ABSTRACT

ROBINSON, THOMAS JUSTIN. Adventitious Rooting of *Eucalyptus benthamii* Maiden and Cambage and *Eucalyptus camaldulensis* Maiden Stem Cuttings. (Under the direction of Barry Goldfarb).

This study examined the production of stem cuttings and their rooting success of *Eucalyptus benthamii* Maiden and Cambage and *Eucalyptus camaldulensis* Maiden in response to family variation (5 families), fertilizer level (15.6 ml L^{-1} $week^{-1}$, 31.25 ml L^{-1} $week^{-1}$), pruning height (10 cm, 20 cm) on the stock plants, auxin application (0 mg L^{-1}, 2000 mg L^{-1}), and mist level (13.71 ml day^{-1}, 10.6 ml day^{-}1). The number of cuttings *E. benthamii* produced was positively influenced by 20 cm pruning height in 2012 and 2013 and by high fertilizer in 2013. No treatments significantly influenced number of cuttings in *E. camaldulensis*. Overall rooting percentage was 49.9% for E. benthamii and 79.9% for E. camaldulensis. Rooting varied significantly by family x pruning, family x fertilization and family x auxin for *E. benthamii*. Rooting varied significantly by family x mist level and pruning height for *E. camaldulensis*. The number of primary roots produced varied significantly by family in *E. benthamii* and by fertilization x auxin x pruning in *E. camaldulensis*. Auxin application had a positive effect on number of primary roots in *E. calmaldulensis*, while none of the applied treatments had a significant effect on *E. benthamii* on the number of primary roots produced.

Adventitious Rooting of *Eucalyptus benthamii* Maiden and Cambage and *Eucalyptus camaldulensis* Maiden Stem Cuttings

by
Thomas Justin Robinson

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APPROVED BY:

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Barry Goldfarb — Committee Chair

John King

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David Lindbo

BIOGRAPHY

Justin Robinson, born and raised in Gastonia, NC, has always had an interest in the science of living things. In 2001, he attended the University of North Carolina at Chapel Hill and earned a B.A. in Linguistics. After graduation, he decided to tour the world as a professional musician. In the fall of 2011, he enrolled in the Masters of Forestry program at North Carolina State University. After graduation, he plans to be gainfully employed in the Triangle region of North Carolina.

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INTRODUCTION

Eucalyptus has become one of the most important sources of hardwood pulp (Clarke et al., 2008), charcoal, solid timber and essential oils worldwide (Henry, 2011). Because of the large number of species, fast growth, coppicing ability, and adaptability to varied climatic conditions, productivity is very high, especially in tropical regions. There is also growing interest in Eucalyptus production in sub-tropical and temperate regions of the world for the production of hardwood pulp and saw timber products. *Eucalyptus benthamii* Maiden and Cambage and *Eucalyptus camaldulensis* Maiden*,* along with their hybrids, show promise as commercial species in temperate regions, such as southern Brazil and the southeastern US, given their fast growth and relative cold tolerance (Embrapa Florestas, 2012; Henry, 2011). Clonal production of *Eucalyptus* is widely utilized by forestry companies around the world to decrease heterogeneity, decrease disease incidence, increase desirable traits, and increase commercial productivity (Hartney, 1980; Xavier & Silva, 2010). Like other *Eucalyptus* species, *E. benthamii* shows high coppicing ability which makes it a suitable choice for vegetative propagation. However, *E. benthamii*, like other subtropical members of the genus, often shows recalcitrance to rooting using stem cuttings (Assis et al., 2004; Graça et al., 1999). Mini-cuttings are produced from axillary shoots of stem cuttings and micro-cuttings are produced from shoot apices of micro propagated plantlets and are produced *in vitro* (Assis et al., 2004). Although research has been conducted on propagation of *E. benthamii* and its hybrids by mini-cuttings grown in semi-hydroponic systems (Brondani et al., 2012a; Brondani et al., 2012b; Brondani et al., 2010a, 2010b; Brondani et al., 2008; Kratz et al., 2012; Cunha et al., 2005), very little research has been published on rooted stem cuttings of the species. If *E. benthamii* is planted widely in temperate region, then it will be important to develop an economical, efficient, and reliable method of producing *Eucalyptus* rooted cuttings that is scalable to operational production of reforestation stock.

Adventitious rooting of cuttings is influenced by mineral nutrition, ontogenetic age, and genetic provenance of donor plants, as well as auxin treatment and amount of water applied to cuttings. Management of stock plants has been shown to have an effect on the survival and subsequent rooting percentages of cuttings (Blake 1980; Mankessi et al., 2011; Trueman et al., 2012; Rowe et al., 2002; da Costa et al., 2013). In particular, nitrogen fertilization of donor plants has been shown to positively influence rooting of cuttings in *Eucalyptus globulus* Labillardiére (Schwambach et al., 2005; Fernandez et al., 2007), eastern redcedar (Henry et al., 1992), guaraná (Albertino et al., 2012), *Prosopis alba* Grisebach (De Souza & Felker, 1986), loblolly pine (Rowe et al. 2002), dwarf elephantgrass (Rusland, Sollenberger, & Jones, 1993) and various *Eucalyptus* hybrids (Cunha et al., 2009). High leaf nitrogen concentrations have also been correlated with frost tolerance, reduced mortality, height growth, and increased diameter growth in cuttings (Fernandez et al. 2007; Albertino et al. 2012; Husen & Pal, 2003). Rosa et al. (2009) found that mini-stumps produced more cuttings with increasing levels of nitrogen. Phosphorous and potassium have also been shown to positively influence rooting in conjunction with nitrogen fertilization (Henry et al., 1992, Fernandez et al. 2007, Lopez-Bucio et al., 2002; De Souza & Felker, 1986). DeSouza & Felker. (1986), Henry et al. (1992) and Rein et al. (1991) also note that increasing nitrogen fertilization of stock plants past an optimum level results in decreased rooting in cuttings. High nitrogen fertilization has been observed to decrease the root:shoot ratio in hybrid

Eucalyptus seedlings (Zeng, et al., 2013), in *E camaldulensis* seedlings (Siddiqui et al., 2008) and petunia cuttings (Santos et al., 2011), an undesirable characteristic for the production of rooted cuttings. However, it is not known how *E. benthamii* and *E. camaldulensis* stem cuttings root in response to fertilization levels of donor plants.

