrid leaves changed from being opposite and sessile to alternate and petiolate at a lower node than in the *E. globulus* parent, though not as quickly as for *E. camaldulensis*. In adult trees the hybrid leaves were longer than leaves of the parent species. Flowers of the hybrid were more similar to *E. camaldulensis* being in pedicellate clusters of 3–6. Flower size was intermediate between the parents (operculum height and width, stamen and style length). Fruits were also intermediate in size but valves were exserted as in *E. camaldulensis*. Hybrids were fertile. Hybrids overlapped in flowering time with *E. camaldulensis* so that if planted adjacent to natural stands of *E. camaldulensis* some introgression may occur.

4 Field trials

4.1 General introduction

It is well known that salt tolerance trials for a particular species or genotype under glasshouse conditions can only give an indication of the performance that might be expected under field conditions. Analysis of how well glasshouse and field assessments match, showed that for the genus *Eucalyptus* there is a very large number of species that have been screened in the glasshouse and far fewer that have also been screened in the field (Niknam and McComb 2000). For 16 species there is a good match between the level of tolerance in the glasshouse and the field, three are more tolerant in the field than would have been expected from glasshouse results, and five less tolerant than would have been expected. For 13 species there are conflicting reports between different glasshouse trials or different field trials (Table 4.1).

Table 4.1 Comparisons between glasshouse and field testing for salt tolerance of *Eucalyptus* species reported in the literature

	No. of species
Total number of species screened	166
Salt tolerance in glasshouse and field the same	16
Salt tolerance in glasshouse higher than in field	5
Salt tolerance in field higher than in glasshouse	3
Salt tolerance variable between different glasshouse tests or different field tests	13

Source: Data from Niknam and McComb (2000).

It is also necessary to assess field trials for a number of years, as initial growth may be independent of the saline ground water. To determine whether commercial harvest of trees will be possible growth to around 10 years is desirable. *E. globulus* is known to grow well for the first one to three years on sites that are poor because of low rain fall, restricted rooting depth, waterlogging or salinity. At about age four, growth deteriorates. Extremely few commercial *E. globulus* plantations are on land exceeding 50mS/m for duplex soils or 25mS/m for sand (Justine Edwards, Department of Conservation and Land Management, W.A. pers. comm.)

4.1.1 Site preparation and planting

Sites were selected mainly within the range of commercial *E. globulus* plantations on land considered too salty for *E. globulus* (Mt Barker, WA, Boyup Brook WA and Narrikup WA). However a site in an area with less than the usual rainfall for *E. globulus* was included (Beverley, WA) as well as a site in Tasmania that is unsuitable for *E. globulus* (Cressy), and a site in NSW at Coleambally that was irrigated for the first 3 years.

Sites were ripped and mounded, and rows treated with herbicide before planting. At the time of planting each tree was given two, 50 gm DAP fertilizer tablets (Bailey's). No further fertilizers or insecticides applied except in the Narrikup trial.

4.1.2 Assessment of salinity

Salinity was assessed using an EM 38 to measure the apparent EC (ECa) at the base of each tree. This was converted to an equivalent EC1:5 by applying the following formula:

$$\begin{aligned}\text{ECa Vertical (to 1m depth) EC 1:5} &= 0.42 \text{ ECa} - 1.74 \\ \text{ECa Horizontal (to 0.4m depth) EC 1:5} &= 0.38 \text{ ECa} - 1.04\end{aligned}$$

In the case of the Boyup Brook plot there was too much ironstone in the area for the EM38 to function well. In this case samples were taken from the centre of each 4 tree plot for analysis. Soil samples were taken at 3 depths: 0–10cm, 10–20cm and 20–30cm. From each sample a 1:5 soil:water dilution was made and the pH and conductivity read as described in House *et al.* (1998). The mean EC 1:5 was then calculated. Salinity was assessed as low to severe according to the following range of EC (1:5) values (Table 4.2). To convert these values to EC (se) the soil type in each sample was assessed and the conductivity values multiplied by the values that are dependent on the amount of sand and clay present as given in House *et al.* (1998).

Table 4.2 Soil salinity classification

Severity	EC (1:5) mS/m			
	Clay loams	Light clays	Medium clays	Heavy clays
Low	<10	<17	<25	<37
Medium	10–25	17–40	25–58	37–85
High	25–45	40–67	58–100	85–150
Very high	45–70	67–105	100–158	150–240
Severe	>70	>105	>158	>240

Source: House *et al.* 1998.

4.1.3 Plants

Plants were seedlings raised at Murdoch University, or clones of *E. camaldulensis* or the hybrids raised at Murdoch and North Eucalypt Technologies (NET) (for Mt Barker, Beverley and Pinjarra, trials) or solely produced by NET (Boyup Brook, Narrikup, Coleambally and Cressy). The trials in which the various clones have been tested are shown in Table 4.3.

4.1.4 Form

Form was quantified using the following scale:

0= a straight bole, 1= few branches, 2= many branches, 3= plagiotrophic or mallee (Fig. 4.1)

A score of above 1 indicates an unacceptable form.

4.1.5 Analysis of results

For the field trials at Mt Barker, Beverley, Boyup Brook and Narrikup standard errors were calculated for the means of the height and survival data for the individual lines, and ANOVA used to indicate the differences between the means at $P < 0.05$. Then the data for all the *E. camaldulensis*, all the *E. camaldulensis* x *globulus* (except the plagiotrophic lines) and all the *E. globulus* were combined and assessed against the soil salinity. Analysis of variance was used to assess the growth of different types of plants at each site. Salinity was included as a covariant in the analysis. Heights of plants and salinity levels were log transformed prior to analysis to normalise the distributions. A log linear test was applied to survival at these sites using the factors survival, salinity and line. As the range of salinity was different at each site, site was not included as a factor. For the plot at Narrikup the analysis only included trees on soil above 20 mS/m as there was only a total of 2 trees on soil below this salinity (Fig. 4.13B).

Table 4.3 Clones tested in field trials

Cross	Line	Mt Barker	Beverley			Narrikup	Boyup Brook	Coleambally	Cressy
<i>E. camaldulensis</i> x <i>globulus</i>			L	M	H				
87 x F	1.2						+		
83 x K	5.1	+	+	+	+				
83 x K	5.2	+		+					
85 x K	6.1	+	+	+	+	+	+	+	+
	6.2	+	+	+	+	+	+	+	
	6.3	+				+	+	+	
	6.4					+	+	+	
	6.5						+	+	
	6.9						+		
	6.11					+	+	+	
	6.12					+	+	+	
	7.1	+	+	+		+	+	+	+
	7.2						+	+	
	7.3	+	+	+	+	+	+	+	+
87 x K	7.4	+	+	+	+	+	+	+	
	7.6					+	+	+	
	7.7					+	+	+	
	7.9					+	+	+	
	7.11							+	
	7.12							+	
85 x O	8.1					+	+	+	
87 x O	9.2							+	
	9.6					+	+	+	
87 x T	10.3	+				+	+	+	
	10.4					+	+	+	
	10.5					+		+	
	10.7					+	+	+	
85 x T	11.2			+	+	+	+	+	
<i>E. globulus</i>	F	+	+	+	+				
	K	+	+	+	+	+	+		
	O	+	+	+	+				
	T	+	+	+	+				
<i>E. camaldulensis</i>	42	+	+	+	+	+	+	+	+
	83	+	+	+	+	+	+	+	+
	84								
	85	+	+	+	+	+	+	+	
	87	+							

Note all *E. globulus* were seedlings, plants at Pinjarra not included as they were all seedlings.

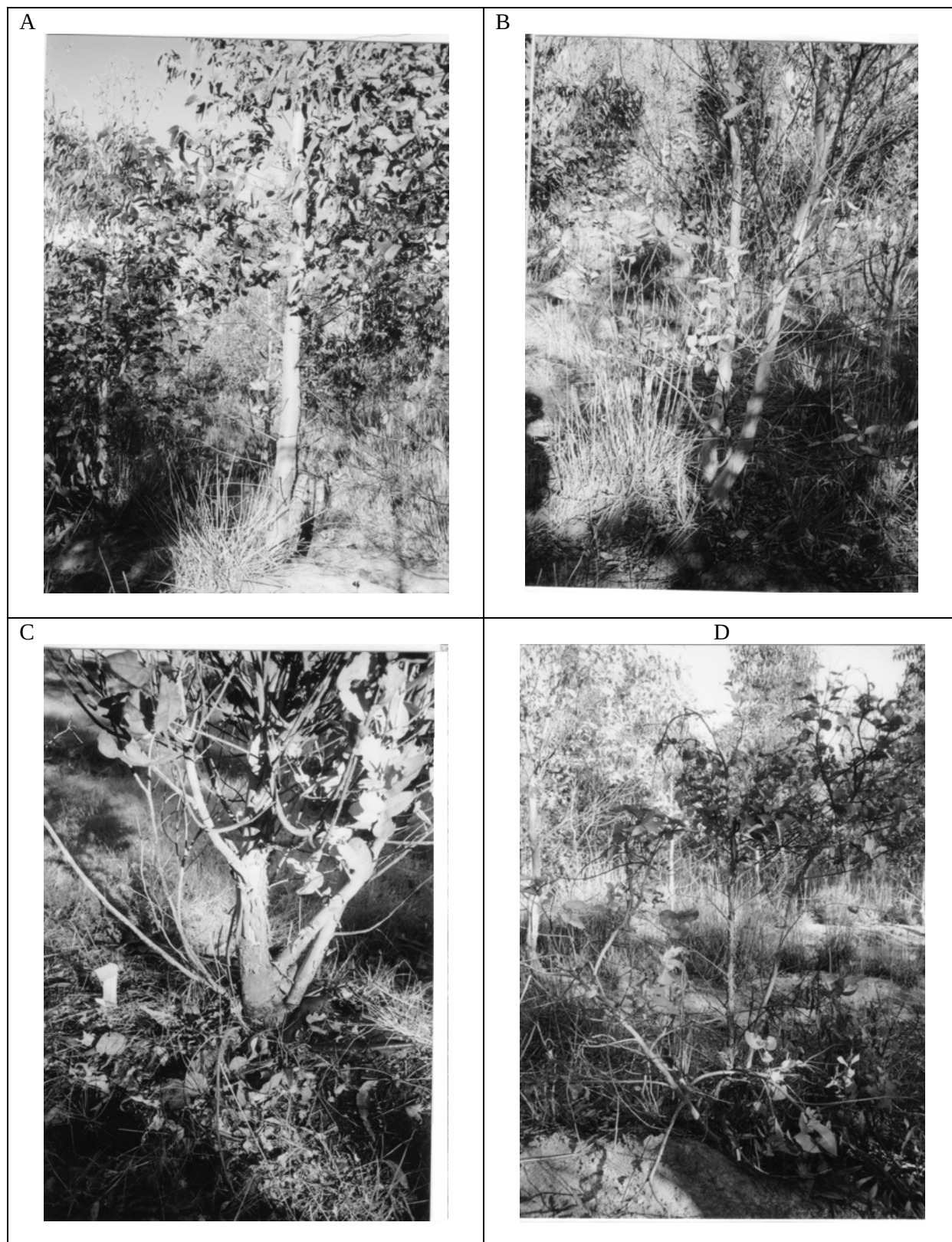


Figure 4.1 Numerical assessment of form

A. form 0 (85 x K 6.2); B. form 1 (*E. camaldulensis* 83); C. form 2 (87 x K 7.3); D. form 3 (87 x T 10.3)

Table 4.4 Mean form of trees on different field sites

Line	Site location				
	Boyup Brook	Mount Barker	Beverley Low salt	Beverley Medium salt	Narrikup
<i>E. camaldulensis</i>					
42	0.7 (0.1)	0.4 (0.1)	0.2 (0.1)	0.3 (0.1)	0.1 (0.1)
83	1.7 (0.1)	0.5 (0.1)	0.9 (0.1)	0.7 (0.2)	0.6 (0.1)
85	1.0 (0.10)	0.4 (0.1)	0.9 (0.1)	0.6 (0.2)	0.3 (0.1)
87		0.3 (0.2)			
<i>E. camaldulensis</i> x <i>camaldulensis</i>					
42 x 87			0.5 (0.2)	0.4 (0.1)	
83 x 84	1.4 (0.1)				
83 x 85		0.4 (0.2)	0.7 (0.2)	1.0(0.2)	
83 x 87					0.5 (0.1)
84 x 85					0.2 (0.1)
85 x 52			0.8 (0.2)	1.0 (0)	
85 x 84	1.2 (0.9)				
87 x 42			0.5 (0.5)	0.6 (0.2)	
87 x 84	0.9 (0.2)				0.9 (0.1)
<i>E. camaldulensis</i> x <i>globulus</i>					
5.1		0.6 (0.3)	0 (0)	0 (0)	
5.2		0 (0)		1.0 (1.0)	
6.1	1.2 (0.1)	0.3 (0.1)	0.5 (0.2)	0.6 (0.2)	0.1 (0)
6.2	0.9 (0.1)	0.3 (0.1)	0.4 (0.2)	0.4 (0.2)	0.1 (0.1)
6.3	1.2 (0.1)	0.5 (0.2)			0.4 (0.1)
6.4	0.9 (0.1)				0 (0)
6.5	1.4 (0.2)				
6.9	0 (0)				
6.11	0.9 (0.1)				0.1 (0)
6.12	0.9 (0.1)				0.1 (0)
7.1	0.8 (0.1)	0 (0)	0.5 (0.5)	(0)	0.1 (0)
7.2	1.5 (0.1)				
7.3	1.2 (0.1)	1.5 (0.3)	1.5 (0.3)	0.3 (0.3)	0.1 (0.1)
7.4	1.2 (0.1)	0.6 (0.1)	0.8 (0.1)	0.5 (0.3)	0.3 (0.1)
7.6	1.2 (0.1)				(0)
7.7	0.7 (1.0)				0.7 (0.1)
7.9	0.9 (0.1)				0.4 (0.1)
8.1	0.5 (0.1)				0.2 (0.1)
9.6	1.0 (0.2)				0.1 (0.1)
10.3	2.3 (0.1)	2.6 (0.3)			2.9 (0.1)
10.4	3.0 (0)				3.0 (0)
10.5					3.0 (0)
10.7	2.6 (0.2)				0.1 (0.1)
11.2	0.9 (0.1)			3.0 (0)	3.0 (0)

Line	Site location				
	Boyup Brook	Mount Barker	Beverley Low salt	Beverley Medium salt	Narrikup
<i>E. globulus</i>					
F		0.4 (0.1)	0.3 (0.2)	0.4 (0.2)	
K	0.3 (0.1)	0.1 (0.1)	0 (0)	0 (0)	0.3 (0.1)
O		0.2 (0.1)	0.4 (0.3)	0 (0)	
T		0 (0)	0 (0)	1.0 (0)	
BG 1	0.6 (0.1)				0.2 (0.1)
BG 2	0.4 (0.1)				0.2 (0.1)

Note: The numerical assessment of form is described in Chapter 4.1, and Fig. 4.1.

