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Influences of seedling size, container type and mammal browsing on the establishment of *Eucalyptus globulus* in plantation forestry

D. C. Close · S. Paterson · R. Corkrey · C. McArthur

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Abstract Rapid early growth of tree seedlings is critical to the success of plantation establishment. We investigated the effects of seedling size (small and large) and container types (small [Lannen 121], medium [Lannen 81] and large [Forestry Tube]) in the nursery and the effects of mammal browsing after planting on growth of *Eucalyptus globulus* in Tasmania's Southern Forests. After planting, seedlings were either exposed to browsing or protected from browsing by wire-mesh cages until age 6 months. Low browsing pressure resulted in around 20% and 5–10% of foliage being browsed in the large and small size categories, respectively, between 1 and 3 months after planting the uncaged treatment. 6 months after planting, height growth increment was lower, and 4 years after planting, mortality was higher in uncaged large than caged large seedling treatments. Six and twelve months after planting, seedlings raised in Forestry Tube containers had significantly greater height increment and root collar diameter relative to other treatments. By 4 years after planting, trees of the small seedling treatment had significantly greater diameter than those of the large seedling treatment, but there was no effect of container type treatment. Four years after planting there was no effect on diameter growth by browsing of <30% of foliage up to 3 months after planting, although there was greater incidence of double leaders in trees that had been browsed as seedlings. Small seedlings produced more growth 4 years after planting than large seedlings of *E. globulus*.

D. C. Close · S. Paterson · C. McArthur
Cooperative Research Centre for Forestry, Private Bag 12, Hobart, TAS 7001, Australia

D. C. Close
School of Zoology, University of Tasmania, Private Bag 54, Hobart, TAS 7001, Australia

D. C. Close
School of Plant Science, University of Tasmania, Private Bag 55, Hobart, TAS 7001, Australia

D. C. Close (✉) · R. Corkrey
Tasmanian Institute of Agricultural Research, Private Bag 54, Hobart, TAS 7001, Australia
e-mail: Dugald.Close@utas.edu.au

C. McArthur
School of Biological Sciences, The University of Sydney, Heydon-Lawrence Building (A08), Sydney, NSW 2006, Australia

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Introduction

The choice of seedling size and container type and their effects on post-planting performance are of economic importance to production forestry (South et al. 1995, 2001). *Eucalyptus globulus* is a species of significant economic importance established in plantations worldwide (Tibbits et al. 1997). Seedling size and the dimensions of container types used in the production of *E. globulus* seedlings for plantation establishment vary widely. Seedlings raised in lower volume containers are less costly to produce per unit, but seedlings raised in larger volume containers can out-perform those raised in smaller volume containers after planting (Sutherland and Day 1988; Aphalo and Rikala 2003; Close et al. 2003a, 2006). Furthermore larger seedlings have been shown to have better performance than small seedlings after planting (South 1993; Dey and Parker 1997), particularly under conditions of competition for resources with weeds (South and Mitchell 1999). Three factors of seedlings raised in larger volume containers may affect post-planting performance: (1) relatively less handling damage at planting; (2) a higher root:shoot ratio, if top growth is similar between plants raised in the different containers or; (3) higher total biomass with a similar root:shoot ratio between plants raised in the different containers. At a physiological level, higher root:shoot ratio may result in more favourable water relations, lower shoot maintenance requirements and thus faster growth rates. Higher total biomass may result in larger total carbohydrate stores available for re-mobilisation for rapid growth soon after planting. South et al. (2005) state that "although the size of (tree seedling) container stock has been increasing, research to show that seedling diameter is positively related to field performance is lacking".

