Available online at www.sciencedirect.com
SCIENCE DIRECT®
Phytochemistry 66 (2005) 215–221
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Differential distribution of leaf chemistry in eucalypt seedlings due to variation in whole-plant nutrient availability

Dugald C. Close [a,b,*], Clare McArthur [a,c,d], Ann E. Hagerman [e], Hugh Fitzgerald [a]

[a] Cooperative Research Centre for Sustainable, Production Forestry, GPO Box 252-12, Private Bag 12, Hobart, Tasmania, 7001 Australia
[b] Schools of Zoology, Plant and Agricultural Sciences, University of Tasmania, Private Bag 05, Hobart, Tasmania, 7001 Australia
[c] School of Zoology, University of Tasmania, Australia
[d] School of Biological Sciences, The University of Sydney, NSW, 2006 Australia
[e] Department of Chemistry and Biochemistry, Miami University, Oxford, OH 45056, USA

Received 21 July 2004; received in revised form 10 November 2004
Available online 5 January 2005

Abstract

We investigated the effects of whole-plant nutrient-availability on the degree of distribution of some plant primary and secondary (nitrogen, fibre, flavonols, gallotannins and cineole) chemicals across young, mature and old leaves of seedlings of *Eucalyptus nitens*. Four treatments that ranged from low to high nutrient-application rates resulted in mean whole-plant foliar concentrations of 0.63%, 0.85%, 1.11% and 1.82% nitrogen dry matter ($N_{\%DM}$) for treatments A, B, C and D, respectively. Within-plant distribution (across the leaf age profile of young, mature and old leaves within a eucalypt seedling) of $N_{\%DM}$ ranged from zero in treatment A to a wide range of distribution in treatment D (low $N_{\%DM}$ concentrations in old leaves to high $N_{\%DM}$ concentrations in young leaves). Similarly, the distribution of fibre ranged from zero in treatment A to a wide range of distribution in treatment D, but with high concentrations in old leaves and low concentrations in young leaves. In contrast, flavonols (weakly) and gallotannins had a wide range of distribution in treatment A (low concentrations in old leaves to high concentrations in young leaves) but were little or not distributed in the other treatments. Again in contrast, cineole was strongly distributed between old and young leaves (low concentrations in old leaves to high concentrations in young leaves) across all treatments while concentrations in mature leaves reflected one or other leaf age depending on treatment. Protein precipitable phenols in treatments A, B and C were high in young, and low in old leaves; whereas in treatment D they were low and similar between leaves of different ages.

Keywords: Antioxidants; Herbivory; Photoinhibition; Protein precipitable phenols; Seedlings; Eucalyptus

1. Introduction

Plant secondary metabolites (PSMs) play an important role in inter-trophic interactions by altering the palatability and/or quality of leaves to herbivores. Tannins, for example, can decrease the nutritive value of plant material by precipitating proteins (Robbins et al., 1987; Bernays et al., 1989; Foley et al., 1999) and essential oils provide a toxic load (Boyle et al., 2000) to large vertebrate herbivores. The availability of nutrients to plants can influence the concentration of plant secondary metabolites (Bryant et al., 1987) and therefore modify the interactions between plants and herbivores.

Allocation of finite amounts of nutrient within plants is strongly regulated via retranslocation (Saur et al., 2000) to maximise plant success through photosynthetic gain and growth (Field, 1983) and/or nutrient storage (Warren et al., 2000; Close et al., 2004). These processes result in correlations between nitrogen and both leaf age

---

[*] Corresponding author. Tel.: +61 3 62267991; fax: +61 3 62267901.
E-mail address: dugald.close@ffp.csiro.au (D.C. Close).

doi:10.1016/j.phytochem.2004.11.004

and light exposure in vertical gradients of tree canopies (Hirose et al., 1989). Older leaves, or those exposed to less light, generally contain less nitrogen than younger, high light-exposed leaves. Gradients in concentrations of PSMs with leaf age have also been reported. Young leaves, for example, can have significantly higher concentrations of PSMs than mature or old leaves (van Dam et al., 1994; Wilkens et al., 1996; Kouki and Manetas, 2002; Wallace and Eigenbrode, 2002).

Within-plant variation (or distribution) in concentrations of PSMs such as flavonols and gallotannins, in relation to light exposure and leaf age, may reflect different requirements for dealing with abiotic stresses. For example, flavonols as a UV radiation screening response (Burchard et al., 2000) and/or gallotannins as an antioxidant response to photoinhibition (Grace and Logan, 2000). Distribution of PSMs can also contribute to differential herbivory (Wilkens et al., 1996; Kouki and Manetas, 2002).

