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Do all possums show the same aversions for genetically resistant seedling stock?

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Contents

Executive summary

A common question and concern raised by tree growers is whether browser aversion for genetically sourced seedling stock is consistent state-wide, or do different populations of browsers show different preferences depending on whether they have coexisted with particular populations of eucalypts?

This project investigated the seedling preferences of two populations of the common brushtail possum (*Trichosurus vulpecula*) that have coexisted with two bluegum (*Eucalyptus globulus*) populations that are of genetic extremes in their susceptibility to mammal browsers. Seed sourced from south east Tasmania (Bluegum Hill; foliage with high levels of defensive chemistry) and north east Tasmania (St Helens; foliage with low levels of defensive chemistry) were grown in standard nursery conditions. Additionally, seed from two Australian mainland populations in Victoria, Jeeralang North and Parker Spur, were selected for use in our trial to include foliage that both possum populations were unfamiliar with. Possums were offered each of the four foliage types as a no-choice feeding trial for four consecutive nights and their browsing preferences were recorded.

Brushtail possums ate significantly more St Helens foliage than any other foliage locality. Possums sourced from the Bluegum Hill population ate more foliage across the duration of the trial than possums sourced from the St Helens population, most likely attributed to differences in body mass between the two populations. There was no significant foliage locality by possum population effect, indicating that foliage susceptibility ranking is stable across the two different possum populations. Levels of defensive chemistry, as predicted using near infrared reflectance spectroscopy (NIRS), showed that the St Helens foliage type (north east Tasmania) had lower levels of cineole (a terpene) and sideroxylonal A (a formylated phloroglucinol compound (FPC)) than the Bluegum Hill foliage type (south east Tasmania); the mainland foliage types had lower levels of cineole than the Tasmanian foliage types; and there were no significant differences between the two mainland foliage types. Terpenes and FPCs have previously been shown to be key defensive compounds in eucalypts and are important in conferring resistance of *E. globulus* to marsupial herbivores.

Our results demonstrate that there are genetic based differences in the susceptibility of seedlings of *E. globulus* to possum browsing. This result is consistent with previous trends in the susceptibility of seedling, coppice and juvenile foliage for Tasmanian *E. globulus*. As both possum populations showed similar intake preferences across the foliage types, our results suggest that the susceptibility ranking of germplasm should remain stable across different browsing populations.

Introduction

Seedlings are vulnerable to mammal browsers from the time they are deployed in plantations, and also remain vulnerable to browsers such as the common brushtail possum (*Trichosurus vulpecula*) throughout latter stages of growth (Gilbert 1961, Cremer 1969). Browsing damage during the early stages of seedling establishment can reduce plantation productivity by reducing seedling growth rate and survival, and may alter tree form (Bulinski and McArthur 1999).

One approach to reduce the levels of browsing damage to seedlings is to increase seedling resistance. As pointed out by McArthur and Appleton (2003), the potential exists to choose eucalypt seedlings for planting with increased resistance to browsing, which could be based on species, seed source within species and on the growing environment in the nursery. Research has shown that there is a genetic basis to variation in the level of resistance of *Eucalyptus globulus* foliage to browsing by mammalian herbivores (O'Reilly-Wapstra *et al.* 2002). This research has demonstrated that some Tasmanian populations of *E. globulus* are more resistant, while others are more susceptible, to marsupial browsers in Tasmania. For example, the *E. globulus* population at Bluegum Hill (near Glen Huon, south east Tasmania) is more resistant and higher in defensive chemistry compared with the *E. globulus* population at St Helens (north east Tasmania) which is more susceptible and lower in defensive chemistry (O'Reilly-Wapstra et al. 2004, 2005a).

Taking this research one step further, we aimed to address a common question and concern raised by tree growers – do browser aversions for genetically sourced seedling stock remain consistent state-wide, or do different populations of browsers show different preferences depending on whether they have coexisted with particular populations of eucalypts? The use of naturally enhanced increase of resistant stock is a very promising strategy for reducing palatability of seedlings prior to planting; however, it is important that tree growers are confident that this level of resistance holds up regardless of geographic location of plantations. We investigated seedling preferences by two different populations of the common brushtail possum (*Trichosurus vulpecula*) that have coexisted with two eucalypt populations that represent genetic differences in their levels of susceptibility to mammal browsers (south east Tasmania (Bluegum Hill) vs. north east Tasmania (St Helens)). Thus, this project aims to give tree growers the knowledge they need to ensure they know whether browser aversions for genetically sourced seed stock remains consistent when exposed to different populations of the brushtail possum.

