Effect of seedling characteristics at planting on browsing of *Eucalyptus globulus* by rabbits

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Summary

We investigated the effect of *Eucalyptus globulus* seedling characteristics, particularly height and diameter at planting, on the extent and severity of browsing damage by rabbits in a plantation field trial. In the first month following planting, browsing was negligible on tall (27 cm), large-basal-diameter (6.2 mm) open-rooted 'half and half' *E. globulus* seedlings compared with normal container stock (height 16–17 cm; basal diameter 1.9 mm) or tall container stock (height 27 cm; basal diameter 2.5 mm). After 4 mo, survival was 96% for the open-rooted seedlings, but only around 75% for the other types of seedling. Survival and growth rate of open-rooted stock decreased between 4 and 14 mo after planting. This was not a result of browsing, but was probably due to drought stress. We also investigated the use of a commercial repellent, 'Multicrop Scat', on rabbit browsing. The repellent did not reduce browsing, but appeared to have a beneficial, though not statistically significant, effect on growth and survival. Results indicate that large open-rooted seedlings could be used to reduce the detrimental impact of rabbit browsing on plantation establishment. Benefits of using these seedlings may need to be balanced against risks associated with reduced survival in periods of low rainfall following planting.

Keywords: seedling; seedling growth; characteristics; browsing; repellents; rabbits; *Eucalyptus globulus*

Introduction

Damage to commercial forestry seedlings by European rabbits (*Oryctolagus cuniculus*) is a major problem in some areas in south-eastern Australia (Statham 1983; Clunie and Becker 1991; Montague 1996), particularly in ex-pasture plantations where rabbit populations can be high (*pers. obs.*). A characteristic of injuries caused by rabbits is clipping of the stem (Gill 1992), which can cause extensive damage, reduced growth and survival. In extreme cases, whole plantations may need to be re-established. The economic cost of browsing can be high: a current estimate of the cost of restocking within a year of first planting is \$680 ha^{-1}, excluding any additional costs of herbicide and fertiliser re-application.

In regions where lethal control of rabbits is not possible or feasible, alternative methods for reducing damage are required for successful plantation establishment. One way of reducing damage is to use seedlings that are less susceptible to browsing. Plants within a species show genetic variation in susceptibility to browsing by mammalian herbivores (Dimock *et al.* 1976; Dickmann 1978; Rousi 1989; O'Reilly-Wapstra *et al.* 2002). This variation is best exploited if it is incorporated into breeding programs, which can be expensive. Nursery conditions are also known to affect characteristics of tree seedlings and their susceptibility to browsing by a range of herbivores (Rodgers *et al.* 1993; Hartley *et al.* 1997; Marks and Moore 1998; McArthur *et al.* 2003). The potential exists, therefore, to manipulate characteristics of eucalypt seedlings in the nursery to reduce browsing by rabbits.

Clipping of tree seedlings by lagomorphs has been related to stem diameter. If diameter is sufficiently large, animals are unable to clip them and damage is reduced. Hartwell and Johnson (1983) reported that snowshoe hares (*Lepus americanus*) rarely clip Douglas-fir (*Pseudotsuga menziesii*) seedlings with diameters larger than 6 mm. Other characteristics of seedlings that have been associated with reduced damage from lagomorphs include age and height. For example, white cypress pine (*Callitris columellaris*) seedlings appear unattractive to rabbits once the seedlings reach 4 mo of age (Johnston 1968), and seedlings of loblolly pine (*Pinus taeda*) less than 15 cm tall are more prone to being clipped by cottontail rabbits (*Sylvilagus floridanus*) than larger seedlings (Hunt 1968). O'Reilly and McArthur (1997) suggested that trials investigating factors affecting the clipping of seedlings by rabbits, such as stem diameter and stem toughness, may be useful in developing methods to reduce damage to plantation seedlings. For example, the use of older and larger seedlings with greater stem diameter may prevent rabbit clipping, in which case the planting of larger seedlings may be an option for reducing rabbit damage.

The main aim of this trial was to test the effect of seedling characteristics, particularly initial height and stem diameter, on browsing damage by rabbits and on subsequent seedling survival and growth. A secondary aim was to determine whether a commercial repellent, applied to one of the seedling types, reduced browsing damage.