4.2 Mount Barker

4.2.1 Trial description

The experimental site was on Manurup, a property that is some 11km west of Mount Barker (34° 40' S 117° 40' E) and is owned by the Department of Agriculture Western Australia. It has 596 mm rainfall per year and the soil is gravel over clay, pH 5.5–7.25. The trial was located on the West side of a commercial plantation of blue gums on land considered too saline for blue gums. A saline seep crossed one side of the trial area and while the upslope area had barley grass. The lower 25% of the site had surface water over most winters of the trial. Trees were planted in rows 4 m apart with 2 m between plants within rows. There were 4 *E. camaldulensis* clones and a seedling lines from the cross 83 x 85, 11 hybrids and seedlings of the four *E. globulus*; a total of 488 trees. Plants were 6 months old at the time of planting in August 1995 and were in one tree plots replicated in parallel blocks proceeding upslope on the site. There was a border of *E. globulus* on the upslope side and of *E. camaldulensis* on the other three sides. Salinity ranged from low on the side nearest the commercial plantation, but most of the site was rated high to very high. Survival and height were assessed annually, and in 2001 DBH (diameter at breast height, 1.3m from the ground) was included.

In March 1997 several trees in the trial were treated with paclobutrazol to enhance precocious flowering and enable some backcrosses to be made. *E. camaldulensis* 85, *E. globulus* K and 6 hybrids were chosen with 2–4 replicate trees of each (Table 4.5) Plants were treated with a soil drench of 2L of solution containing 0.2g ai active ingredient of Cultar® per cm circumference at breast height. Plants were assessed for flower buds in April 1998 and those with no flowers were retreated.

Table 4.5 Trees treated with paclobutrazol at Mt Barker in March 1997

Line	Position	Flowers
<i>E. camaldulensis</i>		
42*	R4	
85*	H3, H4, N6, R3	April '98 (H3, H4)
87*	L9	
85 x K 6.1	J4, Q4, R6	
85 x K 6.2	B4, E3, E5, G2,	
85 x K 6.3	C10,	
87 x K 7.1	D9, I3, P3	April '98 (I3)
87 x K 7.3	B3, C4, L2, O6	July '98 (C4, B3, I17)
87 x K 7.4	C5, D2, T5	
<i>E. globulus</i> K	F4, H5, P4, S3	
<i>E. globulus</i> F		

* untreated trees were also in flower by April 1998

Pollen of the hybrids was examined and assessed for viability using aceto-orcein. The flowers of four trees from the hybrids (7.1 and 7.3) were crossed with pollen from an *E. globulus* selection.

4.2.2 Results and discussion

Survival, height and diameter

The survival of the individual *E. camaldulensis* lines was generally higher than that of the hybrids which in turn was higher than that of *E. globulus* (Fig 4.2). The *E. camaldulensis* were much smaller, more branched trees than the hybrids or the *E. globulus*. The heights and DBH of the best hybrids were slightly smaller than that of the best surviving *E. globulus* with the values not always statistically different in the case of the lines 6.1, 6.2, and 7.1. Some hybrids have excellent form (eg. Line 6.2, Fig. 4.5) but others have poor form (eg. 7.3) that results in low stature. Some hybrids are extremely poor having an almost plagiotrophic growth form, with low multiple branches (Fig. 4.1). This was marked for clones of the crosses 87 x T (cross 10) and 85 x T (cross 11).

The survival and height of the three different types of trees was examined against the salinity of the soil at the base of each tree. There was a significant difference in the percentage survival of the three plant types, with *E. camaldulensis* being higher than *E. camaldulensis* x *globulus* which in turn was higher than *E. globulus* ($\chi^2_{(2)} = 24.0$ $P < 0.01$) (Fig. 4.3). Survival was also significantly affected by salinity ($\chi^2_{(5)} = 14.90$ $P < 0.01$). When the heights of the three types of trees was compared at different salinities (Fig. 4.4), it was shown that they were significantly different ($F_{(2, 205)} = 61.35$, $P < 0.01$) and that salinity influences the height response ($F_{(1, 205)} = 26.26$, $P < 0.01$). Although the relative reduction in height due to salinity was greater in the *E. globulus* than for the *E. camaldulensis* x *globulus*, the *E. globulus* were significantly taller than the other lines at low salinity and not equivalent to the height of the hybrids until a soil salinity above 120 mS/m (Fig. 4.4).

Paclobutrazol

A year after application of paclobutrazol three of the trees were in flower. Some untreated *E. camaldulensis* were also observed to be in flower, but none of the untreated hybrids. The leaf shape and size was not noticeably affected, and by 2001 there was no difference in height between the treated and untreated trees so they are bulked in the graphs.

Crossing

Pollen of the hybrids was 80% viable. The mean seed set was 17 seeds per capsule which is higher than the mean of 9 obtained from the initial *E. camaldulensis* x *globulus* crosses. This confirms that the hybrid clones are reproductively fertile as well as the hybrid seedlings (Chapter 3.3.2).

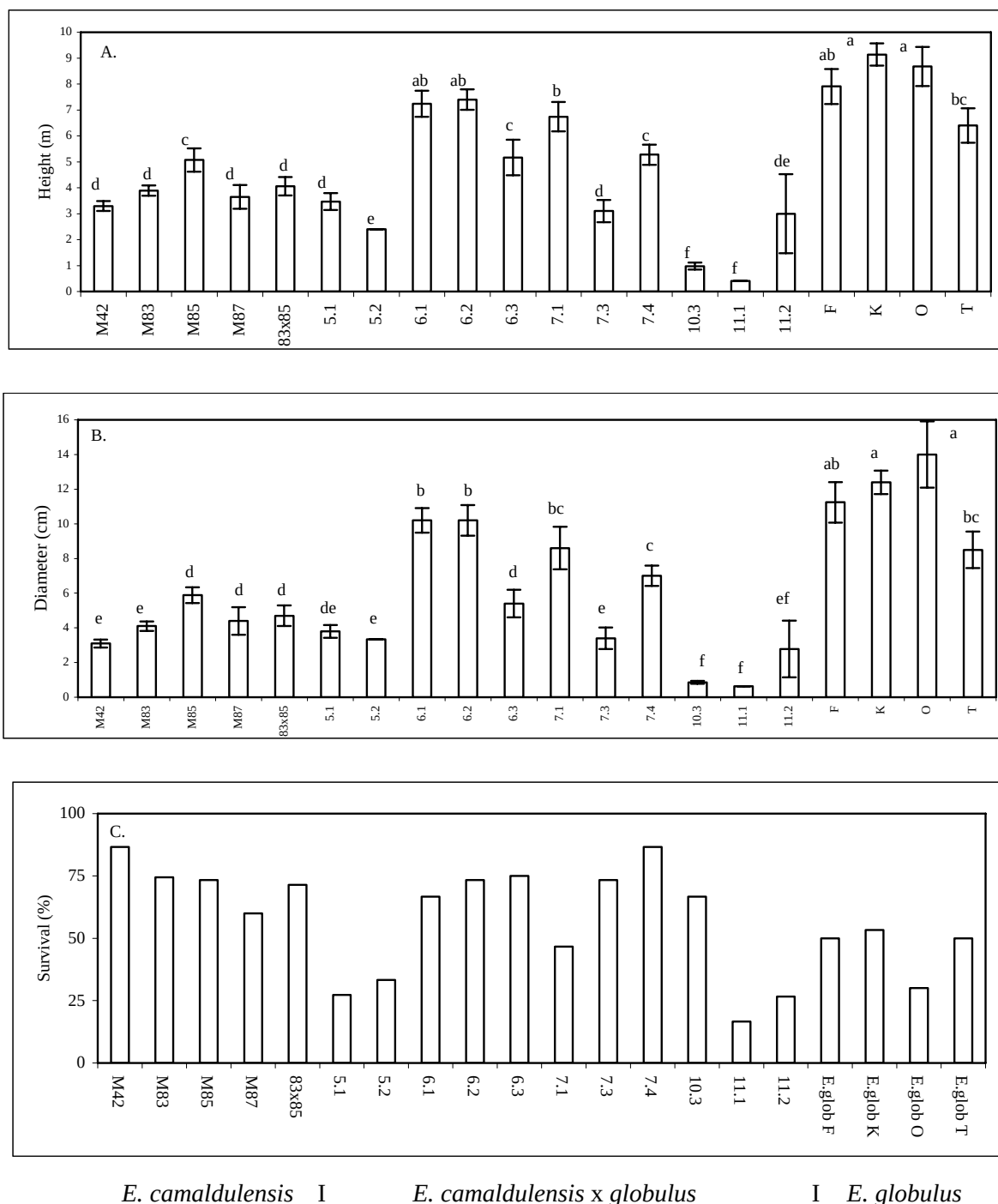


Figure 4.2 Mount Barker 2001: height, diameter and survival of trees

A. Mean height of trees. B. Mean diameter of trees. C. Percentage survival of trees. Bars indicate SE of means. Bars within the same assessment, with the same letters are not significantly different at the 95% confidence level.

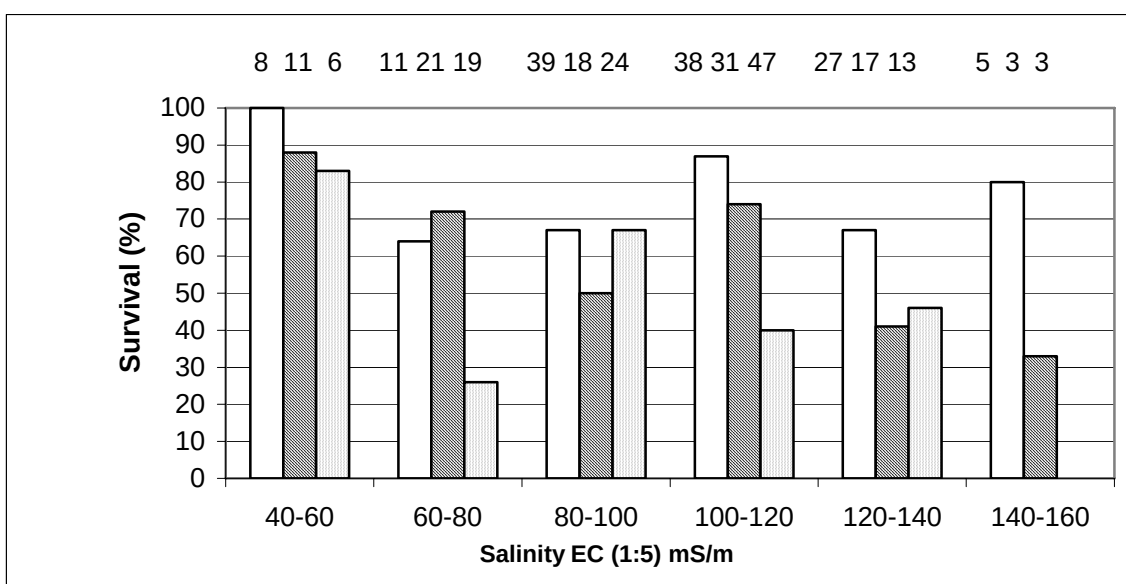


Figure 4.3 Mount Barker 2001: relationship between survival of trees and soil salinity

Clear columns all *E. camaldulensis*, slashed columns all hybrid lines (except the plagiotrophic 10.3, 11.1 and 11.2), stippled columns all *E. globulus*. The small numbers at the top of each column indicate the total number of trees planted.

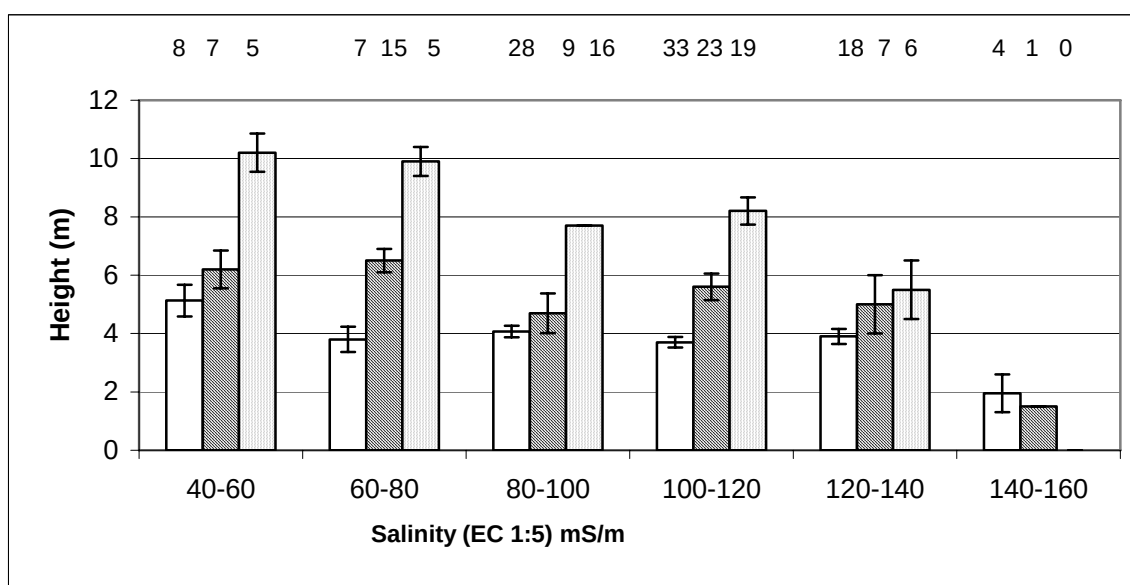


Figure 4.4 Mount Barker 2001: relationship between height of trees and soil salinity

Clear columns all *E. camaldulensis*, slashed columns all hybrid lines (except the plagiotrophic 10.3, 11.1 and 11.2), stippled columns all *E. globulus*. The small numbers at the top of each column indicate the number of living trees. Bars show SEs of the means.



Figure 4.5 *E. camaldulensis* x *globulus* 85 x K 6.2 at Mount Barker July 2001

Height 5.4m. Salinity EC (1:5) 114mS/m.