Materials and methods

Plant material

Seed sourced from two native Tasmanian *Eucalyptus globulus* populations; Bluegum Hill (Glen Huon; collected December 2006/January 2007) and St Helens (collected January 2007) were selected to represent two geographically and genetically different populations of *E. globulus* in Tasmania (see O'Reilly-Wapstra *et al.* 2002, 2004, 2005b). Additionally, seed sourced from two Victorian populations, Jeeralang North and Parker Spur (Victoria; CSIRO seed collection 1987) were also selected for use in our trial to include foliage that both possum populations were unfamiliar with. Previous research suggests that coppice foliage of the two Victorian populations differs slightly in their levels of genetic susceptibility to mammal browsers (O'Reilly-Wapstra et al., unpublished data). Thus, we selected seed stock to represent four geographically different populations of *E. globulus*. A total of 10 families per locality were selected, using 40 seeds per family, to give a total of 400 seeds sown per eucalypt population. Seed was planted in March in 6 x 7 cell plastic seed trays (Rite Gro Kwik Pot 42's, Garden City Plastics (GCP), Waratah Wholesale, Hobart) filled with slow release fertiliser potting mix (Permium + CRF, low P; Horticultural Supplies, Brighton). Seed from each of the 40 seedlots (four *E. globulus* populations by 10 families per locality) were randomly distributed in seed trays with one seedlot per tray and grown in a common environment (glasshouse, School of Plant Science) for 24 weeks to an approximate height of 30 cm. Individual seed trays were randomly allocated a position in the glasshouse and were moved monthly to reduce potential environment effects. The seedlings were moved to an outdoor fenced enclosure in September to harden for four weeks, and were fertilised once in October with an all purpose fertiliser (~1.25 mg Peters® Excel® Water Soluble Fertiliser; N:P:K 20:2.2:6.6, solution concentration 1 g l^{-1}; Scotts Australia, Castle Hill, NSW, Australia).

Animal maintenance

Eight brushtail possums were wild-caught from each of two geographically separated locations: south east Tasmania (Bermuda Road (Bluegum Hill), Glen Huon, 43^{o}03'S, 146^{o}52'E) and north east Tasmania (St Helens, 41^{o}15S, 148^{o}19'E) in September-October. The 16 possums (11 males, 5 females, mean body weight 3.46 kg, s.d. 0.47) were transported to the University of Tasmania's zoology animal enclosures and kept in individual mesh pens (4.3 m long, 1.7 m wide, 2.5 m high) with concrete floors and polycarbonate roofing. Each possum was provided with a nest box and climbing logs, with fresh drinking water always available. Possums were fed daily, being offered a maintenance diet of freshly ground apple, carrot, silver beet, Lucerne and sugar (McArthur et al. 2000; Wiggins et al. 2003). Possums were weighed weekly to monitor their body condition and acclimated to captivity for a minimum of two weeks and a maximum of five weeks prior to the commencement of feeding trials.

Feeding trial

The experimental design consisted of four treatment diets (foliage types), detailed in Table 1. Foliage types were presented to possums as a no-choice feeding trial, where each possum received one bunch of foliage type each night to feed on, over four consecutive nights. With the four foliage types and eight possums per population, the feeding trial was run using two 4x4 Latin square designs across each possum population. The feeding trial was run in November.

Table 1. Treatment diets offered to brushtail possums as a no-choice feeding trial. The source and locality of each *Eucalyptus globulus* foliage type is given. Each possum population's experience with the foliage type is termed as familiar or unfamiliar, and the predicted defensive chemistry levels of the seedlings are termed as higher or lower.

Foliage		Treatment	Possum population	
Source	Locality	(foliage type)	Bluegum Hill experience/defensive chemistry	St Helens experience/defensive chemistry
Victoria	Jeeralang North (JN)	A	Unfamiliar / higher	Unfamiliar / higher
Victoria	Parker Spur (PS)	B	Unfamiliar / lower	Unfamiliar / lower
South east Tasmania	Bluegum Hill (BGH)	C	Familiar / higher	Unfamiliar / higher
North east Tasmania	St Helens (SH)	D	Unfamiliar / lower	Familiar / lower

Each foliage bunch consisted of 20 seedlings (Two seedlings per family from each source population) which were cut at the stem base and tied together with a rubber band. Each bunch was weighed in the evening prior to being offered to possums and again the following morning as grams fresh matter (g FM). Possum intake of each foliage type was calculated by subtracting final bunch mass from initial bunch mass. The assigned foliage bunch was placed in a cylinder with fresh water in each individual pen and offered to possums from 8:00pm until 6:00am the following morning. Foliage bunches were prepared fresh each evening of the trial as required. While each foliage bunch allowed for *ad libitum* feeding, possums were also offered 80 per cent maintenance diet nightly to ensure that hunger was not the predominant driving factor of intake, but rather true preferences reflecting their intake behaviour.

Two control bunches per foliage type were set up per night (i.e. n = 8), with the exception of nights three and four for Jeeralang North and Parker Spur (where total n = 6) due to logistical constraints. Control bunches were placed in individual cylinders with fresh water and kept in a spare pen overnight to account for natural changes in bunch mass overnight. Control bunches were weighed in the evening and again the following morning (g FM) and any changes in bunch mass were incorporated into calculations of possum intake. Sub-samples of each control bunch were taken and oven dried to calculate grams dry matter (g DM) which was used to calculate total intake (g DM). The remaining seedlings were fresh frozen for later chemical analyses.