Materials and methods

Nursery treatments

We compared four nursery treatments to *Eucalyptus globulus* seedlings. Seedlings for Treatments 1 and 2 (T1 and T2) were grown in Hyco containers (40 cells per tray, each 4 cm wide by 8 cm deep) following standard practice. Seeds of a bulked open-pollinated seedlot from a seed orchard were mechanically sown in January 2000 into a potting mix containing Mini Osmocote, a 3–4-month slow-release fertiliser, so that seedlings were hardened before planting in July. Treatment 2 then had 'Multicrop Scat' bird and animal repellent (Multicrop (Aust.) Pty Ltd, Bayswater, Vic.) applied as a foliar spray in the nursery before planting. For Treatment 3 (T3), every second seedling was removed from each tray in March 2000, and the remaining seedlings were staggered to allow greater light penetration and stem growth. No additional nutrients were applied. For Treatment 4 (T4, open-rooted 'half and halfs'), stock of the same seed source had been grown in Hyco containers the previous year (1999), then planted out in nursery beds for 12 mo. During this period, plants received periodic lateral root pruning and under-cutting as roots developed, and were topped about a month before lifting. Those used in the trial had thick, woody stems and many had multiple shoots as a result of the topping process.

Trial site and design

The trial (VRG058) was established in an area within an ex-pasture plantation at Yallourn North (38°08'19"S, 146°21'41"E), north of Morwell in Central Gippsland, Victoria. The plantation had previously been planted in 1999, but seedlings in the area used for the trial had been completely browsed by rabbits and needed to be replanted. The site was strip sprayed with Roundup CT® (a.i. glyphosate 45%) at 2.5 L ha^{-1} and Simazine Flowable (a.i. simazine 50%) at 12 L ha^{-1} before planting, to remove long grass encroaching on the planting ridges. Tree seedlings received a spot application of 11:14:14 (NPK) fertiliser at 147 g $tree^{-1}$, four weeks after planting.

Seedlings were planted in a randomised block design, with 20 seedlings in each treatment plot (two rows of 10 seedlings), four treatments and five replicate blocks. The plots were close to a gully full of blackberries where there was ample evidence of rabbits, including burrows, scats and runways.

Seedling measurements over time

Seedlings were planted in July 2000. Initial measurements were taken of height and stem diameter at ground level. Subsequent measurements of height were made at 2, 3, 4, 8 and 14 mo after planting. Diameter was measured at planting, then at 8 and 14 mo after planting. Dead seedlings were recorded at each time period. Severity and type of browsing damage by mammals was measured at planting then at 2, 3, 4 and 8 mo.

Severity of browsing was given a score of 0–6, which was a visual estimate of the fraction of foliage removed by herbivores:

Score	0	1	2	3	4	5	6
Range (%)	0	1–5	6–25	26–50	51–75	76–95	96–100

Table 1. Height, and diameter at ground level, of the four treatments of *E. globulus* seedlings at planting. Values are least-squares means (± s.e.). Within each column, means lacking a common superscript differ (α = 0.05 after Tukey–Kramer adjustment for multiple comparisons).

Code	Treatment	Height (cm)	Diameter (mm)
T1	Normal container stock	16.1^{a} (± 0.6)	1.9^{a} (± 0.1)
T2	Normal container stock with repellent	16.8^{a} (± 0.6)	1.9^{a} (± 0.1)
T3	Wide-spaced container stock	26.9^{b} (± 0.6)	2.5^{b} (± 0.1)
T4	Open-rooted 'half and halfs'	26.8^{b} (± 0.6)	6.2^{c} (± 0.1)

Only new browsing damage was recorded at each assessment. Seedlings that had been pulled out and were found lying beside the planting hole were given a score of 6, and were also included in the 'dead' category. Presence or absence of browsing damage to the main stem was recorded.