4.3 Beverley

4.3.1 Trial description

The Beverley plantation was located on farmland near Bally Bally, 10km south east of Beverley Western Australia (32° 06' S 116° 55' E). There were three separate plots. One plot was on a waterlogged salt scald which had no vegetation, the second 50 m upslope and the third a further 100 m upslope. They were designated high, medium and low salt plots. The salinity of the high salinity plot (to 1.2m) was highly saline (EC (1:5) 377 mS/m). The low plot had most readings in the 0–20 mS/m range and medium plot had most readings in the 20–40 mS/m range (maximum 51mS/m). The annual rainfall is 432mm and the soil was loam to light clay with pH 5–6.4 on the low to medium salt plots and 7–8.6 on the high salt plot.

Trees on the low and medium sites were within an oil mallee plantation and the planting design for that trial was continued in the hybrid plots. This was two rows 2 m apart, separated from the next two rows by 8 m. Trees within rows were 4 m apart.. There were 3 *E. camaldulensis* parent clones, 4 *E. camaldulensis* x *camaldulensis* seedling lines, 11 hybrids and the 4 *E. globulus* parental seedling lines. Trees were planted as one tree plots replicated up to 10 times on each site. There was a border of

two trees of *E. camaldulensis*. Survival and height were assessed annually, and in 2001 DBH (diameter at breast height, 1.3m from the ground) was included.

4.3.2 Results and discussion

All trees on the highly saline site died within a year. On the medium and low salt plots, survival of individual *E. globulus* lines was markedly lower than for *E. camaldulensis* and the hybrids, the best of which had 100% survival (Fig. 4.6, 7). The plagiotrophic clones of line 11 had no survival on the low salt plot and only one plant survived on the medium salt plot. The best clones were from crosses 6 (85 x K) and 7 (87 x K) with clone 6.2 averaging 7.25m height by 2001. The highest *E. globulus* were only 5.5 m tall. The hybrids had achieved considerably more girth than the *E. globulus*. The wider spacing of this trial may have been the cause of the hybrid clones having poorer form here than in other trials, particularly the one at the Narrikup. However, even at this spacing the *E. globulus* retained good form (Table 4.4).

Height and survival of the different types of plants were analysed after combining data from the low and medium salinity plots (Fig 4.8). The survival of the lines was different ($\chi^2_{(3)} = 83.73$ $P < 0.01$), but there was no effect of salinity ($\chi^2_{(2)} = 0.21$ $P > 0.05$). *E. camaldulensis* and the hybrids showed higher survival than the *E. globulus*. The heights of the three types of plants were significantly different ($F_{(2,182)} = 52.73$, $P < 0.01$), but there was no significant effect of salinity on height ($F_{(1,182)} = 1.69$, $P > 0.05$) (Fig. 4.8). It is evident however that the *E. camaldulensis* x *globulus* were taller than the *E. globulus* at all salinities. This suggests that hybrids may do well on drier sites. A commercial *E. globulus* plantation 20 km west of the trial site was badly affected by the drought of 2000 and 2001 with 90% of the trees killed.

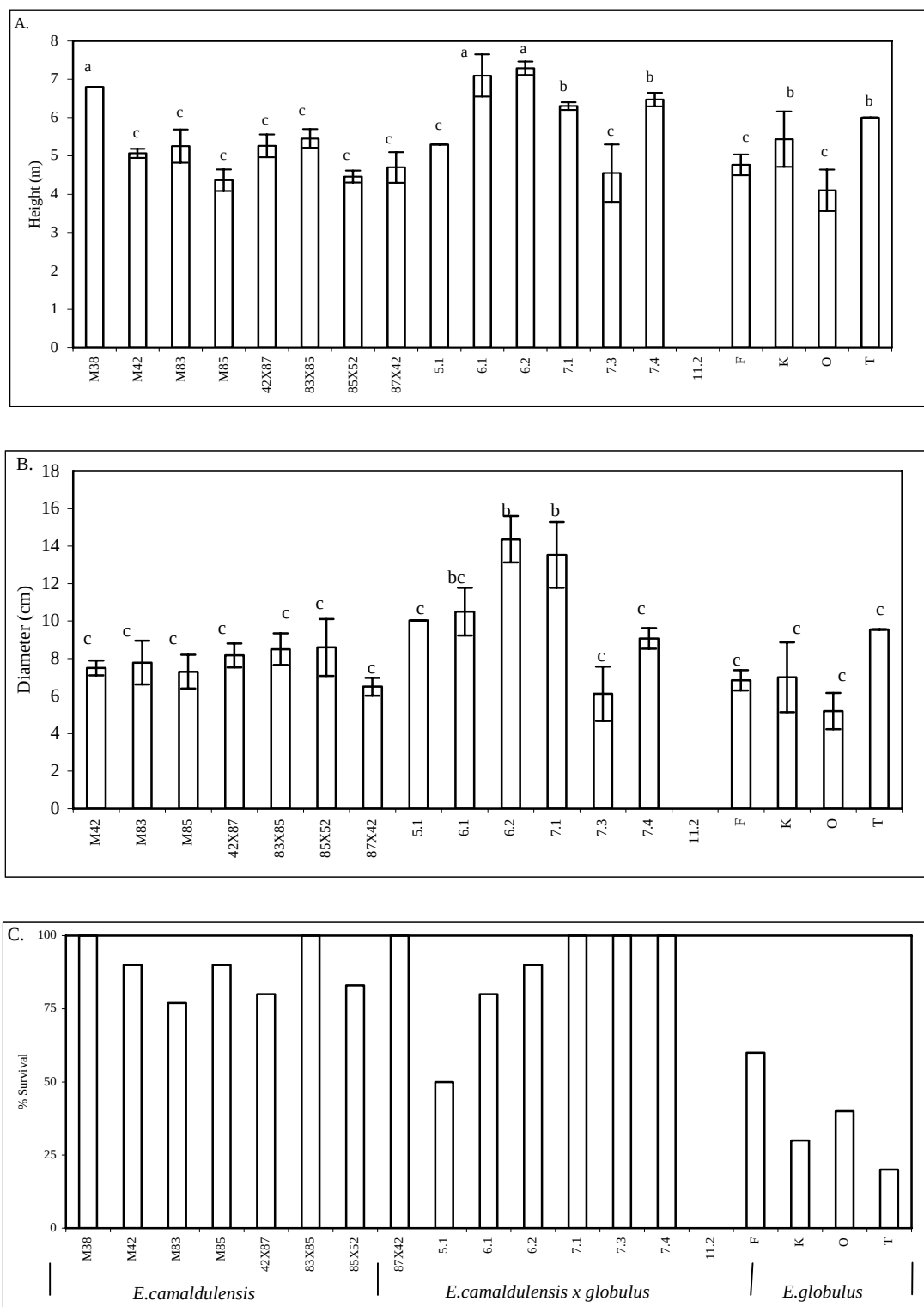


Figure 4.6 Beverley low salt plot 2001

A. Heights, B. DBH, C. Percentage survival. Bars indicate SE of means. Bars within the same assessment, with the same letters are not significantly different at the 95% confidence level.

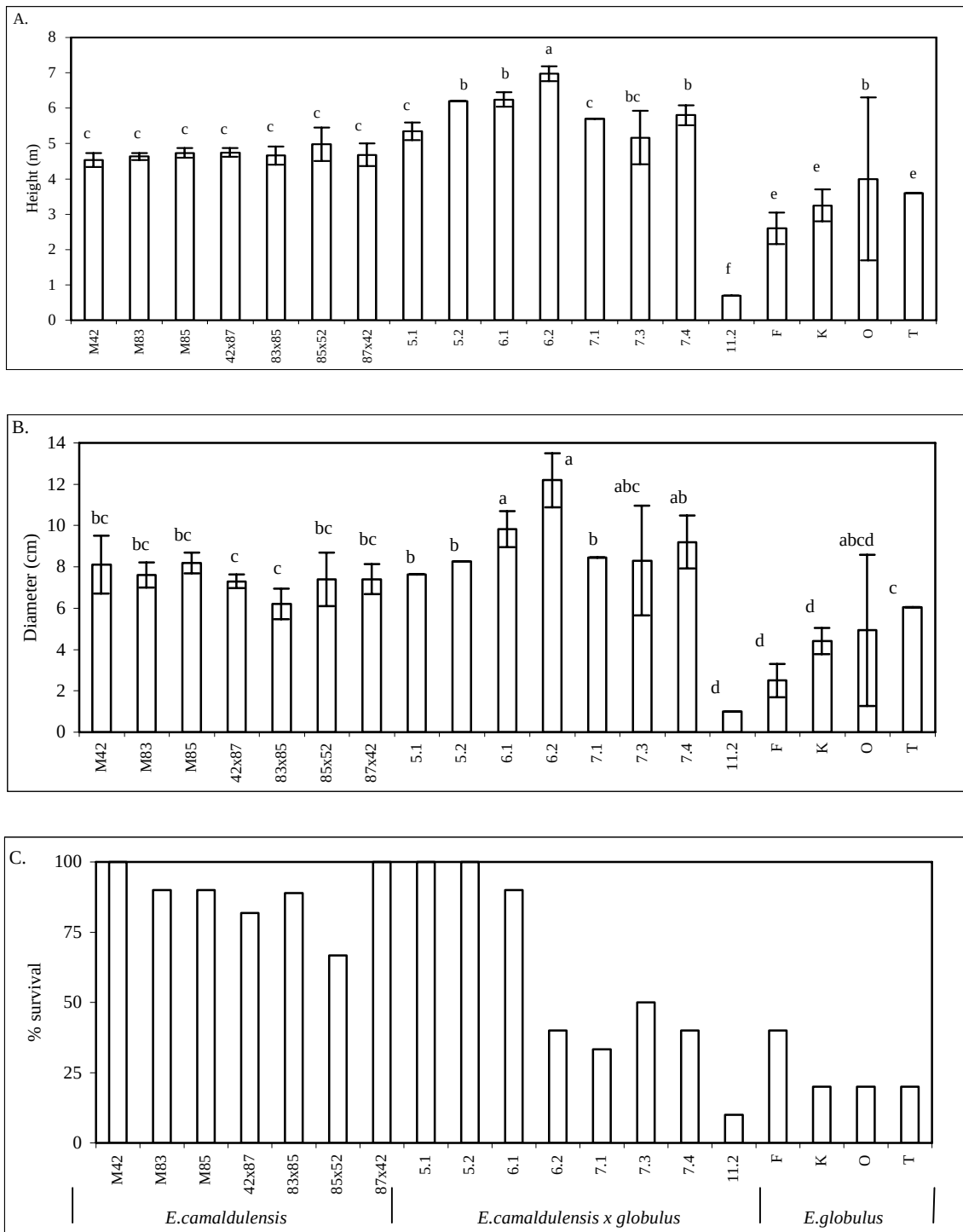


Figure 4.7 Beverley medium plot 2001

A. Heights, B. DBH, C. Percentage survival. Bars indicate SE of means. Bars within the same assessment, with the same letters are not significantly different at the 95% confidence level.

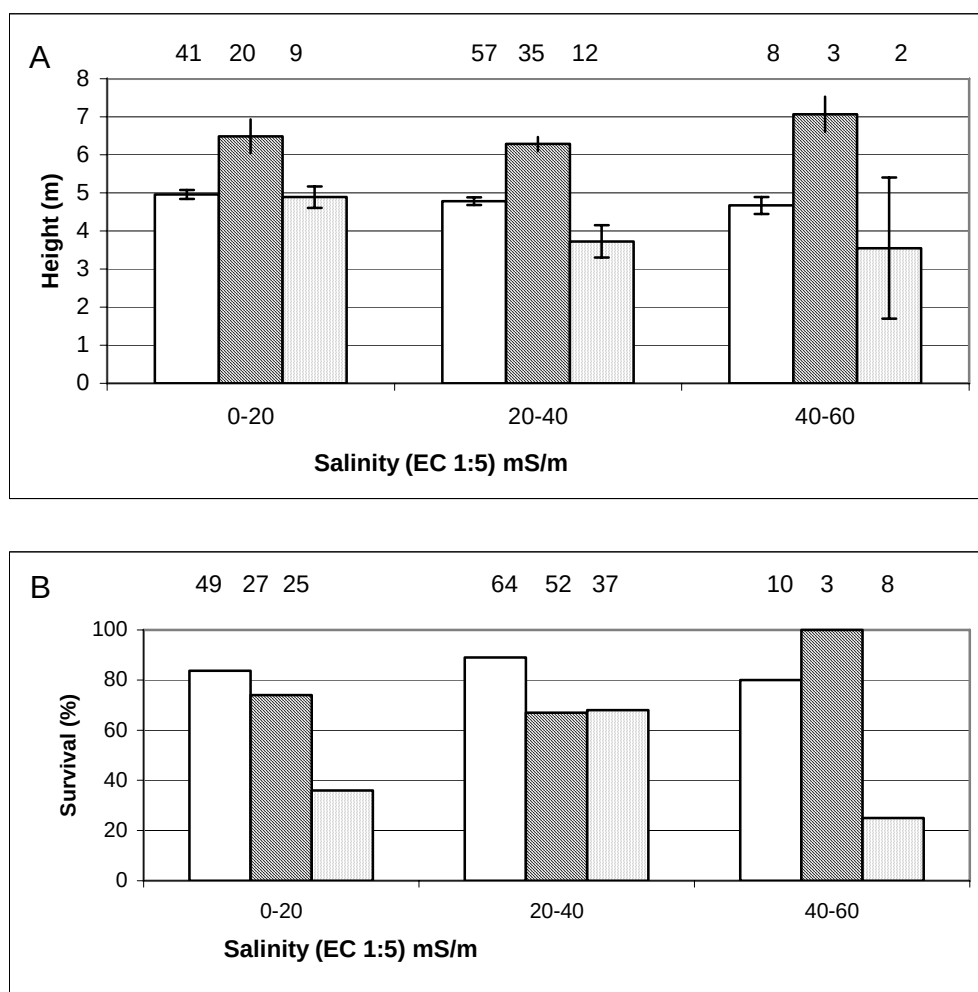


Figure 4.8 Beverley 2001: A. Relationship between soil salinity and height of trees; B. Relationship between survival and salinity

Data from the low and medium salt plots have been combined. Clear bars all *E. camaldulensis*, slashed bars all hybrids (except plagiotrophic ones), stippled bars all *E. globulus*. A. Low salt plot; B. Medium salt plot. Bars indicate SEs of means. Numbers above the columns in A are the numbers of living plants in each salinity range; in B they indicate the total number of trees planted in each salinity range.