At the completion of the feeding trial, possums were fed 120 per cent maintenance diet and fresh eucalypt foliage prior to being transported back to their site of capture and released.

Chemical analyses of seedlings

Two control bunches per foliage type per night were sub-sampled for chemical analyses. Samples were fresh frozen immediately after being taken each morning of the trial and were later freeze-dried for four days to prepare them for chemical analyses. A sub-sample of 20 whole freeze-dried leaves per control bunch was scanned for levels of defensive chemistry using near infrared reflectance spectroscopy (NIRS) (Foely et al. 1998, O'Reilly-Wapstra 2004). Individual leaves were scanned to create individual spectra for each of the 20 leaves per control bunch, which were then pooled and used to predict the levels of defensive chemistry for each foliage locality. The two control bunches taken per locality per day were averaged to predict levels of defensive chemistry per foliage locality per trial day. Foliar chemistry predictions of terpenes (cineole, total oils), formylated phloroglucinol compounds (FPCs) (sideroxylonal A, macrocarpal G) and nitrogen were made using NIRS.

Statistical analyses

The dependent variable of intake was tested against the independent variables of possum population, foliage type, foliage type * possum population, possum (population), night and night * foliage type using the Mixed Models fitted using the restricted maximum likelihood (REML) approach (PROC MIXED; SAS Version 9.1, SAS Institute 2002). The variables of possum (population), night and foliage type * night were random effects in the model, with all remaining variables being fixed effects. Type III sum of squares were used to test fixed effects and the Z test was used to test the significances of random terms. The error term for testing the possum population effect was the random possum (population) term, the fixed foliage type was tested with the random foliage type * night term and the fixed foliage type * possum population interaction was tested against the residual. The Wilk-Shapiro statistic, normal probability plots and plots of standardised residuals all indicated normality of the data. When a multiple-level fixed effect was significant, pairwise *a posteriori* comparisons of least-squares means were made using the Tukey-Kramer adjustment. Additionally, our *a priori* expectations of differences in chemistry levels between the Tasmanian treatment populations gave us reason to directly compare the possum population intake between the foliage types from within Tasmania and the mainland and between areas (contrast 'BGH vs. SH', 'JN vs. PS', 'mainland vs. Tasmania', PROC MIXED, SAS Version 9.1, SAS Institute 2002). Differences in body weight between possum populations were tested using a paired *t*-test (TTEST, SAS Version 9.1, SAS Institute 2002). Results for each chemical constituent were analysed for each chemical variable (PROC MIXED) with foliage type as the fixed effect in the model. Residuals were checked for normality and homoscedasticity. Data for sideroxylonal A was exponentially transformed. If an effect was significant, pairwise *a posteriori* comparisons of least-squares means were made using the Tukey-Kramer adjustment, and we performed *a priori* contrasts (contrast 'BGH vs. SH', 'JN vs. PS', 'mainland vs. Tasmania', PROC MIXED).

Results

Intake: foliage type

There was a significant foliage type effect (Table 2) where possums consumed significantly more of the St Helens foliage type than any other foliage type (Figure 1). There was no significant difference in intake between the Jeeralang North, Parker Spur or Bluegum Hill treatment diets (Figure 1).

Table 2. Results of the mixed model analysis of brushtail possum intake of *Eucalyptus globulus* foliage types. The table indicates the *Z* and *F* values for the random and fixed effects, respectively, and degrees of freedom (*df*).

Effect	*df*	*Z* or *F*[a]	*P*
Possum population	1	11.2[a]	0.007
Foliage type	3	11.6[a]	0.002
Foliage type * possum population	3	0.8[a]	0.520
Possum(Population)	11	1.1	0.133
Night	3	1.1	0.146
Night * foliage type	9	n.e.	n.s.

[a] *F* value. *Note:* Where values are represented as n.e., values are at the boundary of the parameter space.

