

## FOLIAR NUTRITION, SITE QUALITY, AND TEMPERATURE INFLUENCE FOLIAR CHEMISTRY OF TALLOWOOD (*EUCALYPTUS MICROCORYS*)

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**Abstract.** The distribution of marsupial folivores in Australian eucalypt forests is extremely patchy and, although distribution patterns are known to be partly determined by nutritional factors, the role of plant secondary metabolites (PSMs) has been little studied. We investigated variation in the foliar concentrations of five constituents: total phenolics (TP); the foliar component bound by polyethylene glycol (PEGBC); the formylphloroglucinol compound, sideroxylonal (a strong deterrent of mammalian herbivory); the terpene, cineole; and nitrogen (N). These constituents were analyzed using near infrared spectroscopy in 501 individual tallowood (*Eucalyptus microcorys*) trees at 42 sites across a broad geographic range in eastern Australia. We tested three hypotheses: (1) trees with lower foliar N produce higher PSM concentrations; (2) PSM concentrations are greater at colder sites; and (3) PSM concentrations are higher at sites with low resource availability. Hypothesis 1 was true for all PSMs considered and was the result of variation that occurred within sites. This is consistent with the carbon/nutrient balance hypothesis and with the hypothesis that PSMs protect leaves from photodamage. Hypothesis 2 was upheld for all constituents. This was consistent with both the growth–differentiation balance hypothesis, and with the hypothesis that PSMs represent an evolutionary adaptation to greater risks of photodamage and frost damage in cold environments. Hypothesis 3 was rejected, with one PSM, sideroxylonal, being affected by resource availability in a manner opposite to that predicted. The optimal defense hypothesis offers an explanation for this result (as sideroxylonal concentrations increased along with the apparent nutritional quality of the foliage, possibly to provide defense against higher rates of herbivory). We found that trees associated with koala fecal pellets had lower concentrations of sideroxylonal and cineole. We discuss this finding and further implications for the herbivores of *Eucalyptus* of the patterns of plant defense that we detected.

**Key words:** Australia; carbon–nutrient balance; *Eucalyptus*; formylphloroglucinol compounds; graphical vector analysis; herbivory; koala; LMA; near infrared spectroscopy; phenolic; plant defense; sideroxylonal.

### INTRODUCTION

Despite the fact that Australian forests and woodlands are dominated by a single genus, *Eucalyptus*, the distribution of forest-dependent fauna is extremely patchy. For example, in the forests of south-eastern New South Wales, 63% of arboreal marsupials sighted by Braithwaite (1983) were present in only 9% of the area, and none were sighted in 52% of the surveyed forest. High abundance of marsupial folivores is often associated with eucalypt communities that have high average foliar nutrient concentrations and which grow on fertile soils (Braithwaite et al. 1983, 1984, Johnson et al. 2001, McIlwee 2001). Abundance and density of folivores also vary along other environmental gradients, such as elevation and rainfall (Braithwaite 1983,

Bennett et al. 1991, Kanowski et al. 2001, McIlwee 2001).

The evolution of eucalypts on Australia's ancient, nutrient-poor soils has typically resulted in low concentrations of foliar nutrients (Attiwill and Leeper 1987), which presents a major nutritional challenge to folivores. A further challenge is posed by high concentrations of plant secondary metabolites (PSMs), which reduce the availability of nutrients to herbivores, or act as toxins. PSMs can force animals to feed selectively so as to avoid or regulate their intake of these compounds (Foley et al. 1999). Although landscape-scale studies have usually identified nutritional parameters as the key to explaining folivore distributions, PSMs have usually been identified as the strongest explanation of diet choice in comparisons of individual trees of particular *Eucalyptus* species. In particular, the amount of foliage that marsupials eat from many species is primarily determined by concentrations of PSMs known as formylated phloroglucinol compounds (FPCs) (Lawler et al. 1998; B. D. Moore, W. J. Foley, I. R. Wallis, A. Cowling, and K. A. Handasyde, *unpublished manuscript*).

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Numerous theories offer explanations for variation in the type and amounts of PSMs that plants produce under differing environmental conditions (Berenbaum 1995, Koricheva 2002). The carbon–nutrient balance hypothesis (CNBH; Bryant et al. 1983) proposes that both low nutrient availability and abundant sunlight cause the C:N ratio in plant tissue to increase, thereby increasing the production of carbon-based secondary compounds (CBSCs). Since its conception, the CNBH has been narrowed and refined. It is now used only to predict plastic responses of particular plant genotypes to variations in resource supply, and only for some types of CBSC (Lerdau and Coley 2002). It has been dismissed by some ecologists for its narrow scope, its failure to consider adaptive changes in PSM production, and because of perceived empirical failures (Koricheva et al. 1998, Hamilton et al. 2001, Koricheva 2002, Nitao et al. 2002). Nonetheless, products of the shikimic acid pathway, especially tannins, consistently respond as predicted by the CNBH, and the effect of carbon–nutrient balance on FPC concentrations has not been tested.

The growth–differentiation balance hypothesis (GDBH; originally proposed by Loomis 1932) encompasses the CNBH, but has a broader scope. It predicts that any factor that slows plant growth more than photosynthesis will lead to greater cellular differentiation, including increased synthesis of carbon-based secondary compounds. According to this theory, cold temperatures, moderate drought, and moderate nutrient limitation might all increase CBSC production (Herms and Mattson 1992).

Evolutionary models of plant defense consider trade-offs between growth and defense and are appropriate for comparing allocation to PSMs across species, genotypes, and populations (Herms and Mattson 1992, Tuomi 1992, Lerdau and Coley 2002). The resource availability hypothesis (RAH; Coley et al. 1985) proposes that in environments with poor availability of water, nutrients, and light, plant growth should be slower and investment in defense greater, because plants are less able to compensate for the loss of tissue to herbivory. Investment in defense is reduced at resource-rich sites, because greater competition favors plants that are fast-growing. Thus, the RAH assumes that the availability of resources is constant and unaffected by plants, and that fitness is determined by potential growth rate. However, Loreau and de Mazancourt (1999) questioned this assumption, because many plants can accumulate enough biomass to control resource concentration. In this situation, fitness is determined by the ability to deplete limiting resources. Consequently, they suggest that ecologists may lack the knowledge to predict the direction of investment in antiherbivore defenses as a function of resource availability and maximum growth rate.

In some cases, PSMs may be adaptive responses to environmental stress. For example, tannins can protect

plants against damage from ultraviolet light, can enhance nutrient recovery from litter, and may contribute to drought tolerance (Herms and Mattson 1992, Nitao et al. 2002). When leaves absorb light energy in excess of the capacity of photosynthetic and photoprotective processes, highly reactive chemical species can form, leading to severe photodamage. Close and McArthur (2002) have suggested that the main role of phenolics may be to act as antioxidants, which can quench these reactive species. If so, plants should synthesize more phenolics when photosynthesis is suppressed, but light is still abundant. Two such situations are nitrogen deficiency and cold-induced photoinhibition, the latter of which results from cold temperatures co-occurring with high levels of solar irradiance.

We used PSM concentrations in a single eucalypt species to test three hypotheses arising from these theories: (1) that foliar concentrations of nitrogen and of CBSCs are inversely related, as predicted by CNBH and by the photodamage protection hypothesis; (2) that CBSC concentrations are greater in foliage from colder (higher elevation) environments, as predicted by the GDBH and by the photodamage protection hypothesis; and (3) that foliage should contain less CBSC at sites with abundant resources for growth, compared to resource-poor sites, in accordance with the RAH.

The subject of our investigation was tallowwood (*Eucalyptus microcorys* F. Muell.), a medium-sized to very tall forest tree that is widely distributed in north-eastern New South Wales and southeastern Queensland, Australia. It occurs from sea level to an altitude of 750 m, and occasionally as high as 1000 m. Throughout most of its range the climate is warm-humid and, although it prefers fertile soils, tallowwood can grow on relatively poor sands, provided that adequate subsoil moisture is available (Boland et al. 1984). Tallowwood is relatively slow growing and shade tolerant (Florence 1996) and is recognized as an important food tree for the koala, *Phascolarctos cinereus* Goldfuss, a specialized folivore of *Eucalyptus* (Martin and Handasyde 1999).