The importance of juvenile plant material as the donor source for rooted cuttings has been studied in *E. camaldulensis* (Bindumadhava et al., 2011), *Eucalyptus urophylla* Blake x *Eucalyptus grandis* W. Hill ex Maiden (Mankessi et al. 2011), *Eucalyptus grandis* (Patton et al., 1970), *Eucalyptus obliqua* L'Héritier (Blake & Carrodus1970), various *Eucalyptus* spp. (Higashi et al., 2000; Assis et al. 2004) and is the basis for current large-scale Eucalypt production. Ageing is a complex process that involves many factors, which may include the production of root inhibiting substances (Patton et al. 1970). Blake and Carrodus (1970) detected root inhibiting substances as early as 10 days after coppicing *Eucalyptus obliqua*. Stem, mini- and micro-cutting techniques make use of the higher rooting ability of juvenile material by taking cuttings from plants that are kept ontogenetically young by means of a series of pruning known as hedging or coppicing (Awang et al., 2011; Grossnickle & Russell 1993; Assis et al., 2004; Husen & Pal, 2006). However, a study by Bindumadhava et al. (2011) observed that *Eucalyptus camaldulensis* mini-cuttings rooted earlier and had greater dry weights than stem cuttings. Mankessi et al. (2011), did not observe rooting differences in cuttings taken from mature and juvenile portions of *Eucalyptus urophylla* x *Eucalyptus grandis* donor trees, though he did observe survival differences based on ontogenetic age and season. The ageing of shoots is related to its position on the plant. Plants further away from the roots are ontogenetically older than their counterparts that are nearer to the root

(Mankessi et al., 2009), therefore, pruning can induce rejuvenation of stock plants resulting in juvenile physiological characteristics (Hackett & Murray, 1992), such as ease of rooting. It is not known how *E. benthamii* stem cuttings root in response to differences in ontogenetic age.

The application of exogenous phytohormones, namely auxins, is widely used in clonal propagation (Assis et al., 2004), the most common being idole-3-butyric acid (IBA), to stimulate adventitious rooting in cuttings of various species (Fogaça & Fett-Neto, 2005). Several studies assessed the effect of varying concentrations of IBA on survival and rooting success of *E. benthamii* (Brondani et al., 2012b), and *Eucalyptus benthamii* x *Eucalyptus dunnii* Maiden mini-cuttings (Brondani et al., 2008; Brondani et al., 2010a) and *Eucalyptus saligna* Smith and *Eucalyptus globulus* micro-cuttings (Fett-Neto et al., 2001). Mini-cuttings studies of *E. benthamii* and its hybrids conducted by Brondani et al. (2010a, 2012a), in which basal portions of the cuttings were immersed for 10 seconds, exhibited the highest rooting rates at IBA concentrations between 2000 and 6000 mg $L^{-1.}$ Goulart et al. (2008) observed toxicity in IBA concentrations above 2000 mg L^{-1} (15s immersion) in *Eucalyptus grandis* x *Eucalyptus urophylla* mini-cuttings. In a technical paper on *Eucalyptus* spp. clonal propagation, xavier and Silva (2010) suggest an IBA concentration of 6000 to 8000 mg L^{-1} for stem cuttings. *Eucalyptus camaldulensis* mini-cuttings have been shown to root equally well with or without the application of auxin (Bindumadhava et al., 2011) though the method of application was not reported. We do not know how the rooting of *E. benthamii* nor *E. camaldulensis* stem cuttings respond to auxin application.

As cuttings do not have root systems to take up water, cuttings must be kept moist during the root initiation phase and this is often accomplished by misting. The literature regarding misting regimes for the rooting of Eucalyptus cuttings is scarce, however, Sasse and Sands (1996) observed that height and diameter growth rates were reduced in water-stressed *Eucalyptus globulus* cuttings. In loblolly pine cuttings, the highest rooting percentages occurred when the water potential of cuttings were between -0.5 and -1.2 MPa while the poorest rooting occurred at severe or no water deficit (Lebude et al., 2004). Greenwood et al. (1980) studied shortleaf and loblolly pines and also found an optimal mist level for rooting, citing lower rooting with higher mist levels. It is not known how rooting of *E. benthamii* and *E. camaldulensis* stem cuttings responds to different mist levels.

Genetic variation has also been shown to be an important factor in the rooting of both stem and mini-cuttings. Greenwood and Weir (1994) found that rooting varied significantly in half- and full-sibling families in loblolly pine hypocotyl and woody cuttings. Similar results were reported in white spruce (Gravel-Grenier et al., 2011) and in *Eucalyptus tereticornis* Smith (Ginwal, 2009). In studies in which clones were evaluated for rooting, clones were significantly different from at least one other clone in *Eucalyptus benthamii x Eucalyptus dunnii* Maiden mini-cuttings (Brondani et al. 2010a; Brondani et al. 2008; Brondani et al. 2010b; Brondani et al. 2012a) and *E. grandis* x *E. urophylla* mini-cuttings (Goulart et al., 2008; De Melo et al., 2011). This is corroborated by Hartmann et al. (2002) who observes that individual clones may have specific propagation requirements. It is not known how genetic differences influence rooting in *E. benthamii* and *E. camaldulensis*.