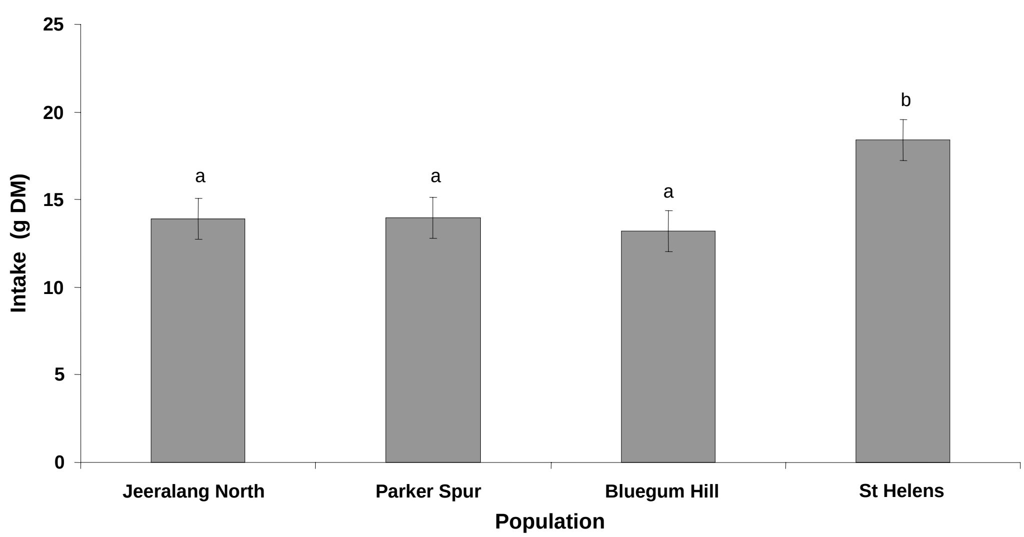


Figure 1. Average nightly intake (leaf dry matter (g DM)) of *Eucalyptus globulus* foliage by brushtail possums across foliage types from which seed has been sourced from four geographically different populations. Values are least-squares means ± standard errors. Bars with different letters are significantly different (α = 0.05 after Tukey-Kramer adjustment for multiple comparisons).

Intake: possum population

Possums sourced from the Bluegum Hill population ate significantly more than possums sourced from the St Helens population for the duration of the feeding trial (16.4 ± 1.1 g DM and 13.3 ± 1.1 g DM, respectively; $F_{1,11} = 11.2$, $P = 0.001$; Figure 2). This was most likely attributed to the significant difference in body weight between the two possum populations (SH = 3.3 ± 0.2 kg; BGH = 3.6 ± 0.1 kg; $t_{14} = -217.0$, $p = 0.001$). There was no significant foliage type*possum population interaction (Table 2). Both possum populations showed similar intake preferences across all foliage types (JN=PS=BGH<SH) (Figure 2).

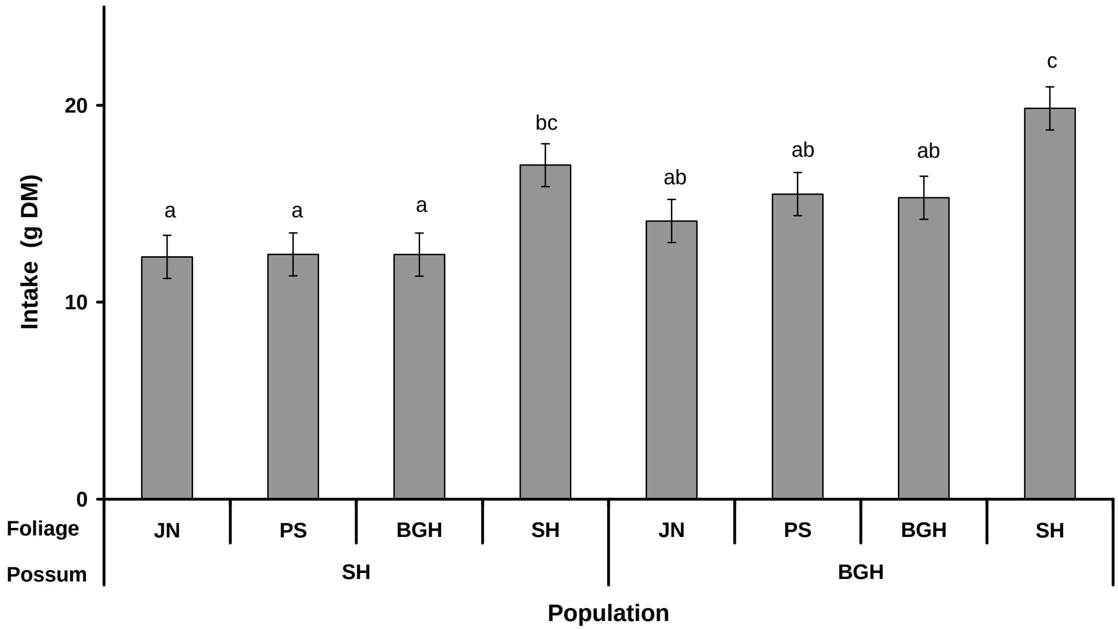
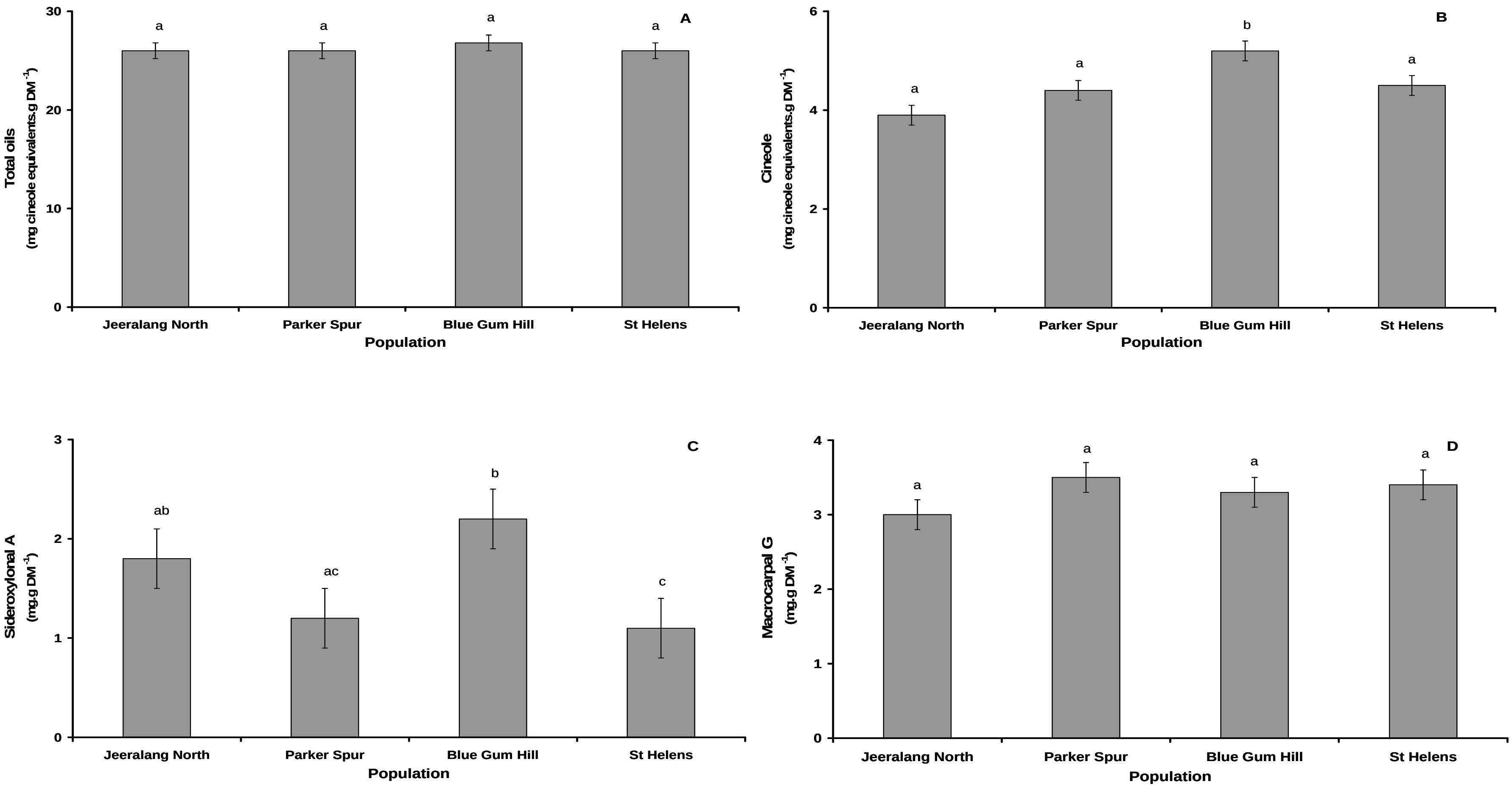


