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TCFA research into alternatives to the use of 1080:

Manipulating seedling palatability for non-lethal browsing management

by Alison Miller[1],
Julianne O'Reilly-Wapstra[1,2],
Brad Potts[1,2], Clare McArthur[3]

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[1] School of Plant Science, University of Tasmania, Private Bag 55, Hobart, TAS 7001
[2] Cooperative Research Centre for Forestry, Private Bag 12, Hobart, TAS, 7001
[3] School of Biological Sciences, Building A08, University of Sydney, NSW, 2006

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COOPERATIVE RESEARCH CENTRE FOR FORESTRY
College Road, Sandy Bay, Tasmania
Private Bag 12, Hobart, Tasmania 7001 Australia

www.crcforestry.com.au

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Summary

Browsing by marsupial herbivores is a major problem in plantation forestry. This has traditionally been controlled through a reduction in herbivore numbers achieved by lethal means. The mammal browsing group at the University of Tasmania and the CRC for Forestry has been researching non-lethal alternatives for over a decade and found that the most effective methods involved manipulating seedling palatability prior to planting and in the field. Specifically, the use of naturally resistant seedling stock, chemical repellent, modification of nursery fertiliser regime and use of natural vegetation on coupes have all proven successful in deterring feeding. Additionally, since 2007, the use of mesh stockings to protect plantation seedlings has become quite popular within the forestry industry, but data to confirm their effectiveness in reducing browsing is lacking.

This study combined extensive browsing research to operationally test the most effective combination of non-lethal methods listed above. Treatment combinations were planted in eight field sites across Tasmania. Experimental seedlings were planted in replicated blocks spread in a single row around the perimeter of operational coupes. Seedlings were monitored regularly for browsing damage, with seedling height and characteristics of the surrounding vegetation being assessed periodically. We found that the most effective treatments at reducing the severity of browsing damage in the short term were seedling stockings and a combination of chemical repellent (Sen-Tree) and low nursery fertiliser.

Stockings and repellent were then tested in further trials to demonstrate the effectiveness of these treatments across a range of sites and during winter, as opposed to spring plantings. Here we used six field sites and followed the same basic design as the initial trial. Stockings and repellent were tested in isolation, in combination, and with versus without field application of repellent. We found that the combination of stockings and repellent was the most effective, and resulted in a significant delay in browsing and a reduction in browse severity over 24 weeks, compared with control seedlings.

These results have important and immediate implications for tree growers. Stockings and/or repellent can be applied to seedlings in the nursery to significantly delay the onset of browsing and reduce its severity when planted in the field. In areas with low browsing intensity, this could be enough to reduce browsing in itself; in other areas the browsing delay could be enough to allow alternative controls to be implemented. The long-term effectiveness of stockings needs to be determined (e.g. effects on seedling growth and form) and the issue of continued repellent re-application needs to be addressed.

Introduction

Browsing by marsupial herbivores is a key factor affecting the success of eucalypt plantations in Tasmania (Coleman *et al.* 1997). It has been well documented that marsupial browsing damage on seedlings significantly reduces the net growth rate of plants (Bulinski and McArthur 1999) and can even result in complete loss of plantations (Bulinski 2000). Browsing at later life stages of the tree can also affect tree form, reduce growth rates and can lead to tree death (Volker and Orme 1988; Scott *et al.* 2002). Methods for controlling Tasmanian native herbivores, such as the common brushtail possum (*Trichosurus vulpecula*), red-bellied pademelon (*Thylogale billardierii*), and Bennett's wallaby (*Macropus rufogriseus*), and the introduced European rabbit (*Oryctolagus cuniculus*) have mostly been lethal, using the poison compound 1080 and shooting. For over a decade the forestry industry in Tasmania has supported research into mitigating browsing damage with a strong emphasis on non-lethal strategies due to social and political pressure to move away from lethal methods of control such as poisoning.

Fourteen years of research by the mammal browsing group at the University of Tasmania (UTAS) and the CRC for Forestry has consolidated that one key area of research offers a non-lethal mechanism for reducing browsing damage on plantations; the manipulation of seedling palatability prior to planting. Specifically, the use of naturally resistant seedling stock, chemical repellents, modification of fertilisation regime in the nursery and use of natural vegetation on coupes have all proved successful in deterring feeding.

Resistant stock

Eucalypts are rich in plant secondary metabolites (PSMs) which enhance natural resistance and protection from browsing by native mammals (Lawler *et al.* 2000; Close *et al.* 2003; O'Reilly-Wapstra *et al.* 2004). Natural plant resistance can be utilised as a means of reducing the amount of damage herbivores cause in many agricultural plant systems (Kennedy and Barbour 1991) and plantation forestry in Tasmania is no exception. Previous research (O'Reilly-Wapstra *et al.* 2002; O'Reilly-Wapstra *et al.* 2004; O'Reilly-Wapstra *et al.* 2005) has identified genotypes of *Eucalyptus globulus* that are naturally more resistant to browsing than other populations. These genotypes are naturally higher in key secondary metabolites, particularly sideroxylonal, which enhances resistance to the browsers (O'Reilly-Wapstra *et al.* 2004). Genotypes with elevated resistance have been tested in captive feeding trials on the common brushtail possum and the red-bellied pademelon but are yet to be tested in operational style plantings.

Chemical repellents

Repellents generally act by deterring the herbivore from approaching the seedling (due to an unpleasant odour), and/or by making the seedling unpalatable to the herbivore. Repellents have been shown to deter mammalian herbivores such as deer, elk, possum, rabbit, wallaby, and beaver from feeding on a range of plants, including plantation trees, fruit trees, vegetables and ornamental plants (Gillingham *et al.* 1987; Epple *et al.* 1993; Andelt *et al.* 1994; Marks *et al.* 1995; Woolhouse and Morgan 1995; Kimball *et al.* 2005). Commonly used repellents have active ingredients including carnivore urine or faeces, egg, bitter-tasting compounds, and capsaicin.

Miller *et al.* (2008) conducted a series of captive feeding trials to test the effectiveness of three chemical repellents for reducing browsing on *Eucalyptus nitens* foliage by red-bellied pademelons and common brushtail possums. These revealed that Sen-Tree (formally WR-1) is the most effective, commercially available browsing deterrent of young eucalypt seedlings; it reduced feeding by 98% compared with non-treated seedlings. Sen-Tree is an egg-based repellent sprayed onto seedlings, with a sandy grit (carborundum) sprinkled onto the egg base. It acts as both an odour repellent (the egg odour) and a palatability repellent (the grit). Carborundum is a hard, non-toxic grit which replicates naturally-occurring silica found in plants. This wears down the animal's teeth, and is something the animal naturally avoids when encountered in high concentrations (Delbridge and Lutze 1998). Sen-Tree (as WR-1) has also been shown to be repellent to captive European rabbits and swamp wallabies (*Wallabia bicolor*) (Marks *et al.* 1995; Harman 1996; Delbridge and Lutze 1998). It is not harmful to humans or animals and does not affect plant growth (Marks *et al.* 1995; Johnston *et al.* 1998; Witt 2002).

Nursery fertiliser

Resistance of eucalypt seedlings can be modified in the nursery through the application of different fertiliser regimes (Close *et al.* 2003; McArthur *et al.* 2003; Miller *et al.* 2007). In terms of plant chemical characteristics, intake is often related to a trade-off between beneficial primary constituents, such as nitrogen, and costly secondary metabolites, such as phenolics (Bergeron and Jodoin 1987; Edenius 1993; Lawler *et al.* 1998; Villalba *et al.* 2002). Nitrogen content is often used as an index of plant palatability because it is positively correlated with protein content, dry matter digestibility, and digestible energy (Marell *et al.* 2002). Plant secondary compounds reduce intake by either reducing digestibility, through interfering with nitrogen availability (e.g. phenolics, (Mattson 1980)), or by providing a toxic load (e.g. formylated phloroglucinol compounds (FPCs), (McLean *et al.* 2004; O'Reilly-Wapstra *et al.* 2004); oils, (Wiggins *et al.* 2003; Boyle *et al.* 2005)). The balance of these, and therefore the palatability of seedlings to herbivores, in terms of the comparative extent to which they are eaten, can be altered by fertiliser application. Manipulation of seedling palatability to herbivores has been well tested in captive and small scale field trials (Close *et al.* 2004; Miller 2006; Miller *et al.* 2007) but is yet to be tested in larger scale operational plantings.

Vegetation management

As highlighted above, browsing on a focal plant, such as a tree seedling, is most commonly related to characteristics of that plant, such as its morphology and chemistry. However, because herbivores can select at multiple levels within habitats (e.g. patch, plant, plant-part), the probability that a focal plant will be attacked by a herbivore depends not only on its own characteristics but also on the relative quality and abundance of its neighbours (Atsatt and O'Dowd 1976; Hjältén *et al.* 1993). The associational plant refuge hypothesis predicts that a plant species of a given quality should gain protection from herbivory when it is associated with species of lower quality, and should be more at risk when occurring alone or in association with species of higher quality (Pfister and Hay 1988). This has been demonstrated in terrestrial systems, with a range of both insect (e.g. Holmes and Jepson-Innes 1989; Hambäck *et al.* 2000; White and Whitham 2000) and mammalian (e.g. McNaughton 1978; Hjältén *et al.* 1993; Frid and Turkington 2001) herbivores. Consistent with this, Miller (2006) found that when pademelons were allowed to select at the patch scale (in this case an area of $12m^2$), seedlings were more vulnerable when surrounded by high quality grass. However, when selection at the patch scale was not possible, seedlings were less vulnerable to browsing when surrounded by high quality grass that could act as an alternative food source (Miller 2006; Miller *et al.* 2007). In addition, it has been shown that seedlings gain protection from browsing through being visually screened by tall unpalatable vegetation (Miller 2006; Miller *et al.* 2006). These studies highlight the importance that coupe vegetation could play in providing protection or alternative food. Site preparation that involves using herbicide to minimise the amount of vegetation, and thus competition for limiting resources between tree seedlings and other vegetation, could lead to increased browsing of tree seedlings because a) they become the main food source present and b) they may be more apparent to potential browsers.

Tree guards/physical protection

Tree guards are something that we had not examined previously, but which industry is increasingly incorporating into their management systems. Tree guards have been used to protect seedlings for several decades. Most published work on tree guards or shelters involves the use of rigid materials that are staked into place over a seedling (Baer 1980; Montague 1993; Stange and Shea 1998; Devine *et al.* 2007). Such guards can work extremely well to reduce browsing, and often have the added bonus of increasing seedling growth, possibly due to the production of a miniature greenhouse effect (Bendfeldt *et al.* 2001). As far as operational plantations are concerned, however, such guards are inappropriate for regular use due to their very high cost per seedling (often upwards of $3/seedling).

The guards in current usage are quite different to the traditional tree guards. They are made of flexible polyethylene netting which is cut to fit individual seedlings and clings to the seedling rather than requiring external support. These have the advantages of being much cheaper (≤$0.15/seedling), and being able to be applied in the nursery. Although in regular use, these guards have not been formally tested and their cost-effectiveness remains unknown. For this reason, tree guards, hereafter referred to as “stockings” were added to the trials. As this treatment was not part of the original plan, their incorporation into the experimental design is not complete, i.e. they are not tested in combination with other nursery treatments.

Aims

To date, research has not tested the effectiveness of the full compliment of seedling manipulation strategies in the field. Our research shows the effects of these characteristics are generally additive (Miller et al. 2006; Miller et al. 2007), so it is plausible, but untested, that “seedling resistance” can be augmented incrementally by each of these mechanisms. Consequently, we aimed to:

1. In field trials, fast-track the identification of the best combination of alternative non-lethal strategies (enhanced resistant planting stock, Sen-Tree repellent, fertiliser regime, coupe vegetation, and stockings) to deter feeding of seedlings by browsing mammals. These were tested on sites that had been both shot and un-shot to investigate the role of these non-lethal approaches in combination with current lethal strategies.

2. Plant operational demonstration field sites using the “best practice” combination.

This project therefore consisted of two distinct trials. The first trial (Trial 1) was designed to find the best combination of seedling manipulation strategies to reduce browsing. The second trial (Trial 2) was designed to operationally demonstrate the effectiveness of these combinations. Because Trial 2 was based upon results from Trial 1, we present methods and results in two parts, beginning with Trial 1.

TRIAL 1

Methods

We began with a trial to find the best combination of seedling manipulation strategies to reduce browsing. Many of these had been tested individually in previous work through the CRC for Forestry and UTAS and were found to be effective, but their combined action was unknown. In addition we added a treatment at the request of industry partners (stockings), which we had not previously tested.

Field sites

We used eight sites across the state. All of these were owned by Forestry Tasmania (FT) and were set up as ex-native forest, first rotation operational *E. nitens* coupes. There were two sites in the far south near Hastings Caves; two sites in the north-east near Scottsdale; and four central sites in the Florentine Valley (Figure 1). Sites were on average 30 ha in area and ranged from 50-650 m above sea level (Table 1). Sites were geographically paired, with one of each pair receiving standard pre-plant shooting, while the other was not shot prior to planting. However, operational issues resulted in neither of the sites in pair C (sites 5 and 6) being pre-plant shot, and these sites were not shot until 5 months after planting.

Browse control data provided by Forestry Tasmania (not presented) shows that all three main herbivore species (Bennett's wallaby, red-bellied pademelon and brushtail possum) were present at each of the eight experimental sites. There was no evidence of fallow deer (*Dama dama*) or rabbit, two other potential pests, at any of these sites. This data cannot be used to compare herbivore numbers or composition between sites or over time because each coupe was subject to a different control regime.

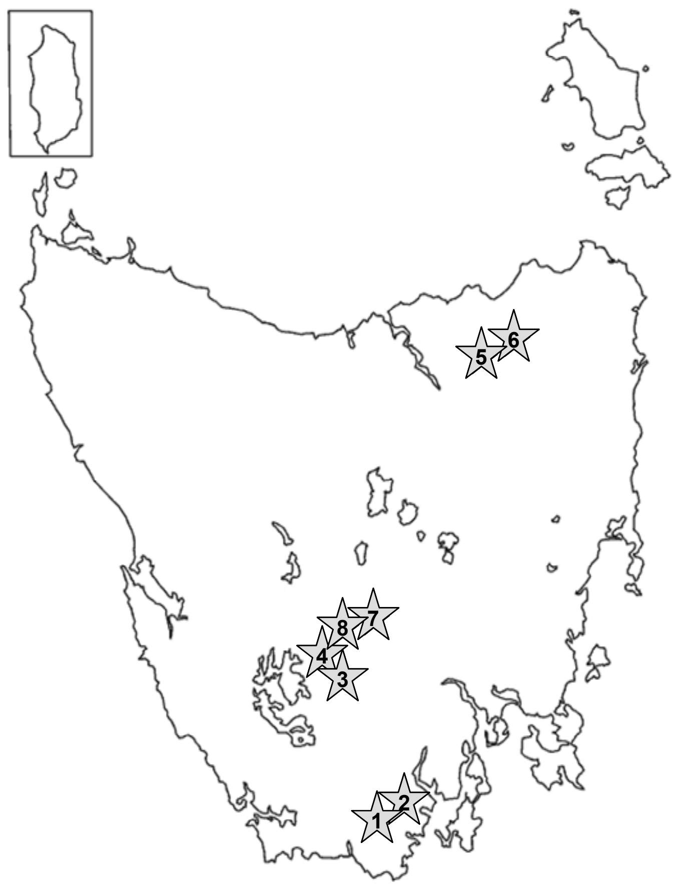

Figure 1. Approximate location of the eight field sites used in Trial 1

Table 1 Characteristics of the eight field sites used in Trial 1

Site no.	Coupe ID	Pair	Location	Area (ha)	Altitude (m)	Pre-plant shot	Planted	Post-plant control
1	HA028C	A	Hastings	35	50	no	03/10/2007	trap
2	SO018A	A	Southport	19	150	yes	05/10/2007	shoot & trap
3	FO033B	B	Florentine	25	450	no	02/10/2007	shoot & trap
4	FO014D	B	Florentine	35	400	yes	04/10/2007	shoot & trap
5	SF143G	C	Springfield	23	300	no	27/11/2007	shoot
6	SF127C	C	Springfield	25	300	no	28/11/2007	shoot
7	RP020D	D	Repulse	38	650	no	10/10/2007	shoot
8	RP026A	D	Repulse	45	650	yes	10/10/2007	shoot & trap

Growing seedlings

All seedlings were raised at the Forestry Tasmania tree nursery in Perth, Tasmania. This ensured uniform growing conditions and allowed for fertilisation treatments to be conducted. Seeds were broadcast sown over 30 x 35 cm seedling trays filled with a mixture of approximately 60% coarse river sand (for drainage) and 40% peat (to retain moisture). The different seedlots used in the trial (Table 2) were each sown on separate labelled trays. The number of seeds sown per tray varied from 300 – 467, depending upon the number of seedlings required and expected germination rates. Generally, the number of seedlings required was doubled, and that amount of seed was counted out and sown. Most seed was less than 12 months old; for seed older than 5 years, four times the amount required was sown due to poor germination rates.

Seeds on trays were then covered with a layer (~1 cm) of vermiculite (*E. globulus*) or sand (*E. nitens*) to retain moisture. Trays were hand-watered with 1.5 ml/L of the fungicide Previcur® (Bayer CropScience Australia). *Eucalyptus globulus* trays were placed straight into a 25°C growth room on wire racks. *Eucalyptus nitens* trays were first placed in a 5°C cool room for 2 weeks for cold stratification, to ensure prompt and uniform germination, before moving to the growth room. Trays were removed from the growth room once a large proportion of seedlings had emerged, and placed out under shade cloth to harden. This generally took between 6 and 10 days. *Eucalyptus nitens* were prepared first to ensure both species would germinate at a similar time.

Table 2. Source and resistance level of *E. globulus* and *E. nitens* seedlots used in Trial 1. BGH = Blue Gum Hill; JN = Jeeralang North; SH = St. Helens

Species	Resistance level	Source	Locality[a]	# seedlots[b]
E. globulus	Operational	FT	.	2
	↑ resistance	UTAS	BGH	5
	↑ resistance	UTAS	JN	5
	↑ sideroxylonal	FT	.	3
	↓ resistance	UTAS	SH	5
	↓ sideroxylonal	FT	.	4
E. nitens	Operational	FT	.	3
	↑ sideroxylonal	FT	.	8
	↓ sideroxylonal	FT	.	6

[a]UTAS localities and FT ↑ and ↓.sideroxylonal groups form five 'populations' for analysis.
[b]See Appendix 1 for details.

After 9-14 days, or once seedlings had reached a height of around 2 cm, individual seedlings were "pricked" out of seedling trays into individual cells of Lännen trays (81 cells per tray, each 41 mm wide by 73 mm deep, with side slots allowing air-pruning of lateral roots). Trays were filled with standard potting mix (mixture of compost, pinebark & peatmoss) and a slow release fertiliser (Osmocote® Mini Controlled Release Fertiliser, Scotts Australia; NPK: 18 + 2.6 + 9.1). This fertiliser provided nutrients for around 10 weeks. Once all seedlings were "pricked" out, all trays were given another drench of Previcur® (1.3 ml/L) due to concern about damp over winter. All trays were moved to a plastic growth house, where they remained until ready for planting. Trays were randomised in order to reduce any position effects while growing; trays from each seedlot were divided evenly into three blocks, and then the position of all trays within each block was randomized. Seedlings were watered twice a day, by either hand or boom, until the soil was saturated.

With experimental seedlings already growing in the growth house, a decision was made, at the request of industry partners, to add two new treatments; stockings and an additional fertiliser treatment (details below). Seedlings for these treatments were therefore sourced from the nursery, including sufficient numbers for controls and chemistry, from stock as close as possible to the size of the other experimental seedlings. Trays were randomized within existing trays two months after experimental seedlings were moved into the growth house. Additionally, seedlings for the *E. nitens* operational treatment were growing slowly (probably because they originated from small seed) and therefore were replaced with seedlings sourced from the nursery around the same time.

Experimental design

Trial 1 consisted of 14 main treatments, as shown in Table 3. There were two eucalypt species; *E. nitens* (17 seedlots) and *E. globulus* (24 seedlots), comprising 41 seedlots, resulting in a total of 92 different treatment combinations. The main treatments involved combinations of seedling resistance level (type), fertiliser treatment, repellent and stockings.

Table 3 Trial 1 treatment summary. S = standard fertiliser; L = low fertiliser; P = high phosphorous fertiliser. N = no; Y = yes

Treat #	Seedling type[a]	Fertiliser	Repellent	Stockings	# globulus seedlots	# nitens seedlots	Treat code
1	operational	S	N	N	1	1	S-R
2	operational	L	N	N	1	1	L-R
3	operational	S	Y	N	1	1	S+R
4	operational	L	Y	N	1	1	L+R
5[b]	↑ resistance / sideroxylonal	S	N	N	10	4	S-R / ↑ resist
6	↑ resistance / sideroxylonal	L	N	N	10	4	L-R
7	↑ resistance / sideroxylonal	S	Y	N	10	4	S+R
8	↑ resistance / sideroxylonal	L	Y	N	10	4	L+R
9[c]	↓ resistance	S	N	N	5	0	↓ resist
10	↑ sideroxylonal	S	N	N	3	4	↑ sider
11	↓ sideroxylonal	S	N	N	4	6	↓ sider
12	operationalB	S	N	N	1	1	Ctrl
13	operationalB	P	N	N	1	1	HighP
14	operationalB	S	N	Y	1	1	Stk

[a]"OperationalB" were seedlings purchased from the nursery (different early growth conditions to other seedlings).
[b]Increased resistance seedlots from BGH and JN
[c]Reduced resistance seedlots from SH

Treatments were grouped into seven experiments to ensure that comparisons of interest were planted in spatial proximity and therefore subjected to similar browsing pressure (Table 4). Treatments were randomised within experiments, experiments within blocks, and blocks were randomised within sites. This procedure was repeated for each of the eight sites.

