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Linking plant genotype, plant defensive chemistry and mammal browsing in a *Eucalyptus* species

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Summary

1. In the field of plant–herbivore interactions a major focus of research is the importance of herbivores as selective agents on the evolution of plant resistance. Evidence to support the role of herbivores as selective agents must demonstrate that the intraspecific variation in plant resistance, and the variation in the plant resistive trait, are under genetic control. Predominantly, research in this field has concentrated on plant–insect systems, with much less emphasis on plant–mammal interactions.

2. In a common *Eucalyptus* species, *Eucalyptus globulus* (Labill.), variation in resistance to a mammalian herbivore, *Trichosurus vulpecula* (Kerr, 1792), is under genetic control.

3. In this paper, plants of known genetic stock grown in a common-environment field trial were used to investigate the plant characteristic responsible for resistance of *E. globulus* to *T. vulpecula* and to determine if there was a genetic basis to variation in the defensive trait.

4. The results demonstrate that a formylated phloroglucinol compound, sideroxylonal, is the dominant plant secondary metabolite that determined intake of *E. globulus* juvenile coppiced foliage by *T. vulpecula*, and that this metabolite is under significant genetic control.

5. These results are discussed in the context of the possible role *T. vulpecula* may play as a selective agent on the evolution of resistance in *E. globulus*.

Key-words: eucalypt, genetic variation, mammalian herbivory, plant defence, plant secondary metabolites

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Introduction

Plants within a species commonly vary in resistance to herbivores (Berenbaum & Zangerl 1992), and the role of plant secondary metabolites as plant defensive strategies, have been well documented (Fraenkel 1959; Freeland & Janzen 1974; Harborne 1991; Foley, Iason & McArthur 1999). Intraspecific phenotypic variation in plant resistance and in the plant traits that confer resistance can be under genetic and/or environmental control (Bryant *et al*. 1991; Berenbaum & Zangerl 1992; Kennedy & Barbour 1992; Kopper & Lindroth 2003). In the past two decades a major focus of research in the field of plant–herbivore interactions has been the importance of herbivores as selective agents on the evolution of plant resistance. If a plant defensive trait is correlated with herbivore damage and plant fitness, and is under some degree of genetic control, then herbivores may act as a selective force on the plant trait (Mauricio & Rausher 1997). Plant–insect systems have provided evidence for the selective role of herbivores on plant resistance (Berenbaum, Zangerl & Nitao 1986; Simms & Rausher 1989; Núñez-Farfán & Dirzo 1994; Pilson 1996; Mauricio & Rausher 1997), but evidence is quite sparse in plant–mammal systems (but see Dimock, Silen & Allen 1976; Mutikainen *et al*. 2000; Pusenius *et al*. 2002; Vourc'h *et al*. 2002).

The tree genus *Eucalyptus* is species-rich (more than 700 species) in Australia (Brooker 2000), and several arboreal marsupials feed on the foliage of eucalypt trees, including the generalist folivore, the Common Brushtail Possum (*Trichosurus vulpecula*) (Landsberg & Cork 1997). Eucalypt foliage contains high quantities of plant secondary metabolites (Boland & Brophy 1993) and is fibrous and highly lignified (Foley & Hume 1987). The chemical basis to herbivore resistance in the genus *Eucalyptus* has been under much investigation in recent years (McArthur & Sanson 1991; Lawler *et al*. 1999a; Wallis, Watson & Foley 2002), but very little is known about the genetic control of plant defensive traits and the relationship between plant genotype, secondary plant chemistry and mammalian herbivore browsing in *Eucalyptus*. In a previous paper

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(O'Reilly-Wapstra, McArthur & Potts 2002) we investigated the intraspecific variation in resistance of different genotypes of a common eucalypt species, *Eucalyptus globulus*, to browsing by *T. vulpecula*. *Eucalyptus globulus* is an important pulpwood species in temperate regions worldwide (Dutkowski & Potts 1999) and grows naturally in south-eastern Australian native forests ranging from a medium-sized tree in woodlands (15–20 m high) to a tall tree in open forests (up to 60 m high) (Cameron 1994). It is a dominant tree species in Tasmania and is browsed by *T. vulpecula*. In our previous paper we detected clear genetic variation between different populations of *E. globulus* in resistance to browsing by *T. vulpecula*, however, the underlying mechanism explaining the genetic variation in browsing resistance was not known (O'Reilly-Wapstra *et al*. 2002).