Figure 2. Total intake (leaf dry matter (g DM)) of *Eucalyptus globulus* foliage by brushtail possums across four foliage types and two possum populations; where JN = Jeeralang North, PS = Parker Spur, BGH = Bluegum Hill, SH = St Helens. Values are least-squares means ± standard errors. Bars with different letters are significantly different (α = 0.05 after Tukey-Kramer adjustment for multiple comparisons).

Chemistry

Across the foliage types, there were significant differences between foliar defensive chemistry levels of cineole and sideroxylonal A, and no significant differences between total oils, macrocarpal G and nitrogen (Figure 3, Appendix 1). The St Helens foliage type (north east Tasmania) had lower levels of cineole and sideroxylonal A than the Bluegum Hill foliage type (south east Tasmania); the mainland foliage types had lower levels of cineole than the Tasmanian foliage types; and there were no significant differences between the two mainland foliage types (contrasts (Appendix 1)).


A
Total oils
(mg cineole equivalents.g DM-1)
30
20
10
0
a
a
a
a
Jeeralang North
Parker Spur
Blue Gum Hill
St Helens
Population
B
Cineole
(mg cineole equivalents.g DM-1)
6
4
2
0
a
a
b
a
Jeeralang North
Parker Spur
Blue Gum Hill
St Helens
Population
C
Sideroxylonal A
(mg.g DM-1)
3
2
1
0
ab
ac
b
c
Jeeralang North
Parker Spur
Blue Gum Hill
St Helens
Population
D
Macrocarpal G
(mg.g DM-1)
4
3
2
1
0
a
a
a
a
Jeeralang North
Parker Spur
Blue Gum Hill
St Helens
Population