The question of whether to produce large or small seedlings in large or small volume containers is complicated further when vertebrate herbivore browsing is considered (Ward et al. 2000). Browsing is a significant problem in eucalypt plantation establishment in Tasmania (Bulinski and McArthur 1999). European rabbits (*Oryctolagus cuniculus*) tend to 'clip' seedlings of relatively low root collar diameter where stems are not yet 'woody' (McArthur and Appleton 2004). The effects of seedling size and container type on browsing by native mammalian herbivores (Common Brushtail Possum, *Trichosurus vulpecula* and Red-Bellied Pademelon, *Thylogale billardierii*) have not been investigated. Seedlings with relatively greater top-growth may be more visible or 'apparent' (Palmer and Truscott 2003; Rao et al. 2003) to browsers, and thus suffer relatively large biomass losses. Alternatively, seedlings with large tops may recover more rapidly from browsing given their relatively greater carbohydrate reserves. Alternatively, seedlings with less top-growth may be more susceptible to browsing due to their relatively younger leaves being more attractive to browsers due to lower levels of 'toughness' or unpalatable secondary compounds (Close et al. 2003b).

The objective of this study was to assess the post-planting performance of large and small seedlings (early- and late-germinated, respectively) raised in three container types of varying volume. The seedlings were planted at a site in the Southern Tasmanian Forests where high browsing pressure was expected. An additional treatment of browsing exclusion, using wire mesh cages, was included to allow the assessment of growth independent of early mammal browsing.

Materials and methods

Plant material and treatments

Seedlings of *E. globulus* were raised from Worrolong Seed Orchard seedlot (Forestry Tasmania improved seed). Germinants were pricked out from germination trays and transplanted into either Lannen® 121 (73 mm deep, 50 cm^3 volume), Lannen® 81 (73 mm deep, 85 cm^3 volume) or Forestry Tube (120 mm deep, 115 cm^3 volume) containers at Forestry Tasmania's tree nursery, Perth, Tasmania (41°34′40.70″S, 147°10′56.99″E, 160 m above sea level). Seeds were germinated in early and late February 2003 to produce large and small sized seedlings, respectively. Seedlings were raised in a potting mix (75% pinebark, 25% peat, pH adjustment with dolomite, microelements added) and were fertilised with Scotts® Special Hi–N soluble fertiliser weekly from the age of 6 weeks.

Trial site and design

The trial site (coupe 'SO13A') was selected from the suite of sites prepared for commercial planting by Forestry Tasmania in the spring of 2003 and was approximately 30 km south of Geeveston. Average annual temperature is 12.05°C and rainfall is 890 mm at Geeveston (43°09′53.08″S, 146°55′37.00″E, 32 m above sea level, recorded 1971–2004, Australian Government Bureau of Meteorology). The sub-section of SO13A used for the trial ran parallel to extensive native regrowth forest. It was selected in order to maximise the chances of browsing, given that herbivory is most intense around the edge of coupes that are closest to native forest (While and McArthur 2005). It was an area of homogenous soil type and had effective weed control (glyphosate at 1 l ha^{-1} prior and post mounding). Planting rows were ripped to 600 mm and wide-profile mounded. The site was north facing on a slope of approximately 6°.

The trial consisted of six randomised blocks each containing 12 plots. Blocks were arranged two-deep and parallel to the coupe edge. Plots consisted of four rows of five seedlings for uncaged treatments and one row of five seedlings for caged treatments, with between- and within-row spacing of 3.5 and 2.5 m, respectively. All six nursery treatments (two seedling sizes in three container types) were both caged and uncaged. The trial was planted and the caged treatments applied between the 10th and 13th of October 2003. The cages consisted of wire netting, 1.8 m in height, clipped into a 'cage' of approximately 0.75 m diameter, clipped across the top edge to form a 'roof' and secured at the base with long wire pegs. The remainder of the coupe was planted by contract planters between the 17th and 20th of October 2003. All cages were removed at age 6 months. Professional shooters, employed by Forestry Tasmania, shot on 18th December 2003, 3rd and 12th January 2004, and 3rd February 2004 for mammalian browse control over the entire SO13A coupe as per their usual operational procedure.

Field measurements

Seedling height of all seedlings within the trial was measured at planting, and at 6 and 12 months after planting. Seedling root collar diameter was measured on the first row of five seedlings of all uncaged plots and was measured for all caged seedlings at planting, and at 6 and 12 months after planting.