Therefore, we hypothesized that fertilizer application, hedge height, auxin application, mist level and family variation will influence rooting. In addition, we hypothesized that the number of primary roots and cuttings produced by each species would be influenced by fertilizer application, hedge height, auxin application, mist level, and genetic variation.

We studied the relationship between rooting percentage and family, mist level, auxin application, fertilization level, and hedge height to determine if these treatments, alone or in combination, would influence adventitious rooting percentage, number of primary roots and cuttings produced by *Eucalyptus benthamii* and *Eucalyptus camaldulensis*. We also investigated the relationship between fertilization level and hedge height and number of cuttings produced for both species. Because *E. camaldulensis* is not considered recalcitrant to rooting, we imposed the same treatments to this species as a reference species.

MATERIALS AND METHODS

Stock plant management

Seeds of *Eucalyptus benthamii* and *Eucalyptus calmaldulensis,* from five families per species, were germinated in the misthouse in January 2012 at the Horticulture Field Lab at North Carolina State University (35°47' N, 78°39' W). Seedlings were transplanted (June 2012) to 7.5 l containers consisting of a 3:1:1 mixture of pine bark, sand, and perlite and grown outside from June to November, after which plants were moved to the misthouse. Plants were fertilized at time of transplantation with 59.1 ml of 4 month slow-release Osmocote ™ (19-6-12) and supplemented with 100 ppm Peters' Professional® fertilizer (21-7-7) weekly until plants were moved to the misthouse. For *E. benthamii,* each family had 12 plants while *E. camaldulensis* had four plants per family. 8 months (Sept 2012), plants were hedged to three heights: 0 cm, 10 cm and 20 cm above the soil surface in order to produce cuttings. The number of cuttings, at least 7 cm in length, produced by each plant was counted. Plants were later hedged to 30 cm to prevent excessive growth in the misthouse. Between November and February 2013, plants were fertilized with the above fertilizer solution twice. Plants were also treated with Strike® 50 WDG and later Neem Oil (7.81 mL L^{-1})to stop and prevent growth of powdery mildew on stock plants. Plants were monitored for insect pests and foliar spray was applied when required.

Cuttings management

In April 2013, stock plants were hedged to 10 or 20 cm height depending on their previous designation. We omitted the 0 cm hedge treatment since these plant produced no shoots. Plants were fertilized with Peter's Professional® fertilizer (21-5-20) at a concentration of 500 ppm. High fertilizer treatments were fertilized twice per week while low fertilizer treatments received fertilizer once per week. Stock plants resprouted soon after, and terminal shoots that were greater than 7cm long, contained at least two nodes, and one pair of expanded leaves were collected as cutting material. Cuttings were wrapped in moist paper towels and stored in Styrofoam™ coolers to prevent loss of turgor pressure. Foliar area was reduced by half and 1 cm of basal portions of cuttings were dipped in 2000 mg L^{-1} IBA solution or 1:1 ethanol-water control solution for 10 seconds. Cuttings were then planted at 2 cm depth in pre-soaked containers (Ray-Leach “Cone-Tainers”, Stuewe and Sons, Corvallis, OR) containing a medium of 60:40 perlite to peat ratio. The environmental management software (Q-Com, Irvine, CA) managed the misting frequency and triggered a traveling gantry (boom) system (ITS, McConkey, Mt. Puyallup, WA) to apply mist using a variable frequency according to the time of day and relative humidity in the misthouse (LeBude et al., 2004). Mist levels were created by adjusting boom traveling speeds to apply high mist (53 ml ft^{-2} per pass) and low mist (41 ml ft^{-2} per pass). Each tube received approximately 13.71 ml of water per day for high mist treatment or 10.6 ml per day for the low mist treatment. After nine weeks, rooting percentage and number of primary roots were counted.

The experiment was a split-split plot design with four blocks, mist level as the main plot, species at the sub-plot, and all other factors as the sub-sub plots. Data were analyzed using a generalized linear mixed model (PROC GLIMMIx) with a logit link function for rooting percentage because of the binary nature of the response variable. Although, ANOVA analysis of arcsine transformations of such data are common, we found the PROC GLIMMIx procedure to be more appropriate (Jaeger, 2008). Because of the count nature of the data, negative binomial regression model (PROC GENMOD) was used to analyze number of primary roots and cuttings produced in SAS (version 9.3; SAS Institute, Cary, NC, USA).

RESULTS

Cuttings

E. benthamii

In 2012 the number of cuttings obtained from donor plants varied significantly by pruning height (Table 1). A mean of 36 cuttings was produced from 20 cm plants and 26 cuttings from 10 cm plants (Fig. 1A).

The same pattern was observed the following year (Fig 1B) with 20 cm plants producing significantly greater numbers of cuttings (22) than 10 cm plants (15).The number of cuttings produced also varied by fertilization level. Donor plants subjected to the high fertilization treatment produced significantly more cuttings (23) than those subjected to low fertilization (15) (Fig 1C).

E. calmaldulensis

There was no significant difference in number of cuttings produced during both 2012 and 2013 (Table 2).