Figure 4.9 Trees at Beverley low salt plot 2001

Smaller tree to the left of people *E. camaldulensis* 85 (Height 5.8m), the one to the right is 85 x K 6.2 (Height 7.9m). Salinity at this position EC (1:5) 17 mS/m.

4.4 Boyup Brook

4.4.1 Trial description

This trial was planted in August 1997 on a property 8 km south of the town of Boyup Brook, (33° 50' S 116° 25' E) in an area with 650 mm annual rainfall. It was planted on farmland with light to medium clay pH 4.98–5.93 with a saline seep. There was a commercial blue gum plantation some 100 m further upslope. The land was ripped and mounded and herbicide applied to the rows for weed control. The spacing was 2 m within rows and 4m between rows and there were 2 rows of *E. camaldulensis* as border plants on all sides. The bluegum lines planted on this site included seedlings of the King Island provenance and two western bluegum lines (designated BG1 and BG2) suitable for the area, provided by the Department of Conservation and Land Management (Dr Liz Barbour). In years 1 and 2 the trees were badly damaged by parrots. No remedial pruning was undertaken. Also in years 1 and 2 there was considerable weed competition, particularly in the less saline areas of the plots. Trees were planted in 4 tree plots replicated 10 times over the site. Trees were assessed annually. The ironstone content of the soil in some areas of the site prevented Em38 readings being used. Soil samples were taken and analysed as described in 4.1 for every 4 tree plot. Survival and height were assessed annually, and in 2001 DBH (diameter at breast height, 1.3m from the ground) was included.

4.4.2 Results and discussion

Survival, height and diameter

Survival was high on this site, except for one *E. camaldulensis* x *camaldulensis* line (87 x 84) that was rather small at the time of planting and one of the plagiotrophic hybrids (10.4) (Fig.4.10). Many of the hybrids showed 100% survival compared with the mean survival for the *E. globules* of 79%. The best hybrids had similar height and diameter to the *E. globulus* lines. There were excellent lines from the crosses 6 (85 x K), 7 (87 x K), 9 (87 x O) and 10 (87 x T) with the greatest height being line 6.5 (8.9 m tall DBH 10 cm). Most lines from the crosses 10 and 11 were very poor and plagiotrophic except for 10.7 that was one of the best lines.

The salinity of the site ranged from non-saline to medium (EC (se) up to 3.4 dS/m). The different types of plants showed different levels of survival ($\chi^2_{(9)} = 508.52$ $P < 0.01$), but there was no significant interaction with salinity ($\chi^2_{(3)} = 1.85$ $P > 0.05$); all lines showed high survival over the full range of soil salinities (Fig. 4.11B). There was a significant difference between heights of the different types of plants ($F_{(2,943)} = 228.72$ $P < 0.01$), but no effect of salinity on height ($F_{(1,943)} = 11.58$, $P > 0.05$) (Fig 4.11A).

Form

The form of the trees at this site was poorer than elsewhere because of the early parrot damage. For example line 6.1 which had good form on the other trial sites had unacceptable low branches at Boyup Brook.

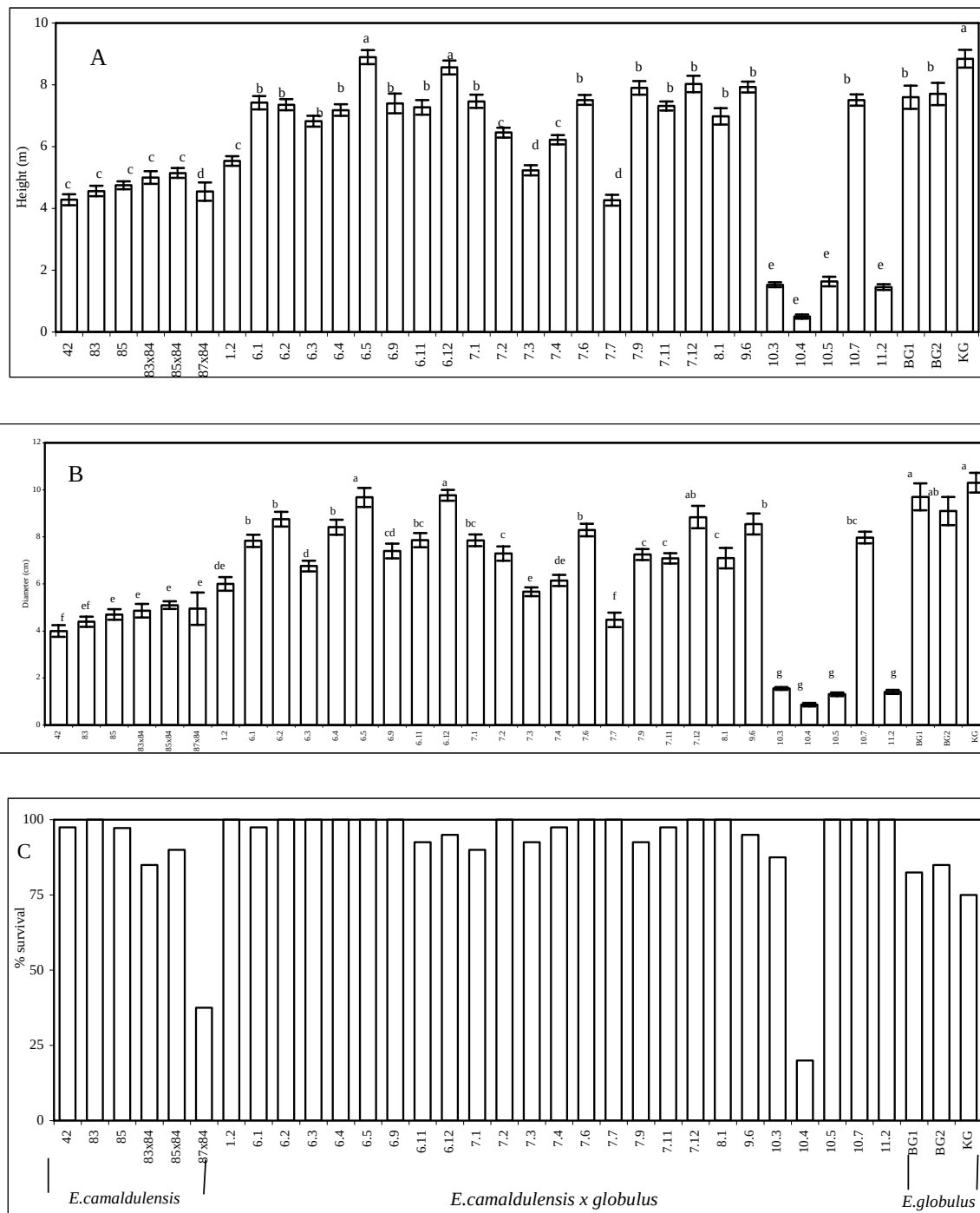


Figure 4.10 Boyup Brook 2001: height, diameter and survival of trees

A. Height, B. DBH, and C. Survival. Bars indicate SE of the means. Bars within the same assessment, with the same letters are not significantly different at the 95% confidence level.

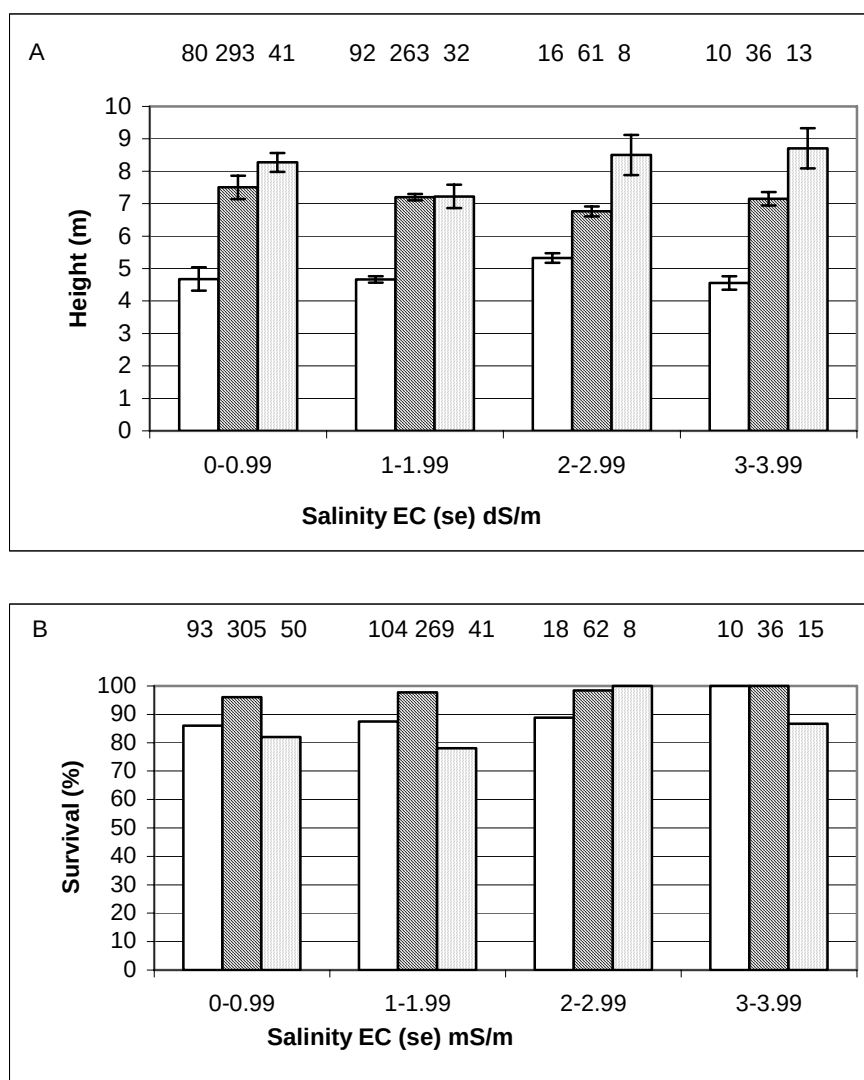


Figure 4.11 Boyup Brook 2001: height and survival assessed against soil salinity

A. Height B. Survival of all *E. camaldulensis* (clear columns), all hybrids (except plagiotrophic lines) (slashed columns), and all *E. globulus* (stippled columns) assessed against soil salinity. Bars indicate SEs of means. Numbers above the columns in A are the numbers of living trees at each salinity range; in B they indicate the total number of trees planted in each salinity range.

4.5 Narrikup

4.5.1 Trial description

The Narrikup (34° 46' S 117° 42' E) trial was located 20 km south east of Mount Barker Western Australia and immediately south of the Porongurups. It has the highest rainfall of all clonal trial sites; average 735 mm over last 10 yrs (John Edwards, Albany, pers. com.). The soil was medium to heavy clay pH 5.5–5.6. The western 25% of the site was waterlogged in most winters. The site was farmland mostly planted to blue gums by South Coast Sharefarms. Trees were planted 2 m apart in rows 4 m apart in July 1997. There was a row of *E. globulus* along the eastern border and the north and south borders were abutted by environmental plantings of *E. camaldulensis* and other salt tolerant species. Trees were initially fertilised with 2 Bailey plant fertilizer tablets and in 1999 and 2001 given 300kg/ha of South Coast Sharefarms' custom mix which provided 38 kg P (12.7%), 21.1 kg S (7%), 50.9 kg Ca (17%), 2.3 kg Cu (0.75%) and 2.8 kg Zn (0.92%).

There were 3 lines of cloned *E. camaldulensis* parent, 3 of *E. camaldulensis* x *camaldulensis* seedlings, 19 hybrid clonal lines seedlings of the King Island *E. globulus* parent and two *E. globulus* commercial lines planted in the area (designated BG1 and BG2). The plants were 6 months old at planting in July 1996, and the design was 4 tree plots replicated up to 10 times over the area. Survival and height were assessed annually and in 2001 DBH was included.

EM38 readings indicated soil salinity mostly in the slight to moderate range (EC (1:5) 20–60 mS/m) with a few patches of soil having high salinity (60–80 mS/m).

4.5.2 Results and discussion

Height, survival and diameter

Survival was high on this well-watered site (Fig 4.12). The only lines that showed significantly less than 100% survival were the *E. camaldulensis* x *camaldulensis* (84 x 87) that were small when planted, the plagiotrophic clones and some *E. globulus*. The *E. camaldulensis* were consistently smaller than the hybrids or the *E. globulus*. The best hybrids were significantly taller than the *E. globulus*. The tallest hybrids were clones 6.1, 6.2, 6.11, 10.7. Clone 6.1 reached a mean height of 8.2 m and 9.7 cm DBH after 4 years.

The survival of the three different types of plants was significantly different ($\chi^2_{(6)} = 412.81$ $P < 0.01$) with *E. camaldulensis* x *globulus* showing consistently high survival and the *E. camaldulensis* and *E. globulus* similar but slightly lower levels of survival (Fig. 4.13B). There was no effect of salinity on survival ($\chi^2_{(2)} = 3.75$ $P > 0.05$). There were significant differences between the heights of the three types of plants ($F_{(2,868)} = 224.05$, $P < 0.01$) (Fig. 4.13A). There was also a statistically significant effect of salinity on heights ($F_{(1,868)} = 21.68$ $P < 0.01$) but inspection of Figure 4.13A shows that this has no biological significance—salinity only resulted in a small decrease in height for *E. camaldulensis* and there was no effect on *E. globulus* or the hybrids.

Form

The high survival and the four tree plot design made the plot more similar to a plantation situation than some of the other trials and assessment of form more reliable. The overall form on this site was better than elsewhere (Table 4.4).

