DEVELOPING CLONES FROM *EUCALYPTUS GLOBULUS* AND HYBRID SEEDLINGS BY STEM CUTTINGS PROPAGATION

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ABSTRACT

Clones were developed by stem cuttings propagation from 1700 potted seedlings of *Eucalyptus globulus* Labill. ssp. *globulus* and various *E. globulus* interspecific hybrids. Propagation traits were evaluated and the 10% of clones that were easiest to propagate were planted in clonal field trials. Ease of propagation varied widely between clones, families, and hybrids, although (overall) the hybrids were not dissimilar from the pure species. Rooting ability also varied widely between occasions within a clone, largely owing to management factors, increasing the error in clonal rankings and leading to under-estimates of clonal mean rooting ability. Over all harvests of cuttings, rooting was higher than 70% of survivors in 3–4% of all *E. globulus* and hybrid clones.

Keywords: rooting; selection; improvement.

INTRODUCTION

Rooted clonal cuttings of tropical and sub-tropical eucalypt species and their interspecific hybrids are planted on a large scale in Brazil (Zobel & Ikemori 1983), the Congo (Souvannavong 1992), and South Africa (Denison & Kietzka 1993). *Eucalyptus globulus* is the most widely planted species in Mediterranean climates but is relatively difficult to propagate, both by stem cuttings (Borralho & Wilson 1994) and by micropropagation (Willyams *et al.* 1992). Propagation techniques are desired both for the pure species and its interspecific hybrids. The latter would be useful on sites which are marginal for the pure species—for example, owing to the severity of winter cold or summer drought.

An important and discrete part of clonal forestry is the development of new clones, which are selected from (variable) progenies of interest whether of seedlings or mature trees. Individual genotypes are propagated vegetatively (generally by stem cuttings), evaluated for propagation traits, and established in clonal field trials. Clones selected in the trials are then further multiplied from a clonal archive (Wilson 1996) to give the number of plants required to initiate large-scale propagation (about 10^4 for *Eucalyptus grandis* Hill ex Maid., Denison & Kietzka 1993).

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In forestry, new clones are often developed from plus-trees growing in the field. However, clonal development from seedlings may be advantageous because:

(a) It saves time, since traits of primary interest are evaluated mainly in clonal field trials rather than from the original phenotype;

(b) Seedlots newly created in a breeding programme are of the best existing pedigrees and therefore contain the best potential clones;

(c) Seedlings can be screened for propagation traits with less effort per potential clone than mature trees, reducing the cost of clonal development if clonal variation in these traits is high.

Clones were developed from seedlings of *E. globulus* ssp. *globulus* and several of its interspecific hybrids by stem cuttings propagation. Data were collected in a management (non-experimental) context.

MATERIALS AND METHODS

Propagation Details

The work was done in the development nursery of Celulose Beira Industrial (CELBI) S.A. in central coastal Portugal. Seedlings grown from seed in store, and rooted cuttings derived from them, were planted in 10-*l* pots containing peat : styrofoam (or perlite) : sand 1:1:1 (v:v:v) with slow-release fertiliser (SRF) (N:P:K 15:10:12) at 3 kg/m^3. They were grown either in a double-skin plastic greenhouse (DSPG) with minimum night temperatures of 10°–15°C and day temperatures 5°–10°C higher than the ambient of 10°–35°C, or outdoors without shade. The plants were harvested for cuttings every 2–4 weeks when the longest principal shoots were 20–30 cm long. Shoots shorter than 15 cm were left to facilitate renewed growth. New shoots arose either from the leafy axils of decapitated shoots or from the woody framework. The plants were periodically treated with 3 kg SRF/m^3 and occasionally form-pruned to maintain a low globular crown.

One apical cutting 12–15 cm long was prepared per shoot; it bore two leaf pairs three-quarter size to full-size which were trimmed, and one pair of immature leaves. The shoot apex was removed and leaf area per cutting was 25–40 cm^2. Cuttings were immersed for 30 seconds in Benomyl fungicide solution (2 g/*l*) then set in peat:perlite 3:2 (v:v) containing 3 kg SRF/m^3.