Table 4. Summary of experimental treatment groupings for Trial 1

Experiment	Treatments	Species.	Summary
1	1, 2, 3, 4	*E. globulus*	Operational fertiliser/repellent combinations
2	1, 2, 3, 4	*E. nitens*	Operational fertiliser/repellent combinations
3	5, 6, 7, 8	*E. globulus*	↑ resistance fertiliser/repellent combinations
4[a]	5, 6, 7, 8	*E. nitens*	↑ sideroxylonal fertiliser/repellent combinations
5	5, 9, 10, 11	*E. globulus*	↑ and ↓ resistance/ sideroxylonal seedlots
6	10, 11	*E. nitens*	↑ and ↓ sideroxylonal seedlots
7	12, 13, 14	*E. globulus* & *E. nitens*	OperationalB with high P and stockings

[a]Experiment 4 later changed to Operational2 fertiliser/repellent combinations (see Analysis section)

Treatments were tested as single tree plots planted in a single row around the perimeter of each coupe. The rest of the coupe was standard operational *E. nitens* planting. The perimeter was used because this is generally where the most intense browsing occurs, and the single row meant that we could cover the entire perimeter. It was important to cover the entire perimeter to ensure that we sampled all areas where browsing could be a threat.

This design resulted in a total of 747 seedlings per site, and these were divided into 20 blocks. Exceptions were sites 7 and 8 which had only 327 seedlings. Problems in the nursery, including an attack by the pathogenic stem fungus *Botrytis cinerea*, reduced the number of *E. globulus* seedlings available for planting. Consequently, rather than reducing replication at all sites, or cutting back on the number of sites, a decision was made to remove *E. globulus*-only experiments (1, 3 and 5) from the two highest altitude sites (sites 7 and 8) where they were least likely to survive.

Genetic stock

Eucalyptus globulus seedlots were sourced from both FT and UTAS seed stores. Unless specified otherwise, each seedlot was open-pollinated seed collected from a single tree. There were ten relatively more resistant seedlots (↑ resistance) from UTAS (Appendix 1). These came from two native populations known to have higher levels of defensive chemistry than other populations and increased resistance to browsing: Blue Gum Hill (hereafter BGH) in southeast Tasmania and Jeeralang North (hereafter JN) in Victoria (5 seedlots per population). JN seed was from the CSIRO 1987/88 seed collection. There were five relatively less resistant (↓ resistance) UTAS seedlots from a native population at St. Helens (hereafter SH), northeast Tasmania. These were known to have reduced levels of sideroxylonal A and reduced resistance to browsing (O'Reilly-Wapstra *et al.* 2002; O'Reilly-Wapstra *et al.* 2004). There were two operational FT seedlots, produced through Mass Supplementary Pollination (MSP), containing a mixture of seed from different seed orchard trees of unknown chemistry. There were also three FT seedlots predicted to contain high sideroxylonal levels and four predicted to contain low sideroxylonal levels. Predictions were based on near-infrared reflectance spectroscopy (NIRS) scans of foliage from seedlings from the same or sibling trees used in this trial, four months after planting out in the field (J. Humphries unpublished data). These seedlots had not been tested in browsing trials, and so their resistance levels were unknown, but it was predicted that, as with the UTAS seedlots, higher sideroxylonal levels would translate into increased resistance to browsing.

All *E. nitens* seedlots were sourced from Forestry Tasmania. This included three operational seedlots of unknown chemistry, eight single-tree seedlots that were predicted to contain high levels of sideroxylonal A and six single-tree seedlots predicted to contain low levels (Appendix 1). These predictions were based on NIRS scans from a previous trial (N. Glancy unpublished data), using foliage collected from the nursery: some seedlots were seed left over from the original collection for this trial, some were from the same tree but a different seed year and some were from a clone of the tree in the original study (same mother genotype but a different graft in the orchard and a different seed year). As for *E. globulus*, there had been no browsing tests performed on any of these FT seedlots, and so their resistance levels were unknown, and the NIRS predictions were unvalidated. All operational/control seedlots were a mix of seed from a number of seed orchard trees, as is used for standard operational plantings around the state.

Fertilisation treatments

All seedlings received slow-release fertiliser (Osmocote® Mini Controlled Release Fertiliser, Scotts Australia; NPK: 18 + 2.6 + 9.1) to begin with, and were then fertilised bi-weekly with "standard" fertiliser via a watering boom until three months before planting, when treatments commenced. This initial fertiliser ensured that seedlings would reach an appropriate height, preferably 20 cm or greater, before planting. "Standard" fertiliser is what is routinely used by the nursery for plantation seedlings: Peat-Lite Special© Hi-N (NPK: 20 + 4.4 + 16.6; Peters® Professional® Water Soluble Fertiliser, Scotts Australia) @ 1 g/L.

Three fertiliser treatments were used: standard, low, and high phosphorous (hereafter high P). These treatments differed primarily in nitrogen and phosphorous levels, and were chosen on the assumption that nitrogen is the main primary constituent influencing seedling palatability, while phosphorus has a strong influence on growth. The high P treatment was added due to industry concerns about slower growth in low fertiliser treatments resulting from a lack of phosphorous. Due to its last minute addition, this treatment was not used in combination with other treatments.

When fertilisation treatments commenced, "low" fertiliser seedlings received no further fertiliser, "standard" fertiliser seedlings continued to receive "Peat-Lite Special© Hi-N", and "high P" seedlings received "Blossom Booster" (NPK: 10 + 13.1 + 16.6; Peters® Professional® Water Soluble Fertiliser, Scotts Australia). Fertiliser was applied weekly by hand. It was ensured that seedlings were not watered within a few hours of fertilising, and that foliage was dry at the time of fertilisation. Both were applied, as per instructions, at a rate of 2 g/L. This is double the amount that is applied when done using boom, but ensures saturation, and is based on the hand fertiliser method used by nursery staff (C. Cox pers. comm.).

Repellent application

Sen-Tree™ browsing deterrent was obtained in kit form from Sure Gro, Dingley, Victoria (www.suregro.com). Each kit arrives in a 15 L bucket, consisting of 7.5 L glue (acrylic polymer adhesive), 1.45 kg egg powder, and 5 kg grit (silicon carbide), and makes enough repellent to treat approximately 6000 seedlings. Due to a very limited shelf-life (24 hours if refrigerated), repellent was prepared as required and used within a few hours. Repellent was applied to seedlings in the nursery shortly before planting. Foliage was dry at application, and then kept undercover for a minimum of 24 hours after application to allow repellent to dry.

Repellent was applied to a total of 1600 seedlings, or 200 at each site. To prepare enough repellent for each site, we combined 50 g egg powder with 250 ml water and blended, then added 250 ml of the glue solution. This was poured into a hand sprayer and sprayed as a fine mist over eucalypt foliage (Plate 1a). Seedlings were spread out in seedling trays so that they were not touching one another and therefore not glued together. Grit was immediately sprinkled onto this glue base using a hand shaker to obtain an even coverage.

Stocking application

Rolls of red polyethylene stocking material (Venet netting), similar to orange or onion stacks, were obtained from Crisp'n'Clean, Somerset, Tasmania. Lengths of stocking material were fed onto a pipe (~10 cm diameter); seedlings were inserted into the pipe foliage first, material was gathered around the root ball, and seedling was pulled out with stocking on, which was then cut to length (a few cm above seedling tip) using scissors (Plate 1b). Only one seedling at a time could be prepared in this way. Stockings enclosed entire seedlings, including roots.

Plate 1. a) Application of repellent & b) application of stocking to eucalypt seedlings

Planting/field operations

In preparation for planting, seedlings were selected at random from trays of the relevant seedlot, individually labelled with fluorescent yellow tags (Tytags Australia, Morisset NSW) to designate treatment and order, and sorted into planting order. Repellent and stocking treatments were applied to the appropriate seedlings several days before planting.

Seedlings were planted out in field sites in spring 2007. Planting was done by UTAS staff within a week of operational planting, using the same methods (i.e. pottaputkis or spades and 3 m spacing). All sites were planted by the same staff members to ensure consistency.

After planting, experimental seedlings were treated the same as operational seedlings. Most seedlings were fertilised using 100 g Di-Ammonium Phosphate (DAP 18-20-0-1) around 5 weeks after planting. SF coupes were fertilised within a week of planting due to being planted quite late in the season.

After planting, browsing control was performed by contractors on a site by site basis, as deemed necessary by forestry operations staff. A combination of trapping and shooting was used on sites 2, 3, 4 and 8; sites 5, 6 and 7 were only shot, and site 1 was only trapped over the study period.

Data collection

Monitoring included assessment of browsing damage, seedling heights, vegetation characteristics and scat transects. Seedlings were monitored fairly intensively for 6 months (24 weeks) after planting, followed by an assessment of height, damage and form 12 months (48 weeks) after planting. Seedling heights were measured at planting (Week 0) and then monthly, from ground level to the base of the apical bud, to the nearest 0.5 cm. Seedlings were monitored for browsing damage weekly for 6 weeks, then fortnightly for 4 months, and monthly up until 6 months after planting, before a final assessment after 12 months. Browsing damage was assessed in three parts: whether damage was caused by mammalian or insect herbivores, whether the apical bud was removed, and the amount of foliage removed. The latter was scored as percentage foliage removed on a scale from 0-6, where 0 = 0%, 1 = 1–5%, 2 = 6–25%, 3 = 26–50%, 4 = 51–75%, 5 = 76–95%, 6 = 96–100%.

The vegetation within a 30 cm radius around each seedling was assessed on a monthly basis. This assessment looked at three variables: vegetation cover, height and type. The percentage of living ground cover of vegetation was scored using the same 0-6 scale as used for browsing damage. The height of vegetation relative to seedlings was scored as either L – shorter than seedling; M – similar height to seedling; or H – taller than seedling. The type of vegetation was scored by category, e.g. grasses, sedges, ferns, shrubs, and this was converted to predominantly palatable (grasses or forbs) or unpalatable (bracken, thistles, fireweed, sedges etc.) for the purpose of statistical analysis.

There were four 25 m scat transects set up permanently at each site. Transects were positioned at roughly equal distances around the coupe perimeter. Monthly monitoring involved counting, identifying and clearing any scats found within 1 m on the coupe side of each transect.

Foliage chemistry

In order to ensure that defensive chemistry related to resistance of seedlings in the field, a sub-sample of seedlings was harvested from each of the seedlot/fertiliser combinations prior to planting. Samples consisted of the foliage from 10 seedlings (or more if small) of a particular combination. Foliage was stripped, placed in labeled bags and frozen. These were then sub-sampled to set aside fresh material for analysis of essential oils, and the rest of the sample was dried in a BREDA scientific freeze-drier (LY-S-FM) until dry. Total oils and 1,8-cineole were assayed using fresh foliage by gas chromatography-mass spectrometry (GCMS). The method was modified from that reported in O'Reilly-Wapstra *et al.* (2004) by sonicating samples in an ultra-sonication bath for 30 minutes and repeating the extraction procedure three times for each sample to increase the proportion of oils extracted. Freeze-dried samples were scanned using NIRS (Foley *et al.* 1998) to determine the defensive chemistry profile of the seedlings. This involved scanning five leaves per sample, using two spots on the top side of each leaf and recording the average value of four scans/spot. Unfortunately data was a poor fit for our current model (NIRS data therefore not presented), and subsequently all samples had to be analysed using traditional chemical assays. These were conducted for total phenolics, condensed tannins, formylated phloroglucinol compounds (FPCs), nitrogen and carbon. Total phenolics and condensed tannins were assayed with the modified prussian blue assay for total phenolics using gallic acid standards (Graham 1992), and the acid butanol assay for condensed tannins using purified sorghum tannin standards (Porter *et al.* 1986). Foliage for these assays was prepared and extracted following the method outlined in Hagerman (2002). Two FPCs (sideroxylonal A and macrocarpal G) were assayed by high performance liquid chromatography (HPLC) following Wallis and Foley (2005). Nitrogen and carbon were determined by Dr Thomas Rodemann at the Central Science Laboratory, UTAS, using a Thermo Finnigan EA 1112 Series Flash Elemental Analyser.

Analysis

We examined eight main variables for this trial:

1. *browsing delay* - the week when browsing first occurred and when browsing first reached a severity of 25% or more of foliage removed.
2. *browsing extent* – percentage of seedlings browsed; calculated as the number of seedlings with damage, divided by the total number of seedlings.
3. *browsing severity* - percentage of foliage removed from seedlings; calculated by converting browsing scores to median values of percentage foliage removed by mammals (e.g. a score of 2 = 15.5%).
4. *browsing recovery* – browse score at week 10 minus browse score at week 16; >0 = recovery; ≤0 = no recovery.
5. *seedling height* - the height (cm) of seedlings at planting (week 0) and at weeks 24 and 48 after planting, and the change in height over the trial (week 0-48).
6. *seedling form* – occurrence of multiple (>1) leaders at week 48.
7. *seedling survival* - the percentage of seedlings alive at the end of the trial (week 48).
8. *foliage chemistry* – content of nitrogen, carbon, condensed tannins, total phenolics, total oils, cineole, sideroxylonal A, macrocarpal G and total PSMs in foliage at planting, where total PSM = sum of all but nitrogen and carbon (expressed in mg/gDM).

The seven experiments of interest were analysed separately to examine field results. All seedlings within each experiment were raised under identical conditions allowing direct comparisons. Experiments 2 and 7 contain seedlings purchased from the nursery and therefore care should be taken when comparing results from these experiments to others. All analyses, except those using binary data sets (see below), were conducted in SAS 9.1 (SAS Institute Inc. 2004). For all statistical tests, residuals were checked for homoscedasticity and normality, and transformations were performed where required and have been indicated (Zar 1996).

Browsing delay was analysed using PROC LIFETEST (SAS Institute Inc. 1989). Analysis was performed on the week that seedlings were first browsed, and the week where browsing first reached or exceeded 25% of foliage removed. This level was chosen to represent a level at which herbivores have shown clear preferences for feeding on seedlings and where seedlings are likely to be influenced by this amount of foliage loss. Because a large proportion of seedlings in some treatments never "failed", where failure was being browsed or reaching a severity of 25% or more, analysis of browsing delay takes this into consideration by censoring this data. Models initially included site and treatment(s) as strata, and vegetation cover and height as covariates. Site was always highly significant, and was removed from the model to examine the average browsing delay in response to treatment across sites.

Browsing severity, seedling height and foliage chemistry were examined by fitting a linear mixed model with the PROC MIXED procedure of SAS using the restricted maximum likelihood (REML) approach. Where significant ($P < 0.05$) treatment effects were found, pairwise comparisons were made using the Tukey-Kramer adjustment for multiple comparisons.

For all analyses (except chemistry), replicate (site) was a random variable in all experiments; experiment 5 also used population(seedlot) as an error term to allow more power for testing across experiments (this experiment is actually a combination of two experiments). Fixed variables were dependent upon experiment; all included site; also species, treatment, population, resistance, fertiliser and/or repellent. Vegetation index (cover x height) was included as a covariate in tests of recovery from browsing and seedling height. Vegetation heights of L, M and H were converted to values of 0, 6 and 8 respectively for statistical analysis to reflect the greater influence of vegetation that is at least as tall as seedlings. Vegetation index was calculated as vegetation cover multiplied by the numerical value assigned to vegetation height. Analyses of the severity of browsing were conducted on data from week 6, 24 and 48; data was arcsin sqrt transformed.

In order to examine the effect of fertilisation treatment on chemical profile, seedlots receiving both standard and low fertiliser treatments were compared. Analysis used species and fertilisation treatment as fixed factors to test the effect on each chemistry component individually. In order to examine the effect of resistance level on chemical profile, seedlings receiving standard fertiliser treatments were compared. Paired contrasts were performed between comparisons of interest for each chemical component, using group as the fixed effect. All chemistry data was log transformed.

Binary analysis of seedling recovery from browsing, and of seedling survival and occurrence of multiple leaders at week 48 were conducted using a logit link function and fitting mixed models using ASREML (Gilmour *et al.* 2006), using vegetation index and seedling starting height as covariates. Week to first browsing was also fitted as a covariate in the analysis of seedling recovery. In these binary analyses site and replicate within site were treated as random effects and the treatments and their interactions were treated as fixed effects.

The *E. nitens* seedlots supplied by FT were not as expected with regard to foliage chemistry. Seedlots placed in the high sideroxylonal group based on NIRS predictions actually had slightly lower levels of sideroxylonal A than those in the low group. A lack of significant difference with regard to sideroxylonal levels makes comparisons of browsing vulnerability between these groups redundant. For this reason, results for experiment 6 are not presented and they are not discussed further. Additionally, experiment 4 is simply presented to illustrate the consistency of treatment effects, rather than to show how fertiliser and repellent treatments affect high sideroxylonal versus operational seedlots. Seedlots in experiment 4 can therefore be regarded as a repeat of the operational experiment 2 and renamed “operational2”.

The number and identity of scats did not relate well to browsing damage or to browse control data, and did not seem representative of scat density on the coupes as a whole. For this reason this data is not presented and will not be discussed further.

Unfortunately, due to vastly different site characteristics, browsing pressures and control regimes, we are unable to meaningfully compare the effectiveness of non-lethal approaches under differing lethal strategies, namely with and without pre-plant shooting.

Results

Browse damage

Browsing pressure and resultant damage differed markedly between the eight sites. The extent of browsing was noticeably different between sites initially, but after 14 weeks over 80% of seedlings from seven of the eight sites were browsed (Figure 2). The exception was site 8, where browsing was much slower. Browsing severity showed a lot more variation between sites (Figure 3). While the majority of seedlings were browsed, browsing was not always severe. The apparent decline in browsing severity over time seen for some sites is due to new growth.

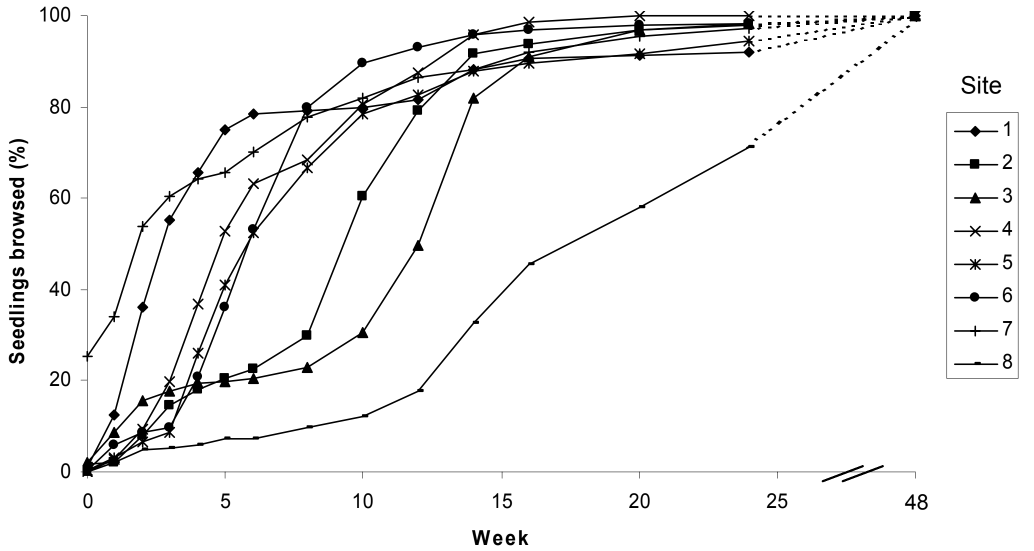

Figure 2. Browsing extent (percentage of seedlings browsed) across 8 sites over the 48-week study period. Note the broken x-axis due to no data between weeks 24 and 48

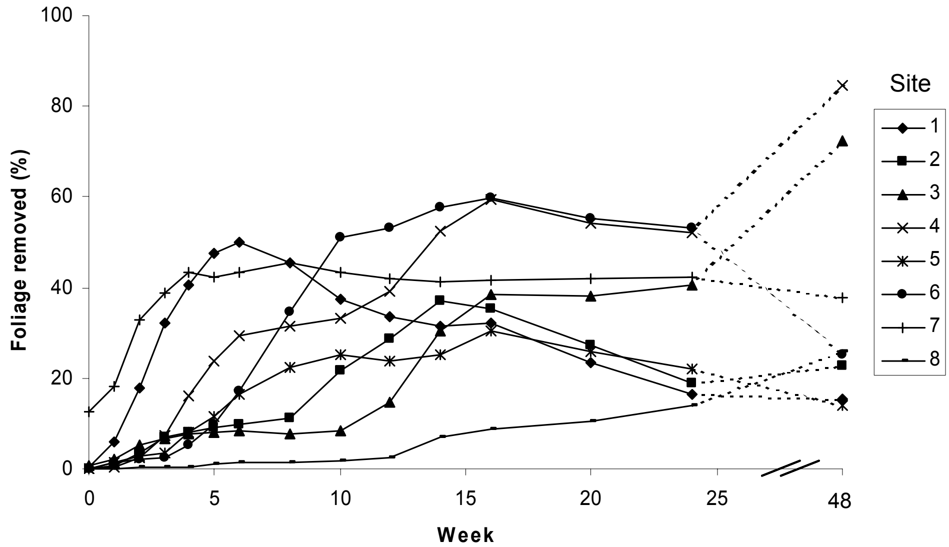

Figure 3. Browsing severity (percentage of foliage removed from seedlings) across 8 sites over the 48-week study period. Note the broken x-axis due to no data between weeks 24 and 48

Due to the large number of sites and treatments, only browsing severity will be presented in detail. This was chosen over browsing extent (percentage of seedlings browsed) because it more clearly shows between-treatment differences. There were often site*treatment interactions for browsing severity, but these were mainly due to the magnitude of the differences between treatments depending on the browsing intensity of the site. For the purpose of conciseness, figures therefore show results averaged across sites. Interactions are discussed where applicable.