Percent Rooting and Primary Roots

E. benthamii

Rooting percentage varied significantly by family x auxin, family x fertilization, and family x hedge height. There was a significant family x auxin interaction. Individual families demonstrated different responses to auxin presence or absence. Although every family, except 1393, had higher rooting without auxin, these differences in rooting were not statistically significant within families (Fig 2A). Family 1393, with auxin, had the highest rooting percentage (73.3%), but had a lower rooting response without auxin (43.7%). There was also a significant family x fertilization level interaction. Families 1391 and 1393 exhibited the highest rooting with low fertilization, 75% and 71.8% respectively (Fig. 2B). Family 1389 had an opposite response with 67.7% rooting with high fertilization and 31.2% with low fertilization. There was a significant family x hedge height level interaction ($p = .0007$). Families 1391 and 1393 at 10 cm hedge height exhibited the highest rooting at 75%

and 72%, respectively. The next highest rooting family was 1389, with 68% rooting success at 20 cm hedge height.

The number of primary roots varied significantly by family (Table 4). Families 155 and 1391 produced the highest number of primary roots at 2.88 and 2.55 (Fig. 4) and were significantly different than the remaining three families. There was no significant main effect from mist level, fertilization, auxin presence, nor pruning.

E. camaldulensis

Rooting percentage varied significantly by pruning and mist level x family (Table 5). Pruning had a significant effect on rooting with 10 cm plants rooting at 85 % and 20 cm rooting at 74.2% (Table 5). Rooting also varied significantly (Table 5) by the mist level x family interaction with higher misted plants rooting at higher percentages across families (Fig. 3C). There was no significant effect of auxin or fertilization on rooting percentage.

The number of primary roots varied significantly by auxin treatment (Table 6). Cuttings without auxin treatment had amean 6 primary roots while cuttings that received auxin treatment had an average 7.3 primary roots. Primary roots also varied by family x fertilization. Families 374 and 411 had the highest number of primary roots under the low fertilization treatment, 9.8 and 7.8, respectively (Fig. 5). The remaining three families exhibited the opposite trend and produced more roots under high fertilization. There was a significant pruning x fertilization interaction in which cuttings from high fertilizer, 10 cm produced more roots than low fertilized plants hedged to 10 cm (Fig. 5C). Cuttings from 20 cm plants had the opposite response and produced more roots under low fertilizer. There was no significant effect of mist, fertilization, family nor pruning alone in relation to number of primary roots.

DISCUSSION

Overall, mean rooting percentage for *E.benthamii* was 49.4% which is higher than previous studies have found at 33% (Graça et al. 1999). We observed significant interactions of family by auxin, family by fertilization, and family by hedge height treatments. Family variation has not been well-studied in *Benthamii*, however, several clonal studies have been conducted revealing differing clonal responses to treatments with *E. benthamii* hybrids (Brondani et al. 2012a, Brondani et al. 2008, and Brondani et al. 2010b) and with other species of *Eucalyptus* (De Melo et al. 2011, Goulart et al. 2008). The number of primary roots produced per cutting was >2 and this also varied by family. Schwambach et al. (2005) found that zinc increased rooting percentage and the application of calcium during the induction phase increased root number in *E. globulus* mini-cuttings. Perhaps, the low phosphorous in the fertilizer solution could be increased in future studies as it has been correlated with primary root growth in *Arabidopsis* root systems (Lopez-Bucio et al. 2002). While overall rooting was higher than previous studies, *E. benthamii* produced relatively few roots. Further studies are needed to determine if there is a meaningful relationship between number of primary roots, survival and growth.

The data show a general, though not significant, trend of higher rooting percentages without auxin. Fett-Neto et al. (2001) found similar results in their study of *Eucalyptus saligna* Smith and *Eucalyptus globulus* micro-cuttings by adding IBA to root induction media. Goulart et al. (2008) found that with concentrations of IBA greater than 2000 mg L^{-1}

toxicity was observed in certain clones. Family 1393 showed the opposite trend and had the highest rooting percentage with auxin treatment. Using talcum powder treated with auxin, Iwasaki et al. (2012) showed higher rooting percentages with auxins than without in *E. globulus* seedlings. Future studies should investigate the effect of differing concentration levels of IBA and application method on rooting success.

Low fertilizer level had a positive effect on rooting percentages in all families except 1389, where high fertilizer level increased rooting. Henry et al. (1992) reported that eastern redcedar cuttings, taken from stock plants at various fertilization levels, had fewer number of roots beyond 20 ppm N. High fertilization of petunia stock plants resulted in lower root dry weight and the lowest rooting percentage of three fertilization levels (Santos et al. 2011). Similar results were also found by Rein et al. (1991) with holly stem cuttings. While these studies of unrelated species demonstrate a negative rooting response due to increased fertilization, Cunha et al. (2009) found varied responses to fertilization of mini-stumps in various *Eucalyptus* species. Therefore, we conclude that general responses to *E. benthamii* fertilization and auxin level should not be the basis for future propagation protocols and should be individually assessed for each family or clone. Although there are no other studies that have investigated family variability in *E. benthamii*, our data supports the hypothesis that there are inherent rooting differences among families and that families respond differently to fertilization and auxin levels.

Family x mist level also played a role in *E. camaldulensis* rooting. Because *E. camaldulensis* is not considered recalcitrant to rooting, the overall rooting percentage of 80% was not surprising. Like E. *benthamii*, little research has been done on the genetic variability of this species. Perhaps due to its high rooting ability and response to established propagation methods, such studies have not been conducted. *Eucalyptus camaldulensis* rooted better under the shorter height (10 cm) and rooted better by under high mist conditions. Mankessi et al. (2011) found differences in survival based on collection in the rainy season and ontogenetic age of plant material where juvenile material survived better in the dry season and mature material in the rainy season. Juvenility of donor plant is important to the subsequent rooting of cuttings. Grossnickle and Russell (1993) found that rooting percentage, speed of rooting and mean root length was higher in cuttings obtained from donor plants that had been hedged than those left intact in yellow-cedar. Therefore, the results from our data were consistent with other findings. Little has been investigated regarding the effect of mist levels on cuttings in *Eucalyptus* spp., though rooting studies in shortleaf and loblolly cuttings found that misting at .05mm/h was more effective than higher levels. Lebude et al. (2004) also found that maximum rooting was achieved when cutting water potential was maintained between -0.5 and -1.5MPa, citing less negative water potential as an impediment to rooting. Thus, the increased rooting due to higher mist levels is an unexpected result, though comparison of pines to Eucalyptus may not account for important physiological differences between the genera.