All PSMs in tallowwood foliage are carbon based. We measured the concentrations of four of them. We analyzed 1,8 cineole because it dominated the terpene fraction in tallowwood (Brophy and Southwell 2002) and was a good indicator of total oil yield (B. D. Moore, unpublished data). We measured sideroxylonals because they are the only FPCs produced by tallowwood. Our total phenolics (TP) assay captured both hydrolyzable and condensed tannins and other water-soluble phenolic compounds, but not FPCs, which are lipid-soluble. We also quantified the subset of water-soluble phenolic compounds that actively bound polyethylene glycol (PEG). Dietary supplementation with PEG allows many herbivores to increase their intake of tannin-rich diets, presumably because PEG blocks the physiological effects of tannins (Marsh et al. 2003b). The concentration of the PEG-binding component (PEGBC)

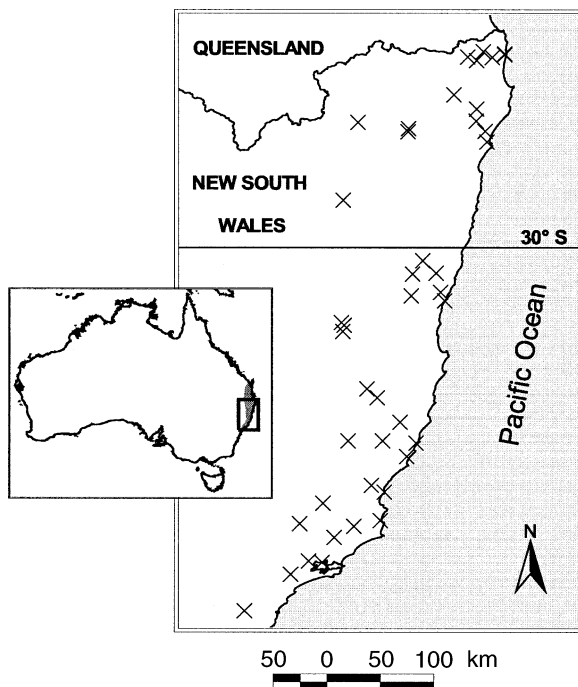


FIG. 1. Location of study sites and geographic range of *Eucalyptus microcorys* (inset).

may indicate the proportion of phenolic compounds that confer defense against herbivory. As PEG binds to the same hydroxyl groups on phenolic molecules that are responsible for their anti-oxidant activity, PEGBC concentration may also reflect the potential antioxidant activity of the phenolic fraction. Finally, because of its relevance to the predictions of the CNBH, we also measured foliar nitrogen concentrations.

## METHODS

### Study sites

Our study area incorporated the southern half of the geographic range of tallowwood, from 28.34° S to 33.08° S (Fig. 1). Annual mean temperatures ranged from 12.6°C to 19.6°C, and annual precipitation from 900 mm to 2100 mm, with a distinct summer maximum. Between 24 July and 28 August 1999, we visited 42 sites, selected so as to maximize variation in elevation between sea level and 980 m. We also maximized diversity of vegetation and soil types. Anthropogenic influence varied, from relatively untouched old-growth forest sites, to historically selectively logged sites, to a few extensively altered sites where a large proportion of trees and understory had been removed and the native ground-level vegetation replaced by exotic grasses. At all but two sites the trees had established naturally. We performed all statistical analyses both with and without significantly altered sites and because our inferences were unaffected, we present results from analyses including all trees.

### Foliage sampling

At each site, we selected 12 tallowwood trees with a diameter at breast height (dbh) of at least 10 cm, using a random walk procedure. We collected ~100 g (fresh mass) of mature, fully expanded leaves from a single branch from each tree, which we immediately froze in a sealed plastic bag at -20°C. We recorded the collection position in the canopy (aspect and height) and the occurrence of new growth, flowers, and flower buds on each branch sampled, using a scale from 0 (absent) to 2 (abundant).

### Tree data

We measured the dbh and estimated total height of each tree using a Suunto (Vantaa, Finland) clinometer and measuring tape. To estimate the density of each crown, we compared it from directly below with a set of reference photographs (Walker and Hopkins 1990). We also described the landform element (e.g., flat, slope, crest, vale, etc.) dominating an area of radius 20 m around each tree and measured the aspect and steepness of the slope. Except where dense vegetation prevented it, we systematically searched the ground around the base of each tree for koala fecal pellets for 2 min, starting at the base of the tree then spiraling outwards.

### Site data

We described vegetation structure in terms of dominant tree species, and the height of the tallest forest stratum and mid- and lower strata, where present. We classified the degree of crown separation in each stratum as dense, mid-dense, sparse, or very sparse. For an area of 300 m radius around each site, we classified relief (maximum elevation—minimum elevation) and modal terrain slope using the scheme of McDonald et al. (1990). We ascertained precise geographic coordinates and elevations from 1:50 000 or 1:100 000-scale topographic maps.

*Site-quality classes.*—Where sufficient information was available, we assigned four site-quality (SQ<sub>i</sub>) classes: from 1 (highest quality) to 4 (lowest) to sites. We intended that this measure would capture overall suitability for plant growth and would be determined primarily by an interaction between nutrient and water availability (i.e., resource availability sensu Coley et al. 1985). This inclusive measure was more suitable than any single, directly measured property, because plants have multiple requirements for growth. The availability of nitrogen to plants, for example, is influenced not only by its concentration, but also by the availability of phosphorous and water and by evolved plant characteristics (Beadle 1954, Adams 1996). We took into account the floristic composition of sites, tree morphometrics, vegetation structure, and soil characteristics. In some cases, we used forest community composition as a key indicator. For example, *Lophos-temon confertus* (R. Br.) Peter G. Wilson & Waterhouse

TABLE 1. NIRS modified partial least-squares regression models predicting foliar concentrations of N, sideroxylonal, total phenolics (TP; quebracho equivalents), cineole, and polyethylene glycol binding component (PEGBC).

Constituent	Foliar concentration <sup>†</sup>		$r^2$	SE, cross validation	Wavelengths used (nm)	Math <sup>‡</sup>	Scatter correction <sup>§</sup>
	Mean	Range					
N	1.25	0.86–1.68	0.96	0.04	800–2492	2,4,4,1	none
Sideroxylonal	23.46	10.4–45.4	0.96	2.37	800–2492	2,4,4,1	none
TP	338.4	276.9–435.9	0.97	10.99	800–2492	2,4,4,1	detrend, SNV
Cineole	8.24	2.2–13.8	0.99	1.20	800–2492	2,4,4,1	none
PEGBC	18.95	6.8–34.8	0.98	1.10	750–2492	2,6,4,1	SNV

<sup>†</sup> N is measured as percentage of dry mass; all other constituents are measured as mg/g dry mass.

<sup>‡</sup> Math describes the mathematical treatment applied to the spectra (stored as  $\log[1/\text{reflectance}]$ ). The first two numbers describe the derivative used; the third and fourth numbers indicate the degrees of primary and secondary smoothing performed on the derivative. Thus 2,4,4,1 indicates that the second derivative was calculated with a gap size of 4 nm and that a maximal primary smooth (4) but no secondary smooth (1) was used.

<sup>§</sup> SNV and detrend transformations are described by Barnes et al. (1989).

and *Eucalyptus saligna* Smith are typical of “fertile soils” and *Eucalyptus umbra* R. Baker, and *Eucalyptus planchoniana* F. Muell. typically occur on infertile soils (Boland et al. 1984, Florence 1996). In many instances, this approach allowed us to eliminate certain quality classes but not others. Forests dominated by *Eucalyptus pilularis* Smith (blackbutt) and *Corymbia maculata* (spotted gum) occur over a broad range of soil types, except that blackbutt is absent from extremely poor and very fertile sites, and spotted gum is absent from very poor sites (Florence 1996). We used the presence of rainforest elements as an indicator of high site fertility while the height of the largest mature trees partially reflected site quality. We took soil properties into account only when the broad soil type was unambiguously identified and characterized. We assigned site-quality ratings to 27 of the 43 sites, rather than accepting classifications that were poorly supported. When we performed analyses on reduced numbers of sites (e.g., only low-elevation sites), we combined the two high-quality classes and the two low-quality classes to produce a two-level (SQ<sub>2</sub>-HIGH and SQ<sub>2</sub>-LOW) scheme.

**Bioclimatic data.**—Bioclimatic data were generated from the geographic coordinates and elevation for each site by the BIOCLIM computer program, a component of the ANUCLIM 5.0 software package (Centre for Resource and Environment Studies, Australian National University, Canberra, ACT, Australia). BIOCLIM is a bioclimatic prediction system that generates bioclimatic surrogate parameters from climate surfaces derived from mean monthly climate estimates, to approximate water and energy balances at a given location (Houlder et al. 1999). The 35 parameters produced describe aspects of temperature, precipitation, radiation, and moisture. Radiation parameters were adjusted for the slope and aspect of each site.

#### Laboratory analyses

We took subsamples from frozen foliage samples and freeze dried and then ground them to pass a 1-mm sieve in a Tecator cyclone mill (Foss Tecator, Höganäs, Swe-

den). This was used for NIRS scanning and for all chemical analyses except cineole concentration. All concentrations were calculated on a dry mass basis and all analyses were performed in duplicate.