Cuttings were set in a glasshouse or outdoor shadehouse and were kept well wetted with intermittent mist. In the glasshouse, temperatures were 15°–20°C at night and 20°–32°C during the day, and relative humidity was generally over 90%. Cuttings were shaded on clear days with 85% shade and unshaded when overcast. The shadehouse had 85% shadecloth at the sides and 25% above, making it permeable to the weather.

Phase 1

Seedlings of 10 superior full-sib families of *E. globulus* ssp. *globulus*, belonging to the Portuguese land race, were planted into pots and grown in family groups in the DSPG. Family relationships are shown in Table 1.

At 15–25 cm height, the seedlings were rogued for poor growth and cut back, leaving 29–151 seedlings per family. They were harvested for cuttings up to five times (up to five

TABLE 1–Relationships among 10 full-sib families (A–J) of *Eucalyptus globulus* from which clones were developed.

Seed parent	Pollen parent						
	1	2	3	4	5	6	7
1							I
6							H
8		B					
9	A	C	D	E	F	G	
10							J

cuttings per plant per harvest) from March to July 1991 (Harvests i–v, Phase 1). Harvests i–iii were set in the glasshouse while Harvests iv–v were set in the shadehouse, which was a more favourable propagation environment during the summer (Wilson 1998a).

There were 30 *E. globulus* interspecific hybrid families, 12 of which were created in the CELBI breeding programme. Each of these had one *E. globulus* parent and several parents of these other species—*E. globulus* Labill. ssp. *bicostata* (Maid. *et al.*) Kirkp., *E. dalrympleana* Maid. ssp. *dalrympleana*, *E. macarthurii* Deane et Maid., and *E. globulus* Labill. ssp. *maidenii* (F. Muell.) Kirkp. *Eucalyptus globulus* was the pollen parent except in the × *bicostata* hybrid. The crosses were abbreviated to GB, DG, HG, and MG respectively. The other families were full-sib families of *E. grandis* × *E. globulus* (RG), created by the South African Forest Research Institute using CELBI pollen and superior *E. grandis* mothers. Hybrid seedlings were planted in pots outdoors and after roguing they were harvested for cuttings twice, in April/June and in July. Harvest (i) of the RG hybrids was set in the glasshouse while Harvest (ii), and both harvests of the other hybrids, were set in the shadehouse.

Phase 2

Clones were selected on the basis of rooting ability in Phase 1 and on family ranking in the breeding programme, and were propagated from groups of up to 20 mother plants per clone in the DSPG from December 1991 to April 1992 (Phase 2, Harvests 1–8). There were 92 clones in four families of *E. globulus* and 82 hybrid clones, which were treated identically throughout Phase 2. All clones were harvested in each harvest, 16 cuttings per clone per harvest in Harvests 1 and 2, and 40 cuttings per clone per harvest thereafter. The cuttings were set in the glasshouse. In Harvests 2–8, natural daylength of 10–12 hours was supplemented with high-pressure sodium lamps giving a further 8 hours of 40–60 $\mu E/m^2.s$ at cutting level from sunset onwards.

The clonal groups of mother plants were in rows of seven extending from next to the central aisle to next to the windows of the DSPG. There was more air movement near the windows while the middle of the greenhouse was relatively hot and humid during the day. Survival and rooting of cuttings from 18 rows and seven perpendicular "columns" of clones were analysed for mother plant position effects.

Survival and Rooting

After 35–40 days, the survival of cuttings was recorded as the number surviving as a percentage of the original number (surv(%)). Cuttings were "dead" if they had no surviving

stem below the level of the substrate or no remaining foliar area. The cuttings were weaned and moved to the DSPG to grow. After a further 30 days, unrooted cuttings were usually dead or moribund whereas rooted cuttings were usually growing actively. Borderline cuttings were eased upwards from the tray until it could be seen whether roots were present or not. The number of cuttings rooted as a percentage of those surviving at Days 35–40 (root(%)surv) and the number rooted as a percentage of the original number (root(%)orig) were recorded.