Auxin increased the number of primary roots as did an interaction between family x fertilization level and fertilization x hedge height. While IBA application has been shown to speed up rooting times and rooting percentages (Fett-Neto et al., 2001) and to increase survival (Brondani et al., 2010a), we are unaware of studies that have measured the effect of

IBA on number of primary roots in either *E. benthamii* and *E.camaldulensis*. Further studies are needed to determine the effect of IBA on root number.

The production of cuttings for *E. benthamii* was positively influenced by higher hedge height during both years and by high fertilization in 2013. The greater number of cuttings on 20 cm donor plants may be due to the increased amount of lateral buds on longer stems and stems with a greater diameter. Fertilization has been long shown to have a positive effect on height growth, stem mass, and stem diameter (Fernandez et al. 2007; Husen & Pal 2003) consistent with our results. *Eucalyptus benthamii* branches freely and regularly which results in many shoots that are suitable for cutting material. The higher number of cuttings produced may be more important in a species that is recalcitrant to root than in one that is easy to root, given that larger numbers of cuttings may need be taken to produce an adequate supply of material. For example, in 2013 *E. benthamii* produced an average of 31.2 cuttings per plant. If we apply the mean rooting percentage of 49.9% to number of cuttings produced per plant, we end up with 15.5 rooted cuttings per donor plant. Plants hedged to 20 cm produced an average of 22.5 cuttings per plant while 10 cm plants produced 15.5 cuttings per plant. With a rooting percentage of 49.9%, we end up with 11.2 and 7.7 rooted cuttings per donor plant for high and low hedge heights, respectively.

CONCLUSIONS

The objective of our study was to investigate the viability of vegetative propagation by rooting stem cuttings in producing clonal material of *Eucalyptus benthamii* and *Eucalyptus camaldulensis*. Both species revealed genetic variability in rooting percentage and number of primary roots produced. This is a beginning step toward further investigation of genetic variation in *E. benthamii*. In general, it responded less positively to treatments than did *E. camaldulensis*. In attempting to establish propagation protocols, family should be taken into account if choosing fertilization and auxin regimes for this species.

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TABLES AND FIGURES

Table 1. Negative binomial regression analysis of the number of cuttings produced From *E. benthamii* in 2012 and 2013. Statistical significance (α = 0.05) is denoted in bold.

Year	Source	DF	Chi-Square	Pr > ChiSq
2012	**Fert**	1	3.18	0.0746
	Height	1	32.42	**<.0001**
	Fert*height	1	0.40	0.5281
2013	**Fert**	1	15.66	**<.0001**
	Height	1	0.59	**0.0011**
	Fert*Height	1	1.64	0.2003

Table 2. Negative binomial regression analysis of the number of cuttings produced From *E. camaldulensis* in 2012 and 2013. No factors were significant. α = .05

Year	Source	DF	Chi-Square	Pr > ChiSq
2012	Fert	1	0.95	0.3301
	Height	1	0.08	0.7762
	Fert*height	1	0.33	0.5645
2013	Fert	1	1.30	0.2548
	Height	1	0.00	0.9544
	Fert*Height	1	0.11	0.7361

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Table 3. Generalized linear mixed model analysis of the treatment effects on rooting percentage of *E. benthamii*. Statistical significance (α = .05) is denoted in bold. There were no significant three way interactions

Effect	Num DF	Den DF	F Value	Pr > F
family	4	236	26.57	**<.0001**
fert	1	47	1.18	0.2820
family*fert	4	236	3.57	**0.0075**
pruning	1	47	0.20	0.6566
family*pruning	4	236	4.97	**0.0007**
pruning*fert	1	47	1.94	0.1697
auxin	1	47	0.32	0.5727
family*auxin	4	236	12.26	**<.0001**
fert*auxin	1	47	2.24	0.1412
pruning*auxin	1	47	0.99	0.3245
mist	1	6	0.18	0.6822
family*mist	4	236	0.62	0.6515
fert*mist	1	47	0.20	0.6593
pruning*mist	1	47	0.05	0.8325
auxin*mist	1	47	0.12	0.7326

Table 4. Negative binomial regression analysis of the mean number of primary roots produced from rooted *E. benthamii* cuttings. Statistical significance (α = .05) is denoted in bold. There were no significant two nor three-way interactions.

Source	DF	Square	Pr > ChiSq
Family	4	21.30	**0.0003**
Auxin	1	1.57	0.2102
Pruning	1	0.14	0.7071
Fert	1	0.22	0.6420
Block	3	6.57	0.0870
Mist	1	0.55	0.4603

Table 5. Generalized linear mixed model analysis of the treatment effects on rooting percentage of *E. benthamii*. Statistical significance (α = .05) is denoted in bold. There were no significant three way interactions.