**Near infrared spectroscopy.**—We used near infrared spectroscopy (NIRS) to predict the five chemical measures. This technique (reviewed by Foley et al. 1998) uses multivariate statistical models to relate spectral characteristics of samples to attributes that have been determined using traditional laboratory techniques.

Before scanning, we placed samples in a 40°C oven for at least 1 h to remove residual moisture that might otherwise alter the NIR spectra. We recorded the reflectance spectrum of each sample between 400 and 2500 nm using an NIRSystems 6500 scanning spectrophotometer with spinning cup attachment (NIRSystems, Silver Spring, Maryland, USA). Each sample was scanned twice or until the root mean square of two scans (stored as  $\log[1/\text{reflectance}]$ ) was less than  $3.0 \times 10^{-4}$ , and the two spectra were averaged.

We developed calibration equations to predict the five measures, based upon reference values obtained from analyses of subsets of the samples, using the methods described in the following subsections. The calibration process used modified partial least squares regression (MPLS) with cross validation to prevent overfitting of models (Shenk and Westerhaus 1991). For most calibrations, we achieved best results without the use of mathematical transformations on the raw spectra to reduce the influence of particle size. The exceptions were the calibrations for PEG-binding components (standard normal variate [SNV] transformation) and TP (SNV and detrend transformations; Barnes et al. 1989). We performed the calibrations using the software NIRS 3, version 4.00 (Infrasoft International, Port Matilda, Pennsylvania, USA). Our NIRS models are summarized in Table 1. The range of values used to develop the N, cineole, sideroxylonal, and PEGBC equations included >99% of predicted values, while that for TP included 97% of predicted values.

1. *Sideroxylonal.*—We assayed sideroxylonals (hereafter referred to as “sideroxylonal”) using the HPLC

(high performance liquid chromatography) technique described by Wallis et al. (2003).

2. *Cineole*.—We quantified 1,8 cineole from solvent extracts of fresh leaf material using gas chromatography (GC). For each sample, we cut  $100 \pm 3$  mg of leaf pieces, exclusive of the median and lateral veins, from the blades of several leaves, and extracted them at  $100^\circ\text{C}$  for 60 min in 400  $\mu\text{L}$  of hexane containing n-tridecane (Sigma, Castle Hill, NSW, Australia) as an internal standard. We performed the GC analyses on a Varian Star 3400 gas chromatograph (Varian, Inc., Palo Alto, California, USA). We injected 1  $\mu\text{L}$  via a splitless injector at  $50^\circ\text{C}$  onto a 46-m hybrid column (internal diameter 0.32 mm, film thickness 0.25  $\mu\text{m}$ ) comprising a 1-m section with a deactivated stationary phase, a 15-m section with a moderately polar stationary phase (14% cyanopropylphenyl, 86% dimethyl siloxane), and a 30-m section with a nonpolar, dimethylpolysilane stationary phase, using helium as a carrier gas. The column temperature was maintained at  $50^\circ\text{C}$  for 60 s, rapidly raised to  $80^\circ\text{C}$  and held there for 12 min before being raised by  $10^\circ\text{C}/\text{min}$  to  $240^\circ\text{C}$  and held for 10 min. A flame ionization detector (FID) operated at  $300^\circ\text{C}$ .

3. *Total phenolics*.—We extracted TP from  $100 \pm 1$  mg subsamples of dried, ground leaf in 25 mL of 50% (v/v) aqueous acetone, homogenizing the extracts for 60 s at maximum speed (Dix 900 homogenizer; Heidolph Instruments LLC, Cinnaminson, New Jersey, USA), before centrifuging them for 10 min at 13 000 g at  $4^\circ\text{C}$ . We removed a 1.0-mL aliquot from this extract and discarded the remaining supernatant, before repeating the extraction with a further 10 mL of solvent to obtain another 1.0-mL aliquot. We quantified TP in both extracts using the Folin-Ciocalteu method (Singleton and Rossi 1965). After allowing the reactions to proceed in the dark for 2 h, we measured their absorbance at 765 nm, using purified quebracho tannin (obtained from Ann Hagerman, Miami University, Oxford, Ohio) as a standard. Results are quebracho equivalents.

4. *Polyethylene glycol-binding component*.—We developed an NIRS equation to predict the PEG-binding component “PEGBC” using PEG-binding assay results for foliage from 16 eucalypt species, including *Eucalyptus microcorys*. The PEG-binding assay measured the amount of  $^{14}\text{C}$  PEG bound by eucalypt foliage and is described by Marsh et al. (2003a).

5. *Nitrogen*.—We determined the N concentration of dried, ground samples from duplicate  $250 \pm 10$  mg quantities using a semimicro Kjeldahl technique with a Tecator 2012 digester (Foss Tecator, Höganäs, Sweden), selenium catalyst, and a Gerhardt Vapodest-5 distillation and titration apparatus (C. Gerhardt, Bonn, Germany). The method was standardized using ammonium sulfate.

*Leaf size and leaf mass per unit area*.—For each constituent at each site, we determined content per leaf

by multiplying concentration by dry leaf mass for one arbitrarily selected leaf from each of six trees. We also measured the surface area of each leaf, enabling us to plot graphical vector diagrams (Koricheva 1999) illustrating shifts in constituent concentrations per unit leaf area and to calculate leaf mass per unit area (LMA) for each site. LMA is the inverse of specific leaf area (SLA) and provides a measure of leaf thickness and/or density (Cunningham et al. 1999). The response of SLA and LMA to environmental gradients including elevation, water availability, and nutrient availability has been well studied (e.g., Körner et al. 1986, Cunningham et al. 1999, Fonseca et al. 2000).

#### *Statistical design and analysis*

We initially investigated patterns of variation in foliar chemistry using linear regression performed in Genstat 5, release 4.02 (VSN International, Ltd., Oxford, UK). For these models, we summarized response variates and explanatory variables as site means, and considered thematically similar groups (e.g., BIOCLIM temperature variables, or vegetation structure variables) of variables together. We identified the variables with the most explanatory power in each group and identified and disregarded confounded variables. Because LMA was estimated for sites and not for individual trees, we used linear regression to model LMA and to model site means of the five measured leaf constituents when LMA was used as an explanatory variable.

Next, we produced linear mixed models using the residual maximum likelihood (REML) algorithm in Genstat 5. All models included terms for random effects attributable to region (the river or lake drainage system in which sites were located), site, and tree, allowing us to assess and control for variation at these three levels. We produced models to test combinations of effects that we had identified using the regression modeling approach and then sequentially dropped non-significant effects to obtain reduced models. We measured the significance of each term by dropping it individually from the model to produce a submodel and measured the resulting change in deviance, which conforms to the chi-square distribution.

Two powerful explanatory variables, site quality and elevation, were confounded across the 42 sites (i.e., high-quality sites were over-represented at high elevations and vice versa). To isolate the effects of these variables, we produced some models that held elevation constant by including only sites below 200 m elevation and others that controlled for resource availability by including only SQ<sub>2</sub>-HIGH sites. Sites higher than 200 m included too few SQ<sub>2</sub>-LOW sites, and SQ<sub>2</sub>-LOW sites included too few high elevation sites to make useful, balanced comparisons. These linear mixed models included random terms for site and tree.

Changes in nutrient and PSM concentrations in foliage depend on shifts in acquisition, production, and

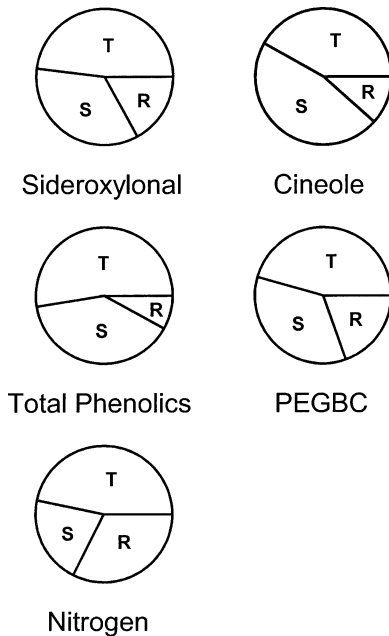


FIG. 2. Proportion of variance attributable to drainage region (R), site (S), and individual trees (T) for all leaf constituents measured. These variance components were obtained from null linear mixed models, i.e., models with only random terms fitted.

changed leaf biomass. Factors such as temperature and water availability can affect leaf biomass via changes in leaf size (area) as well as thickness and density. A technique that allows the effect of each factor on concentration changes to be visually assessed is graphical vector analysis (GVA; Timmer and Stone 1978, Koricheva 1999). Mean concentrations of foliar constituents are plotted against mean content per plant part (or plant) for plants subjected to control conditions and at least one treatment, in a vector diagram. The direction and magnitude of the vector joining the initial (control) point and the treatment point indicates the effect of the treatment. Comprehensive discussions of how to interpret vector diagrams can be found in Haase and Rose (1995) and Koricheva (1999). We used GVA to analyze changes in tallowwood foliar chemistry resulting from, first, an increase in elevation (controlling for site quality by considering only trees at  $SQ_2$ -HIGH sites) and, second, from an increase in site quality (controlling for elevation by considering only trees growing below 200 m).