Browsing delay

At least 95% of seedlings in all treatments were browsed during the trial period (Table 5). There were highly significant differences between sites in all cases. There was no difference in treatment curves for weeks until seedlings were first browsed for experiments 1, 2 or 5. Experiments 3, 4 and 7 exhibited significantly different treatment curves. Seedlings receiving low nursery fertiliser tended to be first browsed later than those receiving standard fertiliser (experiments 3 & 4), while seedlings with stockings were first browsed later than those without (experiment 7).

Table 5. Delay in browsing due to treatments: weeks until first browsed and weeks until browsing reached the biologically/operationally important level of 25% of foliage removed in Trial 1 across eight sites. T25 = time when 25% of seedlings reached level; T50 = time when 50% of seedlings reached level. χ^2 = Test for significant differences in treatment curves within each experiment: P<0.05 = *, P<0.01 = **, P<0.001 = ***. Treatment codes as in Table 3. G = *E. globulus*, N = *E. nitens*

		Weeks until first browsed				Weeks until severity ≥ 25%			
Experiment	Treatment	T25	T50	% never browsed	χ^2	T25	T50	% never ≥ 25%	χ^2
1	L-R	4.0	9.0	0.83	NS	7.5	14.0	21.67	NS
(*E. globulus*)	L+R	4.5	9.0	1.67		9.0	14.0	28.33	
	S-R	4.0	7.5	1.67		5.0	10.5	20.83	
	S+R	4.0	8.0	5.00		8.0	14.0	25.83	
2	L-R	4.0	8.0	1.88	NS	6.0	11.0	19.38	**
(*E. nitens*)	L+R	6.0	9.0	1.88		10.0	14.0	25.00	
	S-R	2.0	5.5	3.75		3.0	8.0	20.00	
	S+R	2.0	6.0	3.75		6.0	11.5	22.50	
3	L-R	4.0	8.0	5.00	*	5.0	10.0	17.50	**
(*E. globulus*)	L+R	5.0	9.0	4.58		8.0	14.0	23.33	
	S-R	4.0	7.0	1.67		5.0	10.5	20.00	
	S+R	4.0	6.0	4.17		6.0	11.0	24.17	
4	L-R	5.0	10.0	1.25	*	8.0	14.0	18.75	NS
(*E. nitens*)	L+R	5.0	10.0	1.88		9.0	14.0	25.63	
	S-R	3.0	8.0	3.13		4.0	9.0	20.63	
	S+R	3.5	8.0	1.25		6.0	14.0	22.50	
5	BGH	3.0	6.0	2.50	NS	5.0	10.5	24.17	**
(*E. globulus*)	JN	4.0	8.0	0.88		5.0	10.5	15.83	
	↑ sider	3.0	6.0	1.67		5.0	11.0	26.67	
	SH	3.0	5.5	0.00		4.0	8.0	5.00	
	↓ sider	3.0	6.0	2.08		4.0	9.0	19.17	
7	G Ctrl	3.0	5.0	0.00	***	3.0	11.0	17.50	***
(*E. globulus*	G HighP	2.5	5.0	1.67		4.5	10.0	15.00	
& *E. nitens*)	G Stk	5.0	9.5	3.33		10.0	16.0	28.33	
	N Ctrl	2.0	5.0	0.83		3.0	9.0	15.00	
	N HighP	2.0	5.5	2.50		3.0	8.0	18.33	
	N Stk	5.0	10.0	1.67		10.0	15.5	27.50	

The time that browsing severity reached a biologically significant level (≥ 25% foliage removed) differed between treatments in experiments 2, 3, 5 and 7 (Table 5). In experiments 2 and 3, browsing was most delayed on low fertiliser seedlings with repellent. In experiment 5, browsing was delayed on increased resistance/ sideroxylonal seedlings. In experiment 7, stockings greatly delayed the time until significant browsing occurred, whereas there was little difference between the operational and high P treatments.

There were significant effects of vegetation height and cover on the time until seedlings were first browsed, but these were all explained by site differences, i.e. some sites with high vegetation also had high early browsing. Experiments 3 and 7 had a significant negative effect of vegetation height on time until browsing severity reached or exceeded 25% that was not explained by site differences (data not shown). This is likely related to vegetation palatability, with browsing occurring earlier amongst palatable vegetation.

Browsing severity

The proportion of foliage removed generally showed greater treatment effects around the middle than at the end of the study period (Figure 4). This is because treatments were becoming less effective due to seedling growth. For example, repellent could not protect new growth, seedlings grew out of the tops of stockings, and nursery fertilisers were wearing off. The apparent decline in browsing severity after around week 15/16 (Figure 4) is due to this new growth. There was a general trend in experiments 1-4 for highest browsing on seedlings receiving standard nursery fertiliser and no repellent, and lowest browsing on seedlings receiving low nursery fertiliser and repellent. Experiment 5 had highest browsing on reduced resistance (SH) seedlings. In experiment 7, seedlings with stockings received substantially lower damage.

Three key periods were selected, and are examined in detail below (Table 6; Figures 5-8) to more clearly demonstrate treatments effects.

1. Week 6 is important because the majority of sites were fertilised shortly before this and this marks the time immediately prior to the flush of new growth. This is therefore the ideal time to look at the effect of nursery fertiliser, repellent and stockings, as new growth after this period is unprotected by repellent and growing out of stockings. Although browsing severity was higher in later weeks, the pattern of treatment differences remains similar.
2. Week 24 marks the end of the intensive monitoring period and there was expected to be little effect of nursery treatments remaining at this stage due to large amounts of growth.
3. Week 48 is the end of the study and a time at which seedlings are thought to have passed the browsing risk (Coleman *et al.* 1997).

Similar browsing patterns were observed in experiments 1 and 3, testing *E. globulus* fertiliser/repellent combinations on operational and increased resistance seedlings respectively, but overall browsing for increased resistance seedlots was often higher than for operational seedlings (Figure 5). The effect of repellent was much stronger and lasted longer than that of nursery fertiliser (Table 6); generally seedlings without repellent were browsed substantially more than those with repellent. Treatment effects lessened over time for both experiments, with no treatment effect for operational seedlings after 48 weeks. Site*treatment interactions were due to the difference in magnitude between treatment effects across sites; sites with very low browsing had no treatment effects. There was a small fertiliser*repellent interaction for increased resistance seedlings at week 6; differences between fertiliser treatments were only obvious on seedlings with repellent.

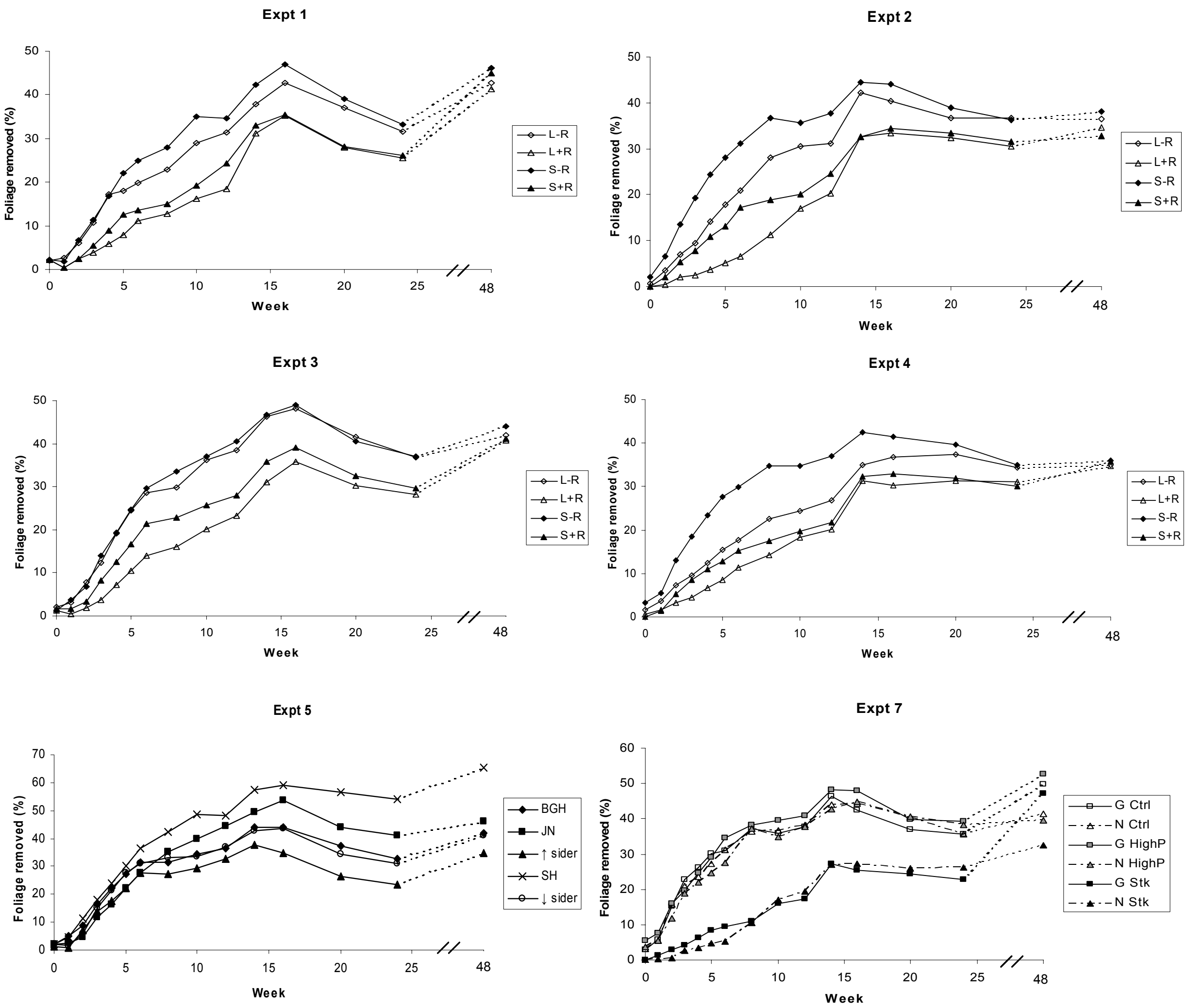

Figure 4. Browsing severity (percentage of foliage removed from seedlings) in the six experiments over time, averaged across sites. L = low nursery fertiliser; S = standard nursery fertiliser; -R = no repellent; +R = repellent; G = *E. globulus*; N = *E. nitens*. Note the broken x-axis due to no data between weeks 24 and 48

Table 6. Results of mixed model analysis of browsing severity (average percentage of foliage removed from seedlings) 6, 24, and 48 weeks after planting in Trial 1. Expt = experiment; Fert = fertiliser; Rplt = repellent; Treat = treatment; Spp = species

Expt	Effect[a]	Num DF	Den DF range[b]	Wk 6 F	Wk 6 P	Wk24 F	Wk24 P	Wk48 F	Wk48 P
1	Site	5	111-114	13.3	<0.001	11.6	<0.001	96.8	<0.001
	Fertiliser	1	283-331	3.8	0.053	0.1	0.762	0.2	0.665
	Site*Fert	5	283-331	2.8	0.017	1.2	0.312	1.8	0.115
	Repellent	1	283-331	15.9	<.0001	10.7	0.001	1.1	0.289
	Site*Rplt	5	283-331	5.7	<.0001	1.8	0.112	1.6	0.157
	Fert*Rplt	1	283-331	0.5	0.484	0.0	0.868	0.6	0.425
	Site*Fert*Rplt	5	283-331	1.3	0.261	0.8	0.529	0.8	0.556
2	Site	7	140-152	19.1	<0.001	17.3	<0.001	17.3	<0.001
	Fertiliser	1	399-444	52.9	<0.001	0.4	0.554	0.2	0.688
	Site*Fert	7	399-444	3.0	0.004	2.1	0.039	1.0	0.464
	Repellent	1	399-444	60.8	<0.001	10.7	0.001	3.3	0.072
	Site*Rplt	7	399-444	8.3	<0.001	1.0	0.468	0.6	0.785
	Fert*Rplt	1	399-444	0.7	0.404	0.2	0.647	0.4	0.550
	Site*Fert*Rplt	7	399-444	1.4	0.212	2.0	0.048	0.5	0.830
3	Site	5	114	16.7	<0.001	22.2	<0.001	107.7	<0.001
	Fertiliser	1	686-792	7.5	0.006	0.0	0.834	1.0	0.323
	Site*Fert	5	686-792	0.4	0.884	0.7	0.597	1.3	0.283
	Repellent	1	686-792	34.2	<0.001	25.4	<0.001	4.1	0.043
	Site*Rplt	5	686-792	4.4	0.001	2.3	0.041	0.6	0.709
	Fert*Rplt	1	686-792	4.0	0.046	0.3	0.613	1.9	0.170
	Site*Fert*Rplt	5	686-792	0.4	0.854	0.3	0.930	0.3	0.931
4	Site	7	147-152	17.7	<0.001	13.5	<0.001	17.8	<0.001
	Fertiliser	1	398-443	22.2	<0.001	0.0	0.997	0.1	0.768
	Site*Fert	7	398-443	3.1	0.003	2.2	0.037	0.9	0.474
	Repellent	1	398-443	23.4	<0.001	5.7	0.017	0.0	0.903
	Site*Rplt	7	398-443	8.0	<0.001	0.7	0.669	0.8	0.575
	Fert*Rplt	1	398-443	4.7	0.031	0.3	0.591	0.2	0.629
	Site*Fert*Rplt	7	398-443	1.1	0.347	0.8	0.619	1.9	0.070
5	Site	5	112-114	17.5	<0.001	23.1	<0.001	83.9	<0.001
	Population	4	17	2.1	0.128	12.7	<0.001	29.7	<0.001
	Site*Population	20	527-607	1.2	0.317	1.5	0.085	3.2	<0.001
7	Site	7	111-112	16.1	<0.001	8.7	<0.001	26.2	<0.001
	Treatment	2	503-549	90.8	<0.001	25.8	<0.001	6.0	0.003
	Site*Treat	14	503-549	7.6	<0.001	1.5	0.117	1.2	0.266
	Species	1	503-549	4.4	0.037	1.4	0.230	32.0	<0.001
	Site*Spp	7	503-549	3.3	0.002	10.5	<0.001	14.2	<0.001
	Treat*Spp	2	503-549	1.3	0.281	0.3	0.750	0.5	0.635
	Site*Treat*Spp	14	503-549	0.4	0.961	2.2	0.006	1.3	0.228

[a]random effect of Replicate(Site) was always highly significant ($P < 0.001$); random effect of Population(Seedlot) was not significant at any stage.
[b]denominator DF range given because value decreases over time due to mortality.

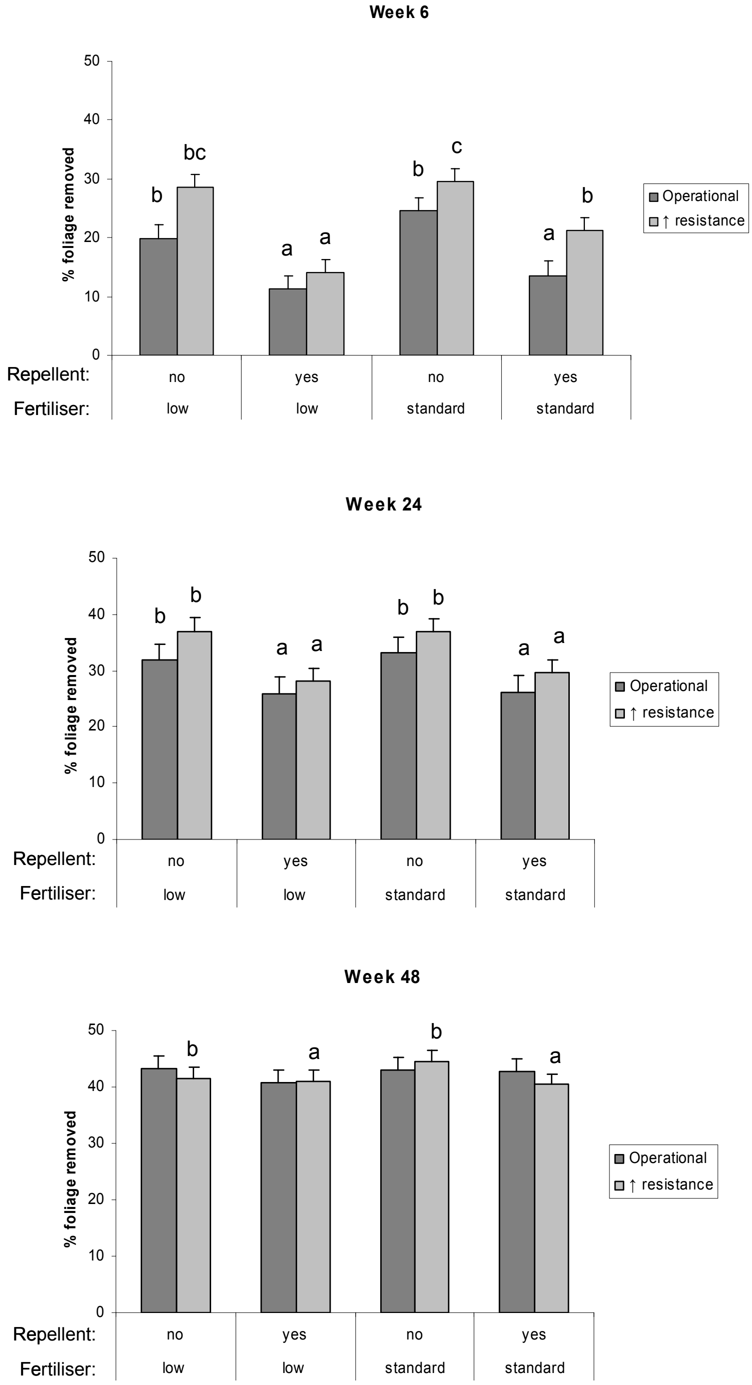

Figure 5. Browsing severity (percentage of foliage removed from seedlings) in experiments 1 (operational) and 3 (↑ resistance), testing fertiliser and repellent combinations on *E. globulus* seedlings, 6, 24, and 48 weeks after planting, averaged across sites. Different letters indicate significant differences within a series at $P < 0.05$; no letters means no significant differences

Eucalyptus nitens fertiliser/repellent combinations (experiments 2 & 4) showed similar patterns to *E. globulus*, but the effects of nursery fertiliser were more apparent during the early stages. For both experiments, seedlings with standard nursery fertiliser and no repellent were browsed significantly more than the other treatments in week 6 (Figure 6). This effect had declined by week 24, and there were no treatment effects for either experiment after 48 weeks (Table 6). As with *E. globulus*, site*treatment interactions were due to a lack of treatment effects at sites with very low browsing. A fertiliser*repellent interaction for operational2 seedlings in week 6 was due to greater differences between repellent treatments under standard fertilisation. A significant site*fertiliser*repellent interaction for operational seedlings in week 24 was due to a repellent effect occurring under standard fertilisation at some sites and low fertilisation at others.

There was no significant difference between five *E. globulus* seed sources 6 weeks after planting, however an effect was apparent after 24 weeks and this remained at week 48 (Table 6; Figure 7). This was due to the reduced resistance seedlot (SH) being browsed to a much greater extent than all other seedlots. There was a site*population interaction in week 48 due to the difference between seedlots lessening on sites with very high damage levels. All seedlings used in this experiment were raised under identical conditions; differences in browsing are due to genetic and not environmental effects.

In experiment 7, seedlings with stockings received significantly less damage in weeks 6 and 24; damage to stocking seedlings was still lower, but not significantly so, in week 48 (Table 6; Figure 8). There was a site*treatment interaction in week 6 because stocking seedlings were not the least browsed at site 8, but this was not biologically meaningful due to atypically low browsing at this site. *Eucalyptus globulus* received significantly more damage than *E. nitens* in weeks 6 and 48 overall, although *E. nitens* was sometimes favoured, resulting in site*species interactions. There was little difference between controls and seedlings with high P nursery fertiliser for either species. A site*treatment*species interaction in week 24 was due to increased browsing on high P relative to standard fertiliser *E. nitens* seedlings at site 5.

Browsing recovery

Seedling recovery from browsing was significantly influenced by the vegetation index in four of the six experiments (Table 7). In each case, seedling recovery was more likely with increasing height and cover of surrounding vegetation. The week seedlings were first browsed also had a significant influence on the likelihood of recovery from browsing in four experiments; the earlier seedlings were browsed, the more likely they were to recover. Interestingly, seedlings treated with repellent appeared less able to recover from browsing than those without, with this being significant in three of the four cases. In experiment 2, fewer seedlings that were shorter at planting were able to recover from browsing.