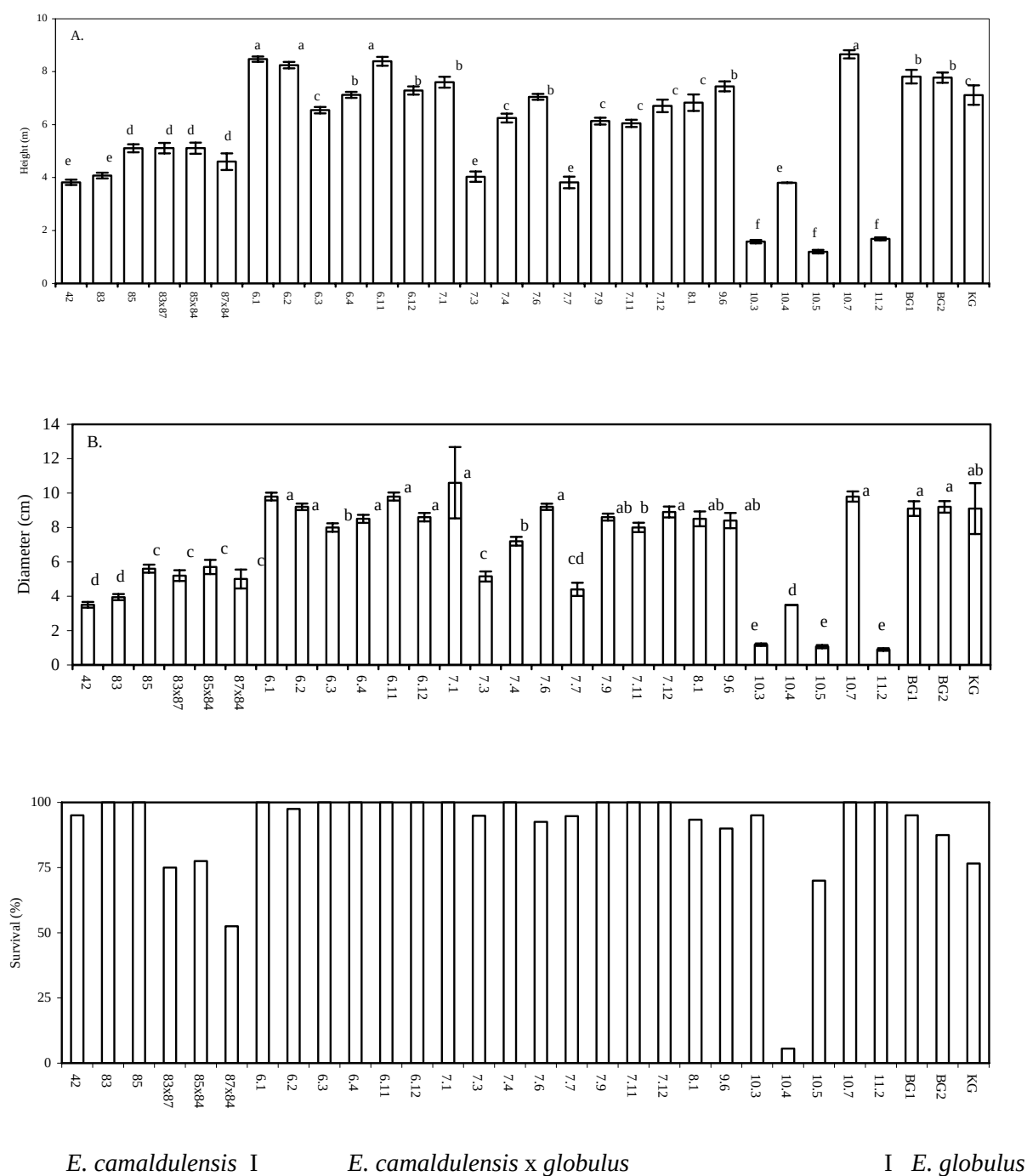


Figure 4.12 Narrikup 2001: height, diameter and survival of trees

A. Mean height of trees. B. Mean diameter of trees. C. Percentage survival of trees. Bars indicate SE of means. Bars within the same assessment, with the same letters are not significantly different at the 95% confidence level.

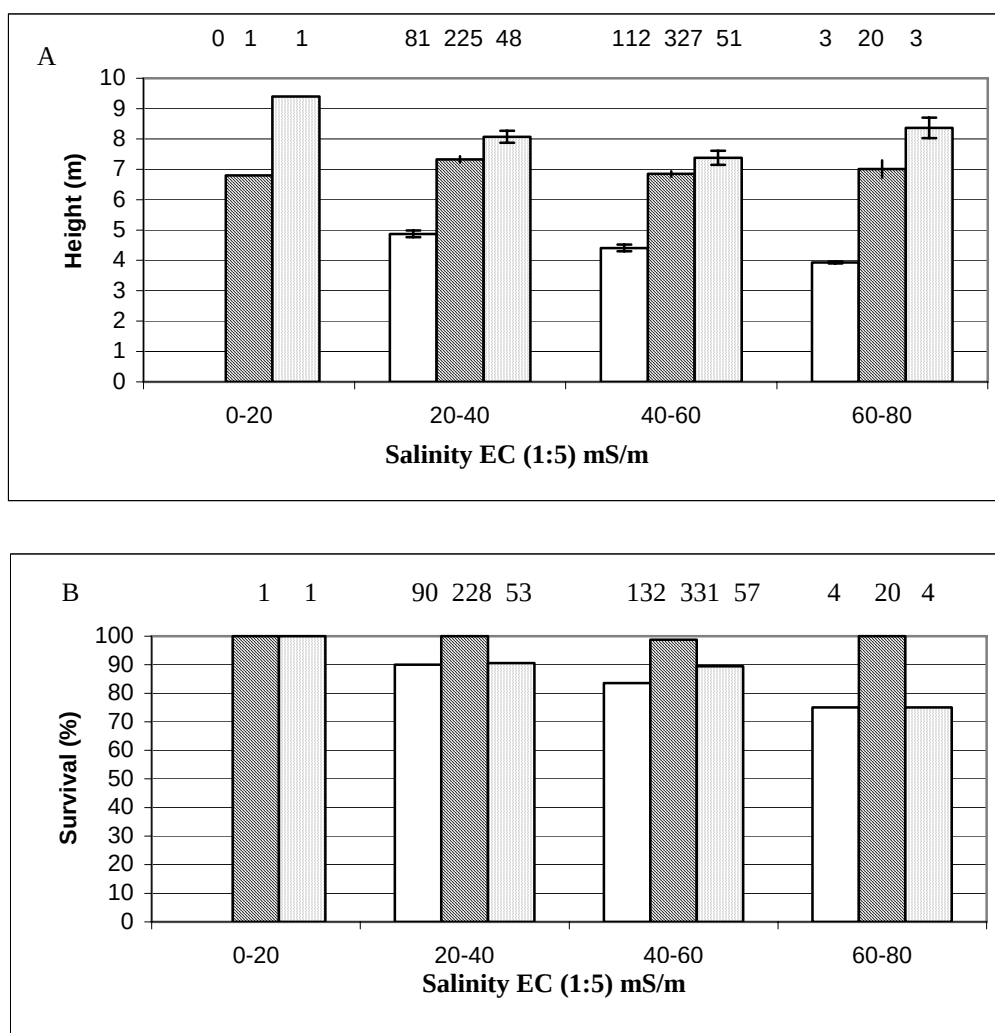


Figure 4.13 Narrikup 2001. A. Relationship between soil salinity and of height of trees. B Relationship between survival and salinity

E. camaldulensis (clear columns), *E. camaldulensis* x *globulus* (except the plagiotrophic lines) (slashed columns) and *E. globulus* (stippled columns). Bars represent SE of the means and the numbers above the bars in A are the numbers of living trees in each salinity range; in B they indicate the total number of trees planted in each salinity range.

A



B



Figure 4.14 Photos of trees at Narrikup August 2001

A. Line 85 x K 6.11 Mean height 9.4m Salinity EC (1:5) 33 mS/m. B. 87 x T 10.7 Mean height 9.7 m Salinity EC (1:5) 40 mS/m.

4.6 Pinjarra

4.6.1 Trial description

This trial was located on the coastal plain near Pinjarra, (32° 35' S 115° 50' E) 87 km S of Perth. The area has 948 mm annual rainfall. A blue gum plantation on this farmland owned by Alcoa World Alumina showed high numbers of deaths over the summer of 1993/4, which was exceptionally dry. All *E. globulus* trees were removed and burned and the area fenced to exclude stock. The land was ripped and mounded and herbicides applied to suppress weeds before planting in July 1995. This site is non-saline and the hybrid plants used were unselected seedlings not clones. They were of two ages. Approximately half the plants were 22 months old when planted into the field. They had been planted as seed in August 1993 and grown in 200 mm pots at Murdoch University for observations of morphology and abnormalities and were rather pot bound. They are designated (93)95. The remainder were 8 month old seedlings designated (94)95. There was a border row of *E. camaldulensis* on the north and south side but the east and west ends had no border plants. Trees were 3 m apart in rows 4 m apart. There were 13 hybrid lines, 5 clonal lines of parent *E. camaldulensis*, 8 seedling lines of *E. camaldulensis* x *camaldulensis*, and 4 seedling lines of *E. globulus* parents. The design was one tree plots replicated 20 times over the site when there were sufficient plants (Table 4.6). Survival and height were assessed annually, and from 1999 DBH (diameter at breast height, 1.3 m from the ground) was included.

These trees were used for several purposes:

1. to check whether further abnormalities developed after the trees reached 18 months old
2. to monitor the growth of trees that had abnormal growth
3. to obtain flowers and fruits for assessment of morphology (Chapter 3)
4. to obtain wood samples for preliminary analysis earlier than is possible from sites where growth was slower due to salinity (Chapter 5)
5. to assess whether the hybrids could survive in an area unsuitable for the *E. globulus* parent
6. to assess growth under non-saline conditions and record the range of performance that might be expected from the genotypes before selection of only those that are salt tolerant.

4.6.2 Results and discussion

Abnormalities

No further hybrid trees developed abnormalities after planting in the field at 22 months old. Only trees that were known to be abnormal at the time of planting showed abnormal growth. The abnormal shoots died back and new shoots sprouted from the base. These were initially normal but then became distorted. The process continued for a maximum of six months before the plants died.

Establishment and survival

There were few establishment deaths. Several trees suffered damage from rabbits and parrots in the first 6 months. The site was clearly unsuitable for *E. globulus* as some 50% of the *E. globulus* had died after 3 years and very few survived for 6 years (Fig 4.15). The clonal *E. camaldulensis* showed 100% survival while the seedlings from the *E. camaldulensis* x *camaldulensis* were variable with 50–100% survival (Fig 4.16, 17). The hybrids were also variable in survival but generally intermediate to the parents. The lines 83 x K and 83 x O had only 2 plants each line amongst the (94)95 trees, but these showed 100% survival and good height growth.

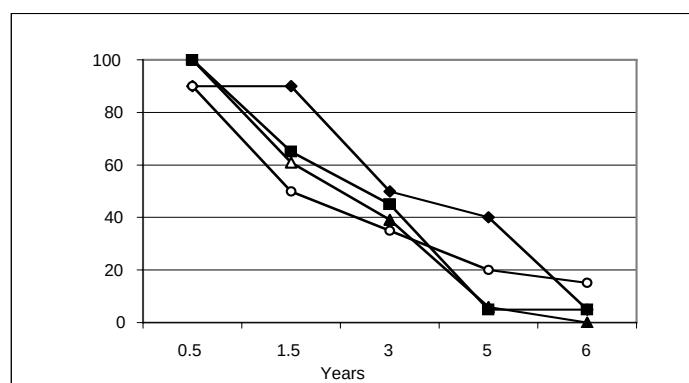


Figure 4.15 Survival of *E. globulus* at Pinjarra

■ *E. globulus* T, ◆ *E. globulus* K, △ *E. globulus* O, ○ *E. globulus* F.

Table 4.6 **Trees planted in July 1995 at Pinjarra**

Line	Code	Total no. of plants	
		(93)95*	(94)95*
<i>E. camaldulensis</i> parents—clones	42	10	
	83	10	
	85	10	
	84	10	
	87	10	
<i>E. camaldulensis</i> x <i>E. camaldulensis</i>	42 x 87	10	10
	83 x 85	10	14
	83 x 87		10
	84 x 85	10	12
	84 x 87	10	10
	85 x 52	10	9
	85 x 84		10
	87 x 42	10	6
<i>E. globulus</i> parents—seedlings	<i>E. glob</i> F	10	10
	<i>E. glob</i> K	10	10
	<i>E. glob</i> O	10	8
	<i>E. glob</i> T	10	10
<i>E. camaldulensis</i> x <i>E. globulus</i> seedlings	42 x F	10 (2)	
	83 x K	11 (1)	2
	83 x F	12 (3)	
	83 x O		2
	84 x F	10 (2)	
	85 x F	10 (3)	
	85 x K	11(1)	
	85 x O	10 (2)	
	85 x T	3 (1)	
	87 x F	9 (2)	
	87 x K	15 (2)	
	87 x T	3 (1)	
	87 x O	14 (4)	
Total		468	

Note: Abnormal seedling nos. in parentheses.