Many studies have investigated the effects of altered nutrient resource-availability on PSM chemistry and its influence on vertebrate herbivory at the whole plant level (Bryant et al., 1983, 1987; Iason and Hester, 1993; Hartley et al., 1995, 1997; Iason et al., 1996; Haukioja et al., 1998). In contrast, effects of nutrient resource-availability on the degree of within-plant distribution of leaf PSM chemistry have been rarely investigated (Wilkens et al., 1996). Such effects should be important, however, since they are likely to influence both the capacity of leaves within strata of plants to acclimate to abiotic stresses and the susceptibility of these leaves to vertebrate, and possibly invertebrate, herbivores.

Eucalyptus nitens naturally occurs on cold sites (600–1600 m above sea level, 145–155°E and 30.5–38°S; Boland et al., 1980) where it is restricted to fertile soils. Precocious seedfall following wildfire results in mass regeneration under high light conditions (Chesterfield et al., 1991) where competition for resources is intense and widespread mortality occurs. Herbivory can cause widespread mortality of *Eucalyptus* seedlings following regeneration after disturbance events (Stefano, 2003). Thus both the regeneration ecology and the plant–herbivore interactions of *E. nitens* may exert significant selection pressure on PSMs.

The aim of this study was to determine the degree to which the distribution of primary and secondary chemistry varies with leaf age in *E. nitens* seedlings as a result of variation in nutrient level applied to the whole plant. We examined nitrogen, fibre, three chemically defined groups of PSMs (gallotannins, flavonols and cineole), and one functionally defined group, the protein precipitable phenols.

2. Results

The nutrient application treatments resulted in foliar nitrogen (N) concentrations ranging from 0.62 to 0.66% DM ($N_{\%DM}$) (Fig. 1) in the lowest nutrient-application treatment (A) to 1.44–2.27 $N_{\%DM}$ in the highest treatment (D). These concentrations are considered to be deficient and above-adequate, respectively, for *Eucalyptus* (Dell et al., 1995). Within-plant variation in nitrogen ranged from zero (no difference between leaf ages) in treatment A to extreme variation in treatment D (Fig. 1; fertiliser × leaf age interaction, $F_{6,35} = 9.91$, $p < 0.0001$). In treatment D, young leaves had 37% more nitrogen than old leaves, with mature leaves intermediate.

Similar to the changing degree of within-plant variation of $N_{\%DM}$, that of neutral detergent fibre ($NDF_{\%DM}$) was zero in treatment A and greatest in treatment D (Fig. 2; fertiliser × leaf age interaction, $F_{6,35} = 6.11$, $p = 0.0005$). In contrast to $N_{\%DM}$, however, $NDF_{\%DM}$ was lowest (12.1 $NDF_{\%DM}$) in young leaves and highest (20.6 $NDF_{\%DM}$, 41% higher) in old leaves in treatment D.

The pattern and degree of within-plant variation of the two phenolic chemical groups across treatments differed to that of nitrogen and fibre. Flavonols showed only a weak interaction between leaf age and treatment (Fig. 3(a); fertiliser × leaf age interaction, $F_{6,35} = 1.98$, $p = 0.1082$). Variation ingallotannins across treatments

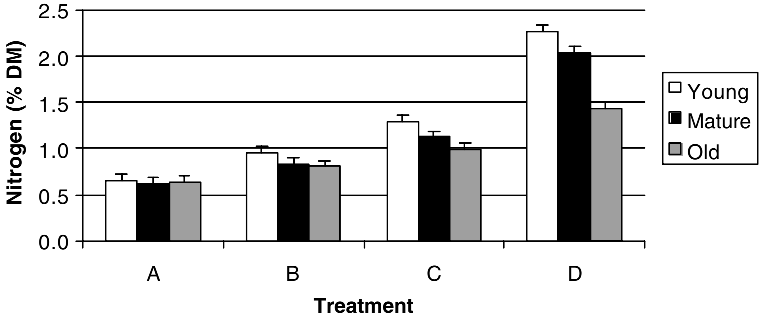

Fig. 1. Concentration of nitrogen (as % DM) in young, mature and old leaves of *Eucalyptus nitens* seedlings raised under Treatments A, B, C and D (not fertilised, fertilised every 14, 7 and 2 days, respectively for 3–4 months). Bars indicate one standard error.

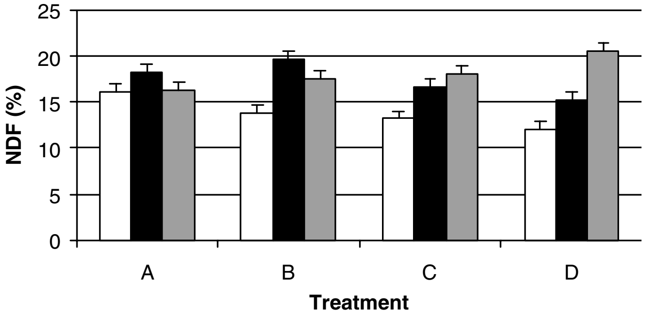

Fig. 2. Concentration of neutral detergent fibre, NDF (as % DM) in young, mature and old leaves of *Eucalyptus nitens* seedlings (see Fig. 1 for treatment details). Bars indicate one standard error.

decreased from high in treatment A (102 and 273 mg tetragalloylglucose equivalents g DM^{-1} in old and young leaves, respectively) to little or none in the other treatments (Fig. 3(b); fertiliser × leaf age interaction, $F_{6,35} = 2.56$, $p = 0.0462$).