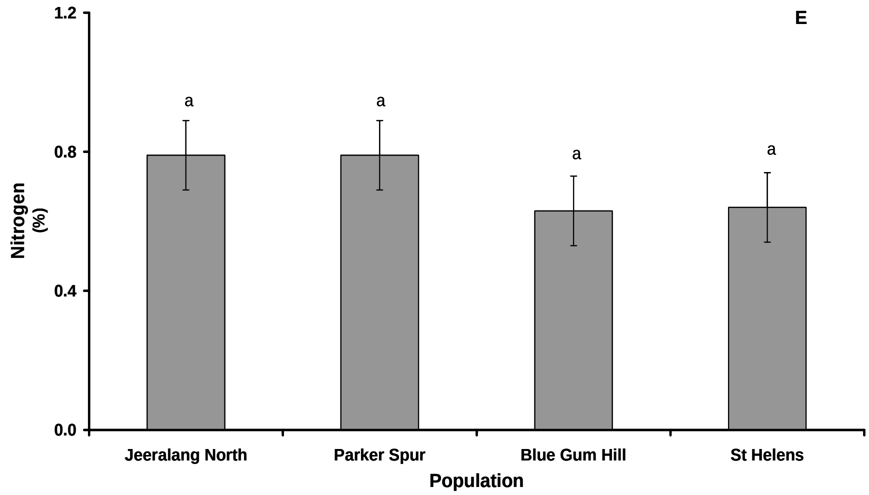


Figure 3. Chemistry of *Eucalyptus globulus* foliage across foliage types from which seed has been sourced from four geographically different populations. Values are least-squares means ± standard errors. $N = 8$ foliage samples for Bluegum Hill and St Helens treatments; $N = 6$ foliage samples for Jeeralang North and Parker Spur treatments. Each foliage sample consisted of 20 individual leaves. Bars with different letters are significantly different ($\alpha = 0.05$ after Tukey-Kramer adjustment for multiple comparisons).

Discussion

Intake preference

The common brushtail possum (*Trichosurus vulpecula*) ate significantly more bluegum (*Eucalyptus globulus*) foliage from seed sourced from the St Helens locality (north east Tasmania) than any other treatment diet. This trend was stable regardless of the possum source population, although there is the possibility that the absolute amounts of foliage consumed differ with possum source population.

Possum intake preferences for the St Helens, Bluegum Hill and Jeeralang North foliage types are consistent with those of previous studies (O'Reilly-Wapstra et al. 2002, 2004, 2005a, 2005b). These intake preferences are most likely attributed to levels of sideroxylonal A, a formylated phloroglucinol compound (FPC) which has been previously shown to be a key defensive compound inferring resistance in *E. globulus* against mammalian herbivores (Lawler et al. 2000, O'Reilly-Wapstra 2004). The higher levels of sideroxylonal A in Bluegum Hill and Jeeralang North seedlings indicate greater levels of resistance of these foliage types to possum browsing than St Helens seedlings, which contained the lowest levels of sideroxylonal A and were clearly selected for by possums. Possum intake preferences for the Parker Spur foliage type are not consistent with our *a priori* expectations or the defensive chemistry levels we measured in these seedlings, which appear to be relatively similar to those found in St Helens seedlings. Our initial *a priori* expectations were based on preliminary results of O'Reilly-Wapstra et al. (unpublished data) which indicate that Parker Spur coppice foliage is more susceptible, and Jeeralang North more resistant, to mammal browsers in a field trial in north west Tasmania. The differences in foliage types (e.g. coppice vs. seedlings) and potential browsing populations (e.g. red-bellied pademelons (*Thylogale billardierii*) and red-necked wallabies (*Macropus rufogriseus*) vs. brushtail possums) may partly explain the deviation in possum intake preferences of Parker Spur seedlings from our *a priori* expectations. However, the intake difference between Parker Spur and St Helens seedlings are more likely to be attributed to differences in defensive chemistry explained by (*a*) slightly, although not significantly, higher levels of the FPCs, sideroxylonal A and macrocarpal G, in Parker Spur foliage than St Helens foliage; and (*b*) differences in other defensive chemicals that we did not measure in this study. For example, it is possible that the levels of defensive phenolics, the condensed tannins, may differ between treatment diets. These compounds may act as toxins, and in some cases digestibility reducers, in mammalian herbivores (Hagerman et al. 1992, Foley et al. 1999). Previous research has shown that *E. globulus* seedling and juvenile foliage from St Helens contains lower levels of condensed tannins than foliage sourced from Bluegum Hill (O'Reilly-Wapstra et al. 2005a, 2005b). Additionally, leaf toughness and fibre levels may offer further insight into subtle differences between the palatability of treatment diets. Predictions of leaf chemical levels of phenolic, tannin and fibre constituents remain in the early stages of development for near infrared reflectance spectroscopy (NIRS) and hence were not assayed in this study. With further verifications and validations we will soon be able to use NIRS as a predictive tool for phenolics, tannins and fibre, in addition to the predictions we can currently make for FPCs, terpenes, and nitrogen.