Statistical analyses

Seedling survival was recorded as the percentage of seedlings per plot that were alive. Severity of mammal browsing damage, seedling height and diameter were all analysed using the arithmetic means for live seedlings in each plot as the unit of replication. Scores of severity of browsing damage were converted to the midpoint of the (percentage of foliage removed) range for each score before analysis. The occurrence of browsing damage to the main stem was analysed using the number of seedlings with main-stem damage per plot as the unit of replication. Least-squares means of dependent variables (survival, severity of browsing, number of seedlings with main-stem damage, seedling height, seedling diameter) were obtained and tested against the independent variables of treatment and block for each month, using the general linear model procedure (PROC GLM) in SAS (SAS Institute Inc. 1989). Data satisfied assumptions of normality and heteroscedasticity so that no transformations were required for analyses. Pairwise comparisons of treatments were made after using the Tukey–Kramer adjustment for multiple comparisons.

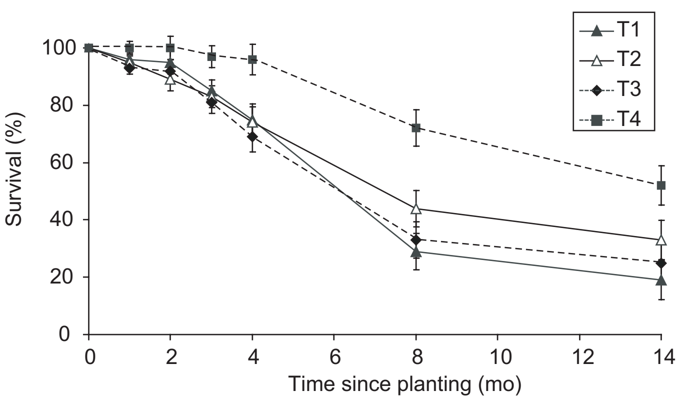

Figure 1. Seedling survival in each of the four treatments over time following planting (least-squares means ± 1 s.e.)

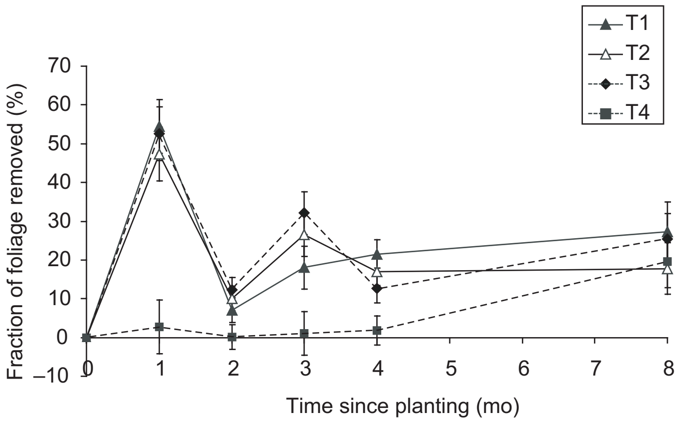

Figure 2. New mammal browsing, recorded as a fraction of the foliage removed (least-squares means ± 1 s.e.) over time following planting

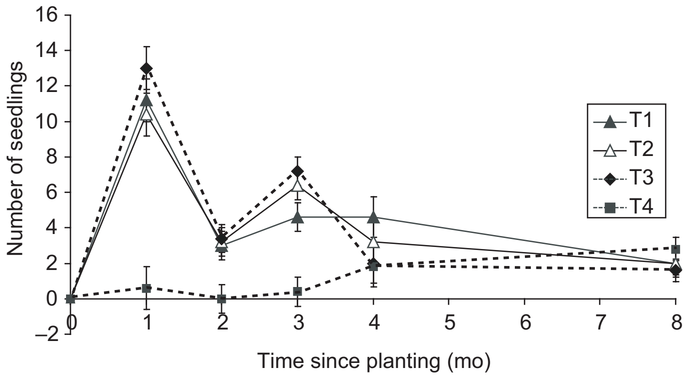

Figure 3. Number of live seedlings per plot (originally of 20 seedlings), with newly-evident mammal damage to the main stem, in each of the four treatments (least-squares means ± 1 s.e.) over time following planting

Results

Seedling characteristics at planting

At planting, seedlings in Treatments 1 and 2 were substantially shorter than those in Treatments 3 and 4 ($F_{3,12}$ = 109.2, P = 0.0001) (Table 1). Seedlings in Treatments 1 and 2 had the smallest diameters (1.9 mm) at ground level. In Treatments 3 and 4, diameters were 1.3 and 3.3 times greater (2.5 mm and 6.2 mm respectively) ($F_{3,12}$ = 675, P = 0.0001) (Table 1).