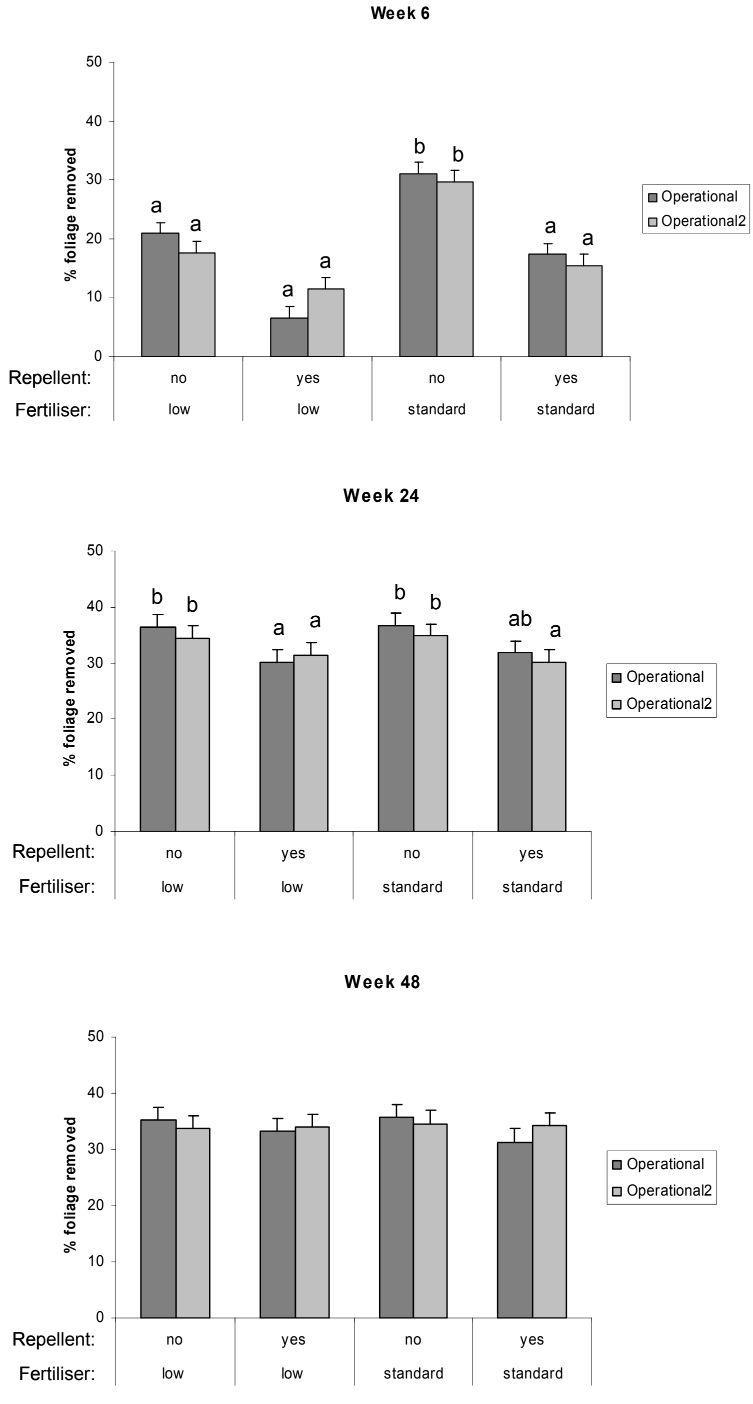

Figure 6. Browsing severity (percentage of foliage removed from seedlings) in experiments 2 (operational) and 4 (operational2), testing fertiliser and repellent combinations on *E. nitens* seedlings, 6, 24, and 48 weeks after planting, averaged across sites. Different letters indicate significant differences within a series at $P < 0.05$; no letters means no significant differences

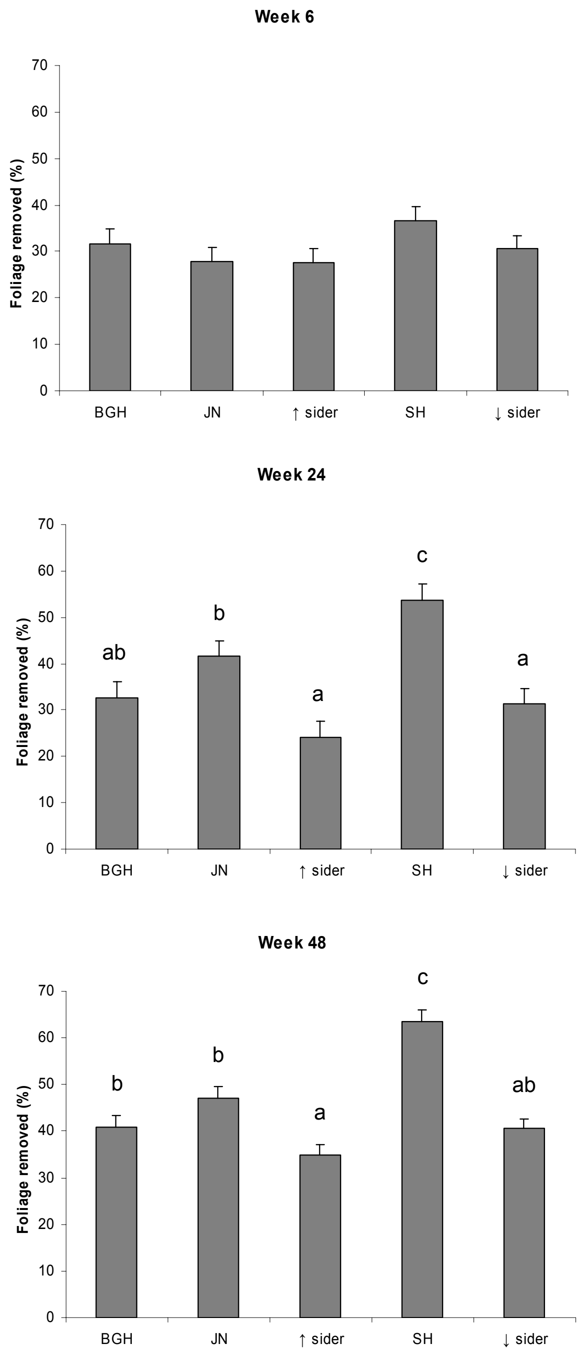

Figure 7. Browsing severity (percentage of foliage removed from seedlings) in experiment 5, testing *E. globulus* seedlot resistance levels, 6, 24, and 48 weeks after planting, averaged across sites. Different letters indicate significant differences within a series at $P < 0.05$; no letters means no significant differences.

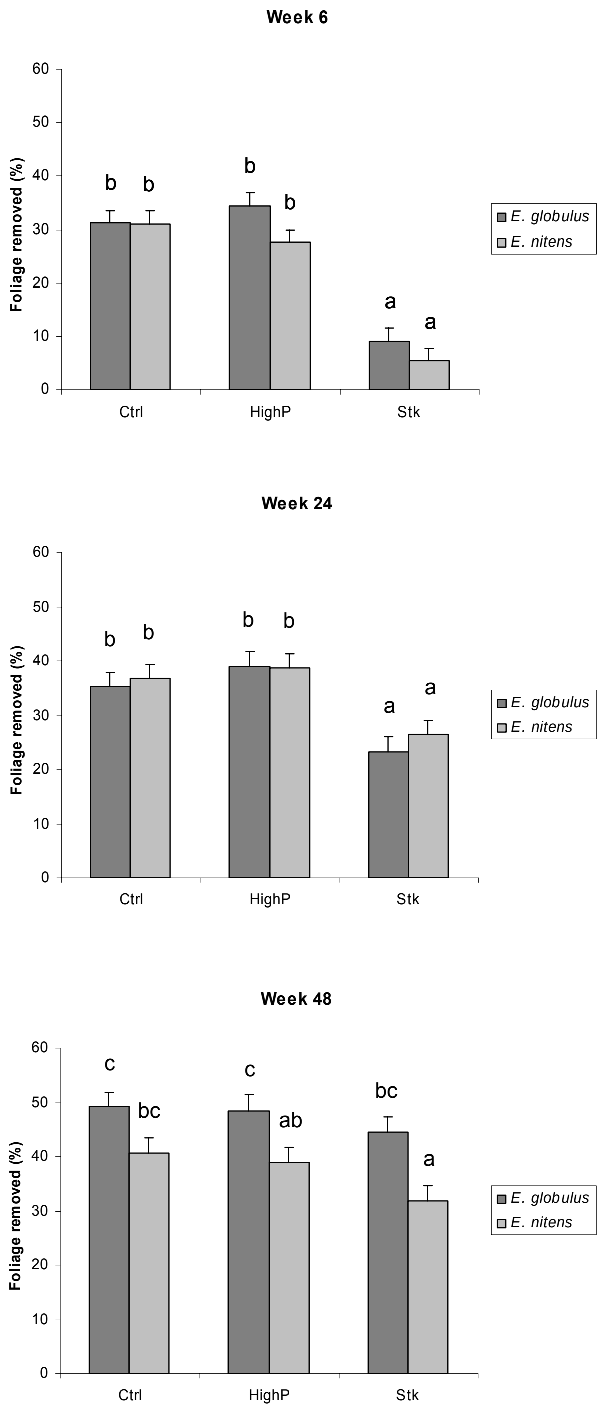

Figure 8. Browsing severity (percentage of foliage removed from seedlings) in experiment 7, testing stockings and high P fertiliser, 6, 24, and 48 weeks after planting, averaged across sites. Different letters indicate significant differences within a week at $P < 0.05$. Ctrl = control; Stk = stocking

Table 7. Summary of analysis of effects on seedling recovery from browsing between weeks 10 and 16, averaged across eight sites. Dataset using only those seedlings that were browsed and therefore had the ability to recover during this period. NS = not significant; '-' = not a valid test; * = P <0.05; ** = P <0.01; *** = P <0.001. Rplt = repellent; Fert = fertiliser; Treat = treatment; Spp = species

	Experiment					
Effect	**1**	**2**	**3**	**4**	**5**	**7**
Start height	NS	*	NS	NS	NS	NS
Veg index	*	*	**	*	NS	NS
Week browsed	*	NS	*	NS	**	*
Repellent	*	**	**	NS	-	-
Fertiliser	NS	NS	NS	NS	-	-
Rplt*Fert	NS	NS	NS	NS	-	-
Population	-	-	-	-	NS	-
Treatment	-	-	-	-	-	NS
Species	-	-	-	-	-	NS
Treat*Spp	-	-	-	-	-	NS

Seedling height and form

As with browsing, there were large differences in seedling height between sites (Figure 9). This was mainly due to the amount of browsing that seedlings received. There was also an effect of planting time, with those planted later in the season (sites 5 & 6) taller than others right from the start.

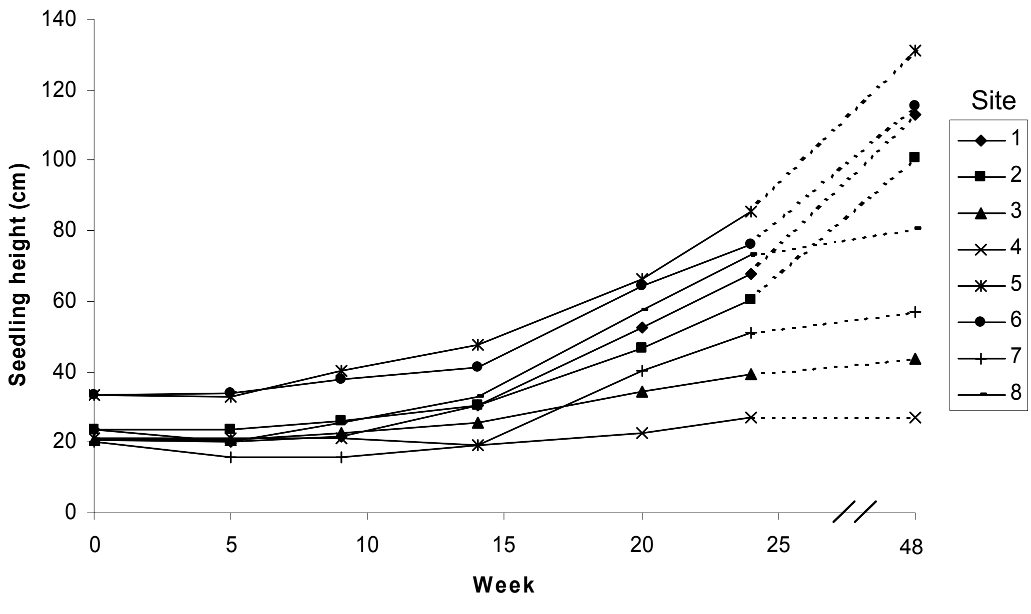

Figure 9. Seedling height in Trial 1 over the 48-week study period by site. Note the broken x-axis due to no data between weeks 24 and 48

Experiments 1-4 all had significantly shorter low fertiliser than standard fertiliser seedlings at planting (week 0) and they remained so until week 48 (Figure 10; Table 8). This initial height difference varied in magnitude between sites, resulting in significant site*fertiliser interactions at planting. A fertiliser*repellent interaction in experiment 3 resulted from repellent seedlings being marginally taller in the standard than low fertiliser treatment at planting. Repellent had no effect on seedling height at planting, but did result in taller seedlings at some sites in experiment 3; it also had more of an effect on the height of standard seedlings compared with low fertiliser seedlings.

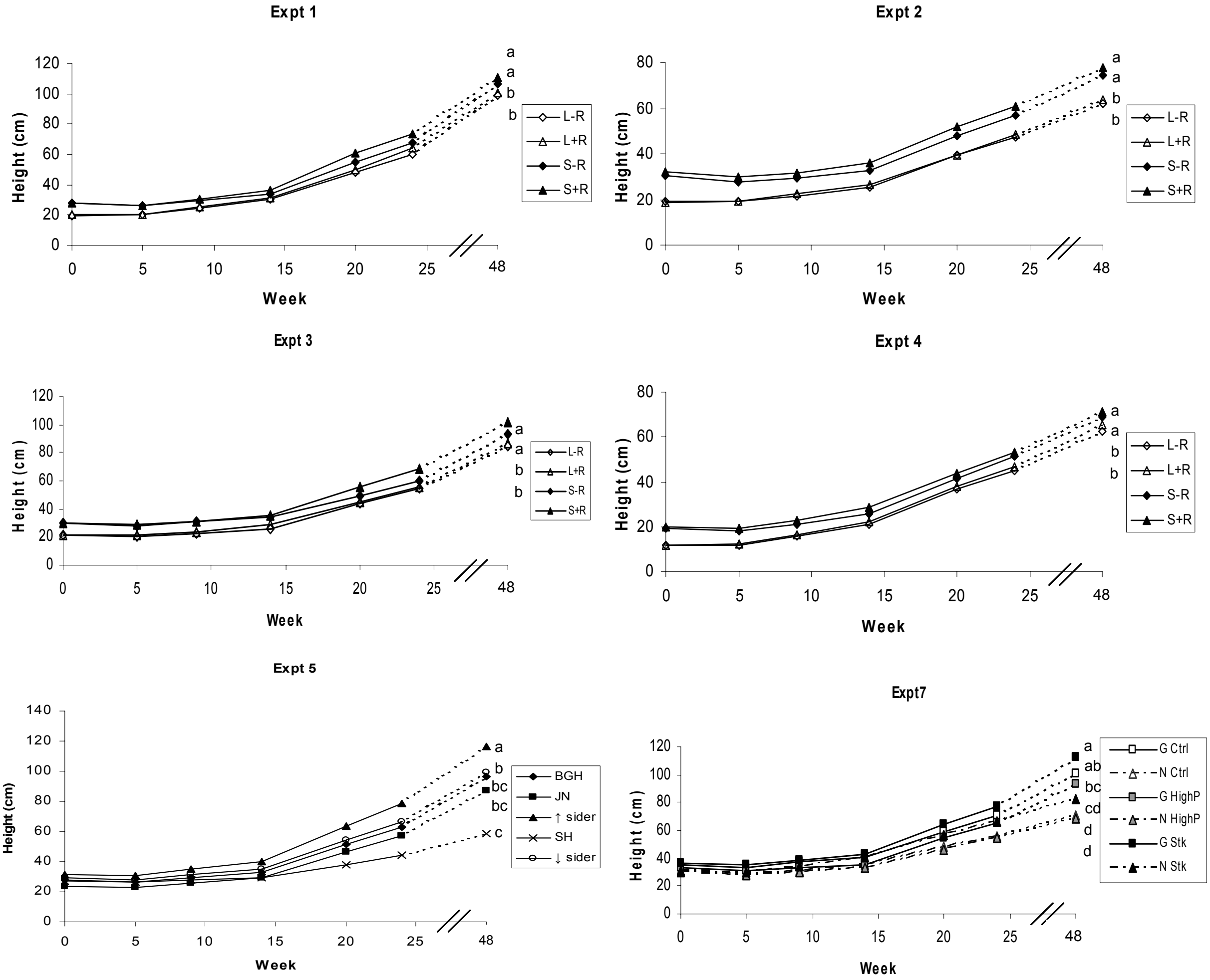

Figure 10. Seedling heights in six experiments in Trial 1 over time. Letters show significant differences ($P < 0.05$) in height between treatments at week 48. L = low nursery fertiliser; S = standard nursery fertiliser; -R = no repellent; +R = repellent; G = *E. globulus*; N = *E. nitens*; Ctrl = control; Stk = stocking.. Note the broken x-axis due to no data between weeks 24 and 48

There was no difference between populations in experiment 5 at planting, but later effects emerged due to the reduced resistance seed source (SH) that was heavily browsed being shorter, and the high sideroxylonal seedlot that was much less browsed being taller than other seedlots. There was a site*population effect at planting, due to JN seedlings at site 5 being shorter than other seedlots. The site*population effect at weeks 24 and 48 was due to the smaller differences between treatments seen on sites with short (heavily browsed) seedlings.

Experiment 7 also had significant treatment effects at planting and throughout the trial. *Eucalyptus globulus* was taller than *E. nitens* and high P seedlings were shorter than those with other treatments at planting. All seedlings used in this experiment were sourced from the nursery; early growth conditions are unknown and any effects include environmental components. The species effect remained throughout the trial and, over time, seedlings with stockings became taller than those without. Site*species interactions at weeks 24 and 48 were due to there being a much larger height difference between *E. globulus* and *E. nitens* at sites 5 and 6.

Table 8. Results of mixed model analysis of seedling height at 0, 24, and 48 after planting, and height change between weeks 0 and 48 in Trial 1. Fert = fertiliser; Rplt = repellent; Treat = treatment; Spp = species

Expt	Effect[a]	Num DF	Den DF range	Wk0 F	Wk0 P	Wk24 F	Wk24 P	Wk48 F	Wk48 P	Change F	Change P
1	Site	5	111-114	50.3	<0.001	23.9	<0.001	31.2	<0.001	27.3	<0.001
	Fertiliser	1	283-342	208.0	<0.001	12.5	0.001	6.0	0.015	0.4	0.530
	Site*Fert	5	283-342	8.4	<0.001	0.6	0.679	0.6	0.669	1.0	0.418
	Repellent	1	283-342	0.3	0.563	3.7	0.056	0.9	0.342	0.7	0.394
	Site*Rplt	5	283-342	1.4	0.231	0.1	0.990	0.6	0.737	0.9	0.481
	Fert*Rplt	1	283-342	0.4	0.533	0.3	0.617	0.2	0.692	0.2	0.630
	Site*Fert*Rplt	5	283-342	0.2	0.969	0.7	0.656	0.9	0.461	1.0	0.437
2	Site	7	149-152	44.0	<0.001	9.4	<0.001	12.1	<0.001	11.4	<0.001
	Fertiliser	1	402-456	766.4	<0.001	37.6	<0.001	26.2	<0.001	0.1	0.781
	Site*Fert	7	402-456	25.5	<0.001	0.6	0.783	1.2	0.287	2.7	0.010
	Repellent	1	402-456	2.5	0.113	2.3	0.127	1.0	0.327	0.8	0.386
	Site*Rplt	7	402-456	0.9	0.498	0.7	0.708	0.5	0.821	0.3	0.964
	Fert*Rplt	1	402-456	4.9	0.028	1.2	0.272	0.2	0.636	0.1	0.785
	Site*Fert*Rplt	7	402-456	1.4	0.227	0.4	0.896	0.6	0.759	0.8	0.598
3	Site	5	114	71.4	<0.001	26.3	<0.001	35.5	<0.001	31.6	<0.001
	Fertiliser	1	686-822	466.6	<0.001	31.5	<0.001	16.4	<0.001	1.4	0.237
	Site*Fert	5	686-822	4.6	<0.001	0.4	0.848	0.6	0.728	0.3	0.904
	Repellent	1	686-822	2.7	0.103	7.5	0.007	2.8	0.094	2.6	0.110
	Site*Rplt	5	686-822	0.8	0.520	2.3	0.041	2.4	0.039	2.1	0.066
	Fert*Rplt	1	686-822	0.1	0.736	4.6	0.033	2.2	0.137	2.1	0.152
	Site*Fert*Rplt	5	686-822	1.0	0.422	1.8	0.118	1.1	0.359	1.0	0.398
4	Site	7	147-152	32.6	<0.001	9.7	<0.001	12.4	<0.001	11.6	<0.001
	Fertiliser	1	398-456	611.8	<0.001	17.8	<0.001	9.3	0.002	0.0	0.888
	Site*Fert	7	398-456	11.1	<0.001	1.5	0.168	1.4	0.193	1.8	0.092
	Repellent	1	398-456	1.5	0.223	0.8	0.382	1.0	0.313	0.7	0.415
	Site*Rplt	7	398-456	0.6	0.733	1.1	0.369	1.2	0.311	1.2	0.302
	Fert*Rplt	1	398-456	0.0	0.842	0.0	0.890	0.0	0.961	0.0	0.959
	Site*Fert*Rplt	7	398-456	0.7	0.712	1.3	0.265	1.3	0.271	1.2	0.303
5	Site	5	112-114	119.9	<0.001	29.6	<0.001	34.2	<0.001	27.4	<0.001
	Population	4	17	1.0	0.424	14.8	<0.001	18.8	<0.001	24.5	<0.001
	Site*Population	20	527-619	1.9	0.013	2.7	<0.001	3.5	<0.001	3.6	<0.001
7	Site	7	111-112	96.0	<0.001	15.4	<0.001	25.0	<0.001	19.5	<0.001
	Treatment	2	505-560	6.3	0.002	12.6	<0.001	10.7	<0.001	9.4	<0.001
	Site*Treat	14	505-560	1.6	0.072	0.5	0.926	0.4	0.965	0.5	0.954
	Species	1	505-560	86.3	<0.001	34.6	<0.001	76.7	<0.001	56.1	<0.001
	Site*Spp	7	505-560	1.1	0.352	8.8	<0.001	17.5	<0.001	17.6	<0.001
	Treat*Spp	2	505-560	1.6	0.205	0.9	0.422	0.5	0.593	0.4	0.672
	Site*Treat*Spp	14	505-560	1.0	0.493	0.8	0.689	0.7	0.735	0.7	0.776

[a]Random effect of Rep (Site) was always highly significant ($P < 0.001$); random effect of Population (Seedlot) NS for weeks 24 and 48 and height change; $Z = 0.003$ for week 0.
[b]Denominator DF range given because value decreases over time due to mortality.