Structural heterogeneity within a broad class of compounds, such as gallotannins, may lead to differences in biological activity that are more suited to functional assays than chemical analysis. We estimated protein precipitation activity of gallotannins, and expressed protein precipitable phenols as a proportion of total phenols (Hagerman and Butler, 1978). The proportion of phenols that are precipitated by protein was higher (1.7- to 2.3-fold) between young and old leaves, but variable in mature leaves, in treatments A, B and C (Fig. 3(c); leaf age effect of $F_{2,35} = 5.53$, $p = 0.0106$). However, percent protein precipitable phenols were low and similar between leaf ages in treatment D.

In contrast to the other chemical constituents, cineole varied greatly (2.5- to 12-fold) between young and old leaves in all fertiliser-application treatments, although the pattern of variation was not identical (Fig. 4; fertiliser × leaf age interaction; $F_{6,35} = 38.13$, $p < 0.0001$). In all treatments, young leaves contained much more cineole than old leaves. However, the relative amount of cineole in mature leaves differed across treatments. At one extreme cineole in mature leaves was similar to old leaves (treatment A), while at the other it was similar

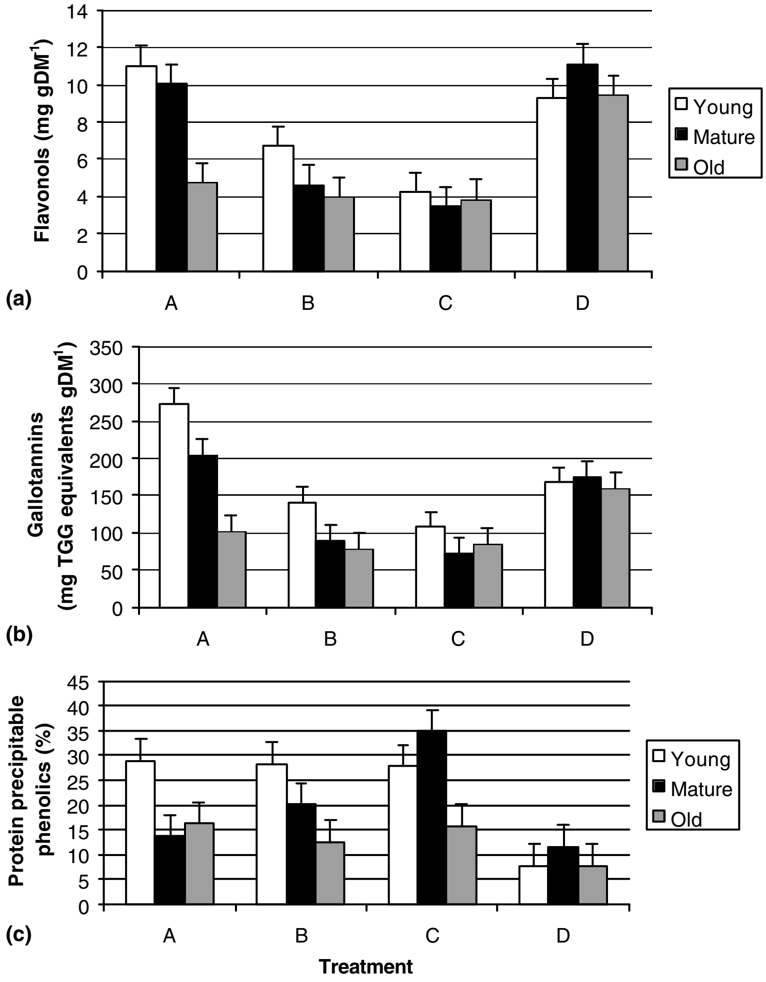

Fig. 3. Concentration of flavonols (mg g DM^{-1}) (a), gallotannins (mg tetra-galloylglucose [TGG] equivalents g DM^{-1}) (b), and protein precipitable phenols (%) (c) in young, mature and old leaves of *Eucalyptus nitens* seedlings (see Fig. 1 for treatment details). Bars indicate one standard error.