We modeled the presence or absence of koala fecal pellets both for sites and for individual trees. At both scales, we produced generalized linear models in Genstat 5, using the logit link function. Models for binary variables at the scale of the tree included a fixed term for region, to remove a significant amount of otherwise unexplained variation.

## RESULTS

### *Variation within and between foliar constituents*

Most variation in chemical concentrations occurred among sites and among trees within sites. Except for N, differences among the drainage regions were less significant (Fig. 2). Concentrations of TP and PEGBC were highly positively correlated. We also found large positive and moderate negative correlations between sideroxylylonal and cineole and between TP and cineole, respectively (Fig. 3). Ignoring PEGBC, which is a subset of TP, TP on average accounted for 91% of PSMs, sideroxylylonal for 7%, and cineole for 2%.

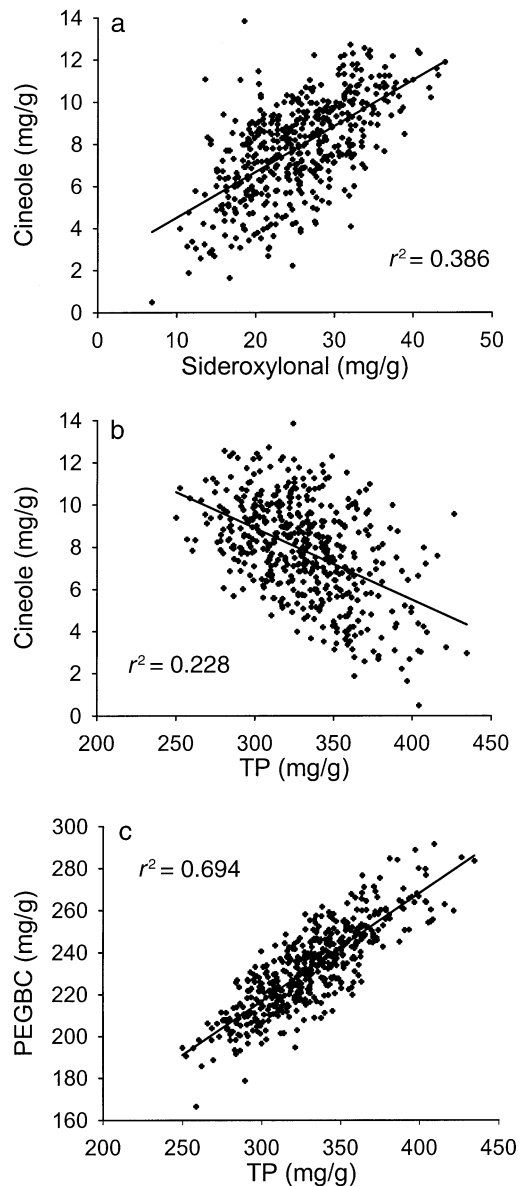


FIG. 3. Coefficients of determination observed between foliar constituents: (a) cineole and sideroxylylonal; (b) cineole and TP; (c) PEGBC and TP.

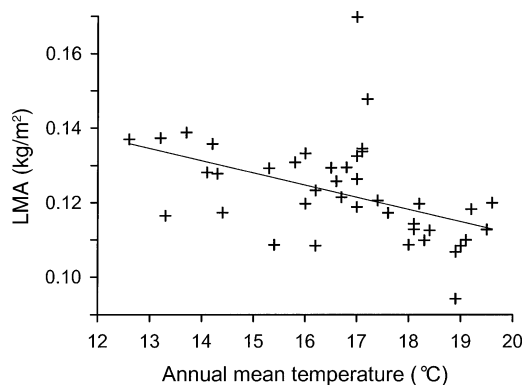


FIG. 4. Linear regression of leaf mass per unit area (LMA) against annual mean temperature of 42 sites ( $r^2 = 15.7$ ,  $F_{1,40} = 8.66$ ,  $P = 0.005$ ).

#### Leaf mass area

LMA was less at SQ<sub>2</sub>-HIGH sites than at SQ<sub>2</sub>-LOW sites (two-tailed test,  $t = -1.86$ ,  $df = 25$ ,  $P = 0.074$ ), a trend that was stronger when only sites below 200 m elevation were considered (SQ<sub>2</sub>-HIGH mean = 0.115, SQ<sub>2</sub>-LOW mean = 0.134 kg/m<sup>2</sup>; two-tailed test,  $t = -2.87$ ,  $df = 13$ ,  $P = 0.013$ ). A linear regression that included only SQ<sub>2</sub>-HIGH sites showed that LMA was directly related to site elevation ( $0.19 \pm 0.06$  mg·g<sup>-1</sup>·1000 m<sup>-1</sup> [mean  $\pm$  1 SE],  $r^2 = 37.5$ ,  $F_{1,14} = 9.99$ ,  $P = 0.007$ ). When all sites were considered, annual mean temperature accounted for 15.7% of variation in LMA ( $F_{1,40} = 8.66$ ,  $P = 0.005$ ; Fig. 4).

#### Foliar nitrogen

A model describing foliar N (total change in deviance [ $\Delta dev$ ] = 47.26,  $df = 10$ ,  $P < 0.001$ ) for all trees is summarized in Table 2. When only sites below 200 m were considered, foliar N was significantly greater at SQ<sub>2</sub>-HIGH than at SQ<sub>2</sub>-LOW sites ( $\Delta dev = 7.240$ ,

$df = 1$ ,  $P = 0.007$ ; Fig. 5b). At SQ<sub>2</sub>-HIGH sites, foliar N declined as elevation increased ( $\Delta dev = 8.952$ ,  $df = 1$ ,  $P = 0.003$ ; Fig. 5b). However, neither site quality ( $\Delta dev = 0.3087$ ,  $df = 1$ ,  $P = 0.58$ ) nor elevation ( $\Delta dev = 1.550$ ,  $df = 1$ ,  $P = 0.21$ ) was significant in models that included all trees. Site differences in LMA explained much variation in mean site foliar N concentrations ( $r^2 = 0.59$ ,  $F_{1,40} = 59.98$ ,  $P < 0.001$ ; Fig. 6). However, a linear mixed model including site as a random term found that LMA did not explain between-tree variation in foliar N within three sites for which we determined LMA of each tree ( $\Delta dev = 0.418$ ,  $df = 1$ ,  $P = 0.5$ ).

Graphical vector analysis showed that higher foliar N at higher quality sites resulted from a concentration effect i.e., leaves of smaller mass contained the same amount of N (Fig. 7a). Leaves from SQ<sub>2</sub>-HIGH and SQ<sub>2</sub>-LOW sites contained similar amounts of N per unit leaf area. Because leaf area did not differ with site quality, changes in leaf mass were attributable to LMA (Fig. 7b). At higher elevations the leaves contained less N and so N per unit mass declined (Fig. 7c). However, leaves became smaller as elevation increased so that N per unit area did not change (Fig. 7d).

#### Sideroxylylonal

Site quality (SQ<sub>4</sub>) alone explained the most variation in sideroxylylonal concentration and indicated that concentrations were higher at better quality sites ( $\Delta dev = 33.41$ ,  $df = 3$ ,  $P < 0.001$ ; Fig. 8a). Another model (Fig. 8b) showed that sideroxylylonal concentrations increased with site elevation ( $\Delta dev = 19.75$ ,  $df = 1$ ,  $P < 0.001$ ), but decreased as foliar N increased ( $\Delta dev = 6.63$ ,  $df = 1$ ,  $P = 0.01$ ). Temperature variables were the only BIOCLIM variables related to sideroxylylonal concentration—the minimum temperature of the coldest period was negatively related to sideroxylylonal con-

TABLE 2. Summary of REML (residual maximum likelihood) model explaining among-tree variation in foliar N concentration.