Effect	Num DF	Den DF	F Value	Pr > F
family	4	235	2.94	**0.0211**
fert	1	47	1.61	0.2102
clones*fert	4	235	1.40	0.2335
pruning	1	47	9.57	**0.0033**
family*pruning	4	235	1.85	0.1195
pruning*fert	1	47	0.29	0.5943
auxin	1	47	0.06	0.8097
family*auxin	4	235	0.53	0.7145
fert*auxin	1	47	0.13	0.7191
pruning*auxin	1	47	0.12	0.7347
mist	1	6	4.16	0.0875
family*mist	4	235	3.79	**0.0052**
fert*mist	1	47	0.00	0.9866
pruning*mist	1	47	0.00	0.9805
auxin*mist	1	47	1.85	0.18

Table 6. Negative binomial regression analysis of the mean number of primary roots produced from rooted *E. camaldulensis* cuttings. Statistical significance (α = .05) is denoted in bold. There were no significant three-way interactions.

Source	DF	Square	Pr > ChiSq
Family	4	7.81	0.0989
Auxin	1	6.31	**0.0120**
Pruning	1	0.22	0.6396
Fert	1	0.01	0.9301
Block	3	19.25	**0.0002**
Mist	1	1.49	0.2222
Family*auxin	4	2.35	0.6712
Family*pruning	4	1.27	0.8671
Family*fert	4	15.46	**0.0038**
Family*mist	4	6.67	0.1544
Pruning*fert	1	13.87	**0.0002**

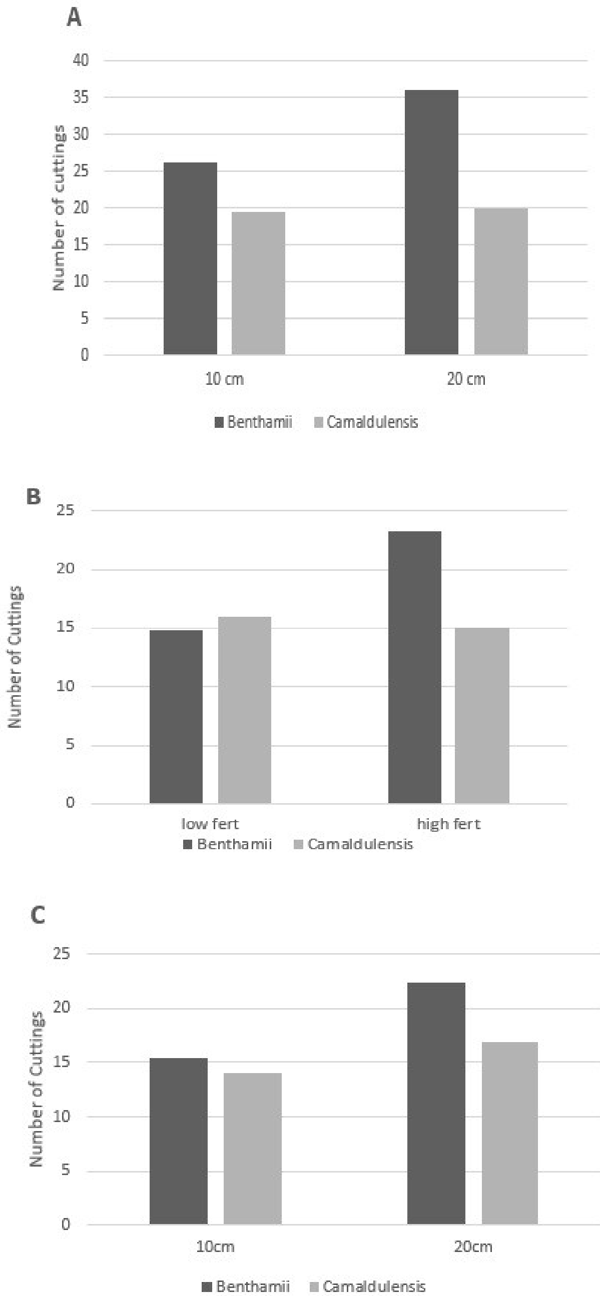

Figure 1. Relationship of number of cuttings produced by (A) hedge height in 2012 $\chi^2(1, N=84) = 32.42$, $p < .0001$, (B) fertilization level in 2013 $\chi^2(1, N=89) = 15.66$, $p < .0001$ and (C) by hedge height in 2013 $\chi^2(1, N=89) = 10.59$, $p = .0011$. The number of cuttings produced by *E. camaldulensis* were not significantly different between treatment.