Seedling survival

One month after planting, seedling survival was greater than 90% in all treatments (Fig. 1). Survival decreased substantially from 2 to 8 mo after planting in Treatments 1, 2 and 3, but decreased only sometime after 4 mo in Treatment 4.

Four months after planting, seedling survival ranged from 69% to 75% in Treatments 1–3, and was lower than in Treatment 4 (96%). The effect of treatment on seedling survival was significant ($F_{3,12}$ = 4.96, P = 0.018), but there was no variation between blocks ($F_{4,12}$ = 0.75, P = 0.58). By 14 mo after planting, there was still a significant treatment effect on survival ($F_{3,12}$ = 4.34, P = 0.027), and the block effect was very significant ($F_{5,12}$ = 5.98, P = 0.007). Survival in Treatment 4 had decreased to 52% after 14 mo, but was still 19–33% higher than in the other treatments.

Loss of foliage due to browsing by mammals

The greatest proportion of foliage was removed by herbivores in the first month after planting (Fig. 2). A significantly smaller proportion of foliage was removed from seedlings in Treatment 4 than in the other three treatments in this first month ($F_{3,12}$ = 12.58, P = 0.0005), and there was no block effect ($F_{4,12}$ = 1.42, P = 0.29). Eight months after planting, there was no difference between treatments in the level of foliar browsing on seedlings that were still alive ($F_{3,11}$ = 0.43, P = 0.73), but there was a significant block effect ($F_{4,12}$ = 4.03, P = 0.030).

Seedlings with browsing damage to the main stem

As with browsing of foliage, damage to the main stem of seedlings occurred mainly during the first month after planting (Fig. 3). One month after planting, there was a significant effect of treatment on number of seedlings with damage to main stem ($F_{3,12}$ = 21.5, P = 0.0001). The main stem was damaged in more than 10 of the 20 seedlings per plot in each of Treatments 1, 2 and 3, that is, in more than 50% of the seedlings originally planted. In contrast, main-stem damage occurred to less than one seedling per plot (3% of the total planted) in Treatment 4. Two and three months after planting, new damage to main stems was still recorded more frequently in Treatments 1–3 than in Treatment 4. By 8 mo, few seedlings from any treatment had damaged main stems.

Growth of live seedlings

During the first 4 mo after planting, mean height of live seedlings was reduced least in Treatment 4 (3 cm), by an intermediate amount in Treatments 1 and 2 (6–7 cm) and most in Treatment 3 (14 cm) (Fig. 4). Despite the variation in height at planting, and the initial differences in height lost through browsing, the height of live seedlings 14 mo after planting was similar for the four treatments ($F_{3,10}$ = 1.54, P = 0.26). There was a strong block effect by this time ($F_{4,10}$ = 4.36, P = 0.027). The height growth curve seemed to be lowest in Treatment 4, intermediate in Treatments 1 and 3, and highest in Treatment 2 (Fig. 4).

As with height, the diameter of seedlings that were still alive 14 mo after planting did not differ significantly between treatments (Fig. 5) ($F_{3,11}$ = 0.81, P = 0.51), but did differ between blocks ($F_{3,11}$ = 5.54, P = 0.011). The diameter of seedlings had increased

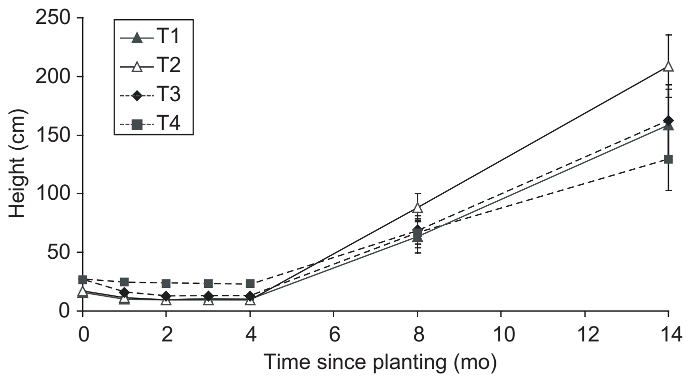

Figure 4. Height of live seedlings (least-squares means ± 1 s.e.) over time following planting