Variable	Unit	$\Delta dev.$	df	$P$	Effect ( $\pm$ 1 SE) or, for factors, predicted means	
Relief†	factor	14.79	3	0.002	extr. low	1.098
					v. low	1.278
					low	1.341
					high	1.390
Modal slope‡	factor	10.97	4	0.027	level	1.306
					v. gentle	1.447
					gentle	1.229
					moderate	1.208
					steep	1.194
Tree height	m	10.57	1	0.001	-0.003 ( $\pm$ 0.001)	
Annual mean temperature	°C	8.06	1	0.005	0.028 ( $\pm$ 0.008)	
New growth	0–2	7.14	1	0.008	0.025 ( $\pm$ 0.009)	
Overall		47.26	10	<0.001		

† Relief is the difference of the maximum and minimum elevations included in a 300-m radius around each site: extr. low, <9 m; v. low, 9–30 m; low, 30–90 m; high, 90–300 m.

‡ Modal slope was considered for an area of 300-m radius around each site: level, 0°–0°35'; v. gentle, 0°35'–5°45'; gentle, 1°45'–5°45'; moderate, 5°45'–18°; steep, 18°–30°.

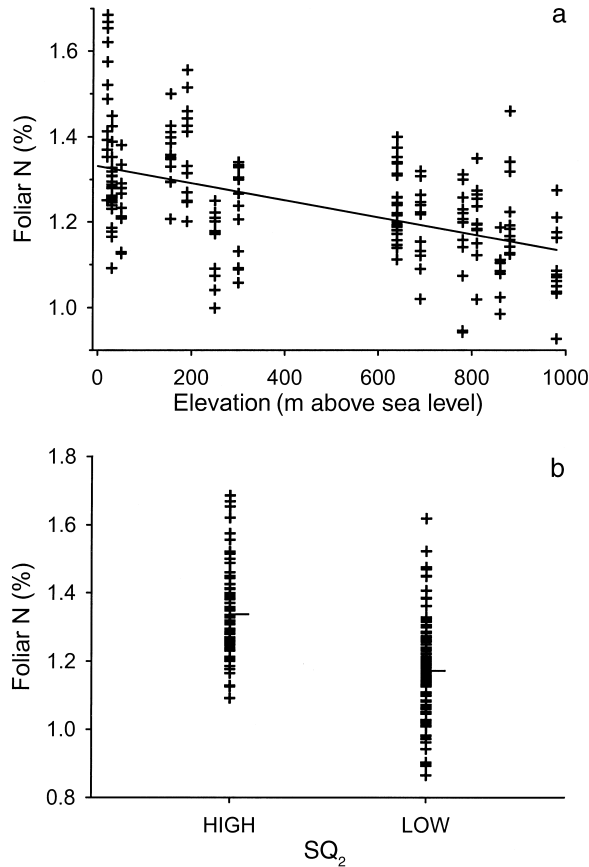


FIG. 5. Linear mixed models of foliar N concentrations after stratification by site quality and elevation. In panel (a), the line indicates a linear mixed model of foliar N in trees at SQ<sub>2</sub>-HIGH sites only, with site elevation as an explanatory variable. In panel (b), the horizontal marks indicate predicted mean foliar N at SQ<sub>2</sub>-HIGH and SQ<sub>2</sub>-LOW sites from a linear mixed model for trees at sites below 200 m elevation. In both panels, crosses indicate observed values.

centration ( $\Delta dev = 20.16$ ,  $df = 1$ ,  $P < 0.001$ ; Fig. 8c). None of the measured morphometric variables of trees, phenology, sampling position in the canopy, or landform element influenced sideroxylonal concentration. However, a model excluding all other variables indicated that the height of the tallest stratum of the forest had a positive effect on sideroxylonal concentration ( $0.426 \pm 0.155 \text{ mg} \cdot \text{g}^{-1} \cdot \text{m}^{-1}$  [mean  $\pm 1$  SE],  $\Delta dev = 6.580$ ,  $df = 1$ ,  $P = 0.01$ ).

Sideroxylonal concentrations in trees at SQ<sub>2</sub>-HIGH sites depended on elevation ( $7.0 \pm 2.3 \text{ mg} \cdot \text{g}^{-1} \cdot 1000 \text{ m}^{-1}$ ,  $\Delta dev = 6.99$ ,  $df = 1$ ,  $P = 0.008$ ). In trees below 200 m elevation, there were higher sideroxylonal concentrations at high than at low quality sites (predicted means: SQ<sub>2</sub>-HIGH = 26.48 mg/g, SQ<sub>2</sub>-LOW = 21.15 mg/g;  $\Delta dev = 14.52$ ,  $df = 1$ ,  $P < 0.001$ ). Sideroxylonal was not significantly influenced by LMA in a regression across all sites ( $F_{1,40} = 0.15$ ,  $P = 0.7$ ) or in a regression considering only sites below 200 m ( $F_{1,23} = 2.18$ ,  $P = 0.15$ ). Because the negative relationship between foliar

N and sideroxylonal in the model described earlier appeared to be at odds with the observation that both N and sideroxylonal were greater at high quality sites, we performed a linear regression of mean site sideroxylonal for sites below 200 m. We found that it was positively related to mean site N ( $r^2 = 17.1$ ,  $F_{1,23} = 5.93$ ,  $P = 0.02$ ; Fig. 9). This suggests that the negative relationship described above occurred among trees within sites, but because different factors affected LMA, and therefore N, at the site level, the relationship was reversed between sites. Graphical vector analysis showed that higher sideroxylonal concentrations at better quality sites were attributable to a concentration effect (which was primarily due to reduced LMA; Fig. 7a, b). In contrast, concentrations increased with elevation due to increased sideroxylonal synthesis per leaf (Fig. 7c, d).

### Cineole

A model (Fig. 10a) including terms for site quality ( $\Delta dev = 11.74$ ,  $df = 3$ ,  $P = 0.008$ ) and foliar N ( $\Delta dev = 4.783$ ,  $df = 1$ ,  $P = 0.029$ ) explained foliar cineole concentrations. Predicted mean cineole concentrations at sites of quality 1, 2, and 3 were not significantly different, but all had higher cineole concentrations than trees at the lowest quality sites. Cineole concentrations were also negatively related to the minimum temperature of the coldest period ( $\Delta dev = 26.18$ ,  $df = 1$ ,  $P < 0.001$ ; Fig. 10b).

We found a trend that cineole concentrations in trees at SQ<sub>2</sub>-HIGH sites were inversely related to the minimum temperature of the coldest period ( $-0.284 \pm 0.154 \text{ mg} \cdot \text{g}^{-1} \cdot ^\circ\text{C}^{-1}$ ,  $\Delta dev = 3.052$ ,  $df = 1$ ,  $P = 0.08$ ). However, site quality did not explain variation in cineole concentrations among trees below 200 m elevation ( $\Delta dev = 1.01$ ,  $df = 1$ ,  $P = 0.31$ ). Graphical vector analysis indicated that cineole concentration and content responded to site quality and elevation in a similar way to sideroxylonal (Fig. 7).

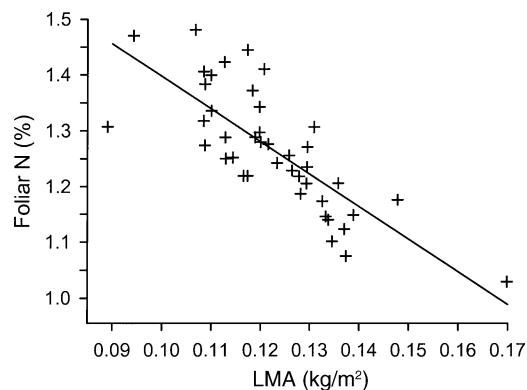


FIG. 6. Linear regression of foliar N (site means) against leaf mass per unit area (LMA) at 42 sites ( $r^2 = 0.59$ ,  $F_{1,40} = 59.98$ ,  $P < 0.001$ ).