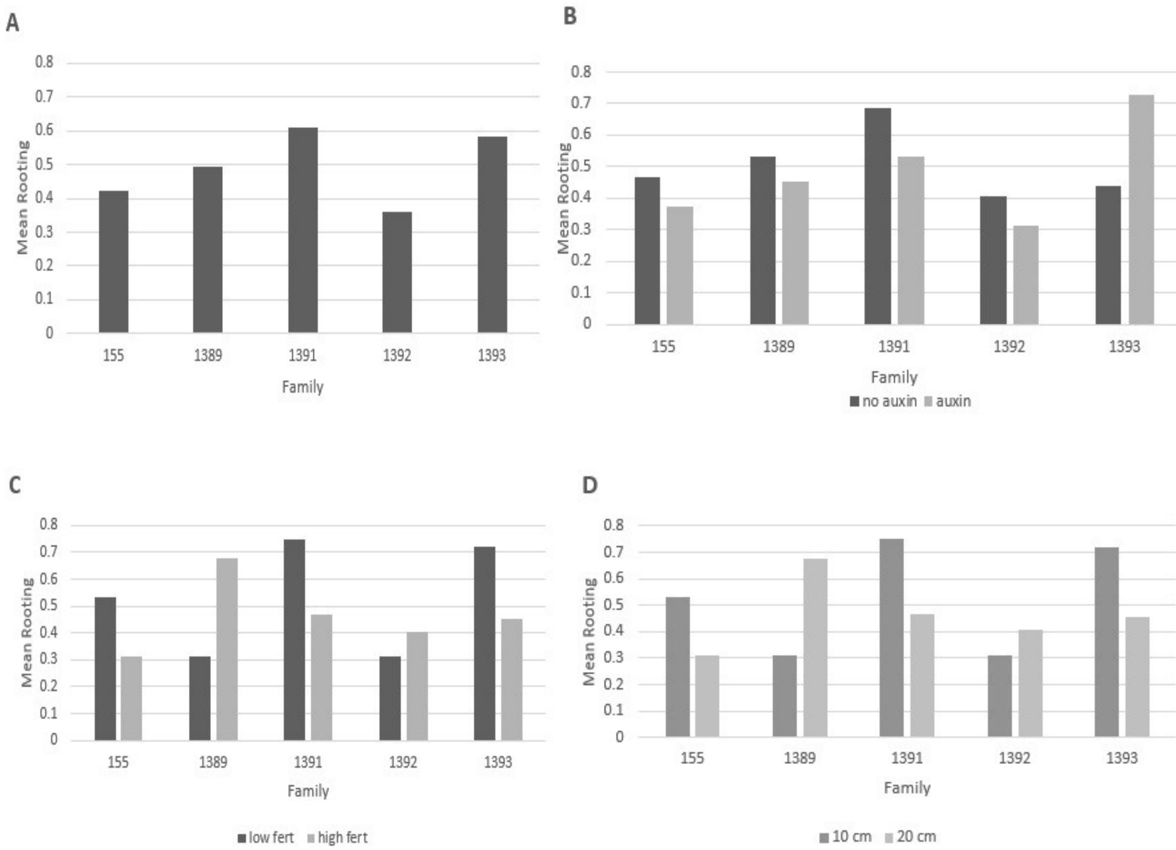

Figure 2. Percent rooting of *Eucalyptus benthamii* by (A) family $\chi^2(4{,}236\ N{=}320) = 26.57$, $p < .0001$, (B) family x fertilization interaction $\chi^2(4{,}236\ N{=}320) = 3.57$, $p = .0075$, (C) family x auxin, $\chi^2(4{,}236\ N{=}320) = 4.96$, $p = .0007$ and (D) $\chi^2(4{,}236\ N{=}320) = 12.26$, $p < .0001$.

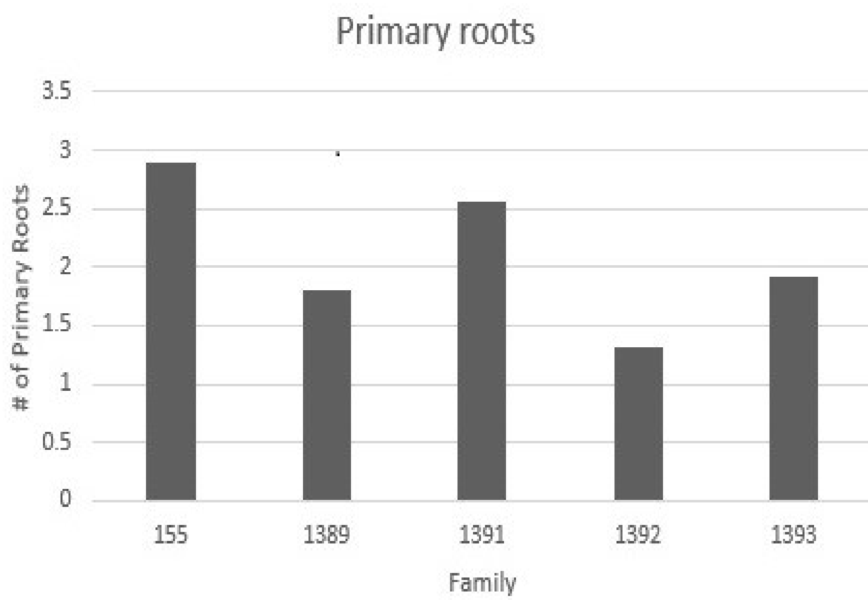

Figure 3. Relationship between family and number of primary roots produced. $\chi^2(4, N=157) = 21.30, p = .0003$.