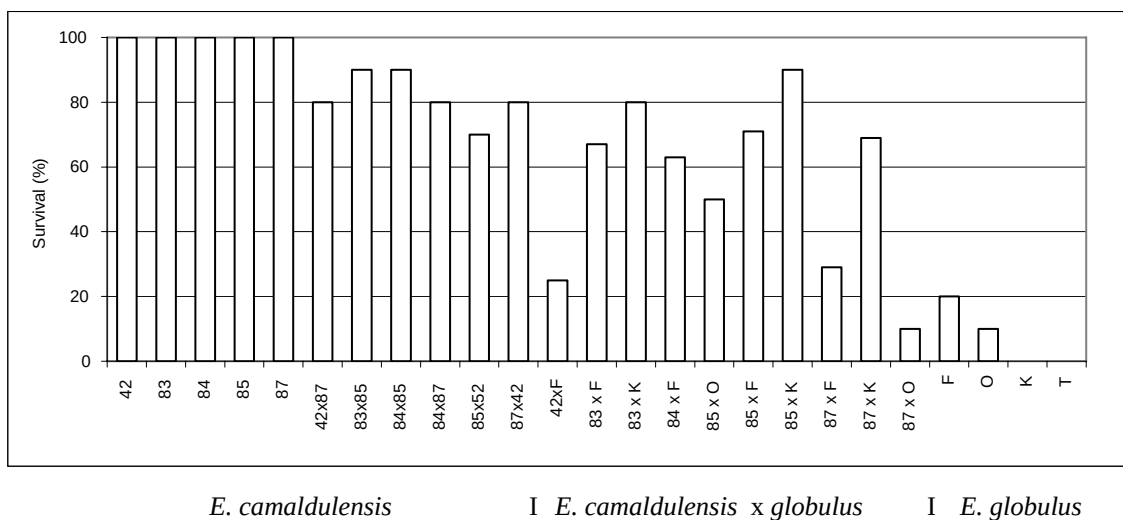


Figure 4.16 Pinjarra 2001: survival of trees planted in (93)95

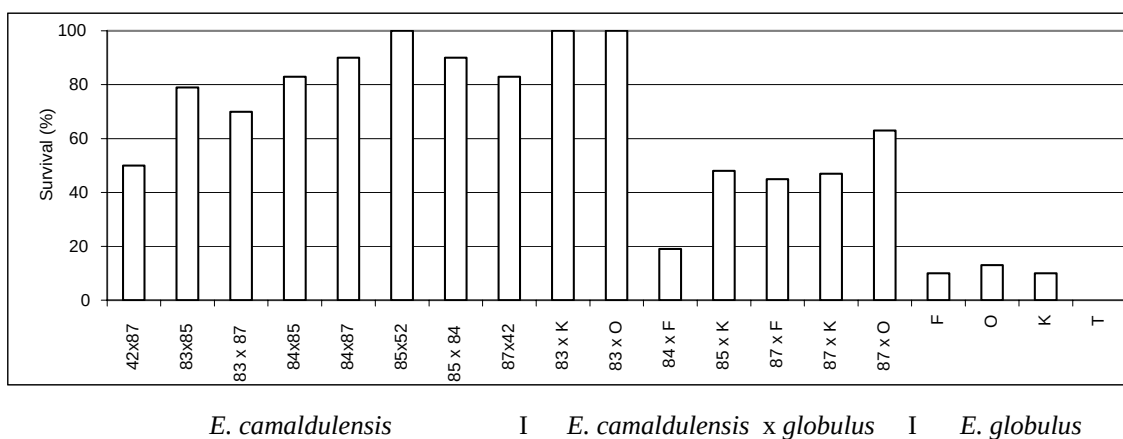


Figure 4.17 Pinjarra 2001: survival of trees planted in (94)95

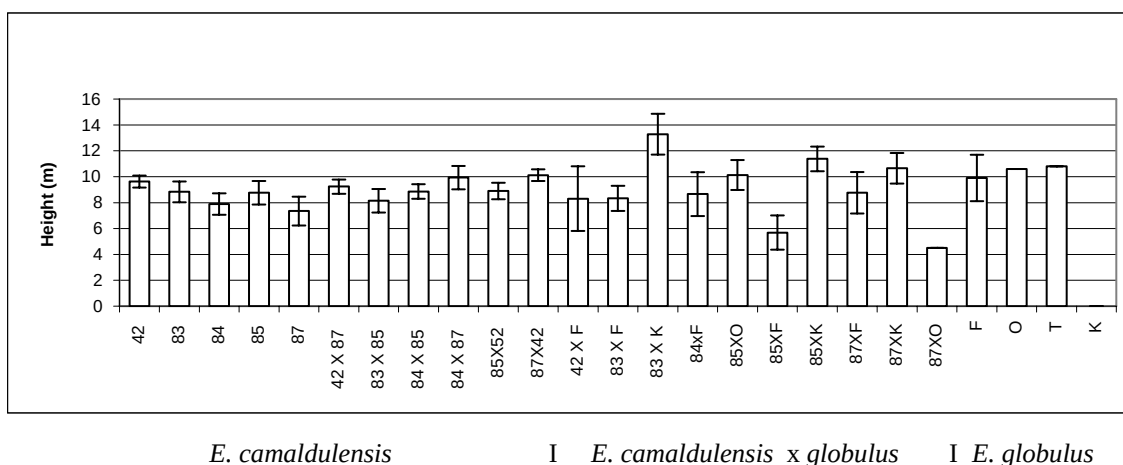


Figure 4.18 Pinjarra 2001: heights of trees planted in (93)95

Note: vertical bars indicate SEs of the means.

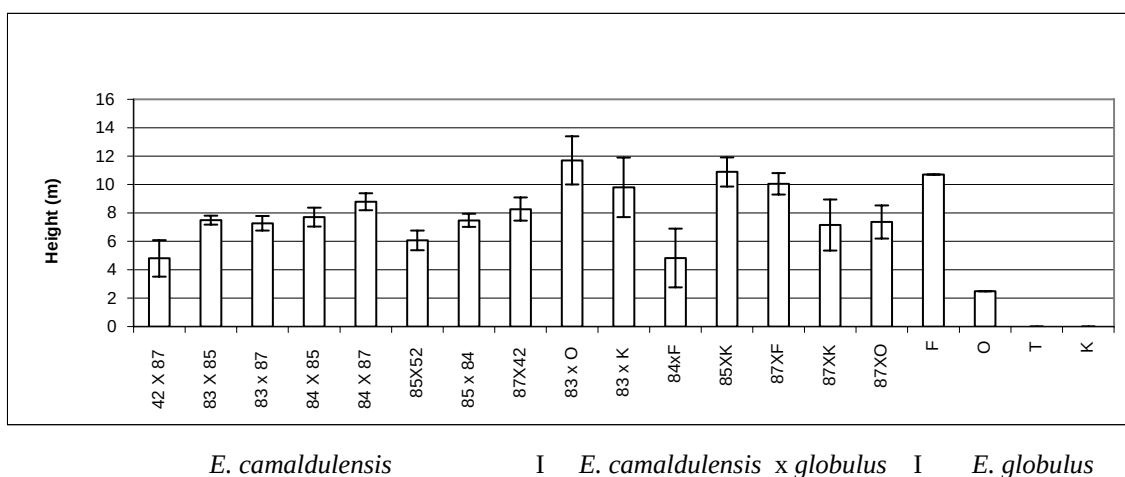


Figure 4.19 Pinjarra 2001: heights of trees planted in (94)95

Note: vertical bars indicate SEs of the means.

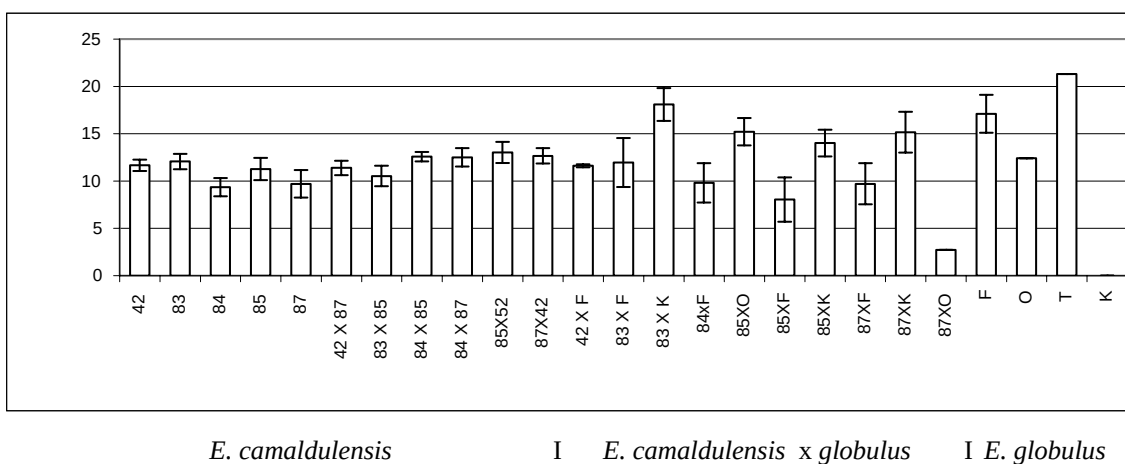


Figure 4.20 Pinjarra 2001: diameters of trees planted in (93)95

Note: vertical bars indicate SEs of the means.

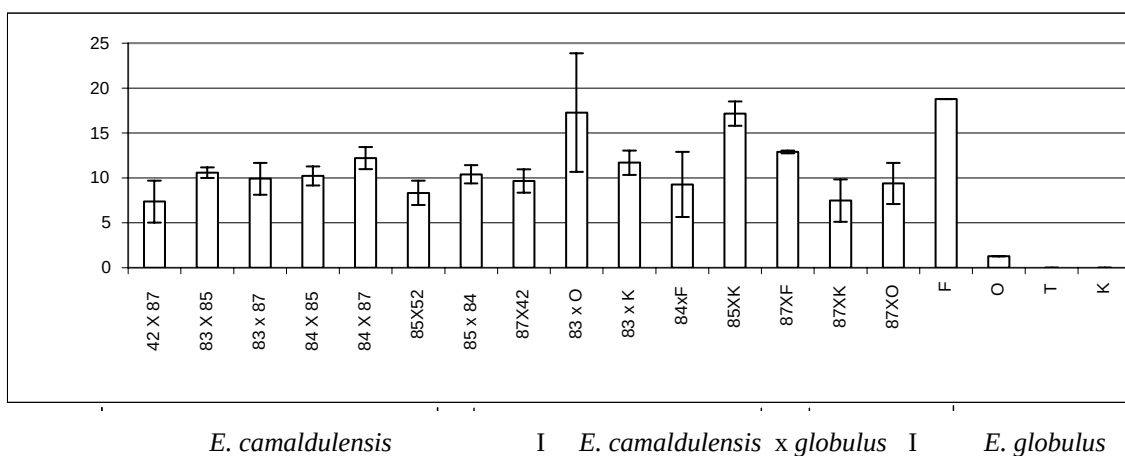


Figure 4.21 Pinjarra 2001: diameters of trees planted in (94)95

Note: vertical bars indicate SEs of the means.

However the survival data should be considered only indicative. The (93)95 plants were pot bound when planted out. The (94)95 seedlings of the hybrid crosses included ones that were abnormal. As it was impossible to monitor the development of abnormalities these individuals are included in the total and would have reduced the percentage of deaths.

Growth—height, diameter and form

There were differences between the heights of trees planted as pot-bound 22 month-old seedlings and those planted as 7 month-old seedlings. When the same lines are compared in 2001 the 7-month old seedlings in general had not reached the same height as those planted out as 22 month old plants (Figs 4.18, 19). The same is true for tree diameters (Fig 4.20, 1). The heights of the best hybrids were greater than those of the *E. camaldulensis*. The few surviving *E. globulus* were of good height but as mentioned above only 1–2 trees of the 20 planted of each line were alive at the end of 2001. The best hybrid lines were 83 x o, 83 x K, 85 x K, 87 x F, 87 x O 87 x K. (Note that some of the best trees from this plantation were removed in 2000 for assessment of wood quality).

Table 4.7 Form of 6-year-old trees grown at Pinjarra in 2001

Line	Code No	Mean Form (SE)	No. of trees
<i>E. camaldulensis</i> clones	42	0.5 (0.16)	10
	83	1.1 (0.18)	10
	84	0.6 (0.16)	10
	85	1.4 (0.16)	10
	87	0.9 (0.27)	10
<i>E. camaldulensis</i> x <i>camaldulensis</i> seedlings	42 x 87	0.9 (0.19)	13
	83 x 85	0.9 (0.14)	2
	83 x 87	1.1 (0.26)	20
	84 x 85	0.8 (0.14)	7
	84 x 87	0.7 (0.19)	6
	85 x 52	0.9 (0.19)	10
	85 x 84	1.0 (0.29)	2
	87 x 42	0.7 (0.16)	19
<i>E. camaldulensis</i> x <i>globulus</i> seedlings	42 x F	1.0 (0)	17
	83 x F	1.0 (0.26)	9
	83 x K	1.0 (0.25)	3
	83 x O	0 (0)	17
	84 x F	1.0 (0.24)	9
	85 x O	1.0 (0.58)	3
	85 x F	0.4 (0.25)	5
	85 x K	1.0 (0.16)	20
	87 x F	1.3 (0.28)	13
	87 x K	1.2 (0.20)	7
<i>E. globulus</i> seedlings	87 x O	1.0 (0.37)	17
	F	0 (0)	6
	O	1.0 (0)	3
	T	0.5 (0.5)	2
	K	0 (0)	1*

Note: Form rankings are described in Figure 4.1. (93)95 and (94)95 trees are bulked for this analysis.

*felled in 2000

The *E. camaldulensis* in general had more low branching than the *E. globulus* parents which had a single straight bole (Table 4.7). Forms scores of more than 1 indicate an unacceptable form for harvest. However these plants were seedlings of variable genotype and the hybrid lines included plants with the best form (0) as well as those with poor form (2). The extremely poor form (3) was never seen amongst seedlings, only from tissue cultured lines in other trials.

On this field site it was found that *E. camaldulensis* and *E. camaldulensis* x *globulus* hybrids could survive while *E. globulus* died. It has been observed that *E. camaldulensis* roots can penetrate clay (Bell *et al.* 1994) and the possibility should be examined that the hybrids have this ability while *E. globulus* does not.

No abnormalities were observed in plants that were scored as 'normal' at 18 months old. Thus after plants reach this age they can be used with confidence in further trials.

It was clear in this trial that the hybrids include numerous elite genotypes. Many of these would be killed if plants were screened for salt tolerance using the screening procedure described under Chapter 1. If hybrids are also to be considered for growth in areas not necessarily saline, but beyond the normal range of environments suitable for *E. globulus*, a screening method that does not kill the salt sensitive plants is desirable. Alternatively, plants could be cloned before the salt tolerance test so that the genotypes of plants killed during the test are retained. Plants could be cloned either in vitro or as cuttings, and abnormal genotypes discarded before the salt trial.

4.7 Cressy

4.7.1 Trial description

Cressy (41° 40' S 147° 05' E) is an agricultural district 175km north of Hobart, Tasmania, with an annual rainfall of 635 mm. The soil is a heavy, sodic clay (solodosed solonetz) and there are increasing soil salinity and waterlogging problems. The trial was planted on the 6th of December 1996. There were 3 blocks of 20 trees of *E. globulus*, *E. camaldulensis* (42 and 83) and the hybrids 85 x K 6.1, 87 x K 7.1 and 7.3. Trees were 2 m apart in rows with rows 3 m apart. The weeds were sprayed before planting and excessive growth of weeds necessitated slashing in years 1 and 2. Salinity in the area was assessed using an EM 31 and at 90 cm was EC(se) 1.5–5.5 mS/m with most reading around 3mS/m. Height and survival were assessed in 1998 and 1999.

4.7.2 Results and discussion

E. camaldulensis and *E. globulus* grew poorly on this site having a large amount of leaf damage from insects and frost. The clones grew well with 85 x K 6.1 having a mean height of 2.75 m after 3 years (Fig. 4.22). Clone 7.3 which was the smallest clone also grew poorly in other field trials.