The main aim of the present study was to identify the foliage characteristics that confer resistance of *E. globulus* to *T. vulpecula*, and to identify the genetic control of these characters. We assessed plant secondary chemistry of 6-month-old juvenile coppiced foliage growing from 54 individual *E. globulus* mature tree stumps, derived from felling 11-year-old trees, and used the ranking for resistance to *T. vulpecula* as determined in a feeding trial with captive animals by O'Reilly-Wapstra *et al*. (2002). The coppiced foliage used in this research was grown in a common-environment field trial, and this trial provided an excellent system to investigate plant resistance and plant defensive traits as all trees were of known pedigree. Three specific questions were addressed.

1. Is there intraspecific variation in leaf chemical characteristics of *E. globulus* trees?
2. Is there a relationship between intake of *E. globulus* trees by *T. vulpecula* and variation in leaf chemistry?
3. Is there a genetic basis to the variation in leaf chemistry?

Materials and methods

PLANT MATERIAL AND DETERMINATION OF INTAKE

The common-environment field trial in north-west Tasmania, Australia (41°05′ S, 145°54′ E) from which foliage was sourced was established from progenies grown from open-pollinated seed collected from nearly 600 families from 49 populations (localities), derived from 13 races throughout the natural range of *E. globulus* in Australia (Dutkowski & Potts 1999). The 54 plants chosen for the feeding trial (O'Reilly-Wapstra *et al*. 2002), and the foliage analysis in this present paper, were from 33 of the 600 families and from seven of the 49 localities (Table 1). It was ensured that the plants chosen encompassed a broad representation of the genetic diversity in *E. globulus*. The localities represented the extremes of browsing resistance in the continual range of *E. globulus* from northern Tasmania (localities from the north-east Tasmanian race) to southern Tasmania (localities from the southern Tasmanian race) (Table 1), covering a distance of ≈250 km. The Jeeralang locality was chosen to represent a mainland Australian locality that is quite differentiated in morphological traits from the Tasmanian populations (Dutkowski & Potts 1999) and is an important population for commercial breeding purposes. The 54 plants were also selected on the basis of containing enough foliage on the plants to offer to mammals in a captive feeding trial (O'Reilly-Wapstra *et al*. 2002).

The common-environment field trial was a resolvable incomplete block design of five replicates, each of 25 incomplete blocks of 24 families. Trees from each family within each block were planted as a two-tree plot in a row and all trees were ≈2·5 m apart in the row and 4 m apart between rows. Any individual trees chosen for the feeding trial and chemical analysis that were represented by the same family were obtained across different replicates, not from the same two-tree plot within a block. At the field site the mean annual rainfall is 999 mm and the mean daily maximum and minimum temperatures are 17·1 and 9·0 °C, respectively (Potts & Jordan 1994), and these environmental conditions fall within the natural range for *E. globulus* throughout south-east Australia.

Branches of the 54 coppiced plants used for foliage analysis in the present study were cut from the common-environment field trial and were previously screened for resistance to *T. vulpecula* in a feeding trial using six captive animals, as detailed by O'Reilly-Wapstra *et al*. (2002). In brief, the resistance of the 54 individual plants was ranked in a no-choice, incomplete-block, cross-over trial design that ran for 9 weeks (foliage was cut from each tree in the field trial at the beginning of each week and stored in a cool room in water). The feeding trial was broken up into four equal periods to balance for any effects of change in leaf characteristics and animal response over time. All 54 individual plants were offered in each period. Plants were allocated to animals randomly in each period, except that no animal received the same plant twice during the trial, and no plant was offered to more than one individual within each animal species on a given night. Each animal received 36 of the 54 total plants during the trial. By the end of the trial each plant had been fed to four of six animals within each species. Intakes of each plant