Untransformed percentages are cited but were angular-transformed before analysis of variance (in RCB designs). F ratio and r^2 values in the text were significant at the $p < 0.05^+$, 0.025^{++}, 0.01*, or 0.005** levels of significance. Least significant differences (LSDs) were sometimes calculated if the overall F value was significant. Initial survival, and the rooting of survivors, are reported and analysed separately because (in this context) they were not correlated, either phenotypically or genotypically (Borralho & Wilson 1994).

RESULTS

Phase 1

The results for *E. globulus* are shown in Tables 2 and 3. The percentage of plants harvested was an index of cuttings productivity because plants were not harvested if growth since the previous harvest had been insufficient. Variation between families in this trait was considerable (Table 2). A low percentage of mother plants was harvested in Harvest (iv) (Table 3) because the preceding interval had been short (14 days).

Variation in survival was non-significant between families (Table 2) but high between harvests (Table 3). Survival was higher in Harvests (iv)–(v), set in the shadehouse, than in Harvests (i)–(iii), set in the glasshouse ($LSD_{0.05}$, transformed data). Harvests (i)–(iii) were also significantly different from each other ($p < 0.05$). Variation in rooting was high between both families and harvests.

On a family basis, in simple linear regression, the percentage of mother plants harvested was unrelated to percentage survival but positively related to rooted percentage of survivors ($r^2 = 0.71$*, 9 d.f.). Percentage survival and rooted percentage of survivors were also negatively related on a family basis ($r^2 = 0.38^+$, 9 d.f.).

Seedlings of seven of the *E. globulus* families were ranked for standing volume 4 years after they were planted in field trials (Families H, I, and J were not represented; C. Deane, CELBI, pers. comm.). The ranking was related in simple linear regression to the family mean propagation traits shown in Table 2: the correlations with mother plants harvested, survival, and rooting of survivors were $r^2 = 0.71$*, 0.08 ns, and 0.56^+ (6 d.f.) respectively.

Among the hybrids, survival was uniformly high (93%–97%) but rooting varied more (Table 4). In Harvest (i) the hybrid seedlings had not previously been cut back. In some RG clones, regrowth after Harvest (i) was poor although the residual leaves (four full-size leaf pairs on the main stem) remained healthy. In the worst-affected family 78% of plants harvested in Harvest (i) were also harvested in Harvest (ii), compared to 92% in the other RG families combined. Rooting in this family in Harvest (i), of clones harvested in Harvest (i) only or also harvested in Harvest (ii), was 7% and 22% respectively ($\chi^2 = 8.7$** 1 d.f.). Thus, plants with poor (subsequent) regrowth ability yielded relatively low-rooting cuttings.

TABLE 2–Phase 1 harvests. (a) The number of seedlings harvested as a percentage of the number per family (Harv(%)), the mean number of cuttings surviving as a percentage of the original number (Surv(%)), and the number of cuttings rooted as a percentage of the survivors (R(%)surv) and of the original number (R(%)orig), in 10 full-sib families (A–J) of *E. globulus*. (b) Frequency distribution of clones in three classes of rooted(%) survivors.

(a) Seedlings harvested, cuttings surviving, and cuttings rooting

	Family										Mean	F
	A	B	C	D	E	F	G	H	I	J		
Harv(%)	88.3	94.9	89.6	84.0	92.3	86.5	86.2	71.9	80.0	70.1	84.4	5.5**
Surv(%)	61.5	68.9	72.8	76.3	64.2	72.8	75.7	71.6	65.8	77.5	70.7	1.9 ns
R(%)surv	45.8	62.9	54.3	21.5	63.6	20.6	18.3	13.2	25.7	7.6	33.3	9.3**
R(%)orig	28.2	43.3	39.6	16.4	40.8	15.0	13.9	9.5	16.9	5.9	23.5	

(b) Frequency distribution of R(%)surv

	A	B	C	D	E	F	G	H	I	J	Total
0–29%	29	17	5	78	3	116	124	46	23	136	577
30–69%	52	59	33	19	55	32	23	8	5	5	291
70–100%	18	61	12	2	40	1	4	0	1	0	139

$p < 0.005$**, 9, 36 d.f.

TABLE 3–Phase 1 harvests. Number of *Eucalyptus globulus* seedlings harvested as a percentage of the total (Harv(%)) and mean survival (Surv(%)), and rooted percentages (R(%)surv and R(%)orig) of cuttings harvested from them.