Browsing damage was assessed 1, 2, 4, 6, 8, and 10 weeks and 3, 4, 5, and 6 months after planting. Browsing was visually estimated (following Bulinski and McArthur 1999) on a 6-point scale by the same worker throughout the trial, as a percentage of the total foliage biomass removed per seedling by herbivores (this was possible given that portions of fully expanded leaves were generally browsed in addition to the apical growing point of seedlings):

Browsing score:	0	1	2	3	4	5	6
Biomass removed (%):	0	1–5	6–25	26–50	51–75	76–95	96–100

Diameter at breast height and presence of double leaders were measured on trees 4 years after planting, except in block one which could not be accurately located.

Statistical analysis

Seedling height increment was calculated as height at a given age minus the height at planting so that it was normalised for size differences at planting as a result of nursery treatment. Seedling height increment, root collar diameter, and browsing scores were analysed by repeated measures ANOVA using the Greenhouse-Geisser epsilon correction. Where there was a significant time effect the data for each time were analysed separately. Tukey's adjustments were used to identify homogeneous groups. Browsing scores (after excluding caged trees) were converted to percentage midpoint values and Arc Sine transformed for repeated measures analysis.

At age 4 years block one could not be accurately located, thus results were analysed for blocks 2–6 only. Diameters of trees with double leaders were excluded from analysis of diameter at breast height. Mortality proportions and proportions of trees with double leaders were Arc Sine transformed. Diagnostic checks such as residual plots were used to check analyses assumptions. All analyses were conducted using Genstat 11 (VSN International Ltd).

Results

Browsing of seedlings

There was no effect of container type on browsing of foliage. There was a significant effect of time ($F(9,270) = 61.05$, $P < 0.001$; Fig. 1) and a significant interaction effect between time and size ($F(9,270) = 8.52$, $P < 0.001$) where browsing of foliage was significantly higher for large than small seedling sizes except at 1 week after planting ($F(1,29) = 1.34$, $P = 0.257$). Differences between browsing of large and small seedlings was marked up to 3 months after planting. Following the shooting of herbivores 2–3.5 months after planting, browsing damage was relatively low.

Root collar diameter

There was a significant effect of time for container ($F(4,120) = 5.90$, $P = 0.003$), but not seedling size ($F(2,120) = 0.35$, $P = 0.596$), on seedling root collar diameters. Root collar

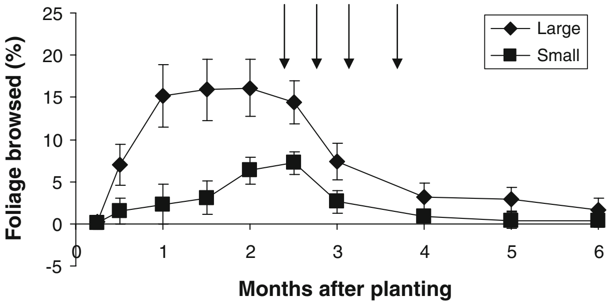

Fig. 1 Percent foliage browsed with time after planting of uncaged seedlings, that were large (average 0.25 m) or small (average 0.13 m) size at planting. Data is pooled across container types for clarity and given a lack of significant effect of container type on percent foliage browsed. *Arrows* indicate operational shooting for browse control of the coupe. *Bars* represent standard error of the means

diameters did not differ significantly at planting or 3 months after planting (results not shown), but at 6 and 12 months there was a significant effect of container (Table 1). Using 95% Tukey adjustments, at 6 and 12 months after planting the mean root collar diameters for seedlings raised in Lannen 81 and 121 containers were not significantly different but were significantly lower than root collar diameters of seedlings raised in the Forestry Tube (Fig. 2).

Seedling height growth

Height was significantly affected by time, container type ($F(4,120) = 6.06$, $P < 0.001$), seedling size ($F(2,120) = 22.96$, $P < 0.001$) and cage ($F(2,120) = 5.76$, $P = 0.010$). At 3, 6 and 12 months after planting, the effect of container type was significant with seedlings raised in Lannen 81 and 121 container types being significantly shorter than seedlings raised in Forestry Tubes (Table 1; Fig. 3a). At 3 and 6 months after planting there was a significant interaction of seedling size and cage on height (Table 1; Fig. 3b), with large uncaged trees having less height growth than the homogeneous group formed by large caged and small caged and uncaged seedlings. At 12 months after planting there was no significant interaction of seedling size and cage (Fig. 3b). Smaller seedlings had greater height growth than larger seedlings (Table 1).