Although seedlings receiving standard fertiliser started taller and finished taller than those receiving low nursery fertiliser (treatments 1 to 4), there was no difference in the absolute growth rate (the difference between start and end height; Table 8, Figure 10). There was a significant difference between sites, with some sites (e.g. sites 3 and 4) experiencing much lower growth. There was a significant site*fertiliser interaction for height change in experiment 2 due to some sites having higher growth on low fertiliser seedlings and others on standard fertiliser seedlings. There were significant differences in growth among treatments for experiments 5 and 7. In experiment 5, SH seedlings experienced significantly lower growth, and increased sideroxylonal seedlings experienced significantly higher growth than all others. There was a significant site*population interaction because increased sideroxylonal seedlings did not experience the greatest growth on site 1. In experiment 7, growth of *E. globulus* was significantly higher than that of *E. nitens*, and stocking seedlings grew more than controls or those with high P fertiliser, which did not differ from each other. *Eucalyptus nitens* seedlings grew more than *E. globulus* on site 8 (resulting in interaction), a site where *E. globulus* was not expected to survive.

There was no effect of vegetation index on seedling height at any stage during the trial period. There was, however, a significant positive relationship between seedling growth and vegetation index for experiment 2; increased vegetation cover and height resulted in an increase in seedling growth. Vegetation index had no effect on growth in any of the other experiments.

A large proportion of seedlings across all experiments and sites (average 41%) exhibited multiple leaders by week 48. The incidence of multiple leaders was significantly influenced by surrounding vegetation in five of the six experiments (Table 9). In each case, the proportion of seedlings with multiple leaders increased with decreasing height and cover of surrounding vegetation (vegetation index). Experiments 2 and 7 showed significant effects of seedling height at planting, with smaller plants less likely to develop multiple leaders. There was a significant repellent*fertiliser interaction in experiment 1, with seedlings with low fertiliser and no repellent more likely to develop multiple leaders. The incidence of multiple leaders in experiment 7 was significantly higher on *E. nitens* (50%) than *E. globulus* (40%).

Table 9. Summary of analysis of effects on proportion of seedlings with multiple leaders at week 48, averaged across eight sites. NS = not significant; '-' = not a valid test; * = P <0.05; ** = P <0.01; *** = P <0.001. Rplt = repellent; Fert = fertiliser; Treat = treatment; Spp = species

	Experiment					
Effect	**1**	**2**	**3**	**4**	**5**	**7**
Start height	NS	*	NS	NS	NS	**
Veg index	**	**	*	*	**	NS
Repellent	NS	NS	NS	NS	-	-
Fertiliser	NS	NS	NS	NS	-	-
Rplt*Fert	*	NS	NS	NS	-	-
Population	-	-	-	-	NS	-
Treatment	-	-	-	-	-	NS
Species	-	-	-	-	-	*
Treat*Spp	-	-	-	-	-	NS

Seedling survival

Survival over the main period of the trial was generally quite high. There were few deaths due to environmental conditions, with the majority due to severe browsing and some due to forestry operations. In the earlier stages of the trial, sites 6 and 7 had notably higher mortality than the other sites (Figure 11). In both cases, this was due to intense repeated browsing. By 48 weeks, site 4 had very high mortality, again due mainly to intense browsing as seedlings had grown little over winter and were still well within the browsing zone.

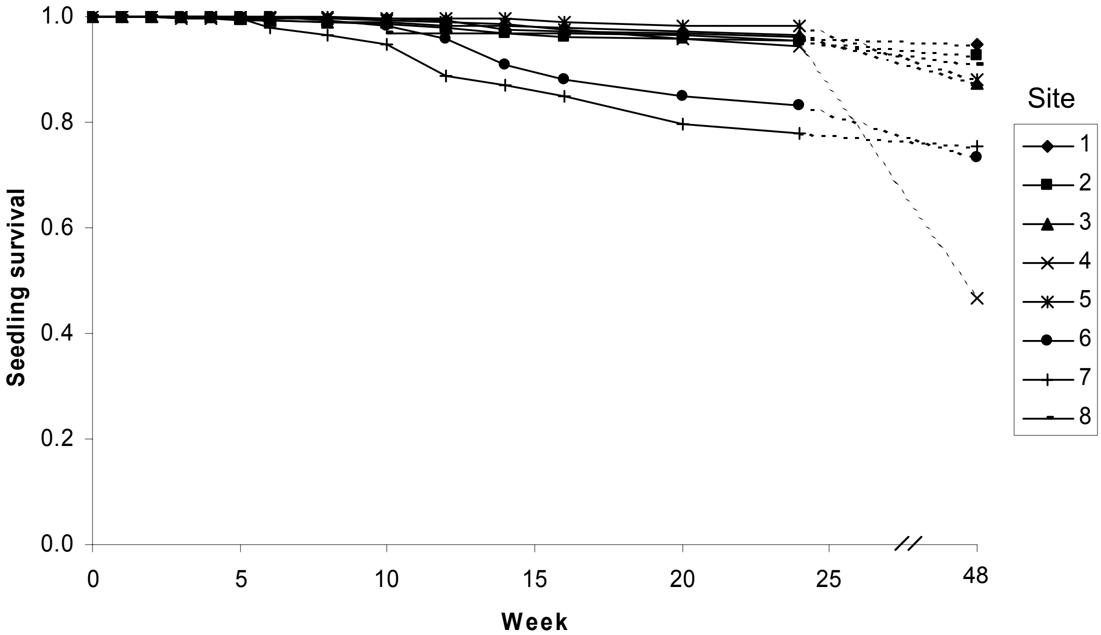

Figure 11. Seedling survival (proportion alive) in Trial 1 across 8 sites over the 48-week study period. Note the broken x-axis due to no data between weeks 24 and 48

By week 48, there were no effects of nursery treatments on seedling survival with one exception (Table 10). There was a significant repellent*fertiliser interaction in experiment 4, due to the presence or absence of repellent causing a greater difference in survival on seedlings with standard than low fertilisation. Increasing vegetation index significantly increased survival in experiment 3. There was a highly significant difference in survival between species seen in experiment 7, with survival of *E. globulus* (77%) being much lower than that of *E. nitens* (92%).

Table 10. Summary of analysis of effects on proportion of seedlings surviving to week 48, averaged across eight sites. NS = not significant; '-' = not a valid test; * = P <0.05; ** = P <0.01; *** = P <0.001. Rplt = repellent; Fert = fertiliser; Treat = treatment; Spp = species

	Experiment					
Effect	**1**	**2**	**3**	**4**	**5**	**7**
Start height	NS	NS	NS	NS	NS	-
Veg index	NS	NS	**	NS	NS	NS
Repellent	NS	NS	NS	NS	-	-
Fertiliser	NS	NS	NS	NS	-	-
Rplt*Fert	NS	NS	NS	**	-	-
Population	-	-	-	-	NS	-
Treatment	-	-	-	-	-	NS
Species	-	-	-	-	-	***
Treat*Spp	-	-	-	-	-	NS

Foliage chemistry

Fertilisation treatments

There were no species*treatment interactions showing that, for our samples, responses to fertiliser treatments were consistent among species. There was an effect of species on carbon content, total phenolics, total oils, cineole and sideroxylonal A (Table 11). Carbon and total phenolics were significantly higher in *E. nitens*, while oils, cineole and sideroxylonal A were significantly higher in *E. globulus*. There was a significant effect of fertiliser on nitrogen content, condensed tannins and total phenolics. Standard seedlings had significantly more nitrogen and significantly less tannin and total phenolics than seedlings receiving low nursery fertiliser.

Table 11. Chemistry of *E. globulus* and *E. nitens* foliage from standard and low fertiliser treatments. Results are averaged across all relevant seedlots; the majority of which were of ↑ resistance. Values are least-squares means (+ 1 SE) for seedlots receiving both fertiliser treatments. '-' = not quantified. Superscript letters within a row indicate a significant difference at P < 0.05

Species		***E. globulus***		***E. nitens***	
Fertilisation		**Standard**	**Low**	**Standard**	**Low**
Nitrogen	%	1.58 (0.06)^{b}	0.83 (0.06)^{a}	1.39 (0.10)^{b}	0.88 (0.10)^{a}
Carbon	%	44.64 (0.24)^{a}	44.41 (0.24)^{a}	45.11 (0.35)^{b}	45.66 (0.35)^{b}
Condensed tannin	mg.gDM equiv. ST	3.53 (0.81)^{a}	7.71 (0.81)^{b}	3.44 (1.21)^{a}	7.47 (1.21)^{b}
Total phenolics	mg.gDM equiv. GA	84.72 (4.14)^{a}	100.96 (4.14)^{b}	113.18 (6.14)^{b}	125.30 (6.14)^{b}
Total oil	mg.gDM	16.44 (0.97)^{b}	13.93 (0.97)^{b}	3.94 (1.44)^{a}	4.18 (1.44)^{a}
Cineole	mg.gDM	8.83 (0.51)^{b}	7.21 (0.51)^{b}	1.57 (0.75)^{a}	1.42 (0.75)^{a}
Sideroxylonal A	mg.gDM	2.38 (0.15)^{b}	1.82 (0.15)^{b}	1.62 (0.23)^{a}	1.65 (0.23)^{a}
Macrocarpal G	equiv. mg.gDM	1.71 (0.35)^{a}	1.69 (0.35)^{a}	-	-

The high P nursery fertiliser treatment did not appear to have a large influence on plant chemistry. Due to the low replication (only one seedlot/species – no error term), seedlots receiving standard and high P treatments cannot be statistically compared. In contrast with standard fertiliser, high P fertiliser resulted in a slight increase of PSMs in both species (8% increase for *E. nitens*; 6% for *E. globulus*); a slight decline in nitrogen for *E. nitens* (1.15% vs. 1.08% N), and a slight increase in nitrogen for *E.*

globulus (0.99% vs. 1.13% N), which is interesting considering the high P fertiliser contains half the nitrogen. Because only one seedlot was used for each species, however, differences between species could be due to either the specific seedlot(s) or fertiliser treatment.

Genetic based differences in resistance

There were significant differences between seedlots of different resistance levels for all chemistry except nitrogen content. There was no obvious pattern of nitrogen content in relation to resistance level (Figure 12). As predicted, the *E. globulus* reduced resistance seedlots had lower levels of sideroxylonal A than operational control and increased resistance seedlots (Figure 13). Not all increased resistance seedlots were higher than operational controls. The pattern of total PSMs for *E. globulus* was quite different to that for sideroxylonal A (Figure 14). Not all the increased resistance/sideroxylonal groups had higher levels than the reduced resistance/sideroxylonal groups.

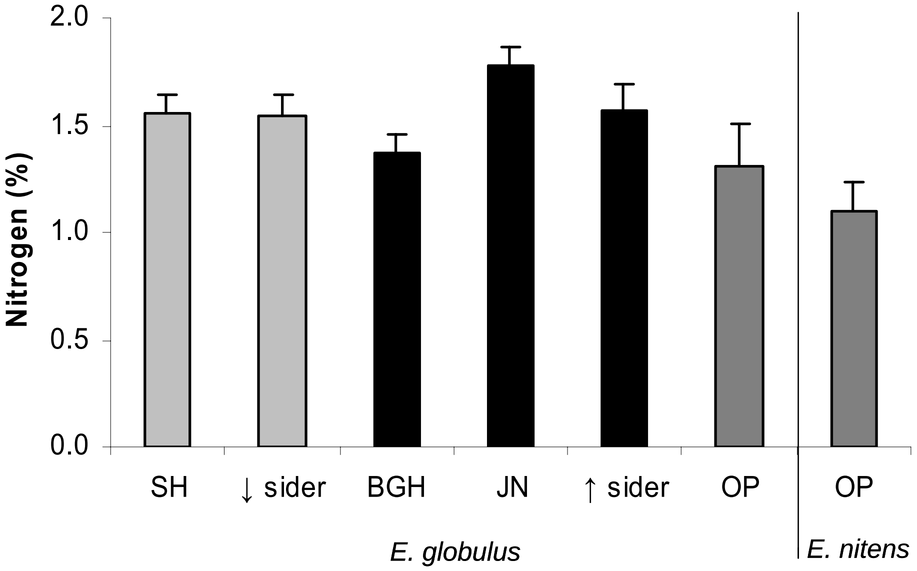

Figure 12. Variation in nitrogen content (% dry matter) across seedling resistance levels. Light grey = ↓ resistance/sideroxylonal; black = ↑ resistance/sideroxylonal; dark grey = operational (OP) controls.

Pairwise comparisons of non-operational seedlots showed that nitrogen content was significantly different in only one of the eight comparisons (Table 12). Sideroxylonal differed in all comparisons. Only half the comparisons differed in total PSMs.

Ranking of all of the individual seedlots used in Trial 1 showed that the five SH seedlots grouped together as the five most susceptible to browsing. These five also had the lowest sideroxylonal levels (Appendix 2).

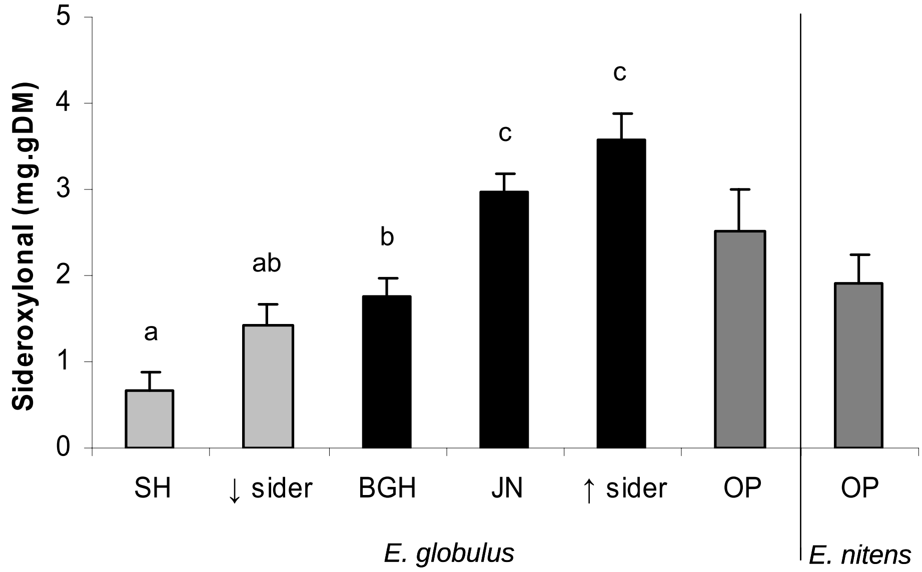

Figure 13. Variation in sideroxylonal A (mg/gDM) content across seedling resistance levels. Light grey = ↓ resistance/sideroxylonal; black = ↑ resistance/sideroxylonal; dark grey = operational (OP) controls. Different letters show significant differences at $P < 0.05$ between non-operational seedlings

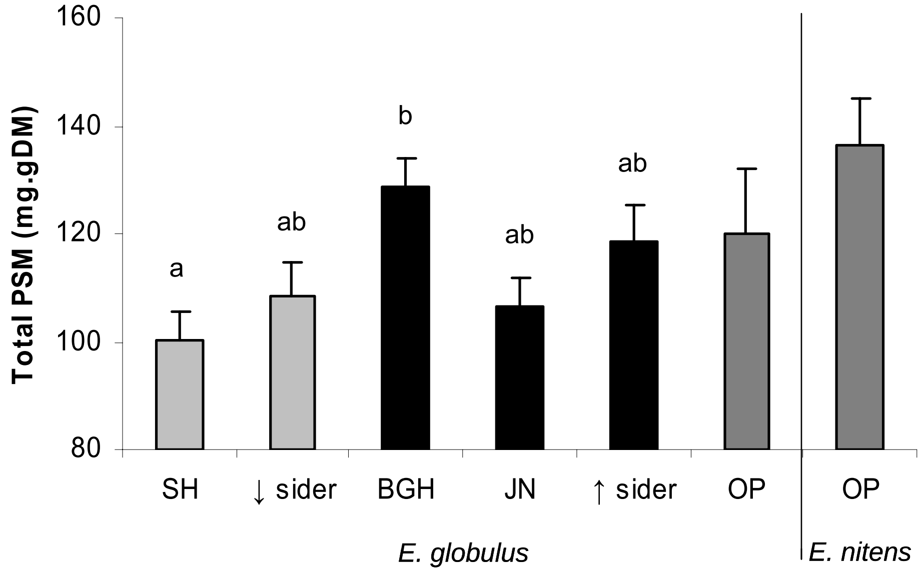

Figure 14. Variation in total plant secondary metabolites content (mg/gDM) across seedling resistance levels. Light grey = ↓ resistance/sideroxylonal; black = ↑ resistance/sideroxylonal; dark grey = operational (OP) controls. Different letters show significant differences at $P < 0.05$ between non-operational seedlings

Table 12. Pairwise comparisons of the nitrogen, sideroxylonal A and total PSM content of experimental seedlots used in Trial 1

			% nitrogen		Sideroxylonal A		Total PSM	
Comparison			**F**	**P**	**F**	**P**	**F**	**P**
SH	vs.	BGH	2.18	0.150	49.38	<0.001	14.00	<0.001
SH	vs.	JN	2.76	0.107	121.55	<0.001	0.79	0.380
BGH	vs.	JN	9.85	0.004	15.98	<0.001	8.13	0.008
↓ sider	vs.	↑ sider	0.02	0.901	31.62	<0.001	1.14	0.294
SH	vs.	BGH/JN	0.01	0.916	108.62	<0.001	7.16	0.012
BGH/JN	vs.	↑ sider	0.00	0.987	10.22	0.003	0.04	0.843
SH	vs.	↓ sider	0.00	0.968	27.51	<0.001	1.36	0.253
SH/↓ sider	vs.	BGH/JN/↑ sider	0.03	0.865	111.51	<0.001	6.45	0.016

Trial 1 results summary

The most effective treatments for delaying and reducing browsing severity were seedling stockings and low nursery fertiliser + repellent (Figure 15). Due to uncertainty about the longer-term effects of reduced nursery fertiliser on seedling growth, industry partners were not interested in using low fertiliser in further tests at this stage. This meant that seedling stockings and repellent were the only treatments attracting sufficient interest for operational use, and these were further explored in Trial 2. It is interesting to note that 48 weeks after planting, the only treatment experiencing notably different browsing severity relative to operational controls was decreased resistance seedlots, showing that genetic effects are longest lasting.