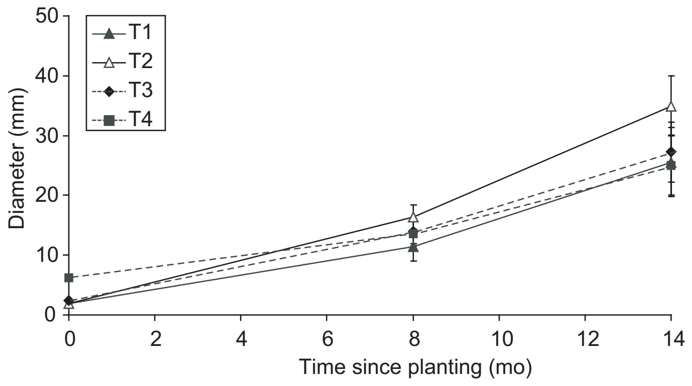

Figure 5. Diameter of live seedlings (least squares means ± 1 s.e.) over time following planting

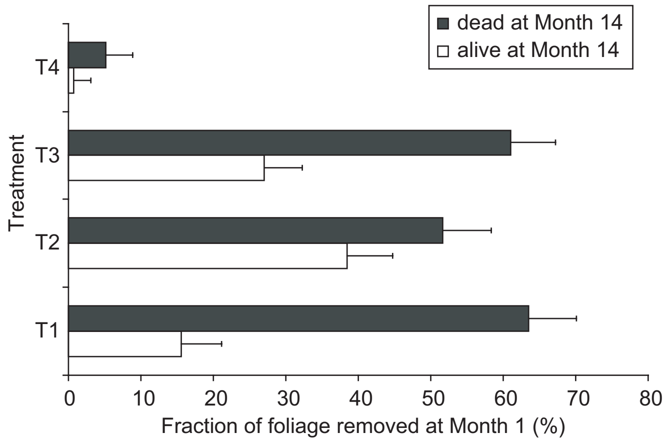

Figure 6. Comparison of the severity of mammal browsing at Month 1, recorded as fraction of the foliage removed (means + 1 s.e.), to seedlings that were subsequently alive or dead 14 mo after planting, for each treatment

11–19-fold in Treatments 1–3, but only 4-fold in Treatment 4 after 14 mo.

Relationship between browsing damage and survival

For Treatments 1–3, there appeared to be a relationship between the severity of browsing within the first month after planting, and subsequent seedling death within the first 14 mo (Fig. 6). Seedlings that were more severely damaged within the first month were more likely to be dead 14 mo after planting. This pattern also occurred in Treatment 4, but early damage in this case was much lower than in the other treatments (Fig. 6).

Discussion

Nursery effects on seedling morphology at planting

Increasing the spacing between container-stock seedlings (T3 compared with T1 and T2) resulted in a relatively small increase in seedling diameter and a large increase in height. Substantial gains in diameter were made only in the open-rooted stock (T4), concomitant with increased woodiness of the stem. There is a trend for forestry nurseries to move away from the use of open-rooted stock to more automated container stock production. It would therefore be worth investigating alternative methods for increasing seedling diameter in containers, perhaps by increasing pot size, because of the advantages of large diameter in relation to rabbit browsing as described below. It is also worth noting that our trials could not differentiate between the effects of stem diameter and woodiness of open-rooted stock. If such characteristics become dissociated during nursery manipulation, then defining an optimal combination would also be useful.

Effect of seedling morphology on browsing and survival

Our results show that the morphological characteristics of seedlings at planting can have a major impact on how severely they are browsed by rabbits, and on their subsequent survival. Browsing damage and mortality were not reduced in taller seedlings *per se* (T3 compared with T1 and T2), but were substantially reduced in tall seedlings with a large-diameter woody main stem (T4 compared with T1 and T2). Both factors, woodiness and large diameter, are likely to reduce the capacity of rabbits to clip seedlings, the latter as predicted by O'Reilly and McArthur (1997) and consistent with results for other lagomorphs (Hartwell and Johnson 1983).