Four year results

There was a significant effect of seedling size on diameter at breast height, with trees raised from the small seedling treatment attaining larger diameters than trees raised from the large seedling treatment (Table 1). Mean diameters of trees raised from small and large seedling treatments were 8.63 and 7.87 ± 0.19 cm, respectively.

There was one marginally significant interaction between seedling size and cage treatment for mortality (Table 1). The large caged seedlings and small caged and uncaged seedlings had lower mortality (average of 7.83% ± 4.25) than large uncaged seedlings (17.2% ± 4.25).

Table 1 ANOVA table of treatment and interaction effects

Factors	Levels	Root collar diameter			Height			4 years after planting		
		3 months	6 months	12 months	3 months	6 months	12 months	DBH	Mortality	Double leaders
Seedling size (S)	2	NS	NS	NS	5.83*	8.66**	30.95***	14.03***	NS	NS
Container Type (T)	3	NS	8.17***	5.76**	26.76***	17.55***	11.29***	NS	NS	NS
SxT	–	NS	NS	NS	NS	NS	NS	NS	NS	NS
Caging (C)	2	NS	NS	NS	20.55***	11.68**	NS	NS	7.23*	5.89*
CxS	–	NS	NS	NS	6.50*	8.96**	NS	NS	4.72*	NS
CxT	–	NS	NS	NS	NS	NS	NS	NS	NS	NS
CxSxT	–	NS	NS	NS	NS	NS	NS	NS	NS	NS

F values and *** $P<0.001$, ** $P<0.01$ and * $P<0.05$. Caging was applied as a split plot treatment and there were two plots for each Seedling size × Container type interaction. There were six blocks with 12 plots per block, except in the case of 4 year data which was five blocks with 12 plots per block

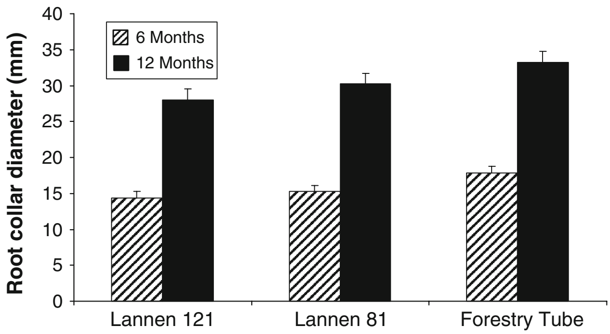

Fig. 2 Seedling root collar diameter 6 and 12 months after planting of seedlings raised in Lannen 121, Lannen 81 and Forestry Tube containers. Data is pooled across seedling size and caging for clarity and given a lack of significant effects of these treatments. *Bars* represent standard error of the means