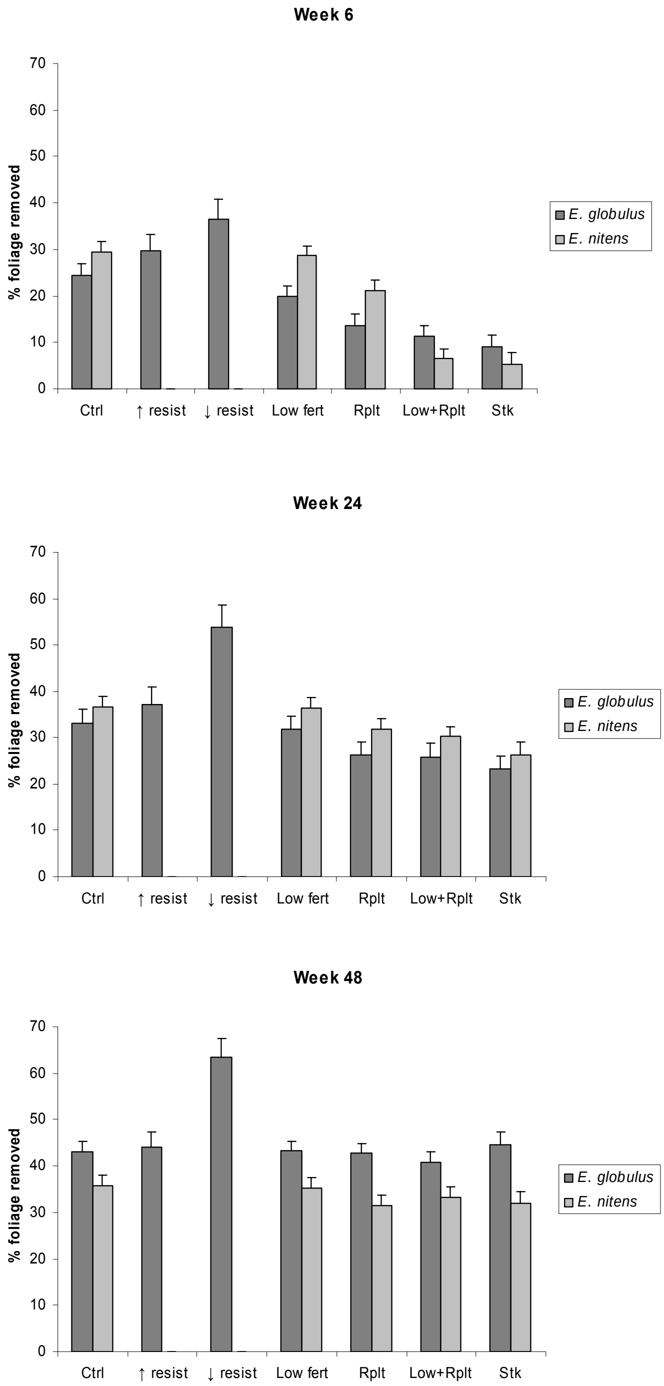

Figure 15. Summary of treatment effects on *E. globulus* and *E. nitens* seedlings in Trial 1 at 6, 24 and 48 weeks after planting, averaged across eight sites

TRIAL 2

Methods

Field sites

This trial was designed to operationally demonstrate the effectiveness of the best combination of seedling manipulation strategies (of interest to industry partners) to reduce browsing, as identified in Trial 1. Six "demonstration" sites were planted in autumn/winter 2008 with *E. nitens*, using the most effective, operationally feasible treatments identified in the Trial 1 (i.e. stockings and repellent).

Due to the last minute addition of stockings to Trial 1, we were yet to have a direct comparison of the effectiveness of repellent and stockings at reducing browsing, or to know if the two could be combined for a more browse-resistant seedling, and we were unable to determine cost-effectiveness. Although stockings are now routinely used by many forestry companies, particularly in high-risk areas, their cost-effectiveness is something that remains largely unknown. In addition, repellents could be a more feasible option under certain circumstances, for example in areas where stockings are prone to removal by wildlife. One of the major drawbacks of both stockings and repellent is their limited lifespan of protection; stockings only prevent browsing until the seedling grows out of the top, and repellent only protects foliage that it is directly applied to, i.e. new growth is unprotected. A potential advantage of using repellent over stockings is that it can be reapplied to seedlings in the field. The feasibility and effectiveness of this was examined.

The eight sites from Trial 1 were all Forestry Tasmania sites and were planted in spring. For financial reasons, however, the private companies generally plant in autumn/winter; a time when there is less alternative food available, and seedlings are likely to remain within the browsing zone for longer due to slower growth. Planting time (i.e. winter vs. spring) could therefore have a strong impact on seedling vulnerability to browsing. In order to ensure that results from Trial 1 are applicable to all industry growers, we used sites provided by private companies and planted over autumn/winter for Trial 2.

We used six sites for these trials, with four operated by Forest Enterprises Australia Ltd and two by Great Southern Plantations Ltd. Two sites were near Beaconsfield; two near Scottsdale; one near Lake Leake; and one near Tunbridge (Figure 16). Sites were a mixture of ex-native forest and ex-pasture sites; all were first rotation (Table 13). Sites were fertilised between 1 and 4 months after planting with 100 g Di-Ammonium Phosphate/seedling. Browse control data (not presented) show that all three herbivore species of interest (possum, pademelon and wallaby) were present on all sites. Fallow deer were also present on site 5, but were not controlled due to insufficient evidence they were causing damage.

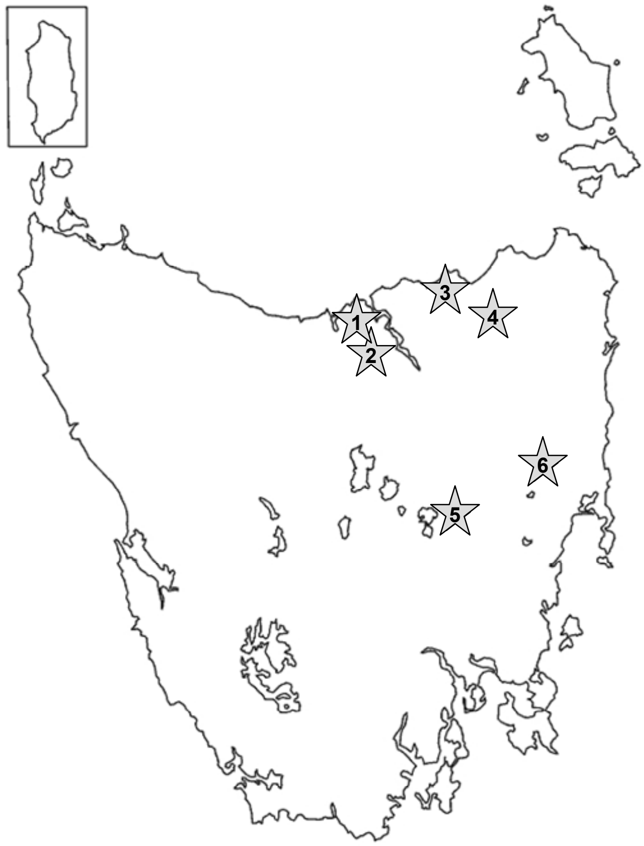

Figure 16. Location map of the six field sites used in Trial 2

Table 13. Characteristics of the six field sites used in Trial 2. FEA = Forest Enterprises Australia; GS = Great Southern Plantations; NF = native forest; P = pasture

Site no.	Coupe ID	Company	Location	Land usage	Area (ha)	Altitude (m)	Planted	Field repellent application
1	Bulls	FEA	Beaconsfield	NF	34	96	8/05/2008	13/08/2008
2	Spencers	FEA	Glengarry	NF/P	52	129	9/05/2008	14/08/2008
3	Halls	GS	Bridport	P	131.1	50	12/05/2008	21/08/2008
4	Gunnadoo	GS	Scottsdale	NF/P	30.1	43	23/06/2008	30/09/2008
5	Tunbridge	FEA	Tunbridge	NF	40	645	4/07/2008	10/10/2008
6	L. Leake	FEA	Lake Leake	NF	15	670	3/07/2008	8/10/2008

Experimental design

Trial 2 consisted of seven treatments, including all combinations of repellent and stocking, with and without repellent application in the field (Table 14). Treatments were applied several days before planting, using the same methods as in Trial 1. For treatments requiring both repellent and stockings, repellent was allowed to dry for 24 hours before applying the stocking. There were only four treatments at planting (1-4), but the other three (5-7) emerged after the field application of repellent.

Table 14. Trial 2 treatment summary. Treatment codes used in later figures

	Treatment	Code
1	Control	Ctrl
2	Repellent	Rplt
3	Stockings	Stk
4	Repellent + Stockings	Rplt+Stk
5	Repellent + Field Repellent	Rplt+FR
6	Stockings + Field Repellent	Stk+FR
7	Repellent + Stockings + Field Repellent	Rplt+Stk+FR

Seedlings were supplied by industry partners, and were the same (e.g. seedlot, fertiliser regime, growth conditions) as those operationally planted on the remainder of each site. Seedlings for site 3 were from Perth nursery, sites 1, 2, 5 and 6 were from Woodley nursery, and site 4 were from Hills nursery. This trial tested only *E. nitens* because this is currently the most common plantation eucalypt in Tasmania.

Seedlings were planted in the same style as Trial 1, and again the remainder of each coupe was an operational *E. nitens* plantation. There were 100 replicates of each treatment on each site, resulting in a total of 700 seedlings per site. Each site had 20 blocks, each containing five replicates of each treatment. Treatments were randomised within a replicate, replicates within a block, and blocks within each site. Experimental seedlings were planted by the same contractors involved in planting the rest of the site, using the same operational methods and over the same period.

Field repellent was applied to seedlings in treatments 5-7 (100/treatment/site), three months (14 weeks) after planting. Dry, calm days were preferred, but seedlings did not always have 24 hours to dry before rain. Weather conditions (particularly wind) provided difficulty in getting good coverage from spray and an even cover of grit. Repellent was applied over the top of stockings for appropriate treatments. For field application, it was necessary to apply repellent to seedlings individually, which therefore used a greater quantity than nursery application (approximately 2.5x more).

Data collection

Seedlings were assessed for browsing weekly for the first six weeks, then fortnightly up until six months (24 weeks) after planting. This was scored on the same 0-6 scale as used for Trial 1. Seedling heights were measured at planting, at three months, and at six months after planting. Heights were not measured as frequently as in Trial 1, as we were expecting slower growth from winter than spring plantings.

Analysis

We examined five main variables for this trial:

1. *browsing delay* - the week when browsing first occurred and when browsing first reached a severity of 25% or more of foliage removed.
2. *browsing extent* – percentage of seedlings browsed; calculated as the number of seedlings with damage, divided by the total number of seedlings.
3. *browsing severity* - percentage of foliage removed from seedlings; calculated by converting browsing scores to median values of percentage foliage removed by mammals (e.g. a score of 2 = 15.5%).
4. *seedling height* - the height (cm) of seedlings at planting (week 0) and at the end of the trial (week 24), and the change in height over this period.

5. *seedling survival* - the percentage of seedlings alive at the end of the trial (week 24).

All analyses, except browsing delay, were conducted on block means by averaging data for each treatment by replicate and block. Browsing delay used individual plants. For all statistical tests, residuals were checked for homoscedasticity and normality, and transformations were preformed where required (Zar 1996). All analyses were conducted in SAS 9.1 (SAS Institute Inc. 2004).

Browsing delay was analysed using PROC LIFETEST, with treatment(s) as strata (SAS Institute Inc. 1989). As with Trial 1, analysis was performed on the week that seedlings were first browsed, and the week where browsing first reached or exceeded 25% of foliage removed.

For all other variables, a linear mixed model was fitted to the block mean data with site (df = 4), treatment (df = 6) and their interaction all treated as fixed effects. This model was fitted with the PROC MIXED procedure of SAS using REML (SAS Institute Inc. 2002). Where significant ($P < 0.05$) treatment effects were found, pairwise comparisons were made using the Tukey-Kramer adjustment for multiple comparisons. Analyses of the extent and severity of browsing were conducted on data from week 12 and week 24. Browsing extent data was arcsin squareroot transformed; browsing severity data was log transformed for week 12 and arcsin squareroot transformed for week 24.

Site 6 experienced extremely high early mortality due to poor planting conditions, i.e. very rocky ground which was frozen at planting, and was then destroyed by road construction. This site has therefore been excluded from the analysis; data is not presented, and it will not be discussed further.

Results

Browsing damage

Although a relatively high percentage of seedlings in all treatments were browsed over the 24 weeks of the trial (Figure 17), the amount of foliage removed from control seedlings was substantially greater than from any other treatment (Figure 18). In-depth data are presented for weeks 12 and 24 below. Week 12 is when the percentage of seedlings browsed first exceeded 10% for all treatments, a level that ensures treatment differences are biologically meaningful, and is shortly before field application of repellent (in week 14). Week 24 was the end of the study and shows the effect of several weeks of the field repellent treatment.

Browsing delay

There was a highly significant effect of treatment on the average time since planting that seedlings were first browsed (log rank test $\chi^2 = 349.5$; $P < 0.001$). The time taken for 25% of seedlings to be first browsed (T25) varied from four weeks for controls to 18 weeks for seedlings with both repellent and stockings (Table 15). This treatment therefore represents a delay of 14 weeks. Repellent and stockings in isolation were less effective; repellent delayed browsing by 3-4 weeks and stockings by 6-8 weeks. Quite a high percentage of seedlings were never browsed (15-43%, depending on treatment; Table 15), but it should be remembered that this data is only to week 24, where Trial 1 is using week 48.

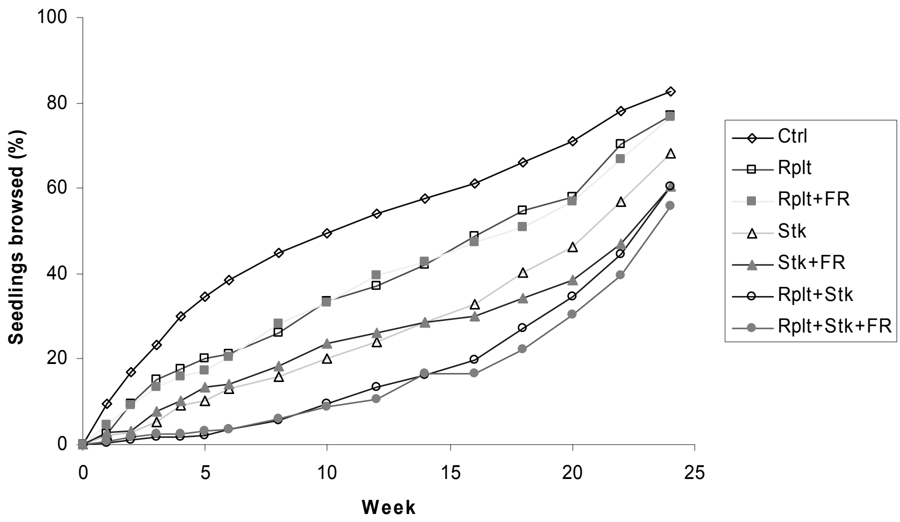

Figure 17. Extent of browsing (mean percentage of seedlings browsed) in each treatment, averaged across sites, over the 24-week trial period in Trial 2. Legend codes as in Table 14.

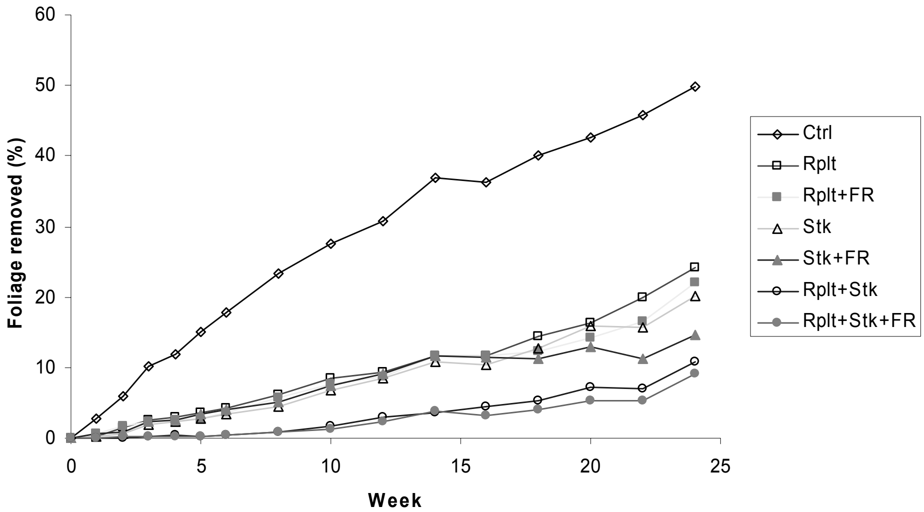

Figure 18. Severity of browsing (mean percentage foliage removed from seedlings) in each treatment, averaged across sites, over the 24-week trial period in Trial 2. Legend codes as in Table 14.

Table 15. Delay in browsing due to treatments: weeks until first browsed and weeks until browsing reached the biologically/operationally important level of 25% of foliage removed in Trial 2 across five sites. T25 = time when 25% of seedlings reached level; T50 = time when 50% of seedlings reached level. Treatment codes as in Table 14.

	Weeks until first browsed			Weeks until severity ≥ 25%		
Treatment	T25	T50	% never browsed	T25	T50	% never ≥ 25%
Ctrl	4	10	15.4	6	16	31.1
Rplt	7	16	19.8	20	-	61.2
Rplt+FR	8	16	20.8	24	-	67.0
Stk	12	22	29.8	25	-	65.4
Stk+FR	10	22	37.0	-	-	75.0
Rplt+Stk	18	24	37.6	-	-	85.4
Rplt+Stk+FR	18	24	43.2	-	-	86.5

Stocking and repellent treatments also resulted in a highly significant delay until browsing severity reached or exceeded the biologically significant level of 25% of foliage removed (log rank test $\chi^2 = 674.0$; $P < 0.001$). While 25% of control seedlings (T25) were browsed to this level after just 6 weeks, the majority of seedlings did not reach this level of browsing severity within the study period (Table 15).

Browsing extent

Twelve weeks after planting, an average of 54% of control seedlings had been browsed compared with only 11% from the most effective treatment (Rplt+Stk+FR), although this was not significant at all sites (Figure 19). There was a significant site*treatment interaction ($F_{24,665} = 4.05$; $P < 0.001$), as well as highly significant effects of site ($F_{4,665} = 131.3$; $P < 0.001$) and treatment ($F_{6,665} = 49.3$; $P < 0.001$). The most notable difference between sites was the substantially lower level of browsing at site 5; levels were so low differences between treatments were not significant or biologically meaningful.

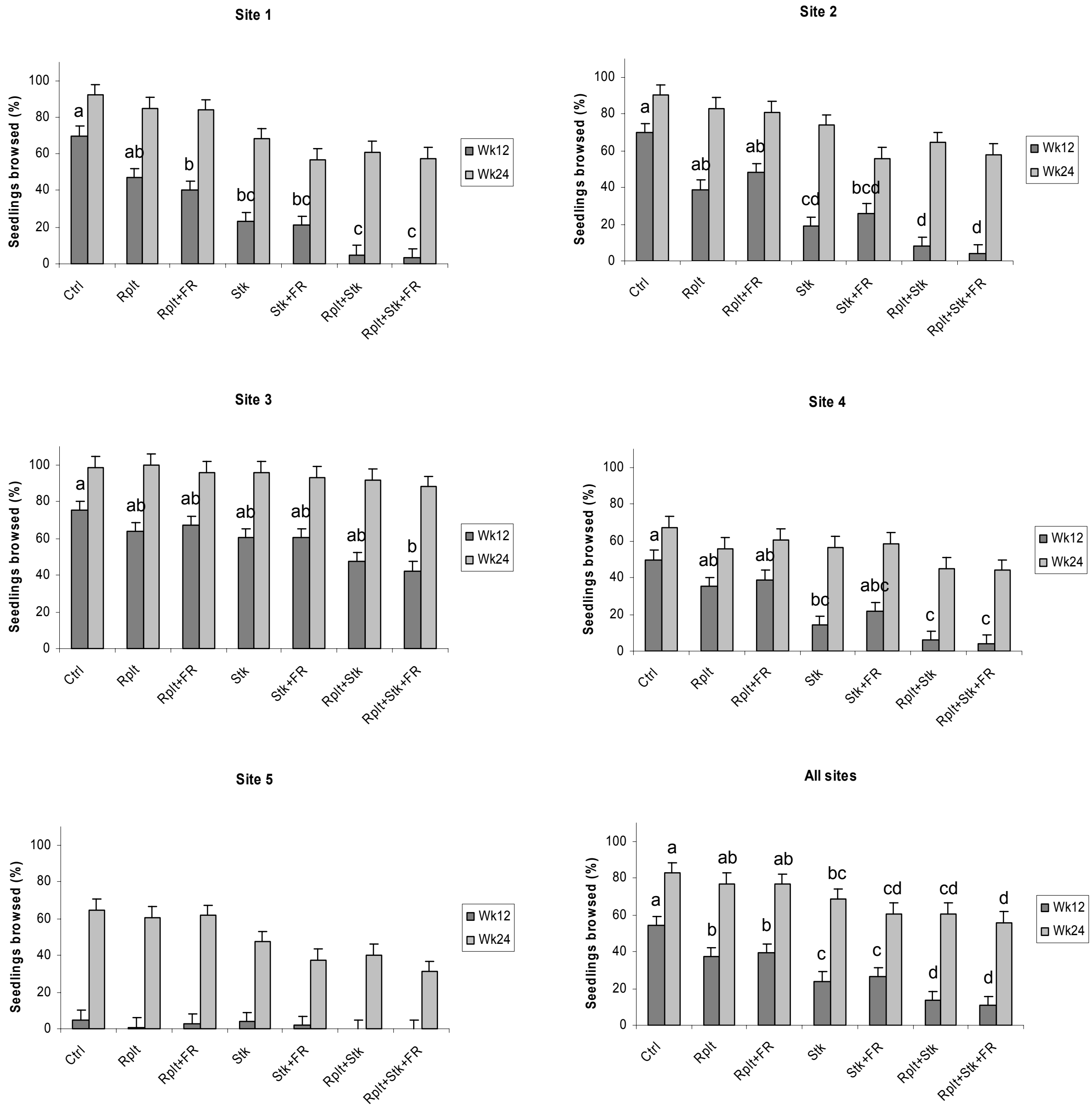

Figure 19. Extent of browsing (percentage of seedlings browsed) across treatments, 12 and 24 weeks after planting at 5 sites. Treatment codes as in Table 14. Different letters within a series indicate significant differences at $P < 0.05$. Differences not shown for week 24 as there was no site*treatment interaction

After 24 weeks, an average of 83% of control seedlings had been browsed, compared with only 56% of seedlings with the most effective treatment (Figure 19; treatment effect: $F_{6,665} = 16.4$; $P < 0.001$). There was no significant difference between the number of control and repellent seedlings browsed, although by this stage there was new growth that was not protected by repellent. Significantly fewer seedlings were browsed in all treatments involving stockings compared with controls. There was a significant effect of site ($F_{4,665} = 72.5$; $P < 0.001$), but no longer a significant interaction between site and treatment ($F_{24,665} = 1.0$; $P = 0.437$). Sites 4 and 5 had significantly fewer seedlings browsed than sites 1 and 2, which in turn were significantly lower than site 3. No additional protection was detected from the application of field repellent to any treatment.