	Harvest					F
	(i)	(ii)	(iii)	(iv)	(v)	
Harv(%)	98.4	95.9	94.4	42.1	91.0	61.2**
Surv(%)	52.3	67.2	56.4	87.7	90.0	48.4**
R(%)surv	34.1	35.3	22.7	42.0	32.7	40.0**
R(%)orig	17.8	23.7	12.8	36.8	29.4	

** $p < 0.005$, 4, 36 d.f.

TABLE 4–Phase 1 harvests. (a) Number of families (N fams), number of seedlings harvested as a percentage of the total (Harv(%)), and the mean survival (Surv(%)) and rooted percentages (Root(%)surv and Root(%)orig) of cuttings harvested from them, in five *Eucalyptus globulus* interspecific hybrids. (b) Frequency distribution of clones in three classes of rooted percentage of survivors.

(a) Families, seedlings harvested, cuttings surviving, and cuttings rooting

	HG	DG	MG	GB	RG	Mean
Nfams	5	3	3	1	18	
Harv(%)	97.8	97.9	97.6	97.1	94.6	97.0
Surv(%)	94.7	93.6	96.6	95.1	92.8	94.6
Root(%)surv	54.7	23.2	58.6	48.9	37.3	44.5
Root(%)orig	51.8	21.7	56.6	46.5	34.6	42.2

(b) Frequency distribution of Root(%)surv

	HG	DG	MG	GB	RG	Total
0–29%	51	46	7	10	138	252
30–69%	73	17	22	17	132	261
70–100%	98	5	26	8	37	174

Phase 2

The results for both *E. globulus* and the hybrids are shown in Tables 5 and 6. There was little variation in survival between families and hybrids (Table 5; overall mean 83%) but high variation between harvests (Table 6). Survival in both the *E. globulus* and the hybrid cuttings was low in Harvest 1, which was made in mid-winter and in which no supplementary light was provided, and in Harvest 5 owing to visible position effects in the glasshouse. Within Families B and E (30 and 29 selected clones), clonal variation in survival was significant but the mean squares for harvests were over 10 times as great.

Rooting was much less in Phase 2 than in Phase 1, especially in the hybrids. For example, in Phase 1, family/hybrid mean rooting (selected clones only) varied from 73% to 91% (Families G and B respectively), and from 72% to 96% (hybrids DG and HG respectively) (cf. Table 5). Similarly, in the two highest-rooting *E. globulus* families (B and E), clonal mean rooted percentage of survivors (selected clones) varied from 82% to 100% (B) and

TABLE 5–Phase 2 harvests. Survival percentage (Surv(%)) and the number of cuttings rooted as a percentage of the survivors (R(%)surv) and of the original number (R(%)orig), in cuttings harvested from selected clones in four full-sib families of *Eucalyptus globulus* and five interspecific hybrids.

	E. globulus family				Mean	F
	A	B	G	E		
Surv(%)	81.6	79.9	84.6	78.5	81.1	1.8 ns
R(%)surv	30.1	65.6	55.0	54.4	51.3	37.5**
R(%)orig	24.6	52.4	46.5	42.7	41.6	

	Hybrids					Mean	F
	HG	DG	MG	GB	RG		
Surv(%)	82.2	75.8	85.1	84.8	91.5	83.9	2.5+
R(%)surv	35.9	31.7	41.7	46.7	56.1	42.4	10.0**
R(%)orig	29.5	24.0	35.4	39.6	51.3	35.6	

\+ $p < 0.05$ 3, 21 d.f. (*E. globulus*); 4, 28 d.f. (hybrids)
** $p < 0.005$
ns not significant

TABLE 6–Phase 2 harvests. Mean survival (Surv(%)), and rooted percentage of survivors (R(%)surv) and of the original number (R(%)orig), in eight harvests of cuttings.