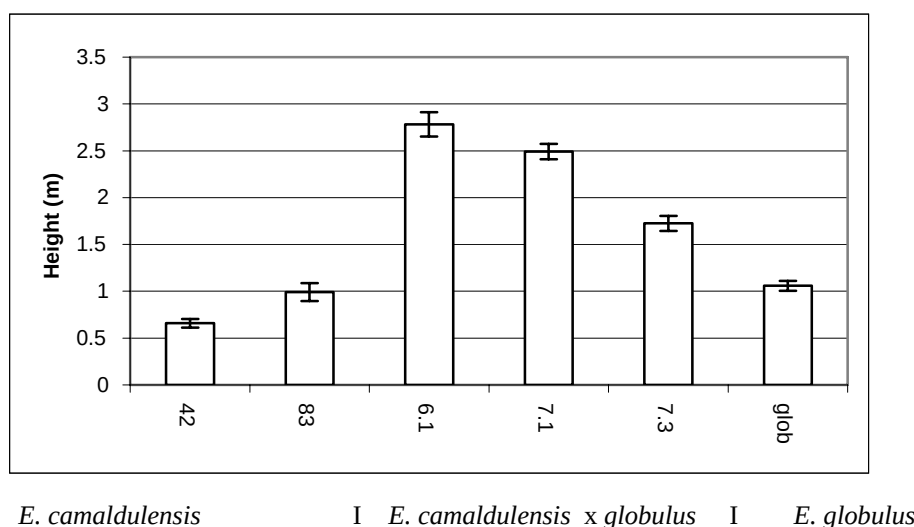


Figure 4.22 Cressy 1999: heights of clones and parent species

4.8 Coleambally

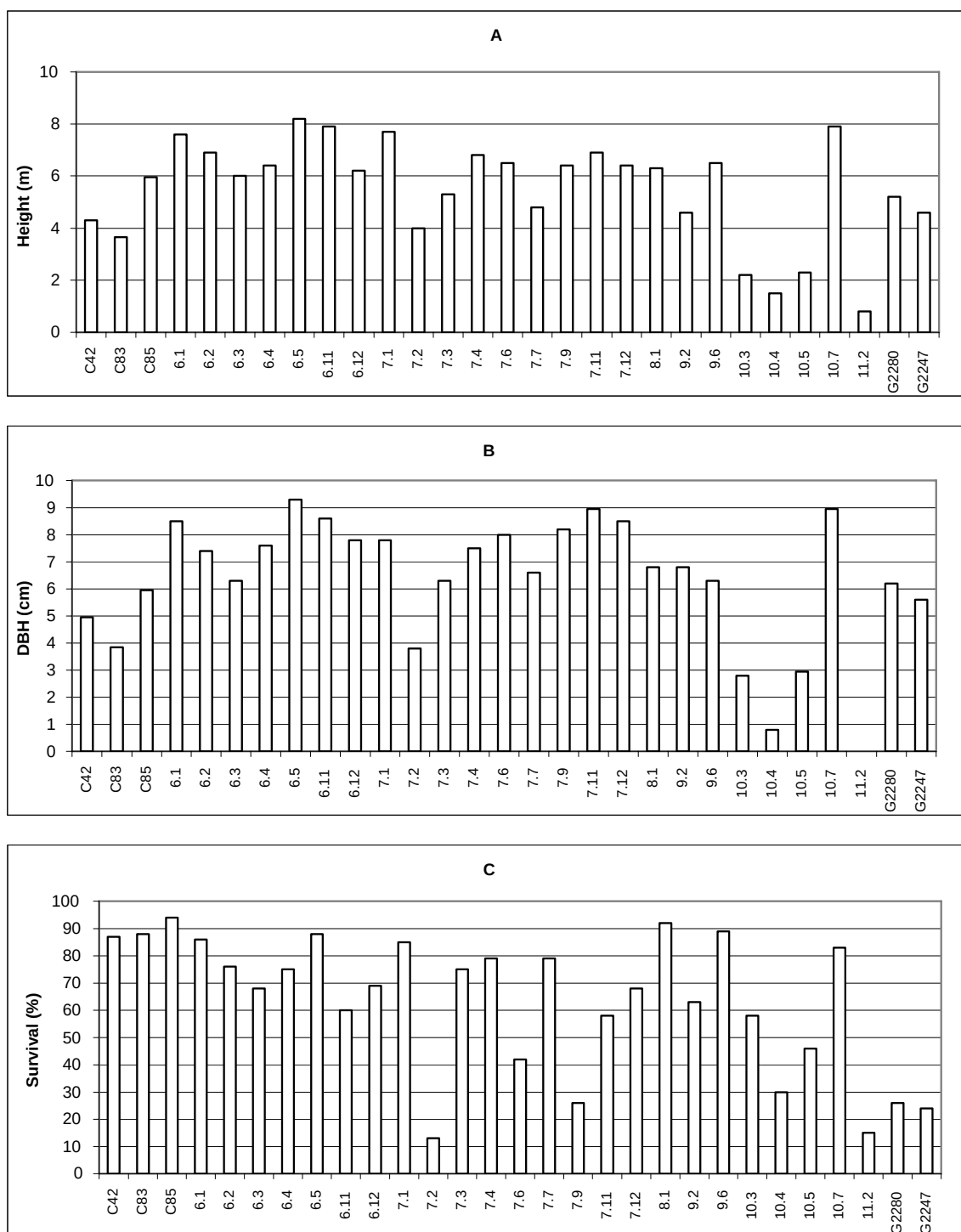
4.8.1 Trial description

Coleambally (35° 00' S 145° 45' E) is an irrigation district south of Griffith and 620 km south west of Sydney NSW that receives an average of 432 mm rainfall. The soil was medium to heavy clay and was non-saline. The trial was sited in the centre of a rice growing district where there is a growing salinity problem. There were 24 hybrid lines, 4 *E. camaldulensis* and 2 *E. globulus*. Trees were planted in 5 tree blocks replicated up to 13 times depending on the number of plants available. There were 3 m between rows and 2.5 m between trees in rows. The planting was in late in the season (September 1997) and was followed by 2 weeks of unseasonably hot weather with temperatures up to 42°C. The caused many deaths, particularly amongst the *E. globulus* and in the following year (September 1998) filler *E. globulus* were planted in the spaces. The survival of these trees was 83% in the milder season of that year. The trial was irrigated over the first two summers with non saline water, given only 3 waterings over 99/00 and no subsequent irrigation. The soil is not saline and the water table is at 4–5m. The trees were assessed annually to 2000.

4.8.2 Results and discussion

Survival was best for the *E. camaldulensis* clones, followed by the hybrids (variable 13–92%) with the *E. globulus* not exceeding 28%. *E. globulus* were the most severely affected by the hot dry weather in summer and by frost in winter.

The height of the best hybrids was clearly superior to that of the *E. camaldulensis* and *E. globulus*. The plagiotrophic lines 10.3, 10.4 and 11.2 were again the poorest lines. The best lines were 6.1, 6.5, 6.11, 7.1, and 10.7 with line 6.5 reaching 8.25 m in height and 9.3 cm DBH by the age of 3 years.



E. camaldulensis I *E. camaldulensis* x *globulus* I *E. globulus*

Figure 4.23 Coleambally: height, diameter and survival

A. Height in 2000, B. DBH in 2001 and C. Survival in 2000 for *E. camaldulensis*, hybrids and *E. globulus*.

4.9 Summary

Field trials of *E. camaldulensis*, *E. globulus* and cloned selected hybrids were established at Beverley and Mt Barker (WA) in 1995, Boyup Brook, Narrikup (WA), Coleambally (NSW) and Cressy (Tas) in 1997. A trial of seedling hybrids and parental species was also set up at Pinjarra (WA) in 1995.

Overall the survival of the *E. camaldulensis* was high, followed by the hybrids with *E. globulus* showing least survival. In 2001 the height of the best clones was equal or better than the height of the surviving *E. globulus*. The poorest clones were plagiotrophic (Having abnormal branched and horizontal growth). This may have been due to the selection of explant buds for tissue culture, but could also be genetic as it was confined to two families. When on each site the heights of the different types of trees were plotted against soil salinity, there was either no effect, or an equal fall in height for the hybrids and the *E. globulus*.

5 Wood properties

5.1 Introduction

Although the hybrid *E. camaldulensis* x *globulus* were initially developed with the objective of harvest for papermaking, as the trials progressed it became apparent that they also had potential as sawlogs, particularly when grown in areas remote from a chipping facility or port. The literature indicates that for *E. camaldulensis* and *E. globulus* up to 5.5 years old there is little difference between the wood properties of trees grown on saline and non saline land although there was a tendency towards greater density on saline sites (Clark *et al.* 1998; Catchpoole, Turnbull and Read, pers. comm.). Some of the 5 and 6 year old trees of hybrids and parent species growing at Pinjarra under non-saline conditions (Chapter 4.6) were sufficiently large for assessment of pulp and paper making qualities, and sawmilling and wood properties of logs.

5.2 Materials and methods

5.2.1 Harvest

Trees were selected for harvest on the basis of height, diameter and form. As mentioned in Chapter 3.3.2 all but one of the *E. globulus* K planted on this site were dead by 2000. This tree was selected together with two from each of *E. camaldulensis* 85 and 87, and *E. camaldulensis* x *globulus* 85 x K and 87 x K.

Trees were felled and samples collected on the 3rd of October 2000 (Figure 5.1). The DBH, total height and merchantable height (height where the stem is a minimum of 8 cm diameter under bark) were measured. Sample disks 2 cm thick were taken at 10%, 30% and 70% of the merchantable height of the trunk (Fig. 5.2). Discs were analyzed by North Forests Products for pulp yield, active alkali and basic density. The data from this analysis is provided to JVAP as Commercial in Confidence. The remaining logs from the four hybrid trees varied in length from 60–149 cm were transferred to the Forest Products Commission Timber Technology mill at Harvey Western Australia. Three of the trees produced both butt and crown logs, and the remaining tree produced only a butt log. The logs were quite severely checked (cracked) upon arrival at Timber Technology. The report below on the milling and sawlog properties of these logs was largely taken from a report prepared by Mr. S. Davis, Forest Products Commission.

5.2.2 Milling and assessment

The logs were sawn on a 'Woodmizer' portable bandsaw. Matched specimens would normally be cut from each side of the heart, but this was not possible in all cases due to checking and internal defect. Where log quality permitted, a 28 mm plank was milled from bark to bark through the heart. The plank was then sawn into 28 mm wide sections for wood density assessment. The density specimens with growth rings parallel to one edge were also used for estimation of tangential and radial shrinkage.

Table 5.1 Details of trees used in the pulping tests

Line	Dates planted*	Position code	Height (m)	Merch Height (m)**	DBH (cm)	Form
<i>E. globulus</i> K	(94) 95	18 F	10.5	5.8	18.0	Good—single trunk
<i>E. camaldulensis</i> 87	(93) 95	3P	9.6	4.6	14.1	Good—1st branch at 3.7m
<i>E. camaldulensis</i> 87	(93) 95	4P	9.3	3.3	12.2	Good—1st branch at 3.6m
<i>E. camaldulensis</i> 85	(93) 95	4O	9.0	3.3	12.6	Trunk crooked but few branches
<i>E. camaldulensis</i> 85	(93) 95	12Q	6.7	3.0	12.8	Several trunks from base—one sampled
Hybrid 85 x K	(93) 95	2W	10.9	6.0	24.0	Good—1st branch at 2.6m
Hybrid 85 x K	(94) 95	14O	11.1	7.0	25.3	Good—single trunk
Hybrid 87 x K	(93) 95	6K	12.5	6.6	20.8	Moderate—1st fork at 2.5m
Hybrid 87 x K	(94) 95	13A	13.7	9.0	28.7	Good—1st branch at 2m

Note: Milling and sawlog trials were done on the hybrids only.

*(93) 95 seeds planted in August 1993 and plants grown in 200mm pots before transfer of 22-month old plants to the field in July 95

(94) 95 seeds planted in Nov 1994, plants 7 months old on transfer to the field in July 1995

** merchantable height = height to trunk 8cm diameter under bark

The sections were then docked to 200 mm length and sanded on four sides using a belt sander to give a smooth, consistent surface, and specimen dimensions of approximately 25 x 25 mm. The properties measured were green density (green mass / green volume), basic density (oven dry mass / green volume), and air dry density (air-dry mass / air dry volume). Lines were drawn across the tangential and radial faces in the centre of the specimens to ensure that the dimensions were measured in the same positions each time. The specimens were then immersed in water for 24 hours to equalize moisture contents prior to taking measurements.

In assessing wood density and shrinkage: mass, length, width and thickness measurements were taken every two days and recorded while the specimens air dried on a wire mesh sheet. Mass was measured on a balance accurate to 0.01 g. The dimensional measurements were taken with a Vernier caliper accurate to 0.03 mm. When equilibrium moisture content of about 12% MC was reached the specimens were oven dried at 103°C to constant weight.



Figure 5.1 Harvest of *E. camaldulensis* x *globulus* 85 x K and extraction of the sample disks

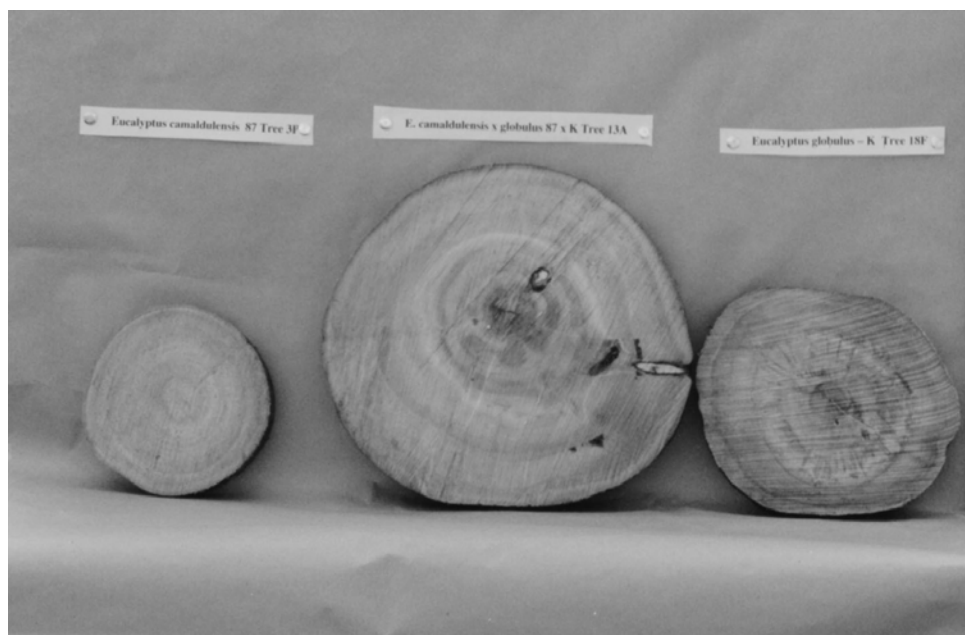


Figure 5.2 Sample disks

Sample disks from *E. camaldulensis* 87 (LHS) *E. camaldulensis* x *globulus* 87 x K (centre) and *E. globulus* K (RHS).