Table 1. Identity and number of individual plants, families, localities and races of *Eucalyptus globulus* used in the chemical analysis

Individuals (*n*)	Families (*n*)	Locality	Race
6	2	Germantown	North-east Tasmania
7	4	Royal George	
8	6	St Helens	
4	3	Blue Gum Hill	Southern Tasmania
9	4	South Bruny	
6	4	South Geeveston	
14	10	Jeeralang North	Strzelecki Ranges

were expressed in terms of grams dry matter per kilogram of body mass of the consumer (g DM kg^{-1} BM). Control plants from each experimental night were either dried to determine percentage dry matter or frozen for later chemical analysis of the foliage.

FOLIAGE ANALYSIS

Four control bunches of foliage (one from each period of the feeding trial) for each of the 54 individual plants offered to *T. vulpecula* in the feeding trial were combined and duplicate random selections of frozen foliage were subsampled for each analysis for each individual plant. Plants were assayed for nitrogen, three plant cell-wall components (neutral detergent fibre, NDF; acid detergent fibre, ADF; and lignin), total tannins, total essential oils and 1,8-cineole. Formylated phloroglucinol compounds (FPCs) in eucalypt foliage have been shown to be important characteristics in influencing intake of foliage by marsupial herbivores (Lawler, Foley & Eschler 2000) and therefore plants were also assayed for six FPCs: sideroxylonal A, sideroxylonal C, macrocarpal A, macrocarpal B, macrocarpal G and grandinol.

PRIMARY CHEMISTRY

For nitrogen and plant cell-wall component analysis, samples of frozen foliage were oven-dried at 60 °C and ground through a 1 mm mesh sieve using a cyclotech mill. Nitrogen was determined using the semimicro-Kjeldahl technique following Lawler *et al*. (1998a), and results are expressed as percentage of total dry matter (% DM). Plant cell-wall components were analysed by sequentially fractioning dried ground foliage. Levels of NDF, ADF and lignin were determined following the ANKOM Technology procedures (ANKOM$^{200/220}$ Technology Operator's Manual 1997). Results are expressed as % DM.

SECONDARY CHEMISTRY

Essential oils (terpenes) were extracted using dichloromethane with heptadecane as an internal standard (100 mg heptadecane was diluted in 1 l dichloromethane; Jones 1999). One gram of thawed foliage, cut into ≈1 cm pieces, was soaked in the dichloromethane solvent for 1 h. Extracts were analysed by combined gas chromatography–mass spectrometry (GC–MS) on a Hewlett-Packard 5890 Ga chromatograph coupled to a Hewlett-Packard 5970B mass selective detector, using a 25 m × 0·32 mm HP-1 column with a 0·17 μm film. Total ion currents were determined separately for 1,8-cineole, the sum of all oil components (referred to as total oils), and for the heptadecane internal standard. Results for 1,8-cineole and total oils were then standardized by dividing by the internal standard. The amount of 1,8-cineole was expressed as mg g^{-1} DM. The amount of total oils was expressed as equivalents of cineole (mg g^{-1} DM).

The phenolic fraction, referred to as 'total tannins', was extracted using acidified (pH 1) methanol (Close, Davies & Beadle 2001). One gram of thawed foliage, cut into fine pieces by hand, was homogenized using a Pro-200 tissue homogenizer (Pro Scientific Inc., Monroe, CT, USA) in 20 ml acidified methanol and boiled in a water bath for ≈1·5 min. These samples were left to extract in the dark at 5 °C overnight. Extracts were then centrifuged at 10 000 ***g*** for 7 min. Extracts were analysed by high-pressure liquid chromatography (HPLC) on a Walters Alliance 2690 with a Waters 996 photodiode array detector (Waters Corporation, Milford, MA, USA). The column was a Waters Novpak C18 column fitted with an Alltech Econosphere 5 μm C18 guard cartridge (Alltech, Melbourne, Australia). This total tannin fraction was the total peak area of the combined group of hydrolysable and condensed tannins detected clearly at 280 nm (Noel Davies, personal communication). Traditional colorimetric assays for total phenolics (Price & Butler 1977) or major groups of tannins such as gallotannins (Inoue & Hagerman 1988) do not differentiate individual compounds. Our assay for total tannins is based on a similar concept. Results for total tannins were standardized by a known response factor of a tetragalloglucose (TeGG) standard and the units were therefore TeGG equivalents (mg g^{-1} DM).