	Harvest								Mean	F
	1	2	3	4	5	6	7	8		
Eucalyptus globulus										
Surv(%)	39.7	87.2	93.3	86.1	74.1	91.3	91.4	86.0	81.1	28.0**
R(%)surv	45.3	59.5	55.5	68.5	39.3	53.0	53.9	35.1	51.3	10.2**
R(%)orig	18.0	51.9	51.8	59.0	29.1	48.4	49.3	30.2	41.6	
Hybrids										
Surv(%)	51.2	93.2	95.9	89.3	73.8	93.0	92.7	82.2	83.9	10.4**
R(%)surv	47.4	38.1	53.2	61.1	32.8	45.0	37.0	24.7	42.4	9.2**
R(%)orig	24.3	35.5	51.0	54.6	24.2	41.8	34.3	20.3	35.6	

** $p < 0.005$ 7, 21 d.f. (*E. globulus*); 7, 28 d.f. (hybrids)

from 72% to 100% (E) in Phase 1, but from 31% to 86% and from 27% to 87% (E) in Phase 2. Within Families B and E, clonal variation in rooting was highly significant ($p < 0.005$), while the mean squares for harvests were approximately 5 times greater.

In clonal analyses (angular-transformed data) of Harvests 3–8 in Families B and E, in which there were 40 cuttings per plot (i.e., per clone per harvest), error variance was 109 and 121 respectively (survival) and 111 and 139 respectively (rooting). If all error variance is binomial it should be about 821/n in the angular scale (Snedecor & Cochran 1980) = 20.5, indicating that variation much in excess of the binomial was present.

Survival and rooting were generally uncorrelated in both the pure species and the hybrids. An occasional positive correlation was caused by a small minority of clones with atypically

high mortality and low rooting ability, probably due to locally unfavourable conditions in the propagation environment.

In Families B and E, over Harvests (i)–(v) selected clones, 1–4, and 5–8, family mean survival increased by about 15% while rooting decreased by about 30%. The first two harvest groups were about twice as different from each other as the second and third. Clonal mean values were generally poorly correlated between pairs of harvest groups and clonal rankings were only moderately consistent (Table 7).

There was no meaningful variation in the survival or rooting of cuttings due to the position of mother plants in the DSPG. Variation in survival percentage in the pure species was weakly significant ($F = 2.1^{+}$ 6, 54 d.f.) but was not associated with a recognisable gradient.

TABLE 7–Phase 2 harvests. Correlation coefficients (r) for clonal rooting and survival (above and below diagonals) in three harvest groups of cuttings and two families (B and E) of *Eucalyptus globulus* (containing 30 and 29 clones respectively). The third cell of the table shows the number of clones common to the 20 top-ranked clones (the top 10 in each family combined) in each pair of harvest groups.

Harvest group	Family B			Family E			n clones		
	i–v	1–4	5–8	i–v	1–4	5–8	i–v	1–4	5–8
i–v		0.17	0.00		0.32	0.29		8	7
1–4	–0.06		0.68*	0.11		0.50*	8		13
5–8	–0.18	0.12		0.22	0.58*		5	9	

* $p<0.01$

DISCUSSION

In developing clones of *E. globulus* and its hybrids from potted seedlings, propagation traits of interest included the cuttings productivity of mother plants, the initial survival of cuttings in the propagation environment, and the rooting ability of the survivors. These traits were suitable as initial criteria of selection because only a minority of clones were easy to propagate. In addition, survival and rooting were at least moderately heritable (Borralho & Wilson 1994) and, on a family basis, cuttings productivity, rooted percentage of surviving cuttings, and growth rate in the field were mutually positively correlated.

Rooting ability of cuttings from small *E. globulus* seedlings was only moderate (50%–70% of survivors, Wilson 1993), already reflecting marked "clonal" variation, so that indiscriminate ("family") propagation would soon be confined to the higher-rooting clones. Thus, correlations between propagation traits should be understood on a clonal basis (even in indiscriminate propagation) before being used to weight selection indices.

In Phase 1 more than 70% of survivors rooted in 14% and 25% of *E. globulus* and hybrid clones respectively, but in only 3%–4% of clones in Phases 1 and 2 combined. Mother plants were of seedling and clonal origin in Phases 1 and 2 respectively, but the form of the two origins, including root development and shoot (hence cutting) morphology, was very similar. Seasonal factors were unimportant since there were no trends in time, and the best and worst harvests (Phase 1 and the end of Phase 2) were both made in spring. The chronological age of the mother plants was also comparable.