In Treatment 4, browsing damage was so low that it is unlikely to have been the major cause of mortality. In Treatments 1–3, however, the low longer-term survival (19–33% at Month 14) observed in this trial could at least partly be linked to early browsing damage (Fig. 6). This link appears to contrast with between-plantation studies (Bulinski 1999; Bulinski and McArthur 1999), in which there was no relationship between survival of *E. nitens* seedlings at 12 mo and severity of browsing of remaining live seedlings at 12 mo. There are several possible explanations for this difference in results. First, in between-plantation comparisons, variation in other factors, such as climate, may overwhelm any effect of browsing on mortality. Second, browsing severity to live seedlings at 12 mo may not reflect browsing of live seedlings one month after planting. Third, early browsing may have less impact on survival of *E. nitens* than *E. globulus* seedlings, though this seems unlikely.

Despite advantages of Treatment 4 in relation to rabbit browsing, the fact that survival did decrease to 52% at 14 mo indicates there may be some cost in using seedlings with these characteristics, particularly in the absence of browsing. We suggest that the rapid reduction in survival of Treatment 4 seedlings following Month 4 was related to drought stress. An extremely dry summer period began in mid-November (4 mo after the trial was planted), and drought deaths occurred from November to February in other trials planted with *E. nitens* in that year (R. Appleton, *pers. obs.*). First-year container stock *E. globulus* seedlings survived in other trials, although their growth was affected. Open-rooted *E. globulus* seedlings survive well in their second year on good sites in normal years (R. Appleton, *pers. obs.*), but are more vulnerable to drought than container stock. On being transferred from field nursery to plantation, open-rooted seedlings may lose up to 25% of their root volume and have poor initial root–soil contact, both of which contribute to water stress (Wilson and Clark 1998). Therefore, these seedlings may be at a disadvantage if planted in a dry year, despite the absence of browsing.

Effect of seedling morphology on long-term plantation success

Three factors are useful for predicting whether a 1-y-old plantation will be successful in the longer term. These are (i) seedling size after 1 y, (ii) stocking rate after 1 y, and (iii) long-term growth rate. One year after planting, seedlings greater than 1 m in height have essentially escaped from browsing and are considered to be viable in estimates of establishment success in commercial plantations. The average height of seedlings that were still alive after 12 mo in our trial equalled or exceeded 100 cm in all treatments (Fig. 4), indicating that all treatments produced viable seedlings. The final stocking rate varied, however, due to differences in survival. Given the initial spacing of 1000 stems ha^{-1} in the trial, densities after 14 mo were 190, 330, 250 and 520 stems ha^{-1}, in Treatments 1 to 4 respectively. There was some suggestion that after 6 mo the seedlings remaining in Treatment 4 grew more slowly than seedlings in the other treatments (Fig. 4). This may represent another potential cost of using such seedlings, although again that cost may be realised only in drought conditions.

Effect of the repellent on browsing, survival and growth

There was no evidence that the 'Scat' repellent reduced browsing damage to seedlings (T2 compared with T1), either in terms of foliage loss or damage to the main stem. However, a surprising observation was that long-term survival and growth rates (height and diameter) tended to be higher in T2 than in the equivalent normal container stock (T1): on average 14% greater survival, 32% or 50 cm greater height and 36% or 9 mm greater diameter after 14 mo. It is unlikely that the repellent provided any direct nutrient benefit, given the single application in the nursery. It may have had an hormonal effect on growth, which could be realised at very low concentrations (D. Close, *pers. comm*.).

Conclusions

Our trial has demonstrated a benefit of using open-rooted 'half and half' *E. globulus* seedlings to reduce browsing damage by rabbits and to promote early survival under conditions of high browsing pressure from rabbits. Potential disadvantages of using these seedlings include decreased longer-term survival under drought stress and possibly reduced longer-term growth rate. The trade-offs between treatments in relation to browsing susceptibility, survival and growth rate under different biotic and abiotic conditions need to be carefully considered when choosing seedlings for plantations. The use of 'Scat' repellent deserves further attention, not least because it appears to have a beneficial effect on growth and survival irrespective of any impact, or lack of impact, on rabbit browsing.

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