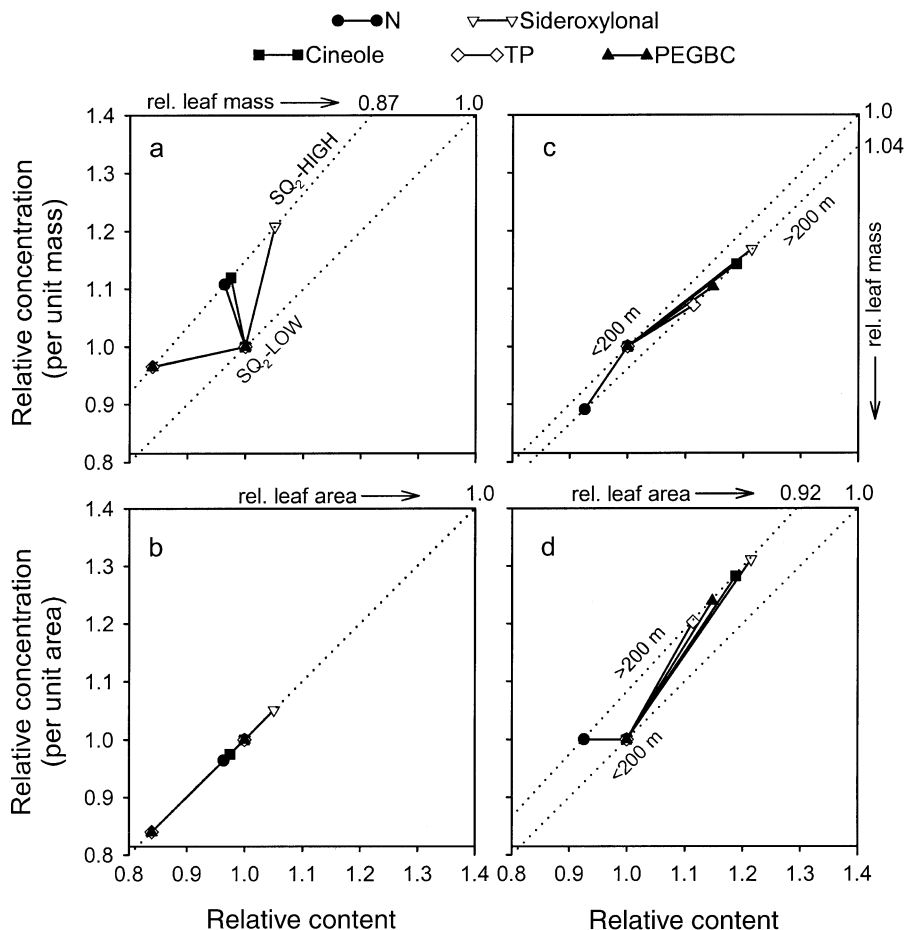


FIG. 7. Vector diagrams for graphical vector analysis (GVA) of the effects of elevation and site quality on five foliar constituents, after stratification of sites by elevation and site quality. Considering only trees growing below 200 m elevation, panels (a) and (b) show the changes in relative concentrations per unit mass and per unit leaf area, respectively, against relative content, going from  $SQ_2$ -LOW to  $SQ_2$ -HIGH sites. Considering only  $SQ_2$ -HIGH sites, panels (c) and (d) show the changes that occur going from trees growing below 200 m to those above 200 m.

#### Total phenolics and PEG-binding component

Only a negative relationship with foliar N explained variation in TP across all trees ( $\Delta dev = 56.09$ ,  $P < 0.001$ ; Fig. 11a). Another linear mixed model (Fig. 11b) showed that PEGBC was negatively related to N ( $\Delta dev = 102.8$ ,  $df = 1$ ,  $P < 0.001$ ) but also increased with elevation ( $\Delta dev = 6.587$ ,  $df = 1$ ,  $P = 0.01$ ). Models including only  $SQ_2$ -HIGH sites showed that both TP ( $-5.88 \pm 2.42 \text{ mg}\cdot\text{g}^{-1}\cdot\text{C}^{-1}$ ,  $\Delta dev = 4.938$ ,  $P = 0.026$ ) and PEGBC ( $-5.62 \pm 1.25 \text{ mg}\cdot\text{g}^{-1}\cdot\text{C}^{-1}$ ,  $\Delta dev = 12.46$ ,  $P < 0.001$ ) were inversely related to annual mean temperature. However, neither TP nor PEGBC were explained by site quality when models were restricted to sites below 200 m. Linear regression models showed that TP ( $r^2 = 6.3$ ,  $F_{1,40} = 3.75$ ,  $P = 0.06$ ) and PEGBC ( $r^2 = 13.9$ ,  $F_{1,40} = 7.64$ ,  $P < 0.01$ ) were directly related to LMA. Graphical vector analysis (Fig. 7) showed that these constituents both behaved in the same way. Their content per leaf was reduced at  $SQ_2$ -HIGH relative to  $SQ_2$ -LOW sites, but their concentration on a mass basis

remained the same because leaf mass was reduced. Content per leaf was greater at higher than at lower sites because of increased synthesis.

#### Koalas

Koala fecal pellets were found at 19 of the 37 sites and at 42 of 451 trees searched. The presence of koala pellets at a site was best explained by elevation ( $P = 0.009$ ). The occurrence of pellets at a tree could be explained by drainage region ( $P < 0.001$ ), dbh ( $P < 0.001$ ), and cineole concentration ( $P = 0.013$ ), or alternatively, drainage region ( $P < 0.001$ ), dbh ( $P < 0.001$ ), and sideroxylylonal concentration ( $P = 0.024$ ). Both models suggested that koala pellets were more common under larger, less chemically defended trees. Fig. 12 presents mean concentrations of cineole, sideroxylylonal, TP, and mean dbh for trees with and without koala pellets.

#### DISCUSSION

Despite the recognized importance of chemistry to animal feeding decisions, this is the first investigation

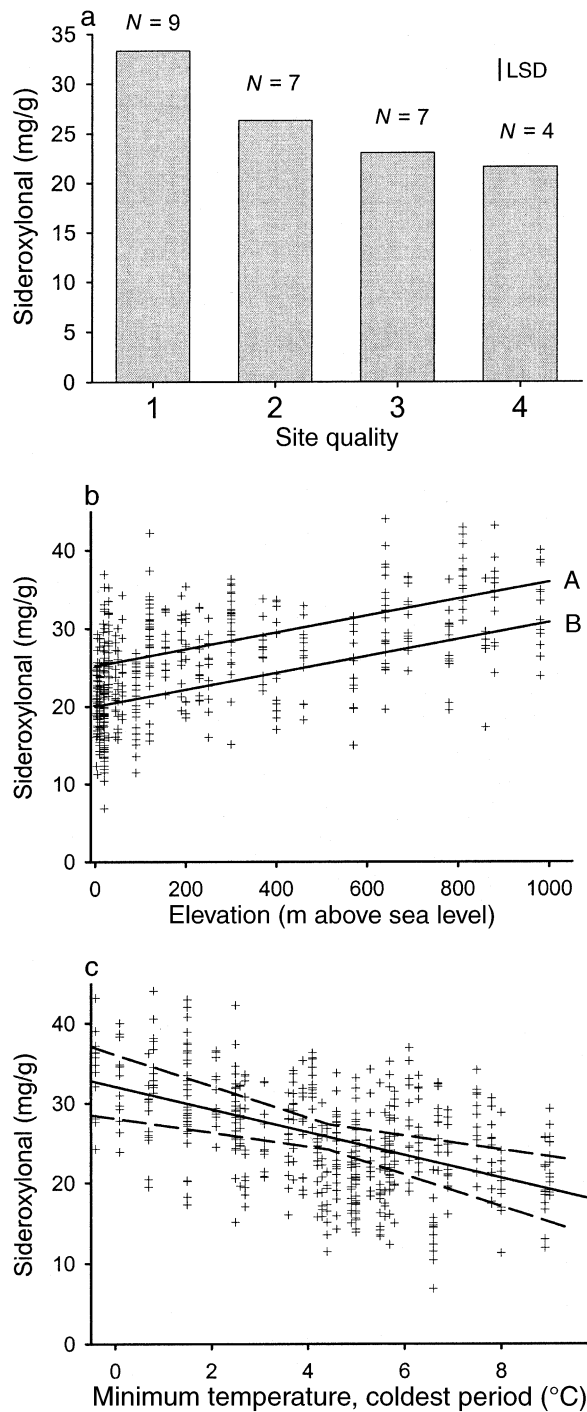


FIG. 8. Three linear mixed models (and observed values, indicated by crosses) of sideroxylonal concentrations in trees at 42 sites. (a) Predicted means at four site-quality levels, where 1 represents the highest and 4 the lowest quality, with the least significant difference indicated by a vertical line;  $N$  = number of sites in each class. (b) Sideroxylonal responses to elevation when foliar N is fixed at 0.8% (line A) and at 1.8% (line B). (c) Sideroxylonal response to minimum temperature of the coldest period (solid line) with 95% confidence interval (dashed lines).

into how allocation to chemical defense varies along environmental gradients in a eucalypt species. We identified strong effects of elevation/temperature and site quality on chemical defense in tallowwood. An understanding of the patterns and processes determining habitat quality for herbivores of *Eucalyptus* has broad application, because of the domination of this genus in Australian ecosystems.

In support of our first hypothesis, there was a negative relationship between foliar CBSC and N concentrations for all CBSCs considered. However, for sideroxylonal and cineole, this relationship was driven entirely by variation within sites. Thus, we found that mean concentrations of cineole were unaffected by mean site N concentrations and that sideroxylonal concentrations were likely to be greater at sites producing foliage with high mean N concentrations.