Browsing severity

Three months (12 weeks) after planting, more foliage had been removed from control seedlings (31%) than any others (range 2-9%), although this was not significant at all sites (Figure 20; site*treatment $F_{24,665} = 4.77$; $P < 0.001$). The repellent + stocking treatment provided the most protection, followed by stockings only and repellent only ($F_{6,665} = 54.41$; $P < 0.001$). Site 5 received very low damage levels resulting in no difference between treatments. At all other sites, control seedlings were browsed significantly more than seedlings with both repellent and stockings. There was generally little difference between repellent-only and stocking-only seedlings. There was no significant difference between the base treatments and those with field repellent, which is good as repellent was not applied until week 14. There was a large and significant difference between sites ($F_{4,665} = 118.44$; $P < 0.001$); sites 3 and 5 were significantly different to all others. Site 3 had less difference between controls and other treatments; site 5 had very low browsing.

By the end of the trial (week 24), control seedlings were still more severely damaged (average of 50% foliage removed) than seedlings in any other treatment, although once again this was not significant at all sites (Figure 20; site*treatment $F_{24,665} = 5.99$; $P < 0.001$). Seedlings with both stocking and repellent received the least damage at most sites. While the field repellent seems to have provided a bit of extra protection in comparison to the equivalent treatments without field application of repellent, this difference was never significant. There were large differences in the severity of browsing between sites ($F_{4,665} = 72.43$; $P < 0.001$). While treatment was highly significant overall ($F_{6,665} = 72.21$; $P < 0.001$), it had no effect on site 4 after 24 weeks.

Seedling heights

Seedling heights differed between sites at planting due to the use of different nursery stock. Planting heights ranged from just 8 cm at site 4, to 29 cm at sites 1 and 2 (Figure 21). There was a significant interaction between site and treatment on seedling height at planting ($F_{24,665} = 3.69$; $P < 0.001$). There were no differences between treatments except at sites 1 and 3 (Figure 21). At site 1 Rplt+Stk seedlings were shorter; at site 3 control and stocking seedlings were shorter than Rplt+Stk seedlings.

Six months (24 weeks) after planting there was still a significant site*treatment interaction on seedling height ($F_{24,665} = 2.4$; $P < 0.001$), and significant effects of both treatment ($F_{6,665} = 23.2$; $P < 0.001$) and site ($F_{4,665} = 215.7$; $P < 0.001$; range 12.4 – 27.0 cm). At four of the five sites control seedlings were shorter than all other treatments, however this was not always significant (Figure 21). Treatments had no significant effect on height at sites 4 or 5.

There was a significant site*treatment interaction for height change between weeks 0 and 24 ($F_{24,665}$ = 4.1; P < 0.001). There were also significant effects of site ($F_{4,665}$ = 399.9; P < 0.001) and treatment ($F_{6,665}$ = 27.8; P < 0.001). Overall, and at sites 1 and 2, control seedlings experienced a significantly greater height change than other treatments; this was a decline in height due to browsing. There was no difference between treatments at sites 3, 4 or 5. Seedlings on sites with heavier browsing reduced in height over the course of the trial. Only site 4 experienced much in the way of growth (Figure 21), but this was not related to treatment.

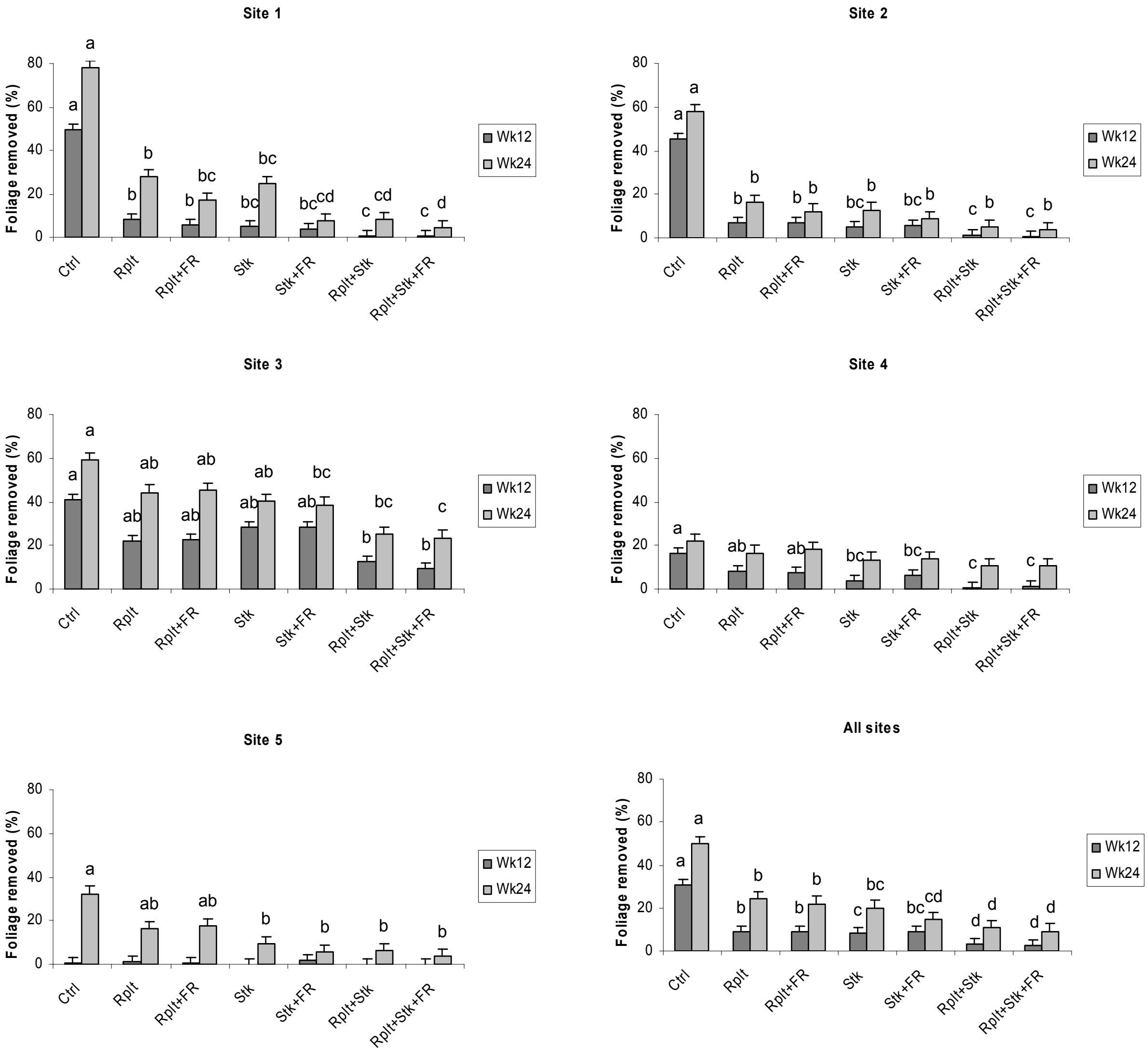

Figure 20. Severity of browsing (percentage of foliage removed from seedlings) across treatments, 12 and 24 weeks after planting at 5 sites. Treatment codes as in Table 14. Different letters within a series indicate significant differences at P < 0.05

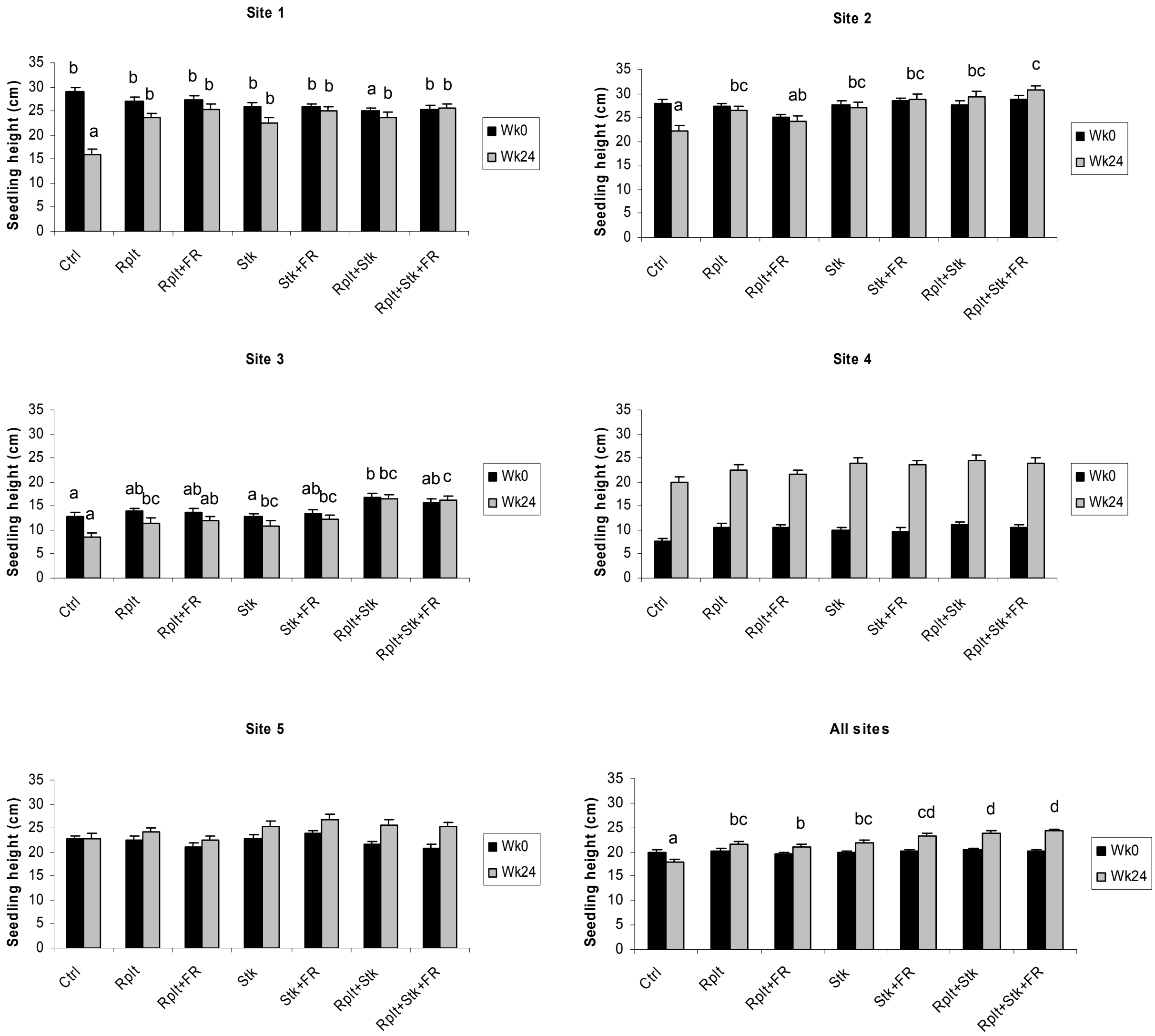

Figure 21. Seedling height at the beginning (week 0) and end (week 24) of the trial period by treatment and site. Treatment codes as in Table 14. Different letters within a series indicate significant differences at P < 0.05

Seedling survival

Survival of seedlings to week 24 was high, and not significantly different between treatments ($F_{6,665}$ = 0.27; P = 0.952; range 95.2 – 96.6%). It should be noted, however, that data could not be normalised; untransformed results are presented. There was a significant difference between sites ($F_{4,665}$ = 23.11; P < 0.001), but no interaction between treatment and site ($F_{24,665}$ = 1.07; P = 0.374). Sites 1 and 2 had over 99% seedling survival which was significantly greater than the other sites. Site 4 had 96% survival, which was significantly greater than the 92% of seedlings surviving at sites 3 and 5.

Discussion

Effectiveness of non-lethal methods of reducing browsing damage

Resistant stock

Unfortunately, the *E. nitens* seedlots that we predicted by NIRS modelling to be higher and lower in sideroxylonal were not. We were therefore unable to test how sideroxylonal levels influenced seedling browsing resistance in *E. nitens* at the "population" level. There are two reasons why there may have been a discrepancy between the predicted and observed sideroxylonal levels. Firstly, the NIRS models used to predict the seedling chemistry were still in the development phase and did not cover the full range of sideroxylonal. These models are under continuing development as part of a separate project. Second, the exact seed stock that were originally predicted to be higher and lower in sideroxylonal were not always available for planting and thus closely related stock was used which would bring variation into the predictions. Nevertheless, our high and low sideroxylonal *E. globulus* seedlots were as predicted, despite the fact that we used seed from a different flowering year and some were untested siblings. Higher sideroxylonal levels translated into increased browsing resistance in the field. The high sideroxylonal deployment seedlots proved more resistant than the increased resistance seedlots from native populations (BGH and JN).

The *E. globulus* seedlots sourced from UTAS differed in their chemistry and browsing severity in the direction expected, with reduced resistance seedlots having less sideroxylonal and receiving more damage. This was particularly obvious for SH seed source. It had previously been reported that SH has significantly less sideroxylonal and was more vulnerable to browsing than BGH or JN in captive feeding trials with pademelon and possum using seedlots from the same locations (O'Reilly-Wapstra *et al.* 2002; O'Reilly-Wapstra *et al.* 2004; Wiggins *et al.* 2008), but this is the first operational-style field test to confirm this.

O'Reilly-Wapstra *et al.* (2005) found that resistance of BGH vs. SH seedlings was related to the level of fertilisation, with genetic differences only expressed when seedlings were not supplementary fertilised. They suggested the lack of difference between fetilised genotypes was due to 50% lower levels of condensed tannins in BGH seedlings when fertilised, reducing to levels similar to those seen in SH seedlings. In Trial 1, we saw this same reduction in condensed tannins under standard vs. low fertiliser (Table 11), but we still saw quite obvious genetic differences in resistance between BGH and SH seedlings under standard fertilisation (Figure 7). In addition, we did not observe the substantially (32%) reduced resistance of BGH seedlings under standard vs. low fertiliser that was shown by O'Reilly-Wapstra *et al.* (2005). These results show that environmental conditions affect the expression of some of the defensive chemistry in *E. globulus* seedlings, but despite changes in chemistry, resistance seen in captive trials holds up in the field.

The fact that we saw differences between naturally more and less resistant seed sources under this experimental design is very encouraging as it suggests the differences must be quite strong. Tests of the ability of PSMs to reduce browsing really need to be planted in large plots rather than the single tree plots tested here as they rely on animal feeding behaviour. For example, in this current design the animals had a choice of food types, whereas in operational plantings of more resistant stock, animals would not have a choice. Additionally, animals need to be able to consume sufficient quantities of secondary chemicals to be deterred (i.e. build up a toxic load which in turn provides a behavioural feedback loop), which was not necessarily happening here with low replication due to a large number of treatments and the single tree plots. It should be noted that, particularly within BGH and JN localities, there were quite large differences between families within localities. Analysis of data at the level of family rather than locality could therefore show even stronger genetic effects. This trial also demonstrated that, unlike a lot of nursery treatments, genetic effects are long-lasting; SH seedlings were still being significantly more severely browsed after 12 months, by which time the fertiliser, repellent and stocking treatments had long since worn off.

Although not formally tested, there does not appear to be an additive effect of combining low fertiliser and repellent with resistant seedlings to further reduce seedling susceptibility to browsing damage. Our "increased resistance" *E. globulus* seedlings were actually less resistant than the operational seedlots provided by FT; only three of seven "increased resistance" seedlots had higher sideroxylonal levels than the operational seedlings. This is not overly surprising considering the background of these seedlots. For the native seedlots, we defined increased resistance at the population level relative to other native populations, whereas the FT seedlots were from seed orchards and have been selectively bred for characteristics beneficial to producing productive plantations, such as growth and survival in the face of field browsing. Research at UTAS continues in order to find more resistant seedlots that may not yet be used in operational plantings. However, it should be noted that while a significant difference in browsing damage was not detected between the high and low sideroxylonal FT seedlots of *E. globulus* at the populations level, the trends were in the right direction and considerable variation been the individual FT seedlots in their browsing resistance was detected under the present experimental design (Appendix 2) for both *E. nitens* and *E. globulus*, and even between the *E. globulus* seedlots classified as having high sideroxylonal levels. This suggests that there is opportunity to improve the genetic resistance of *E. globulus* and *E. nitens* stock used for planting.

Nursery fertiliser

When discussing chemistry results in relation to nursery fertilisation treatment, it is important to look at differences between treatments rather than absolute values because the majority of seedlots which received both standard and low fertiliser were of increased resistance/sideroxylonal. Nursery fertilisation regime had significant effects on seedling chemistry, height, and vulnerability to browsing. The main effect of the low fertiliser treatment was to reduce nitrogen and increase condensed tannins, thus making seedlings less nutritious. This effect was expected, and has been shown by Close *et al.* (2004) and Miller *et al.* (2007).

Despite the effectiveness of low nursery fertiliser at reducing browsing severity, particularly in combination with repellent, this treatment was not examined further due to industry concerns of negative effects on growth. In an earlier trial, Close *et al.* (2000) found no difference in height 23 weeks after planting cold-hardened, non-hardened and nutrient starved *E. nitens* seedlings in the field, but nutrient starved seedlings were taller at planting. At the time of planning Trial 2 we did not have the data to show the effect of nursery fertiliser on seedling growth. We have now shown that, although seedlings receiving low fertiliser started shorter and finished shorter than those receiving standard nursery fertiliser, there was no difference in the absolute growth rate, i.e. seedlings receiving reduced fertiliser in the nursery grew just as well in the field. We would predict that, after a few years, these differences of a few centimetres would disappear or be unimportant. If this height difference were an issue, an option would be to plant taller, low fertiliser seedlings. This could be achieved by keeping them in the nursery for longer, or by reducing the period without fertilisation. It should be noted, however, that seedlings receiving standard fertiliser grew just as well despite higher browsing. It could be that under low browsing, low fertiliser seedlings would do worse, but this is unlikely as nursery effects would have only lasted until nutrients provided by field fertiliser were accessed. In addition to a lack of effect on growth, there was no effect of fertilisation treatment on seedling survival or form after 48 weeks.

The high phosphorous fertiliser was predicted to reduce palatability, through a reduction in foliage nitrogen, without reducing growth potential, and hence be a more viable alternative to low fertiliser. This fertiliser, however, failed to have much impact on seedling chemistry, vulnerability, or growth potential, and any effects seemed to be eucalypt species-dependent. There may have been a more obvious effect of high P fertiliser if it had been applied to the seedlings for longer, but this would require a larger alteration to nursery practises and the lack of negative effect of low fertiliser on growth suggests this is unwarranted as an alternative.

Repellent

Sen-Tree repellent proved quite effective at delaying and reducing browsing. Its effectiveness was increased when combined with either low nursery fertiliser or stockings. In isolation, repellent appeared more effective in Trial 2 than Trial 1. For example, in Trial 1, there was no difference in browsing delay of *E. nitens* standard fertiliser seedlings with and without repellent, whereas in Trial 2 repellent delayed browsing for three weeks compared with controls. The greater effectiveness of repellent in Trial 2 than Trial 1 could be due to the time of year, with repellent comparatively less effective over summer due to rapid growth. The difference in growth between the trials is obvious from both seedling heights and browsing severity graphs (no decline in severity over time in Trial 2). The between-trial differences could also be due to the lower overall browsing intensity in Trial 2. Sen-Tree repellent would be expected to be more effective under lower browsing pressure due to its mode of action; it works by "teaching" herbivores to associate the smell of egg with the gritty taste. If there are large numbers of herbivores, even if they were all immediately deterred after sampling, this would still result in a large amount of damage to seedlings. The fact that repellent seedlings were first browsed several weeks later than control seedlings (Trial 2) shows that animals were capable of perceiving treatment and therefore seedling palatability prior to tasting, i.e. associative learning is apparent. Browsing intensity could also be related to the time of year, however it is believed that browsing is more severe over winter than summer when there is less alternative food available (Coleman *et al.* 1997).

Sen-Tree repellent was not as effective in the field as captive animal trials suggested Miller *et al.* (2008). In captive trials, Sen-Tree reduced browsing on *E. nitens* seedlings by 97% for possum and 99% for pademelon compared with controls. In field trials, Sen-Tree effectiveness varied between sites and trials and decreased over time, but at week 12 there was an average browsing reduction of treated vs. untreated *E. nitens* seedlings of 34% for Trial 1 and 70% for Trial 2. This difference in effectiveness between captive and field trials is not unexpected as there are many variables influencing feeding behaviour in field trials which are controlled in a captive environment; for example hunger level, amount of alternative food available and browsing pressure. In addition, the captive trials were much shorter in duration than field trials, seedlings always had full repellent coverage, and captive trials did not include Bennett's wallaby, which could be less deterred by Sen-Tree than pademelon and possum.