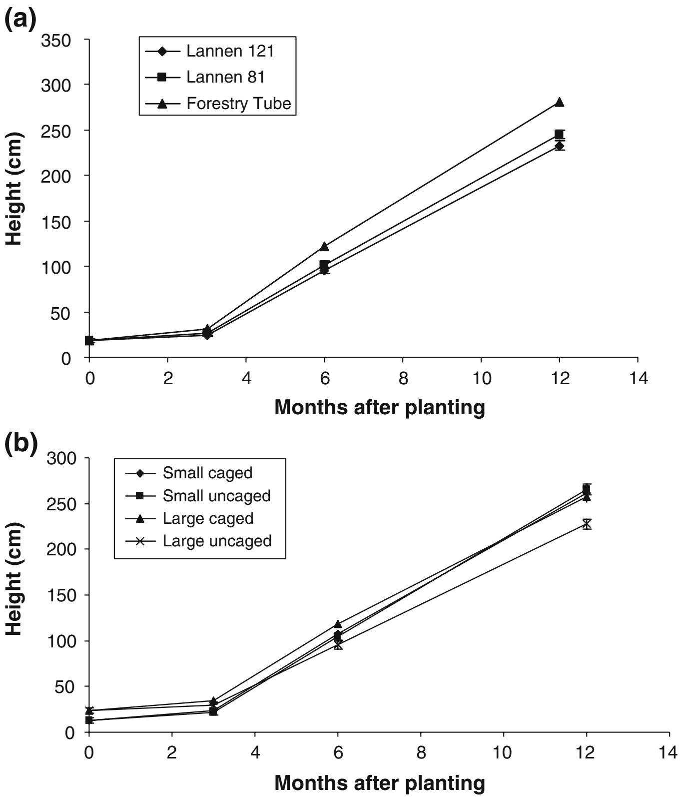

Fig. 3 Height with time after planting for seedlings raised in Lannen 121, Lannen 81 and Forestry Tube containers (**a**) and for small (average 0.13 m) and large (average 0.25 m) sized seedlings at planting either caged by wire mesh (to exclude mammalian herbivores) or uncaged immediately after planting (**b**). *Bars* represent standard error of the means

Cage had a small significant effect on the frequency of double leaders (Table 1) with caged seedlings having fewer trees with double leaders (2.7% ± 1.99) than uncaged seedlings (7.7% ± 1.99).

Discussion

Browsing effects

Despite operational browser control (shooting between 2 and 3.5 months after planting), foliage removal of around 20% in the large and between 5 and 10% in the small seedling size categories occurred during the 3 months after planting. This preferential browsing of the large seedlings possibly contributed to significantly lower height growth and greater mortality in large uncaged, compared with large caged, seedlings 3 and 6 months after planting. Rao et al. (2003) reported that larger saplings of downy birch (*Betula pubescens*) planted into a heathland, were more likely to be browsed and to have more biomass removed by mountain hares (*Lepus timidus*) than smaller saplings. This difference is attributed to the relative ‘apparency’ (or likelihood of sapling discovery by herbivores; Feeny 1976). Larger seedlings provide more consumable biomass per plant, allowing longer browsing, and this has been shown to be independent of effects of chemical composition of the biomass (Hartley et al. 1997). Increased patch residence time with increasing patch size has been reported in studies of plant-herbivore interactions (Shipley and Spalinger 1995). The study of Rao et al. (2003) was conducted within a natural vegetation of heathland, in contrast to the virtual absence of background vegetation (due to herbicide application) on our experimental plantation site. Thus it appears that apparency of tree seedlings to herbivores applies even in an environment requiring little energy expenditure by the herbivore to discover a food plant. It is possible that the greater browsing on large than small seedlings was due to differences in leaf chemistry, but we did not test for this. It is unlikely though, given that seedlings show increased levels of plant defence chemicals as they age (Loney et al. 2006).

There was a pronounced peak in percent foliage browsed that started immediately post-planting and finished at 3 months after planting. Operational shooting, that occurred between 2 and 3.5 months after planting, may be the primary factor that led to the rapid decrease of percent foliage browsed by 3 months after planting. In addition the browsing pattern may be affected by seasonality and alternative food sources, and by nursery versus field effects on physical and chemical characteristics of foliage. Planting was done in early October, a month that averages only 11.1°C (Australian Bureau of Meteorology) at nearby Geeveston, thus there would be little spring-flush growth on surrounding vegetation. Secondly, in the nursery environment, seedlings received daily water application, regular nutrient application and self-shelter from wind. After planting in the field, water stress (Stape et al. 2001), lack of nutrient availability (Close and Beadle 2003) and wind (Telewski and Jaffe 1986) are all factors that could lead to the development of thicker leaves in tree seedlings and leaf toughness is a primary cue for decreased herbivore palatability of eucalypt seedlings (Close et al. 2003b). Thus the development of thicker, tougher leaves in the field may be another factor that explains the rapid decline that we observed in percent foliage browsed 3 months after planting.

Importantly for industry, we found no effect of caging seedlings up to age 6 months on diameter at age 4 years. This indicates that the increased browsing of uncaged, compared to caged, seedlings soon after planting does not have lasting effects on overall growth. This is consistent with findings that early low severity browsing of <30% of foliage of eucalypt seedlings did not affect long term growth (Bulinski and McArthur 1999). However, it should be cautioned that this was on a site with effective browse control and therefore relatively low browsing pressure. If browsing pressure was higher then we may have detected significant effects of the caging treatment. Seedlings that have >30% foliage browsed, or repeated browsing, suffer long term depression in growth, increased mortality and have lower reproductive output at age 10 years (O'Reilly-Wapstra et al. unpublished data).