The FPCs sideroxylonal A and sideroxylonal C were analysed following the method detailed by Wallis *et al*. (2003) and results are expressed as mg g^{-1} DM. Macrocarpals (mg g^{-1} DM) and grandinol (mg g^{-1} DM) were extracted by the same process (Wallis *et al*. 2003) but were separated using the following gradient analysis. Chromatographic separations were carried out on an SGE (NSW, Australia) 250 × 4·0 mm GL Wakosil II 3C18RS 3 mm column connected to a Waters Alliance HPLC system consisting of a Waters 2690 separation module, an autosampler fitted with a 250 ml syringe and a 100 ml sample loop, and a Waters 996 diode array detector. Just prior to HPLC, a known mass of crude extract (≈15 mg) was weighed into a glass vial and dissolved by sonication in 5·0 ml 20% methanol in acetonitrile, containing an internal standard of 2-ethyl phenol (0·300 g l^{-1}). The optimal separation was obtained with a gradient elution using acetonitrile (A) and water (B), both containing 0·1% trifluoroacetic acid. The flow rate was 0·75 ml min^{-1} with a column temperature of 40 °C and a run time of 80 min. The gradient was 0–5 min 60% A, 40% B; increasing linearly to 90% A, 10% B at 60 min, where it remained until 70 min before declining to the starting conditions at 80 min. Typical injection volumes ranged from 10 to 25 ml. Grandinol eluted on this gradient at 16 min.

STATISTICS

Univariate regression (PROC REG, SAS 6·12, SAS Institute Inc. 1990) was used as the first step in identifying which foliage chemical characteristics related to

dry matter intake by *T. vulpecula*. A Bonferroni adjustment was used to take into account the number of individual tests (μ = 0·0038). For the second step, stepwise multivariate regression analysis was used to develop a more complex model to explain the relationship between intake and leaf characteristics (PROC REG, STEPWISE, SAS Institute Inc. 1990). The entry level for variables to be added to the model was 0·1 and the level at which they were removed was 0·05. All independent variables were tested for multicollinearity following Tabachnick & Fidell (1989), using Pearson's correlation coefficients (PROC CORR, SAS Institute Inc. 1990). When variables were highly correlated ($r > 0·7$) only one of the correlated variables was used in the stepwise analysis. Sideroxylonal A and C were highly correlated, as were the three macrocarpals with each other, and the three plant cell-wall components. The independent variables included in the stepwise analysis were therefore nitrogen, NDF, total oil, total tannin, sideroxylonal A, macrocarpal G and grandinol.

Genetic variation in the foliage chemistry of the plants was analysed at the locality and family within locality levels using the mixed model procedure of SAS (PROC MIXED, SAS Institute Inc. 1997). Locality was a fixed effect while family within locality was a random effect. Results for each variable were analysed separately, again with a Bonferroni adjustment to adjust for the number of tests. Residuals were checked for normality and homoscedasticity with the general linear model procedure of SAS (PROC GLM, SAS Institute Inc. 1989). Data for macrocarpal B were square-root transformed, while macrocarpal G data were log transformed. Multiple pairwise comparisons of significant effects (least-squares means) were made using the Tukey–Kramer adjustment. The univariate relationship (PROC REG, SAS) between intake of foliage by *T. vulpecula* and concentration of each chemical characteristic at the population (locality) level was determined. Bonferroni adjustment was implemented to adjust for the multiple number of tests.