The finding that the concentrations of all five CBSCs considered increased in colder environments supported our second hypothesis. We rejected our third hypothesis, that the lower availability of resources for growth at low quality sites would lead to greater investment in carbon-based defenses. Both TP and PEGBC concentrations were unaffected by site quality. However, graphical vector analysis demonstrated that trees produced more of these compounds per leaf and per unit leaf area at resource-poor sites, but that their concentrations were diluted as LMA increased. There was generally a positive relationship between foliar cineole and sideroxylonal concentrations and site quality. The pattern disappeared for cineole when elevation was held constant. GVA showed that synthesis per leaf was not altered by resource availability. With sideroxylonal, increased LMA at poor quality sites effected a decrease in concentration, which is contradictory to RAH predictions.

#### Relationships between foliar constituents

Positive correlations between cineole and sideroxylonal were also found by Lawler et al. (1998) and

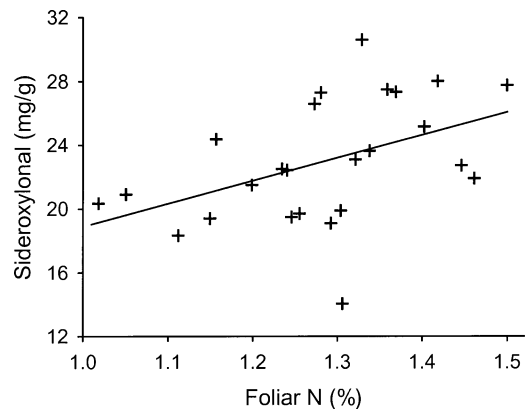


FIG. 9. Linear regression of sideroxylonal (site means) against foliar N (site means) for 25 sites below 200 m elevation ( $r^2 = 17.1$ ,  $F_{1,23} = 5.93$ ,  $P = 0.02$ ).

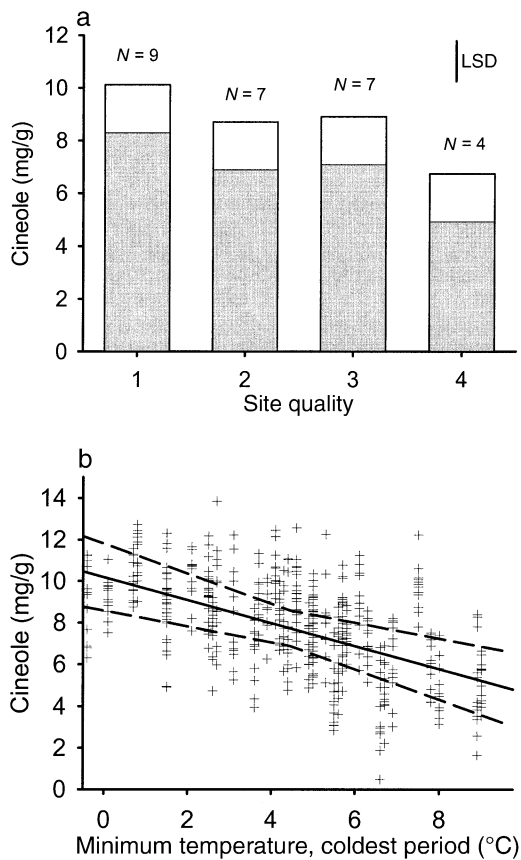


FIG. 10. Two linear mixed models (and observed values, indicated by crosses) of cineole concentrations. (a) Predicted means at four site-quality levels, where 1 represents the highest, and 4 the lowest quality, when foliar N is fixed at 0.8% (unshaded bars) and at 1.8% (gray bars); the least significant difference between predicted means is shown by a vertical line, and the number of sites in each class is indicated. (b) Cineole response to minimum temperature of the coldest period with mean (solid line) and 95% confidence interval (dashed lines).

Moore et al. (2004) but there is no biosynthetic explanation for this relationship. Cineole is a product of the mevalonic acid pathway, or of the mevalonate-independent pathway via deoxyxylulose phosphate (Dewick 2002). Sideroxylyonal (which, unlike many FPCs, is not a terpene adduct) is derived, entirely independently along the shikimic acid pathway. One explanation for the observed relationship is that cineole and sideroxylyonal synthesis are genetically correlated. A negative relationship between phenolic and terpenoid components was unexpected, although Mutikainen et al. (2000) described a similar relationship in silver birch saplings provided with different levels of fertilization. It is possible that the accumulation of flavonoid derivatives, such as condensed tannins, is affected by substrate competition with the synthesis of terpenes and other carbon-based metabolites via malonyl-CoA (Keinänen et al. 1999).

#### Resource availability, foliar nitrogen, and plant secondary metabolites

The RAH is an evolutionary model that assumes a trade-off between growth and defense, where defense is defined by its effectiveness in deterring herbivores. In eucalypts, both FPCs and other phenolics can defend trees against some mammalian herbivores (Marsh et al. 2003b). Terpenes pose detoxification costs for herbivores (Foley et al. 1995). Although there are reports of negative, positive, and null effects of terpenes on marsupial feeding (Betts 1978, Southwell 1978, Hume and Esson 1993, Lawler et al. 1999), terpenes deter mammalian herbivores in other systems (e.g., Tixier et al. 1997, Dearing et al. 2000) and confer resistance to insect herbivory in several eucalypts (Edwards et al. 1993, Stone and Bacon 1994). The relevant estimate of the effect of a PSM as an herbivore deterrent is its concentration.

In contrast to the RAH, the CNBH describes only plastic, nonadaptive responses to variations in carbon-nitrogen balance, which may not be optimal. Its predictions primarily concern the most abundant CBSCs, as they represent the destination of “excess” photosynthate (Herms and Mattson 1992, Lerdau and Coley

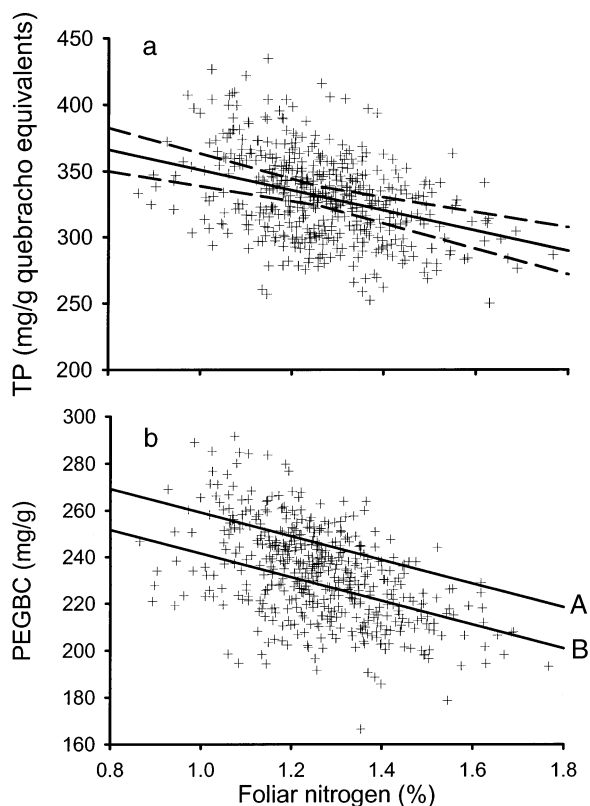


FIG. 11. Linear mixed models (and observed values, indicated by crosses) of TP and PEGBC: (a) TP response to N with mean (solid line) and 95% confidence interval (dashed lines); (b) PEGBC response to foliar N when site elevation is fixed at 1000 m (line A) and at 0 m (line B).

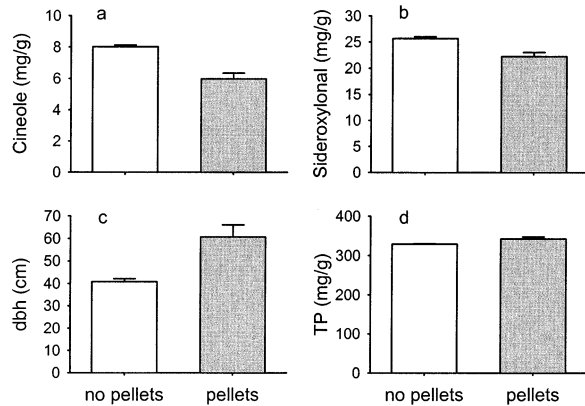


FIG. 12. Mean (+1 SE) cineole, sideroxytonal, and TP concentrations and mean dbh for trees with and without koala pellets.

2002). The CNBH was originally formulated in terms of concentrations of PSMs per unit mass, and is generally tested in these units. The predictions of this hypothesis should be correctly considered by monitoring changes in total PSM content as well (Koricheva 1999).