Seedling size effects

Small seedlings had greater height growth increment at 6 and 12 months after planting, and greater diameter at breast height 4 years after planting, relative to the large seedling treatment. The mechanisms underpinning this result can only be speculated upon but possibly relatively low root:shoot ratios of large seedlings were induced by the 'luxurious' conditions of regular water and nutrient application in the nursery and thus large seedlings suffered greater transplant shock and needed to establish roots to support new top growth (Close and Beadle 2003). South and Mitchell (1999) have cautioned that larger seedlings do not survive better than smaller seedlings when top-growth is "out of balance". Our results at 4 years after planting indicate no difference in survival between small and large caged seedling treatments. An alternative and/or additional factor may be the early effects of preferential browsing of large seedlings given the higher incidence of double leaders. Double leaders were likely caused by browsing damage (Bulinski and McArthur 1999) because the experimental site had infrequent frost and was protected from strong winds by adjacent forest. Note that trees with double leaders were excluded from the analysis of diameter. Whatever the explanation, it is further evidence for industry that planting smaller seedlings does not result in greater risk of crop failure.

Container-type effects

Three, 6 and 12 months after planting we found consistently greater height growth by seedlings raised in the high volume Forestry Tube than those raised in the Lannen 121 and 81 containers. This result is consistent with our previous findings in four trials across the 'Green Triangle' region of Victoria and South Australia (Close et al. 2003a, 2006). The Forestry Tube has significantly greater volume and depth relative to the other containers. The deeper Forestry Tube may enable improved after-planting growth by improving root access to soil moisture at greater soil depth. South and Mitchell (1999) tested planting depth as a trial treatment in the establishment of barerooted *Pinus elliotii* and reported significantly lower survival in shallow (2.5 cm) versus deep (15 cm) planting holes. Burdett (1990) emphasised the critical role of new root growth soon after tree seedling planting in order to avoid water stress, support photosynthesis, and ultimately to ensure the success of plantation establishment. Alternatively or additionally, better root architecture may form in the large relative to smaller container types (Nelson 1996). Larger containers increase the ratio of course:fine roots (Oddiraju et al. 1994) and root volume and the number of first order lateral roots have been recognised as critical determinants of post-planting performance in barerooted seedlings (Jacobs et al. 2005). South et al. (2005) found that survival and early growth of containerised *Pinus palustris* was more closely related to root collar diameter, root growth potential and container depth than with root form per se. However, their study was focused on the development of a root-binding index and in most cases seedlings were over 12 months of age at planting. This contrasts with the shorter growing season (approximately 7 months) for *E. globulus* in Tasmania where root binding is not considered an issue.

We found no effect of container type 4 years after planting, despite the significant early effects of container type. Thus whilst container type may be an important consideration for rapid early growth to reduced early browsing or drought risk, we found that container type does not affect growth in the longer term when browsing is controlled.

Conclusion

Our results indicate that plant apparency theory holds in the plantation context where the influence of background vegetation is low, consistent with the over-riding effects of plant morphology on red deer browsing in artificial feeding trials reported by Hartley et al. (1997). We found no effect of low levels of browsing on longer term growth. Findings of improved performance of seedlings raised in larger, than smaller, volume containers indicates that *E. globulus* in temperate Australia responds similarly to many other tree species. This result has important implications if managers wish to maximize early growth as a risk abatement strategy. Our findings of significantly increased growth of small compared to large seedlings up to 4 years after planting indicate that small seedling size at planting is not a risk to future production. In fact, our results favour the use of smaller seedlings due to the lower incidence of double leaders. Double leaders represent a significant loss in production, particularly if a solid wood product is sought from the plantation. The data of this study can be used by tree nurseries and forestry companies to compile cost-benefit analyses of the production costs and growth gains associated with the range of containers and seedling sizes currently used by industry.

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