Results

There was a wide range in intraspecific variation in concentration of leaf chemical characteristics of the juvenile coppiced foliage obtained from the individual *E. globulus* plants assessed in this study (Table 2). Table 3 illustrates the phenotypic correlations between each of the chemical variables measured in this study, and demonstrates that the individual chemical compounds within each the major groups (plant cell-wall components, sideroxylonals and macrocarpals) are highly correlated ($r > 0·7$), as discussed in Materials and methods. Interestingly, total oils and 1,8-cineole were positively correlated with the six FPC compounds. Nitrogen was positively correlated with the plant cell-wall components but negatively correlated with tannin levels.

Univariate regression analysis identified a significant relationship ($P < 0·0038$) between intake of individual *E. globulus* plants by *T. vulpecula* and six foliage characteristics: sideroxylonal A, sideroxylonal C, macrocarpal A, macrocarpal G, total oils and 1,8-cineole (Table 2). The relationship between intake and these six variables was negative. In the multivariate model, two variables together explained 49% of the variation

Table 2. Primary and secondary foliage chemistry of 54 individual *Eucalyptus globulus* trees [$n = 48$ for neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin]

					Genetic analysis locality effect		Univariate regression	
Variable	Units	Mean	± SE	Range	F_{df}	P	r^2	P
Nitrogen	% DM	2·3	0·03	1·7–2·8	$1·98_{6,26}$	0·105	0·01	0·390
NDF	% DM	35·3	0·86	18·3–49·4	$1·81_{6,25}$	0·137	0·002	0·761
ADF	% DM	18·3	0·37	12·8–25·3	$1·82_{6,25}$	0·135	0·03	0·275
Lignin	% DM	6·7	0·27	2·4–12·0	$2·00_{6,25}$	0·103	0·004	0·671
Total essential oil	Cineole equivalents mg g^{-1} DM	33·7	1·67	12·0–70·6	$2·48_{6,26}$	0·049	0·16	**0·003**
1,8-cineole	mg g^{-1} DM	17·8	0·91	5·8–36·4	$3·50_{6,26}$	0·011	0·18	**0·001**
Total tannin	TeGG equivalents mg g^{-1} DM	153	7·51	90·1–352	$1·20_{6,26}$	0·335	0·03	0·23
Sideroxylonal C	mg g^{-1} DM	1·5	0·08	0·3–2·9	$6·58_{6,26}$	**0·0003**	0·39	**0·0001**
Sideroxylonal A	mg g^{-1} DM	4·8	0·28	0·9–8·5	$7·90_{6,26}$	**0·0001**	0·38	**0·0001**
Macrocarpal A	mg g^{-1} DM	2·17	0·14	0·0–4·9	$2·86_{6,24}$	0·028	0·25	**0·0002**
Macrocarpal G	mg g^{-1} DM	5·58	0·33	0·0–13·0	$1·52_{6,26}$	0·212	0·17	**0·002**
Macrocarpal B	mg g^{-1} DM	1·49	0·10	0·0–3·9	$2·29_{6,24}$	0·066	0·16	0·004
Grandinol	mg g^{-1} DM	0·79	0·06	0·1–2·4	$3·90_{6,26}$	0·006	0·11	0·013
Foliage intake*	g DM kg^{-1} BM	14·9	0·48	6·4–24·8	$4·00_{6,26}$	0·006		

Means (± SE) and range for each variable are presented. Results of the mixed model analysis of genetic variation at locality level for each variable are presented (family within locality was used as the error term) and results of the univariate regression analysis of the relationship between each variable and intake are also presented. Bold type indicates significance ($P < 0·0038$) after the Bonferroni adjustment.

*Foliage intake of the 54 individual plants by *Trichosurus vulpecula* from O'Reilly-Wapstra *et al.* (2002).