Possum population preferences

Possums sourced from the Bluegum Hill population (south east Tasmania) ate significantly more foliage than possums sourced from the St Helens population (north east Tasmania) for the duration of the no-choice feeding trial. Possums sourced from the Bluegum Hill population have coexisted with *E. globulus* foliage which contains higher levels of defensive chemistry than possums sourced from the St Helens population with lower levels of defensive chemistry (O'Reilly-Wapstra et al. 2002, 2004, 2005a). However, the higher total intake levels shown by Bluegum Hill possums are most likely attributed to the significant difference in body weight between the two possum populations - possums sourced from Bluegum Hill were on average 0.3 kilograms heavier than possums sourced from St Helens. While it appears, quite logically, that body weight is correlated with intake (e.g. Clauss et al. 2007), we do not know the driving factor behind the differences in body weights between the possum populations. This may simply be a factor of the random sampling method used when trapping possums. If, however, the sampled possums are a true representative subset of their respective populations, then these differences may be based on a genetic and/or environmental basis driving this geographical difference in body weight. Morphological size differences between 'grey' possum populations sourced from south east Australia and 'black' possum populations sourced from Tasmania have previously been demonstrated, where the black possum populations are larger in body size, possibly associated with genetic differences between the possum populations (Triggs and Green 1989). Previous research has found that black populations of Tasmanian possums are more common in areas of high rainfall than the grey populations of Tasmanian possums (Guiler 1953), with a suggested physiological basis for different climatic tolerances in brushtail possums as a result of differences in water metabolism between the population types (Williams and Turnbull 1983).

Genetic-based resistance of seedlings across possum populations

Firstly, our results demonstrate that there are genetic based differences in the susceptibility of seedlings of *E. globulus* from different populations to possum browsing. This result it consistent with previous trends in the susceptibility of seedling foliage (O'Reilly-Wapstra et al. 2005b), juvenile foliage (O'Reilly-Wapstra et al. 2005a) and coppice foliage (O'Reilly-Wapstra et al. 2002, 2004) for Tasmanian *E. globulus*. More importantly, there was no significant foliage type by possum population interaction, indicating that the levels of foliage resistance are stable across the two different possum populations. Both possum populations showed similar intake preferences across all foliage types (JN=PS=BGH<SH), indicating that the rank order of variation in susceptibility is stable across different possum populations. While we have demonstrated similar within-species preferences for brushtail possums, previous research suggests that there is also stability in foliage susceptibility across pademelon populations sourced from south eastern Tasmania (O'Reilly-Wapstra 2002).

Implications for tree growers

Our results demonstrate that there are genetic based differences in the susceptibility of seedlings of *E. globulus* from different populations to possum browsing. This result is consistent with previous trends in the susceptibility of seedling, juvenile and coppice foliage for Tasmanian *E. globulus* populations (O'Reilly-Wapstra et al. 2002, 2004, 2005a, 2005b). This suggests the use of more resistant stock as a possible management technique to decrease browsing damage. With this strategy in mind, this current study has demonstrated that different possum populations show similar intake preferences across *E. globulus* foliage types (JN=PS=BGH<SH). The genetic variation in susceptibility of *E. globulus* seedlings did not change when exposed to different populations of brushtail possums. Our results suggest that while absolute browsing levels may change, the susceptibility ranking of germplasm should remain stable across different browsing populations.

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References

Bulinski J, McArthur C (1999) An experimental field study of the effects of mammalian herbivore damage on *Eucalyptus nitens* seedlings. *Forest Ecology and Management* **113**, 241-249.

Clauss M, Schwarm A, Ortmann S, Streich WJ, Hummel J (2007) A case of non-scaling in mammalian physiology? Body size, digestive capacity, food intake, and ingesta passage in mammalian herbivores. *Comparative Biochemistry and Physiology A* **148**, 249-265.

Cremer KW (1969) Browsing of mountain ash regeneration by wallabies and possums in Tasmania. *Australian Forestry* **33**, 201-210.

Foley WJ, Iason GR, McArthur C (1999) Role of plant secondary metabolites in the nutritional ecology of mammalian herbivores – how far have we come in 25 years? In 'Nutritional Ecology of Herbivores. Proceedings of the Vth International Symposium on the Nutrition of Herbivores.' (Eds HG Jung, GC Fahey) pp. 130-209. (American Society of Animal Science: Savoy, Illinois, USA).

Foley WJ, McIlwee A, Lawler I, Aragones L, Woolnough AP, Berding N (1998) Ecological applications of near infrared reflectance spectroscopy - a tool for rapid, cost-effective prediction of the composition of plant and animal tissues and aspects of animal performance. *Oecologia* **116**, 293-305.

Gilbert JM (1961) The effects of browsing by native animals on the establishment of seedlings of *Eucalyptus regnans* in the Florentine valley, Tasmania. *Australian Forestry* **25**, 116-121.

Guiler ER (1953) Distribution of the brush possum in Tasmania. *Nature* **172**, 1091-1093.

Hagerman AE, Robbins CT, Weerasuriya Y, Wilson TC, McArthur C (1992) Tannin chemistry in relation to digestion. *Journal of Range Management* **45**, 57-62.