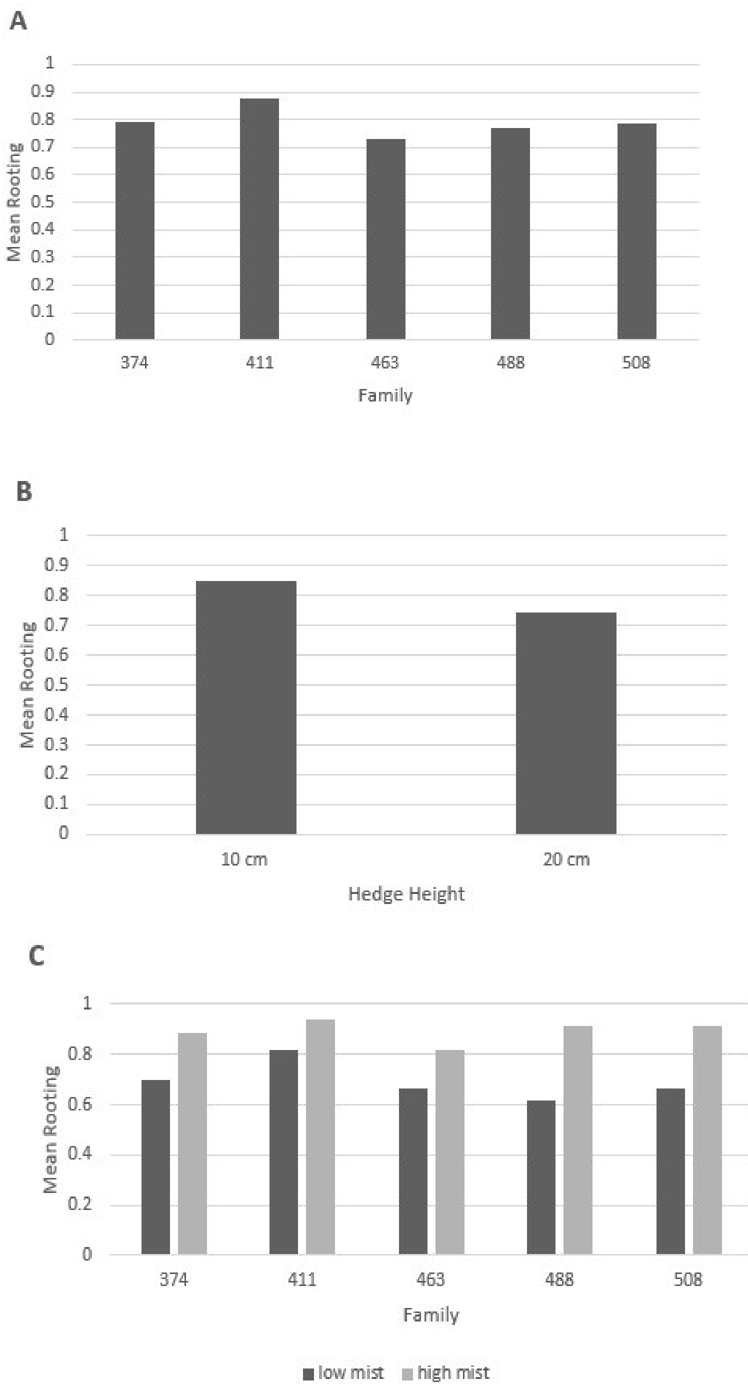

Figure 4. Rooting percentage of *Eucalyptus camaldulensis* by (A) family $\chi^2(4{,}236\ N{=}319) = 26.57$, $p < .0001$, (B) hedge height $\chi^2(1{,}47\ N{=}319) = 9.57$, $p < .0033$, and (C) mist x family $\chi^2(4{,}236\ N{=}319) = 3.79$, $p = .0052$.

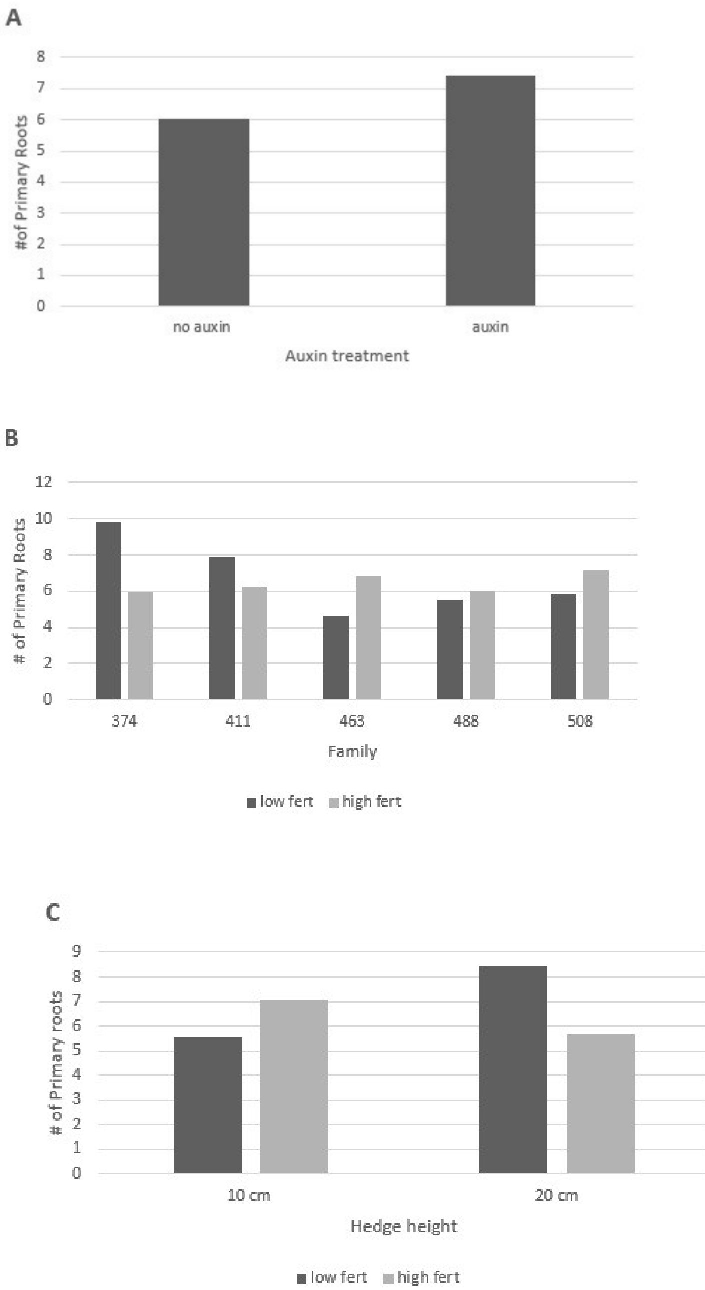

Figure 5. Relationship of number of primary roots produced by (A) auxin $\chi^2(1, N=255) = 6.31$, p=.0120, (B) family x fertilization $\chi^2(4, N=255) = 15.46$, p =.0038, and (C) pruning x fertilization $\chi^2(1, N=255) = 13.87$, p =.0002.