Given these conditions, the RAH clearly failed to predict allocation to CBSCs in tallowwood—TP and PEGBC per leaf and per unit leaf area may have declined as resource availability increased, but their concentrations did not. The RAH may have failed because of the reasons outlined by Loreau and de Mazancourt (1999), which we discussed earlier. However, a further possibility is the failure of one of the key conditions of the RAH: that the comparison between plants in low and high resource environments be made under conditions of equivalent herbivory (Coley et al. 1985). Where this condition is not met, resource allocation is probably better considered in terms of the optimal defense hypothesis.

Evolutionary models of plant defense can explain adaptation to selective forces (e.g., herbivory, climate, soil characteristics) that act on populations over evolutionary time, but cannot explain variation in allocation to CBSCs within tallowwood populations. Genetic variation may account for a large proportion of this within-site variation (R. Andrew, *unpublished data*). However, the CNBH may explain part of it (although our result was also consistent with the photodamage protection hypothesis). In support of the CNBH, Lawler et al. (1997) found that low soil nutrient availability increased the C:N ratio of leaves of glasshouse-grown *Eucalyptus tereticornis* Smith. This resulted in higher levels of TP and condensed tannins, but concentrations of volatile terpenes decreased.

The CNBH predicts that the C:N ratio, and consequently CBSC levels, increase when plant growth is constrained by a lack of nitrogen, i.e., when nitrogen deficiency occurs. Individual trees may encounter nitrogen deficiency for several reasons, for example if (1) their genetically determined ability to acquire ni-

trogen is poor; (2) soil nitrogen concentrations are low because of fine-scale spatial variation; or (3) mycorrhizal symbionts are absent or deficient. One reason that the CNBH cannot predict mean sideroxytonal concentrations at a site as a function of mean foliar N concentration, is that populations of trees are less likely to be N-deficient than are individuals. This is because species and provenances of eucalypts possess numerous adaptations to the soils on which they have evolved, matching their nutrient demands to availability. Those that grow on naturally depauperate soils are more efficient in acquiring soil nutrients (Kriedemann and Cromer 1996), recycle nutrients more efficiently (Adams 1996), and have genetically constrained low maximal growth rates (Grove et al. 1996). The use of GVA confirmed that tallowwood trees at low quality sites were not nutrient deficient. Although they possessed lower N concentrations, N content per leaf and per unit leaf area differed only slightly from those at high quality sites.

Our results support the contention of Lerdaun and Coley (2002) that the CNBH and evolutionary theories of plant defense allocation can usefully coexist because they each describe processes that act at different scales. Other examples exist where defense levels are determined by both genetic and environmental factors. In some daisy species, PSM concentrations are influenced most strongly by evolutionary pressures, but also by resource availability (Hagele and Rowell-Rahier 1999). Although some phenolic components in silver birch are determined primarily by genotype, condensed tannin concentrations are reduced by fertilization (Keinänen et al. 1999). In aspen, an evolutionarily determined basal allocation sets the concentration of phenolic glycosides, while starch and condensed tannins respond passively to resource availability, as predicted by the CNBH (Hemming and Lindroth 1999, Osier and Lindroth 2001).

#### *Temperature/elevation and plant secondary metabolites*

Both the GDBH and the photodamage protection hypothesis explain why all of the CBSCs we considered increased at higher elevations or as the climate became colder. Pyrke and Kirkpatrick (1994) studied four eucalypt species in Tasmania and found that trees increased in height and diameter more slowly at higher elevations. This was partially a direct response to the physical environment, but a glasshouse trial indicated that potential growth rates also declined with increasing elevation of seed source. Similarly, in *Eucalyptus globulus* Labill. plantations in Tasmania, productivity and photosynthetic rate both declined with temperature (Sands and Landsberg 2002). However, to be fully consistent with the GDBH, cold temperatures must reduce the growth rate of tallowwood more than they reduce photosynthesis. Some evidence that this occurred in tallowwood is provided by the increase in LMA, which

might indicate that leaf expansion rate was depressed more than photosynthesis (Tardieu et al. 1999).

Photodamage protection has been proposed as an explanation for both intra- and interspecific differences in allocation to phenolics in several eucalypt species. For example, *Eucalyptus nitens* (Deane & Maiden) seedlings possess higher concentrations of phenolic compounds, including anthocyanins that attenuate excess light, and antioxidant galloylglucoses (tannins) and flavanols, when foliar nitrogen levels are lowered by nutrient deprivation. Concentrations of anthocyanins also increase following photoinhibition (Close et al. 2000, 2001). All FPCs possess phenolic hydroxyl groups, like those that contribute to the antioxidant quenching efficacy of high molecular weight condensed and hydrolyzable tannins (Hodnick et al. 1988). However, the antioxidant effect of macrocarpal FPCs is minor compared to that of other eucalypt phenolics such as gallic and ellagic acid (Amakura et al. 2002). The high UV absorbance of sideroxylonal (B. D. Moore, unpublished data) is consistent with a role in protecting leaves from UV-B radiation.

Apart from avoiding photodamage, plants in cold environments must also resist frost. In eucalypts, this process has been linked to increased levels of monoterpenes (Shimizu 1974), certain growth regulators (Patton 1987), and the FPCs grandinol and homograndinol (Patton 1987, Yoshida et al. 1988, Ghisalberti 1996). Sideroxylonal may be similarly involved in frost resistance by controlling the active electron transport properties of membranes.

#### *Optimal defense theory and plant secondary metabolites*

According to the optimal defense theory (Feeny 1976), herbivory is a primary selective force for PSMs, and investment in defense should reflect the frequency and severity of herbivory over time. Selective pressure from herbivores may explain why defenses in tallowwood, particularly sideroxylonal, did not respond as predicted by the RAH. At high-quality sites, trees produce leaves with low LMA. Apart from being thinner, these leaves contain a smaller proportion of fiber than those at poor sites. Presumably they are easier to puncture, chew, and digest. At these sites, foliar N concentrations are also higher, and more foliage is likely to be produced in these more productive ecosystems. Numerous studies have shown that marsupial folivores are more abundant in eucalypt forests with high foliar N concentrations (e.g., Braithwaite et al. 1984, Cork and Catling 1996, Wormington et al. 2002). However, at some of our sites, mean foliar N was as low as 1.0%, which may be inadequate even to meet the needs of the highly specialized koala (Cork 1986). These sites may escape herbivory by virtue of their poor nutritional quality.

However, optimal defense theory seems unlikely to explain the increased chemical defense we found at

high-elevation sites. In cold conditions, primary productivity is reduced, young foliage is available for fewer weeks of the year, foliar N concentrations are lower and LMA is greater. Furthermore, the major mammalian herbivore of tallowwood, the koala, is generally restricted to low elevations.

#### *What are the implications for folivores?*

Previously, habitat models for *Eucalyptus* herbivores have ignored intraspecific variation in foliage quality. Typically, researchers have characterized each tree species' nutrient and PSM profile from a few samples and then extrapolated the nutritional quality of a forest from its species composition (e.g., Braithwaite et al. 1983, Cork and Catling 1996). However, the significant differences that we found in the nutritional quality and level of chemical defense of foliage within a single species must influence habitat quality, and should be incorporated into future habitat models.

Densities of mammalian folivores have been linked to foliar concentrations of polyphenolic compounds, especially tannins, both in eucalypt forests (Cork and Catling 1996) and in other ecosystems (e.g., McKey et al. 1978, Waterman et al. 1988). Cork (1992) proposed that a threshold level of toxicity (a ratio of foliar nitrogen: phenolics) excluded marsupial folivores from many eucalypt forests, but habitat models that consider tannins and nitrogen together have proved to be no better than those with foliar nitrogen alone (Moore et al., *in press*). FPCs have not yet been incorporated into habitat models for marsupial folivores. In tallowwood, sideroxylonal and TP concentrations responded differently to resource availability, while sideroxylonal showed a greater relative increase along the elevation gradient. Investigating how between-species patterns of allocation to FPCs are affected by environmental gradients should be a research priority.

Koalas are known to reduce their feeding across the range of FPC concentrations we measured in tallowwood (B. D. Moore, W. J. Foley, I. R. Wallis, A. Cowling, and K. A. Handasyde, unpublished manuscript). We found that the tallowwood trees favored by koalas were larger and contained lower concentrations of sideroxylonal and cineole than those that were avoided. It is well known that free-ranging koalas prefer larger trees (Moore and Foley 2000) but this is the first time that their foraging has been linked to concentrations of specific secondary metabolites. Wild common brushtail possums (*Trichosurus vulpecula* Kerr), another eucalypt folivore, also avoid trees with high FPC concentrations (Scrivener et al. 2004). Lower elevations and warmer temperatures have previously been nominated as key predictors of koala habitat (Kavanagh et al. 1995, Cork et al. 1997). Our findings suggest that this pattern may be partially explained by a negative relationship between plant defense and temperature.

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