Table 3. Testing for multicollinearity of all independent variables. Values indicate Pearson's correlation coefficients for each variable

	Nitrogen	NDF	ADF	Lignin	Total oil	1,8-Cineole	Tannin	Sider C	Sider A	Mac A	Mac G	Mac B
NDF	0·43 *											
ADF	0·36 *	0·73 **										
Lignin	0·45 **	0·74 **	0·91 **									
Total oil	−0·02 NS	0·13 NS	0·02 NS	0·08 NS								
1,8-Cineole	−0·02 NS	0·13 NS	0·04 NS	0·10 NS	0·97 **							
Tannin	−0·56 **	−0·34 *	−0·28 NS	−0·33 *	−0·19 NS	−0·18 NS						
Sider C	−0·04 NS	−0·08 NS	−0·29 *	−0·15 NS	0·44 **	0·51 **	−0·03 NS					
Sider A	−0·12 NS	−0·13 NS	−0·29 *	−0·16 NS	0·37 *	0·45 **	−0·01 NS	0·96 **				
Mac A	0·07 NS	−0·02 NS	−0·12 NS	−0·08 NS	0·62 **	0·60 **	−0·12 NS	0·45 **	0·30 *			
Mac G	0·15 NS	0·09 NS	−0·02 NS	0·04 NS	0·56 **	0·55 **	−0·15 NS	0·33 *	0·16 NS	0·95 **		
Mac B	0·19 NS	0·07 NS	−0·08 NS	0·00 NS	0·53 **	0·50 *	−0·12 NS	0·39 *	0·17 NS	0·86 **	0·84 **	
Grandinol	0·01 NS	0·01 NS	0·05 NS	−0·01 NS	0·44 **	0·42 **	−0·22 NS	0·23 NS	0·17 NS	0·43 *	0·39 *	0·29 *

*Significant at $P < 0·05$; **significant at $P < 0·01$.
NDF, neutral detergent fibre; ADF, acid detergent fibre; Sider C, sideroxylonal C; Sider A, sideroxylonal A; Mac A, macrocarpal A; Mac G, macrocarpal G; Mac B, macrocarpal B.

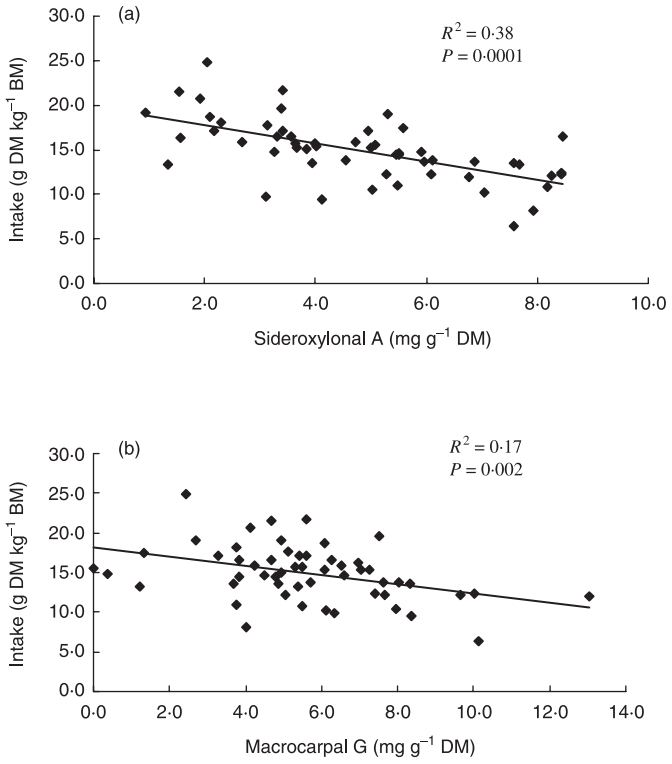

Fig. 1. Univariate relationship between foliage intake of the 54 individual *Eucalyptus globulus* trees (g DM kg^{-1} BM) by *Trichosurus vulpecula* and (a) sideroxylonal A (mg g^{-1} DM) and (b) macrocarpal G (mg g^{-1} DM). Sideroxylonal C showed a relationship to intake similar to that of sideroxylonal A; and macrocarpals A and B showed a relationship to intake similar to that of macrocarpal G, therefore these significant variables are not illustrated.

in intake. These variables were sideroxylonal A (partial $r^2 = 0·41$, $P = 0·0001$) and macrocarpal G (partial $r^2 = 0·08$, $P = 0·009$). Figure 1 illustrates the univariate relationship between intake and sideroxylonal A and macrocarpal G concentrations. No other variables were significant at the multivariate level. In both univariate and multivariate models the sideroxylonals had the strongest relationship with intake.