5.3 Results and discussion

Despite severe checking upon arrival at Timber Technology and the presence of internal defects, sawing the logs on the 'Woodmizer' presented no problems. Growth stresses were not evident, but this could be attributed to stress release caused by the checking prior to milling. The slabs from which the specimens were later prepared were quite knotty and cracked, and it was not possible to prepare a complete set of defect free specimens, as explained previously.

The density specimens dried at a uniform rate, although collapse was evident in some specimens. In a commercial operation using this timber, a steam treatment at approximately 18 % MC would be necessary to recover the wood. Moisture content, green density, basic density and air dry density are given in Table 5.2, and tangential, radial and longitudinal shrinkage values are given in Table 5.3.

The wood density measurements from this assessment are compared with published data in Table 5.4. The results show that the five year old hybrid timber has significantly lower basic and air dry density than 13 year old *E. globulus* (Moore *et al.* 1996), although one hybrid parent (*E. camaldulensis*) is a high density timber. The young age is presumably the reason for this difference.

Also in Table 5.4, shrinkage measurements of the hybrid are compared with published data from Kingston and Risdon (1961). The hybrid shrank 9.6% tangentially and 5.2% radially, compared with 8.9 % and 4.4 % respectively for mature *E. camaldulensis*, and 7.7 % and 6.1 % respectively for mature *E. globulus*. Age difference is presumably the reason for this difference also.

Resprouting from the cut stumps was recorded after 9 months. The *E. globulus* had not resprouted, nor had one 85 and one tree of the hybrid 85x K. The remaining 3 *E. camaldulensis* and 3 hybrid trees were regenerating vigorously.

5.4 Summary

Trees of *E. camaldulensis*, *E. globulus* K and *E. camaldulensis* x *globulus* hybrids growing at Pinjarra were harvested for assessment of wood fibre and saw logs. Trees had been in the field for 5 years at the time of felling. It is expected that under normal growing conditions all properties will improve with age (Jim French, North Eucalypt Technologies, pers. comm.). The wood of *E. camaldulensis* x *globulus* has potential for value-added purposes. Assessment of the timber properties and processing behaviour indicated that there were no problems with saw milling, drying and sanding. Wood density was in the medium range with basic density of 507 kg/m³ and air-dry density of 653 kg/m³. Shrinkage of 9.6 % tangentially and 5.2 % radially (before reconditioning) was greater than the shrinkage of jarrah (*E. marginata*) and river red gum (*E. camaldulensis*), but less than that of 17–23 year old Tasmanian bluegum (*E. globulus* subsp *globulus*). Further tests of wood properties of older trees will be necessary using plantations where replicates of the same genotype can be tested, and where trees are growing surrounded by equally vigorous genotypes. Comparisons of wood properties from plantations on land of a range of salinities will also be of value.

Table 5.2 Moisture content, green density, basic density and air dry density of *E. camaldulensis* x *globulus* wood

Tree	Log no.	No. of samples	Mean moisture content (%)	Mean green density (kg/m ³)	Mean basic density (kg/m ³)	Mean air-dry density (kg/m ³)
Hybrid 87 x K	6K Crown	2	97	975	555	648
	6K Butt	4	101	995	497	685
	13 A Crown	4	86	986	532	666
	13A Butt	11	91(9)	979 (33)	513 (26)	649 (49)
Hybrid 85 x K	140 Butt	5	106(8)	996 (34)	483 (18)	622 (15)
	2W Crown	2	85	944	509	649
	2W Butt	2	87	952	510	677
Weighted mean			94 (10)	980 (34)	507 (28)	653 (45)

Note: The values in brackets denote the average standard deviation given only for five or more samples.

Table 5.3 Tangential, radial and longitudinal shrinkage of *E. camaldulensis* x *globulus* wood

Tree	Log no.	No. of samples	Mean tangential shrinkage (%)	Mean radial shrinkage (%)	Mean longitudinal shrinkage (%)
Hybrid 87 x K	6K Crown	1	12.7	6.5	0.6
	6K Butt	2	8.5	6.7	0.7
	13 A Crown	2	9.3	3.0	0.5 (0.3)
	13A Butt	6	7.4 (4.4)	5.2 (2.9)	0.4 (0.3)
Hybrid 85 x K	140 Butt	5	12.3 (2.2)	4.2 (1.8)	0.3 (0.2)
	2W Crown	2	N.S.	N.S.	0.2
	2W Butt	2	N.S.	N.S.	0.3
Weighted mean			9.6	5.2	0.4

Note: The values in brackets denote the average standard deviation given only for five or more samples.

N.S. =not suitable. Specimens did not have growth rings orientated parallel to the face.

Table 5.4 Comparisons of wood density and shrinkage of *Eucalyptus camaldulensis* x *E. globulus* hybrid timber with parent species and *E. marginata*

Species	Green density (kg/m ³)	Basic density (kg/m ³)	Air-dry density (kg/m ³)*	Tangential shrinkage (%)*	Radial shrinkage (%)*
<i>E. camaldulensis</i> x <i>globulus</i> (5 years old)	980	507	653	9.6	5.2
<i>E. camaldulensis</i> (mature) ¹		710	913	8.9	4.4
<i>E. globulus</i> (mature) ¹		681	900	7.7**	6.1**
<i>E. globulus</i> (17–23 years old) ¹		561	790	14.4	6.9
<i>E. globulus</i> (13 years old) ²	1040	538	737	8.5***	6.9***
<i>E. marginata</i> (mature) ¹		663	855	6.6	3.7

¹ Kingston and Risdon (1961), ² Moore *et al.* (1996),*Before reconditioning,

***Based on backsawn boards and not small specimens ,**Based on one specimen only

6 General discussion

Amongst hybrids between *E. camaldulensis* and *E. globulus* are vigorous genotypes that have potential for planting in saline areas, or in areas beyond the usual climatic limits for *E. globulus*. The wood of the hybrids is of medium density and has potential not only for pulp production but also as sawlogs.

Controlled pollination between *E. camaldulensis* and *E. globulus* produced 50% capsule set and an average of 9 seeds per capsule when *E. camaldulensis* was used as a female parent. Although the cut style method of pollination (Harbard *et al.* 1999; Williams *et al.* 1999) has raised the possibility of using *E. globulus* as a female parent in such crosses, the faster ripening time of the *E. camaldulensis* capsules (3–4 months) compared to *E. globulus* (up to a year) makes it more efficient to use *E. camaldulensis* as the female parent. There were high levels of lethal abnormalities in progenies (50–97%) with abnormality becoming evident in plants up to 22 months old, after which time no further abnormalities were evident. The proportion of abnormal plants varied between crosses and was related to the male parent. The *E. globulus* from King Island gave least abnormalities.

When the proportion of abnormalities in a family was compared with the field performance of that cross, it was seen that crosses that gave high abnormalities (cross 11, 85 x T, and cross 10, 87 x T) produced plants with mainly plagiotrophic or mallee type habit. This could have been an effect of the choice of explant bud for micropropagation, but it is suggestive that this growth form was confined to these two families. Cross 10 produced only one good clone, 10.7. When the number of normal plants per pollination event (Table 1.3) was compared with the number of superior lines in the field (Chapter 4, Table 6.1) it was evident that for unit effort, it is much more effective to concentrate on producing and selecting from progeny of families with high proportions of normal plants.

For plantation use of *E. camaldulensis* x *globulus* hybrids a wider range of genotypes should be produced. It is known that Saltgrow has additional hybrid lines under trial (Dr. Glenn Dale, Tree Crop Technologies, Brisbane pers. com.). The hybrids are fertile, so to generate a broader genetic base backcrosses with *E. globulus* are possible as well as completely new hybrid combinations. When using the superior *E. camaldulensis* trees as female parents, crossing can be facilitated by felling the trees and using the more accessible flowers on the basal coppice.

The hybrids were intermediate between the parent species in many traits. The ease of propagation was more similar to *E. camaldulensis* in that the hybrids will grow well and develop roots in tissue culture; a feature of *E. camaldulensis* which is lacking in *E. globulus*. For rooting of cuttings too, the hybrid is more similar to *E. camaldulensis* and roots well, whereas *E. globulus* cuttings are more difficult. The clonal lines generated by this research are held by Plant Biotechnologies Tasmania and the maintenance is being paid by Gunn's Ltd. Forest Research Centre. More research is needed to improve propagation efficiency in order to reduce the price of the ramets.

As is the case with most eucalypt hybrids the *E. camaldulensis* x *globulus* plants were morphologically distinct from both parents. In vegetative morphology the young seedlings were closer to *E. globulus* than *E. camaldulensis*. Flower and fruit shape and size were intermediate, but hybrids were closer to *E. camaldulensis* than *E. globulus* in having pedicellate flowers in a cluster of 3–6, and exerted valves on the fruit.

Salt tolerance of the hybrid seedlings was tested under glasshouse conditions using a trial design that exposed the seedlings to both salinity and waterlogging. Under these selection conditions the hybrid families showed a level of survival intermediate between the tolerant *E. camaldulensis* parent and the intolerant *E. globulus*. It was evident from the trial of hybrid seedlings at Pinjarra that the salt tolerance screening method used here resulted in the deaths of many non tolerant genotypes that may have shown excellent growth under non saline conditions. It would be better to clone the plants either in vitro or using cuttings before the salt tolerance testing so that the genotypes are not lost. The protocol of growing seedlings in vitro and micropropagating before salt screening was adopted by

Saltgrow (Dr. Glenn Dale, pers. com). This was successful although the abnormal genotypes caused some difficulties in vitro. New in vitro techniques are available that have improved the root strike and transplant survival of several Australian woody species. The technique involves using a sterile soil for the rooting phase, thus producing plants that have normal root structure, and resulting in minimal disturbance when plants are transferred to the glasshouse (Newell *et al.* 2002). It would be beneficial to test this method to reduce the cost of in vitro propagation of the hybrids.

The field trials tested survival and growth of plants on sites ranging from non-saline to highly salinity (EC (1:5) above 150 mS/m), but most plants were on soil with low to moderate salinity. There were 3 trials set up in 1995 and a further 3 large and one small trial set up in 1997. They covered regions with rainfall from 432 to 948 mm (Table 6.1).

E. camaldulensis had the highest levels of survival in most sites. *E. globulus* survived less well than the *E. camaldulensis* or the hybrids on all sites. However the deaths amongst the *E. globulus* and the hybrids were mainly establishment deaths, and apart from the Pinjarra site, survival remained relatively constant after the first year. Total percentage of deaths amongst the *E. globulus* was less than expected in response to salinity. It is possible that the trees will be more affected by salinity in future as the increasing biomass necessitates greater use of saline ground water during summer. The future survival of hybrids and the *E. globulus* should be monitored.

The most marked superiority of the hybrids was seen in the saline sites at Beverley and Cressy, and the non saline sites of Coleambally and Pinjarra. Beverley had the lowest rainfall of any saline site, and at this site the hybrids had better survival and growth than the *E. globulus*. At Cressy, growth of both the *E. camaldulensis* and *E. globulus* parents was severely retarded by frost and insect damage while the hybrids grew well. At Pinjarra almost all the *E. globulus* died, despite being planted with wider spacing than a previous *E. globulus* plantation on the site that had also died. The site in NSW, Coleambally was irrigated over the first 3 summers, and the hybrids were clearly superior both in survival and height.

The expectation was that there would be a reduction in height of trees with increasing salinity and that this reduction would be least in *E. camaldulensis* and greatest in *E. globulus* with the hybrids in between. This expectation was not met on trials where the salinity was low to moderate (e.g. Beverley, Boyup Brook Narrikup). Where salinity was higher (e.g. Mt Barker) the differences in the slope of the plots of height against salinity did show that the hybrids were less affected by salinity than the *E. globulus*. However in terms of absolute growth, even with the reduction of height due to salinity the *E. globulus* were taller than the hybrids in salinities less than 120 mS/m. It is important to continue to monitor growth to determine whether or not there will be a relatively larger reduction in growth of *E. globulus* on saline sites compared to the hybrids as trees become larger.

Fastest growth was obtained at Coleambally (a non saline site), where the best clone reached 8.25m in 3 years. At Narrikup the next best site, the tallest clone was 7.75m in 3 years. It will be interesting to see how the absence of summer irrigation affects the growth rate of the Coleambally site in future. A study of the seasonal water use characteristics of the hybrids and the parent species on saline and non saline sites would be most valuable.

The success of the hybrids in areas outside the normal range for *E. globulus* (e.g. Beverley, Cressy, Coleambally, and a small trial not reported here at Northampton, 450 km north of Perth), make it clear that the hybrids should be tested for growth on both saline and non-saline areas to extend the range of land that can be used for trees for pulping. In areas remote from a chipping mill or port, hybrids might be grown for sawlogs.

Given the time scale of the JVAP funding contract it was only possible to test on wood fibre quality and sawlog properties on a small number of trees. These were 5-year-old seedlings (not salt tolerant clones), and had been grown at Pinjarra, a non-saline site on which nearly all the *E. globulus* had died. These preliminary results suggest that the hybrids may be suitable for wood fibre production and for

sawlogs. However the small number of trees sampled and their young age means that it is necessary to do further tests on the salt tolerant clones growing on both saline and non saline sites. Trials where the planting design has included 4 tree plots rather than single tree plots (as used at Pinjarra) may give results more comparable to those that might be expected from a plantation situation. Three of the four hybrid trees that were felled resprouted from the stumps.

The clones showing the best growth were the same regardless of the environmental conditions of the trial sites (Table 6.1). The predominance of good performers from the crosses 85 x K (cross 6) and 87 x K (cross 7) is evident. These crosses produced the highest numbers of normal progeny so there were more chances of selecting superior ones.

Table 6.1 Superior clones at the different field sites, and seedling families at Pinjarra

Location	Rainfall (mm)	Clonal lines giving best growth, in order of decreasing height
Clonal trials		
Coleambally*	432	6.1, 6.5, 6.11, 7.1, 10.7
Beverley low salt	432	6.2, 6.1, 7.4, 7.1
Beverley medium salt	432	6.2, 6.1, 5.2, 7.4, 7.1
Mt Barker	596	6.2, 6.1, 7.1
Cressy	635	6.1, 7.1
Boyup Brook	650	6.5, 6.12, 7.12, 7.9, 9.6, 7.6, 6.9, 7.1
Narrikup	735	10.7, 6.1, 6.2, 6.11
Seedling trial		
Pinjarra	948	83 x K (cross 5), 85 x K (6), 87 x K (7), 83 x O

Note: not all clones were planted at all sites.

*irrigated for first 3 summers

7 References

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