Despite heavy rain during and shortly after planting the majority of sites in Trial 1, the repellent was not washed off, and in fact was still obvious on seedlings after 12 months (by which stage it was generally restricted to a few leaves at the base of the stem). This shows that weather is not an issue with regards to repellent longevity once repellent is on and dry. One problem is that Sen-Tree is a contact repellent, and as such was only effective on the foliage to which it was applied. Reapplication of repellent to cover new foliage is therefore necessary if it is to protect seedlings for an extended amount of time. Although never significantly different, there was a trend for seedlings with field application of repellent to be less heavily browsed than the corresponding treatments without field repellent. This is particularly encouraging since time constraints meant that the field application of repellent was performed at a time when it was unlikely to be beneficial due to a lack of growth. If applied at a time when there was new foliage requiring protection, field application may have been much more effective.

Field application of repellent is a slow procedure, and due to the larger amounts of repellent required, costs more per seedling than nursery application. Another issue with reapplication is that care must be taken to ensure that excess repellent is not applied to older foliage. Too much grit or glue could clog stomata and retard photosynthesis and respiration. This may have been responsible for the negative effect of repellent on seedling recovery from browsing observed in three of four experiments involving repellent in Trial 1. Grit can also cause foliage to burn by attracting heat. In addition, field application to stocking seedlings often resulted in stockings being glued onto seedlings, although this did not seem to cause a problem unless the stocking top was glued closed (which would not be a problem if reapplication was at a more appropriate time).

The ideal situation would be to reapply repellent at regular intervals or at crucial stages, for example coming into winter to protect current foliage of seedlings that are unlikely to grow for several months. The majority of published studies into contact repellents are either captive animal trials, often on processed foods, or short term field trials comparing the effectiveness of several repellents (e.g. Gillingham *et al.* 1987; Andelt *et al.* 1994; Bergquist and Orlander 1996; Santilli *et al.* 2004). Repellent longevity is not an issue in such tests, and so reapplications are rarely discussed or performed (but see Hygnstrom and Craven (1988) for a failed attempt). The problem of repellent reapplication is avoided by using systemic repellents, e.g. selenium (Rediske and Lawrence 1962; Angradi and Tzilkowski 1987; Moser 2003). These are absorbed into the plant, via either foliage or roots, and translocated to all parts of the plant. Systemic repellents, however, are much less commonly used than contact repellents and as yet none have been produced that can effectively reduce browsing by mammalian herbivores without being phytotoxic.

It is possible that repeated application of repellent would lead to herbivores becoming adapted to it, resulting in reduced effectiveness. There is evidence, however, that Sen-Tree repellent would remain effective in the long term due to an inability of herbivores to adapt to silica, as can occur with repeated exposure to various chemical defenses (Massey and Hartley 2009).

Stockings

Stockings effectively reduced browsing in both trials, but the higher browsing intensity in Trial 1 resulted in stocking seedlings being less effective at delaying browsing than in Trial 2 (Trial 1 T25 = week 5; Trial 2 T25 = week 12). By week 24, stocking seedlings in Trial 1 had an average of 26% foliage removed, while those in Trial 2 had 20%. Under the intense browsing pressure in Trial 1 at site 7, stockings appeared to be more of a problem than benefit. Where seedlings without stockings were browsed quite heavily, they were often able to recover from this, but seedlings with stockings were severely damaged as animals attempted to feed on them, and thus had no chance at all of recovery (A. Miller pers. obs.).

We expected stockings to be more effective than repellent as they provide a physical barrier to browsing, whereas repellent relies on animals learning that it is unpleasant. Trial 1 did not allow for a direct comparison of these treatments, but did suggest that stockings were the more effective of the two. Although there was a slight trend in this direction for Trial 2, there was generally no significant difference between browsing severity on seedlings with stocking vs. repellent. Stockings were, however, able to delay browsing for longer, and can therefore perhaps be said to be slightly more effective.

Perhaps not surprisingly, the combination of Sen-Tree repellent and stockings proved quite effective at delaying browsing and reducing browsing severity, and in most cases, these seedlings received significantly less damage than controls. Although generally not significant, the trend for lower browsing on seedlings with both protective measures, compared to just one, suggests that perhaps the scent of the repellent deterred animals from trying to get inside the stocking. These seedlings were also protected when stockings were removed or damaged.

As with repellent, stockings have a limited life span which is influenced by seedling growth rate. Effectiveness is often extended when using rigid tree guards by making shelters up to 0.5 m taller than seedlings to allow for growth and for animals that can stick their noses and/or tongues into shelters to feed (e.g. Stange and Shea 1998; Bendfeldt *et al.* 2001; Chaar *et al.* 2008). This cannot be done with soft shelters as the top would droop over and prevent escape of the terminal bud. In fact, this problem and the resultant stem deformation was observed in both trials.

Deformation of seedlings in Trial 1, caused by stockings, seemed to have declined after 12 months, and this observation is supported by the lack of difference in the presence of multiple leaders between treatments. Unfortunately, we did not have the resources to examine this in depth in Trial 2, but observations suggest that deformation is related to the size of the stocking relative to seedling, and the time of year. Seedlings in which the stockings were too tall, through either poor planting or poor application, tended to grow around in circles inside the stocking and out through the sides rather than straight up and out the top. This is because, without a supporting structure, the top of the stocking tended to droop over and prevent normal seedling growth. The time of year also seemed to have an influence on the degree of stem deformity. Over summer (spring plant) seedlings grew fast and so escaped stockings relatively quickly. Conversely, over winter growth was slow and seedlings seemed more likely to get tangled inside stockings. For this reason, the lack of significant effect on form in Trial 1 should not be taken to mean that there will be no negative effect on form for winter-plant seedlings. This is particularly important as stockings are perhaps more effective over winter as they are able to protect seedlings for longer periods.

As highlighted above, seedlings that are poorly planted can end up with increased risk of deformity. Poor planting can also reduce the effectiveness of stockings as, during planting, they are often pulled off or down. A badly planted stocking seedling is perhaps worse than an unprotected seedling as the stocking will not protect the seedling from browsing, but is still able to become tangled and damage seedling. Care must be taken to ensure the base of stockings is buried and that stockings extend a few centimetres (but no more) past seedling tips.

Vegetation

We had aimed to examine the effect of existing coupe vegetation on browsing extent and severity. None of the sites had much in the way of vegetation at planting, and not surprisingly there was no effect of vegetation height or cover on browsing delay. What we did observe, however, was that seedlings that had vegetation grow up around them appeared better able to recover from early browsing. This confirms earlier findings that vegetation can have a significant influence on seedling vulnerability to browsing (Miller 2006; Miller *et al.* 2006; Miller *et al.* 2007). Unlike the other aspects examined, however, vegetation manipulation would not be as easy to incorporate into established procedures and cannot always be accurately controlled. In addition, adverse effects on growth through competition have not been fully explored. For these reasons it is not a preferred method of industry.

Seedling performance

Performance of experimental seedlings should only be compared within the trials and not directly compared with those of operational seedlings under operational conditions. This is because the experimental seedlings were frequently planted in sub-optimal locations, particularly in Trial 1 – they were often not on planting mounds, and in fact in cases ended up on tracks and other uncultivated spots and, as a result, soil and water conditions would have been less than ideal. This is because we were initially expecting to remove seedlings after six months and, in some cases, there was little space remaining in which to plant seedlings. In addition, perimeter seedlings rarely do as well as those in the centre of the coupe initially for varying reasons, including increased browsing, increased wind, damage from forestry operations, and often less than ideal preparation.

Height and form

Growth in Trial 1 was much greater than in Trial 2, where it was often negative. This is related primarily to the time of year, with a higher growth potential of seedlings over summer than winter. In Trial 2, control seedlings were generally shorter than others at the end of the study. This was most likely due to the higher level of browsing these seedlings received, but could also potentially be due to both stockings and repellents providing some growth benefit. This is unlikely, however, as control seedlings that were not heavily browsed generally appeared to be healthier than other treatments.

Eucalyptus globulus seedlings were taller than *E. nitens* seedlings at planting, and remained so after 48 weeks, despite more severe browsing. This is a surprising result since the majority of sites were not areas where this species would usually be planted due to its poor frost resistance compared with *E. nitens*. The increased browsing damage could be due to the fact that *E. globulus* was a novelty on *E. nitens* coupes rather than any specific preferences. It will be interesting to compare heights and other productivity measures after two years, to see if differences remain.

The high incidence of multiple leaders observed in Trial 1 was likely due to the intense repeated browsing these seedlings received. Likewise, the reduced proportion of seedlings with multiple leaders amongst vegetation of increasing height and cover in Trial 1 was probably due to the greater recovery from browsing these seedlings experienced. Many of these seedlings could be expected to develop into single-leader trees once browsing declines, which will hopefully be demonstrated when form is reassessed in late 2009.

Survival

Seedling survival was not consistently significantly influenced by any of the nursery treatments. Severe browsing was responsible for a large proportion of seedling mortality, particularly in Trial 1. Survival to week 48 in experiment 7 was greater for operational *E. nitens* than for operational *E. globulus* seedlings. Both these seedlots were purchased from the nursery however, and so initial growth conditions are unknown and may have influenced survival. The greater mortality of *E. globulus* could be due to the heavier browsing this species received. It could also be due to the fact that many sites were areas where *E. globulus* would not usually be planted (e.g. high altitude). Additionally, *E. globulus* seedlings planted in areas that were outside their usual range, and that were stressed going into winter (mostly through browsing damage) were much less likely to survive than those that were healthy.

Cost-effectiveness

Stockings are substantially cheaper than traditional rigid tree guards. The price for seedlings to be "socked" on an operational scale varies between \$0.11 and \$0.15/seedling, depending on seedling size (larger seedlings require more material) and supplier. Some suppliers use a semi-automated system, and some do it by hand. Currently, nursery application of repellent is cheaper, at only \$0.05/seedling (D. Woodlea pers. comm.).

Although repellent is only slightly less effective than stockings and currently much cheaper, it is not a favoured method of industry. This is due largely to the highly variable results obtained from initial tests. Work is underway on a new stocking applicator machine, with the aim of reducing costs down to \$0.05/seedling (H. Cusick, Gunns Ltd pers. comm.), which would bring stocking costs in line with those for repellent application. We therefore feel that it is unlikely that repellent will be looked upon to replace stockings for large-scale use. There are circumstances, however, when it may be beneficial to use repellents instead, or to combine both methods for enhanced resistance. For example, the relatively small cost of adding repellent to stocking seedlings could be worthwhile on sites with heavy browsing pressure (until shooting can be effective) or where there are populations of herbivores known to remove stockings, e.g. deer.

Due to the relatively new incorporation of stockings into forestry, the cost-effectiveness of using them as a means to reduce reliance on lethal controls, e.g. shooting, is as yet unknown. Industry have noted a decline in the need for shooting and the number of infills required where stockings have been used, but this is yet to be quantified. This process should be possible by mid-2009 after coupe survival estimates have been performed.

Future research

The use of seedling stockings at an operational scale has been increasing rapidly for the past few years. In 2008, over 4 million stocking seedlings were planted across the state (combination of FT Perth, Woodlea and Gunns Somerset nurseries and Statewide Plantation Services). Due to the large numbers currently in use, it is of great importance to gather longer-term data of the effect of seedling stockings on growth, form and survival. Stockings are made of polyethylene and so are not biodegradable. There is some evidence that stockings begin to disintegrate after 18 months. While this is good for the seedlings as it means they are unlikely to be ringbarked, it is not ideal for the environment. Due to the increasing usage of stockings, an important area for future research could be into a more biodegradable mesh; it would be ideal if they broke down after 12-18 months. It could be worth assessing the social acceptability of large-scale stocking use. In addition to the issue of degradability, large areas of red stockings are perhaps not aesthetically pleasing. It is also possible that stocking colour could influence effectiveness, for example if different colours attract different animals. Further benefits of stockings on seedlings may be addressed with additional research, for example, there is anecdotal evidence that stockings can provide frost protection through their alteration of the seedlings microenvironment.

Unfortunately, time constraints meant that the field application of repellent was performed at a time when it was unlikely to provide increased protection due to a lack of growth. It would be good to conduct another field application at a time when seedlings actually need it, in order to gauge more accurately the cost effectiveness of such a method. Repellents appear to be less of an environmental concern, but if field reapplication were to become common then it would be beneficial to look at any health effects on the plant of repeated application. Excess repellent coverage could, for example, clog stomata and hinder photosynthesis and respiration.

Although we have clearly demonstrated the effectiveness of stockings and repellents on reducing browsing by wallaby, pademelon and possum, we are unsure of their effectiveness in reducing deer damage. Fallow deer are a species that are not as easily controlled as others, due to game control legislation and permit issues, but that may be an important pest damaging young plantations. There is anecdotal evidence that deer pull stockings off, and the seedlings are often pulled out of the ground at the same time. Work in the northern hemisphere reports that egg-based repellents, such as Sen-Tree, show the most promising results against deer (e.g. Conover 1984; Harris *et al.* 2000; Wagner and Nolte 2001), so it would be useful to confirm this locally.

The strong, long-lasting effect of seedlings with reduced natural resistance to browsing highlights the area of genetic selection as one of importance for future research. Planting seedlots with naturally higher resistance would circumvent problems with longevity seen with other treatments, and could also be used to target resistance against other plantation pests and diseases. Research is required, particularly for *E. nitens*, to identify seedlots with high levels of sideroxylonal, or other chemical characteristics associated with reduced browsing, and test their browsing resistance, growth potential, and wood properties. These seedlings could also be given enhanced early protection through the use of stockings or repellent if required.

Although not investigated further in Trial 2, the use of decreased nursery fertiliser to reduce browsing is perhaps another area warranting further research. The anticipated poor growth rate in the field was not observed, and low fertiliser seedlings are easily produced and incorporated into current practises. We have demonstrated that the combination of low fertiliser and repellent can significantly reduce browsing, and it is possible that low fertiliser could be combined with other treatments (e.g. stockings) to further reduce browsing on these. Heights of seedlings from Trial 1 will be assessed again late 2009 (two years after planting) and this should address the issue of whether or not fertiliser treatments have any longer-term effect on seedling growth.

Implications for tree growers

With our current understanding of nursery manipulation and resultant browsing vulnerability, stockings and/or repellents are the best option to delay browsing onset and reduce browsing severity. Although both these treatments wear off over time with the emergence of new growth, on the majority of sites the delay in browsing should be sufficient to allow alternative methods of control (e.g. shooting) to be established or genetic resistance effects to become expressed.

A further advantage of stockings and repellents is that they are easy to integrate into current practices. This is particularly the case for stockings, which are already in extensive use. Repellents have been applied successfully at the nursery and require no alteration to planting practises. Neither of these methods are particularly expensive, but currently their cost limits their application to the coupe perimeter and other high-risk areas (e.g. small sections). Further research into application methods is likely to reduce these costs, but we would be hesitant in supporting broad scale application until the longer-term effects on seedling performance are more fully understood.

While there is still avenue for research to be done, we are making positive steps towards reduced reliance on lethal methods for controlling browsing damage.

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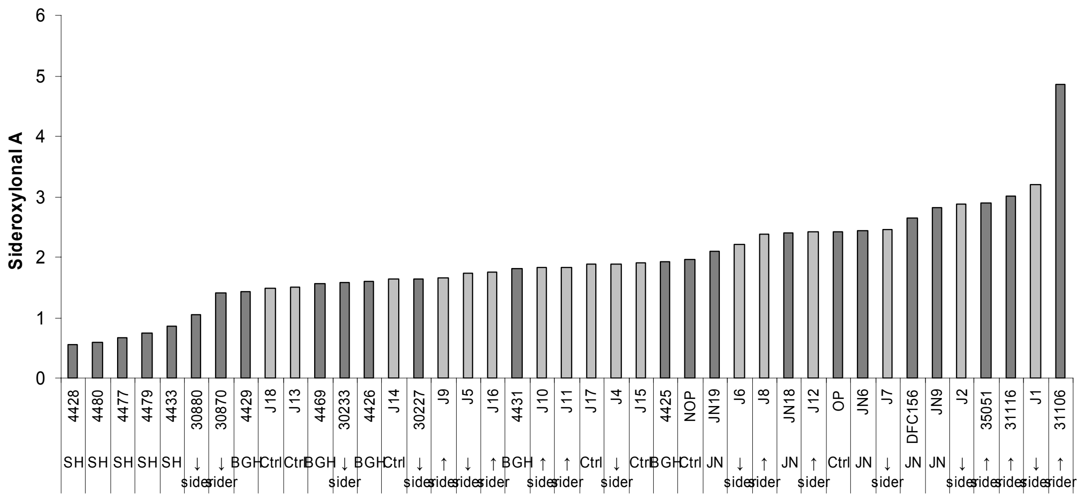
Appendices

Appendix 1. Complete listing of eucalypt seedlots used in Trial 1

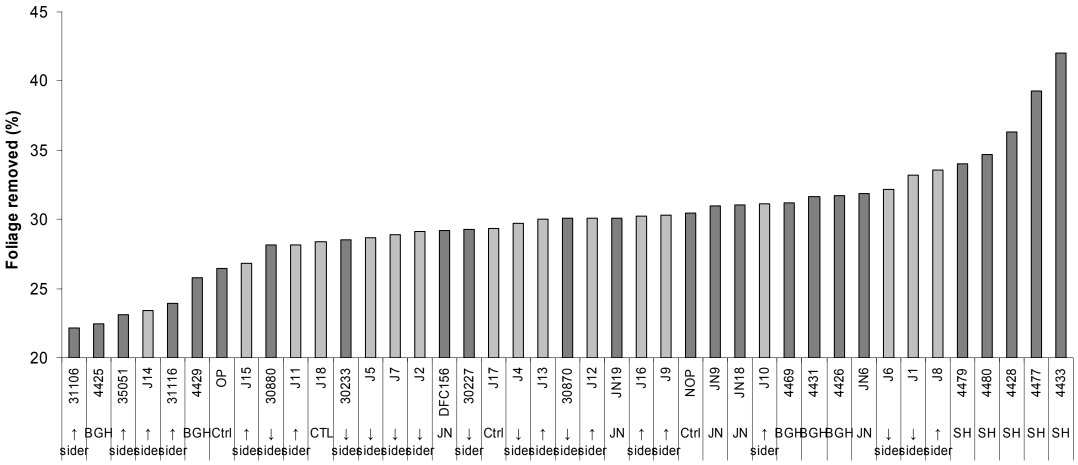
Species	Trial code	Tree ID	Family ID	Resistance level	Source	Locality	Treat
E. globulus	OP[a]	.	.	unknown	FT	.	1 2 3 4
	NOP[a]	.	.	unknown	FT[b]	.	12 13 14
	4425	4425		↑ resistance	UTAS	BGH	5 6 7 8
	4426	4426		↑ resistance	UTAS	BGH	5 6 7 8
	4429	4429		↑ resistance	UTAS	BGH	5 6 7 8
	4431	4431		↑ resistance	UTAS	BGH	5 6 7 8
	4469	4469		↑ resistance	UTAS	BGH	5 6 7 8
	JN6	JN6		↑ resistance	UTAS	JN[c]	5 6 7 8
	JN9	JN9		↑ resistance	UTAS	JN	5 6 7 8
	JN18	JN18		↑ resistance	UTAS	JN	5 6 7 8
	JN19	JN19		↑ resistance	UTAS	JN	5 6 7 8
	DFC156	DFC156		↑ resistance	UTAS	JN	5 6 7 8
	4428	4428		↓ resistance	UTAS	SH	9
	4433	4433		↓ resistance	UTAS	SH	9
	4477	4477		↓ resistance	UTAS	SH	9
	4479	4479		↓ resistance	UTAS	SH	9
	4480	4480		↓ resistance	UTAS	SH	9
	31106	31106	F33	↑ sideroxylonal	FT	.	10
	31116	31116	F33	↑ sideroxylonal	FT	.	10
	35051	35051	F46	↑ sideroxylonal	FT	.	10
	30227	30227	K12	↓ sideroxylonal	FT	.	11
	30233	30233	K12	↓ sideroxylonal	FT	.	11
	30880	30880	K22	↓ sideroxylonal	FT	.	11
	30870	30870	K22	↓ sideroxylonal	FT	.	11
E. nitens	J1	500510	7	↓ sideroxylonal	FT	.	11
	J2	502905	574	↓ sideroxylonal	FT	.	11
	J4	500806	20	↓ sideroxylonal	FT	.	11
	J5	500420	653	↓ sideroxylonal	FT	.	11
	J6	502706	646	↓ sideroxylonal	FT	.	11
	J7	503606	655	↓ sideroxylonal	FT	.	11
	J8	1002866	41	↑ sideroxylonal	FT	.	10
	J9	501319	648	↑ sideroxylonal	FT	.	5 6 7 8
	J10	501319	648	↑ sideroxylonal	FT	.	10
	J11	501006	20	↑ sideroxylonal	FT	.	10
	J12	1001028	41	↑ sideroxylonal	FT	.	10
	J13	504101	574	↑ sideroxylonal	FT	.	5 6 7 8
	J14	1002849	653	↑ sideroxylonal	FT	.	5 6 7 8
	J15	501508	8	↑ sideroxylonal	FT	.	5 6 7 8
	J16[a]	.	.	unknown	FT	.	replaced
	J17[a]	.	.	unknown	FT[b]	.	12 13 14
	J18[a]	.	.	unknown	FT[b]	.	1 2 3 4

[a]Operational groups using mixture of seed from seed orchard trees
[b]Seedlings sourced from stock already growing at the Perth nursery
[c]JN seedlots from CSIRO 1987/88 seed collection

a)



b)



Appendix 2. Summary of a) sideroxylonal A levels and b) browsing severity (% foliage removed) averaged over the 48-week period for all eucalypt seedlots used in Trial 1. Dark grey = *E. globulus*; Light grey = *E. nitens*. Note: average of two technical replicates consisting of pooled foliage from 10 seedlings receiving standard nursery fertilisation