There were significant differences ($P < 0·0038$) between populations (localities) in sideroxylonal A and C concentrations (Table 2). The plants used in this trial were from open-pollinated families grown, randomized, in a common-environment field trial, therefore these results must be due to genetic differences between populations of *E. globulus* in their ability to produce sideroxylonals. No other characteristics showed significant genetic variation between localities ($P > 0·05$). No characteristic showed singificant variation between families within localities ($P > 0·05$), but this was no doubt due to the low number of samples at the family level. Figure 2 illustrates the genetic variation in sideroxylonal A between localities. The mean sideroxylonal A concentration of the seven localities ranged from 2·37 to 6·14 mg g^{-1} DM and sideroxylonal C concentration ranged from 0·79 to 1·92 mg g^{-1} DM. This intraspecific genetic variation in sideroxylonal concentration was strongly correlated with the intraspecific genetic variation in browsing resistance of *E. globulus* to *T. vulpecula*. The population with the lowest concentration of sideroxylonal (St Helens) was the

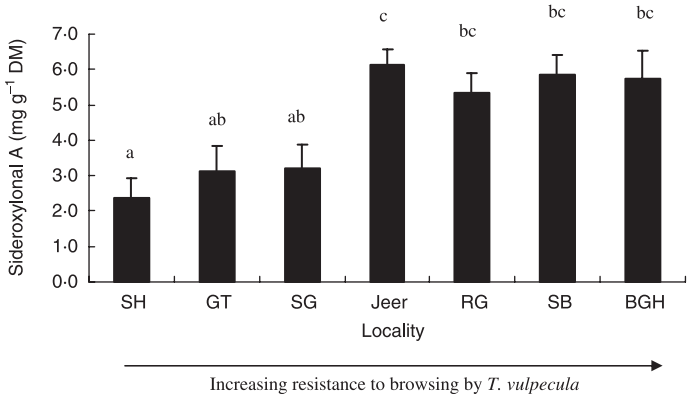

Fig. 2. Concentration of sideroxylonal A (mg g^{-1} DM) in foliage of *Eucalyptus globulus* trees by locality. Localities are presented in ascending order of resistance to *Trichosurus vulpecula*. All results are expressed as least-squares means with SE bars. Letters that differ indicate significant differences ($\alpha = 0{\cdot}05$ after Tukey–Kramer adjustment for multiple comparisons). SH, St Helens; GT, German Town; RG, Royal George; BGH, Blue Gum Hill; SG, South Geeveston; Jeer, Jeeralang North; SB, South Bruny. Sideroxylonal C concentration shows a pattern across localities similar to that of sideroxylonal A and therefore is not illustrated.

most susceptible to *T. vulpecula* (O'Reilly-Wapstra *et al*. 2002), while the more resistant populations had significantly higher sideroxylonal concentrations. There was a significant ($P < 0{\cdot}0038$) negative relationship between intake of foliage by *T. vulpecula* and sideroxylonal A and sideroxylonal C concentrations at the population (locality) level ($r^2 = 0{\cdot}84$, $P = 0{\cdot}0036$; $r^2 = 0{\cdot}90$, $P = 0{\cdot}001$, respectively).