On average, crown size was less in Phase 1 harvests since not all clones had grown sufficiently to be harvestable, while in Phase 2 all clones were harvested in all harvests. Thus, harvested shoots were on average shorter and less lignified in Phase 1, and this was probably advantageous for rooting. In a previous study, as woodiness increased at the base of *E. globulus* cuttings, rooting slightly decreased while survival increased (Wilson 1993); and in Phase 1 rooted percentage of survivors and survival were similarly negatively related (family basis). Other management factors probably contributed to the difference between Phase 1 and 2 harvests. In *E. globulus*, rooting ability within the clone can vary widely from harvest to harvest of cuttings and is sensitive to (for example) the fertiliser treatment of mother plants and their cuttings productivity in the interval before harvest (Wilson 1998a).

Thus, the lower rooting ability of Phase 2 was almost certainly avoidable. The standard of nursery management clearly has a large effect on propagation traits and can easily make the difference between success and failure.

On average, when 70% of survivors rooted, there were approximately 2.8 roots per rooted cutting, and approximately 25% of rooted cuttings had only one root (data not shown). For practical purposes some culling for poor initial growth, reflecting poor root development, was necessary, but most cuttings with one or a few roots grew satisfactorily in the nursery and in the field. A photograph of a clonal plantation of *E. globulus* in inland Portugal, taken at age 3 years and 8 months and established from a single first-generation plus-tree clone with moderate rooting ability, showed very uniform growth (Wilson 1995).

Assuming that at least 70% of survivors root in 3.5% of clones and that this rooting ability within the clone is adequate for practical purposes, one propagatable clone could be developed on average from 29 seedlings. However, there was high variation between families/hybrids. For example, based on the higher-rooting Phase 1 harvests, from three to an estimated minimum of 150 seedlings would be required in different *E. globulus* families (from Table 2). Clearly, an estimate of family mean rooting ability would be helpful at the outset, and could possibly be made from genetic data since the rooting ability of stem cuttings in *E. globulus* is moderately heritable (Borralho & Wilson 1994).

Such selection intensities represent a cost (but not necessarily a high one since the majority of clones can be discarded after very little work), and a constraint if the amount of available seed is limiting. If there is enough seed to give the desired final number of easy-to-propagate clones there is no formal constraint on families, although cost considerations would probably restrict their representation in practice. Within the family, restricting clonal plantings to the small or moderate proportion of clones which are easy to propagate is a risk, since propagation ability is poorly understood (Wilson 1998b), and propagation traits could not be used as criteria of selection in a breeding programme without narrowing the genetic base.

Family × harvest interactions in survival (but not rooting) were noted by Borralho & Wilson (1994), based on analyses of the Phase 1 data. Including the Phase 2 harvests, clonal rankings for both traits were only moderately consistent (Table 7), and in clonal analyses of Phase 2, error variation much in excess of the binomial (which may have been due to interaction) was present to a similar extent.

Within about 13 months of the first harvest of cuttings, clones had been ranked for propagation traits (based on 280–300 cuttings per clone), rooted cuttings from selected

(high-rooting) clones had been planted in clonal field trials, and approximately 50–200 additional rooted cuttings and potted plants per clone were available for a clonal archive. This multiplication rate was slower than an ideal rate (Wilson 1996) largely because the ideal rate was continuous while the selection at the end of Phase 1 created delay.

Potted mother plants were convenient for clonal development from seedlings. They could be kept in a greenhouse (increasing cuttings productivity in the seasonal Mediterranean climate) and were easily moved about at the end of Phase 1. The first selection was also made after very little effort per potential clone. This effort could be further reduced by making the first selection earlier but the rankings would then be more sensitive to error. The optimum timing (and possibly intensity) of selections therefore depends partly on the amount of variation in the propagation system.

CONCLUSION

Clonal development from *E. globulus* and hybrid seedlings by stem cuttings propagation was practicable, and initial selection for propagation traits was appropriate. However, variation in the propagation system has to be controlled before costs can be specified and selection strategy developed.

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