Lawler IR, Stapley J, Foely WJ, Eschler BM (1999) Ecological example of conditioned flavor aversion in plant-herbivore interactions: effect of terpenes of *Eucalyptus* leaves on feeding by common ringtail and brushtail possums. *Journal of Chemical Ecology* **25**, 401-415.

Lawler IR, Foley WJ, Eschler BM (2000) Foliar concentration of a single toxin creates habitat patchiness for a marsupial folivore. *Ecology* **81**, 1327-1338.

McArthur C, Goodwin A, Turner S (2000) Preferences, selection and damage to seedlings under changing availability by two marsupial herbivores. *Forest Ecology and Management* **112**, 1-8.

McArthur C, Appleton R (2003) Effect of seed and nursery conditions on survival, growth and browsing of eucalypt seedlings by swamp wallabies in plantations. Technical Report 122 (Cooperative Research Centre for Sustainable Production Forestry: Hobart, Tasmania).

O'Reilly-Wapstra JM (2004) The phenotypic and genetic basis of browsing resistance of *Eucalyptus globulus* to marsupial herbivores. PhD, School of Zoology and Cooperative Research Centre for Sustainable Production Forestry, University of Tasmania, Hobart, Tasmania.

O'Reilly-Wapstra JM, McArthur C, Potts BM (2002) Genetic variation in resistance of *Eucalyptus globulus* to marsupial browsers. *Oecologia* **130**, 289-296.

O'Reilly-Wapstra JM, McArthur C, Potts BM (2004) Linking plant genotype, plant defensive chemistry and mammal browsing in a *Eucalyptus* species. *Functional Ecology* **18**, 677-684.

O'Reilly-Wapstra JM, Potts BM, McArthur C, Davies NW, Tilyard P (2005a) Inheritance of resistance to mammalian herbivores and of plant defensive chemistry in an *Eucalypt* species. *Journal of Chemical Ecology* **31**, 357-375.

O'Reilly-Wapstra JM, Potts BM, McArthur C, Davies NW (2005b) Effects of nutrient variability on the genetic-based resistance of *Eucalyptus globulus* to a mammalian herbivore and on plant defensive chemistry. *Oecologia* **142**, 597-605.

SAS Institute (2002) SAS, Version 9.1. (SAS Institute: Cary, North Carolina, USA).

Triggs SJ, Green WQ (1989) Geographic patterns of genetic-variation in brushtail possums *Trichosurus-vulpecula* and implications for pest-control. *New Zealand Journal of Zoology* **12**, 1-16.

Wiggins NL, McArthur C, McLean S, Boyle R (2003) Effects of two plant secondary metabolites, cineole and gallic acid, on nightly feeding patterns of the common brushtail possum. *Journal of Chemical Ecology* **29**, 1447-1464.

Williams CK, Turnbull HL (1983) Variation in seasonal nutrition, thermoregulation and water balance in two New Zealand populations of the common brusthail possum, *Trichosurus vulpecula* (Phalangeridae). *Australian Journal of Zoology* **31**, 333-343.

Appendix

Appendix 1. Near infrared reflectance spectroscopy (NIRS) predicted chemistry of *Eucalyptus globulus* seedling foliage across foliage types from which seed has been sourced from four geographically different populations.

Constituent	Units	*Eucalyptus globulus* foliage population				$F_{3,27}$	P	contrast					
		Jeeralang North	Parker Spur	Bluegum Hill	St Helens			BGH vs. SH		JN vs. PS		Mainland vs. Tasmania	
								$F_{1,9}$	P	$F_{1,9}$	P	$F_{1,9}$	P
Total oils[1]	mg.g DM^{-1}	26.0 ± 0.8	26.0 ± 0.8	26.8 ± 0.8	26.0 ± 0.8	0.2	0.875	0.4	0.534	0.0	0.985	0.3	0.618
Cineole[1]	mg.g DM^{-1}	3.9 ± 0.2	4.4 ± 0.2	5.2 ± 0.2	4.5 ± 0.2	5.6	0.019	4.8	0.051	1.9	0.205	10.3	0.011
Sideroxylonal A	mg.g DM^{-1}	1.8 ± 0.3	1.2 ± 0.3	2.2 ± 0.3	1.1 ± 0.3	4.4	0.036	9.9	0.012	2.6	0.141	0.7	0.425
Macrocarpal G	mg.g DM^{-1}	3.0 ± 0.2	3.5 ± 0.2	3.3 ± 0.2	3.4 ± 0.2	1.2	0.365	0.1	0.757	3.0	0.115	0.5	0.520
Nitrogen	% DM	0.8 ± 0.1	0.8 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	1.5	0.276	0.3	0.623	0.0	0.854	4.3	0.069

[1] Cineole equivalents. *Note:* Constituents of nitrogen are measured in units of percentage dry matter (DM). Values are least-squares means ± standard errors. $N = 8$ foliage samples for Bluegum Hill and St Helens treatments; $N = 6$ foliage samples for Jeeralang North and Parker Spur treatments. Each foliage sample consisted of 20 individual leaves.