Discussion

We detected marked phenotypic variation in foliage chemistry between individual plants. In both univariate and multivariate analyses the sideroxylonals accounted for the greatest amount of the observed variation in intake of foliage by *T. vulpecula*. Sideroxylonals are clearly the most important determinant of intake of this *E. globulus* coppice foliage by *T. vulpecula*, and variation in these chemicals explains the significant genetically based differences in browsing susceptibility between different populations within *E. globulus*. There was over a ninefold range in total sideroxylonal concentrations of *E. globulus* foliage between plants, from 1·2 to 11·4 mg g^{-1} DM (Table 3). Wallis *et al*. (2002) demonstrated that *T. vulpecula* regulated their intake of an artificial diet when sideroxylonal concentrations were ≥7 mg g^{-1} DM; similarly, when offered a choice of two diets they preferred to eat a control diet when concentrations were ≥4 mg g^{-1} DM. We found a decrease in intake of *E. globulus* foliage by *T. vulpecula* at similar and even lower levels of sideroxylonal, possibly due to the compounding negative effects of other plant secondary metabolites found in *E. globulus* foliage, such as the macrocarpals as discussed below. Sideroxylonal is an FPC, and it is believed that FPCs act as antifeedants to marsupial herbivores by releasing inflammatory mediators such as serotonin, which stimulate 5HT3 receptors resulting in nauseous sensations that lead to conditioned food aversions (Lawler *et al*. 1998b).

Another group of FPCs, the macrocarpals, also appear to have a secondary role in explaining variation in intake, although they explained a much lower proportion of the variation than sideroxylonal in the multivariate analyses (8 *vs* 41%, respectively). Similarly to sideroxylonals, the importance has been shown of macrocarpals affecting intake by marsupial herbivores in other eucalypt systems (Lawler *et al*. 1998a). Lawler *et al*. (1999a) examined different functional groups of FPCs to examine their effectiveness as antifeedants to the Common Ringtail Possum, *Pseudocherius peregrinus*. They determined that it was the presence of aldehyde groups and free phenol groups that determined the effectiveness of FPCs as feeding deterrents, and both sideroxylonals and macrocarpals possess these structural features.

The phenotypic expression of several groups of compounds were significantly correlated, including nitrogen with the three plant cell-wall components and tannins. Oils were significantly correlated with the FPCs. It has previously been suggested that 1,8-cineole may act as a 'cue' for browsing marsupials to indicate the levels of toxic FPCs in eucalypt foliage (Lawler *et al*. 1999b). In the *E. globulus* foliage used in this study there was a significant, but weak, negative relationship between intake of foliage and 1,8-cineole concentration at the univariate level. The correlations between 1,8-cineole and FPCs were significant and positive (Table 3). This provides some support for the suggestion that the volatile compound 1,8-cineole may function as a cue to the concentration of the more toxic FPC. In a previous study, Close *et al*. (2003) found no correlation between sideroxylonals and 1,8-cineole in *Eucalyptus nitens* seedlings and concluded that the latter could not function as a cue for sideroxylonal. It appears therefore that in some eucalypt species the possible role of 1,8-cineole as a cue remains to be elucidated.

Many studies have identified phenotypic variation in resistance within plant species in plant–mammal herbivore systems (Snyder 1993; Lawler *et al*. 1998a). Similarly, a vast number of studies have documented the importance of plant chemistry in influencing intraspecific variation in intake by mammalian herbivores (Marquis & Batzli 1989; Lawler *et al*. 2000). However, only a few studies have demonstrated the role of plant genetics in determining the variation in chemically based resistance to mammalian herbivores (Dimock *et al*. 1976; Mutikainen *et al*. 2000; Vourc'h *et al*. 2002). If intraspecific genetic variation in plant resistance to herbivores and in the plant resistance trait can be demonstrated, and if the associated herbivory affects plant fitness, then the sum of this evidence is consistent with the hypothesis that defensive chemistry has evolved in response to selection pressures imposed by herbivores (Mauricio & Rausher 1997). As yet we have no evidence that different browsing pressures exist, both currently

and historically, across the range of *E. globulus*. However, we have demonstrated significant genetic variation in browsing resistance of *E. globulus* to *T. vulpecula* (O'Reilly-Wapstra *et al.* 2002) and significant genetic variation in the dominant defensive plant trait, sideroxylonal (present study). Browsing by *T. vulpecula* is known to have significant effects on fitness surrogates of *E. globulus*, such as seedling growth (Bulinski & McArthur 1999) and survival (Wilkinson & Neilsen 1995). It is possible therefore that browsing by *T. vulpecula* has acted as a selective pressure on the evolution of defensive traits in *E. globulus*, although the possibility of correlated selection from other pressures cannot be discounted.

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