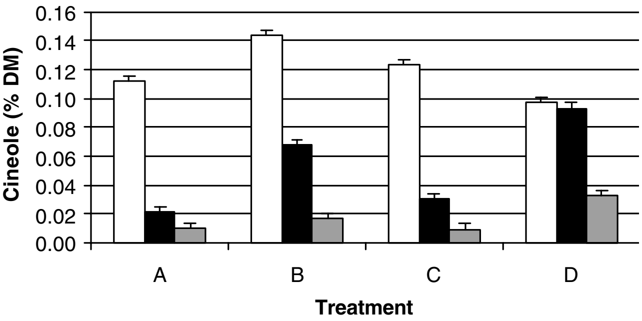

Fig. 4. Concentration of cineole (as % DM) in young, mature and old leaves of *Eucalyptus nitens* seedlings (see Fig. 1 for treatment details). Bars indicate one standard error.

to young leaves (treatment D) (Fig. 4). The high cineole concentrations of mature leaves in treatment D may have occurred because, although the leaves were fully expanded and thus were sampled as mature, they were actually physiologically "young", reflecting the rapid growth rate of this treatment (Close et al., 2003).

3. Discussion

Lack of within-plant variation in $N_{\%DM}$ (across leaf ages) in treatment A (ca. 0.6%) suggests that this nitrogen concentration is the minimum required by *E. nitens* in order for photosynthetic carbon gain to supply maintenance costs. This is consistent with the observation that abscission of old leaves was commonly observed in treatment A seedlings, but not in the other treatments. Increased variation in $N_{\%DM}$ across leaf ages with increased whole plant nutrient-availability presumably represents a balance between allocation to mature leaves, which have the greatest contribution to plant carbon gain (Close et al., 2004) and to young leaves, where N-containing proteins are involved in active leaf expansion.

Interestingly, despite the changing degree of within-plant variation in neutral detergent fibre ($NDF_{\%DM}$) across treatments, concentrations were similar between treatments A–D when expressed on a whole-plant basis (Close et al., 2003). This demonstrates that whole-plant analyses do not provide complete information about the within-plant levels of some constituents.

The distribution of gallotannins in treatment A represented a 2.5-fold difference between young and old leaves. We found no within-plant variation in gallotannin concentration in treatment D but high variation in treatment A when $N_{\%DM}$ was at a critical minimum concentration. One explanation for this pattern in treatment A is that gallotannins were protecting leaves from photoinhibition by functioning as antioxidants (Hagerman et al., 1998) and a higher concentration was required to protect young than old leaves. Flavonols, which have a well established antioxidant function (Miliauskas et al., 2004), showed similar trends, supporting this contention. Young leaves are often more susceptible to photoinhibition than mature or old ones (Krause et al., 1995) due to both morphological and physiological development differences as well as increased exposure to light, in the present case related to their higher location on the seedlings. For similar reasons, self-shading of old leaves may decrease their susceptibility to photoinhibition. We suspect that this susceptibility of young leaves was augmented in treatment A due to the concomitant relatively low photochemical efficiency (F_v/F_m) associated with low $N_{\%DM}$ concentrations (Close et al., 2003). An alternative explanation for the observed distribution of gallotannins, particularly in treatment A, is that it represents within-plant differences in allocation to defence against herbivores (Wilkens et al., 1996). In this case, defence compounds are argued to maximise fitness (McKey, 1974) and young leaves of relatively high "value" are preferably defended (Harper, 1989) as predicted by the optimal defence theory (van Dam et al., 1996). We suggest that this argument is less convincing, because gallotannin concentration would then be expected to be highest in young leaves of all four nutrient-application treatments and this did not occur.

The differences in percent protein precipitable phenols between young and old leaves, up to a critical high $N_{\%DM}$ concentration of treatment D, may indicate chemical defence of young (and mature in the case of treatment C) leaves against herbivores, as protein precipitation can decrease the nutritive value of plant material to vertebrate herbivores (Robbins et al., 1987; Bernays et al., 1989; Foley et al., 1999). In treatment D, however, the high nitrogen status of leaves may negate the value of investment into chemical defence through protein precipitation.

In contrast to the other chemical compounds described above, the reasonably consistent within-plant distribution pattern of cineole across all treatments may indicate a function in chemical defence against vertebrate herbivores, since cineole was consistently high in vulnerable, young (and "mature" in the case of treatment D) leaves across treatments irrespective of $N_{\%DM}$ status. Although *E. nitens* inherently produces low concentrations of cineole and total essential oils relative to other species of *Eucalyptus* (total essential oils of 0.75%, 3.9% and 5.2% DM for *E. nitens*, *E. globulus* and *E. regnans*, respectively; Li and Madden, 1995), its ingestion still represents a toxic load to vertebrate herbivores such as the common arboreal folivore, the brushtail possum (*Trichosurus vulpecula*) (Boyle et al., 2000), and can affect their feeding behaviour at both high (Wiggins et al., 2003) and low concentrations (GR Iason, C McArthur and NL Wiggins unpublished results).

4. Conclusion

Our results show that variation in nutrient availability at the whole-plant scale results in foliar $N_{\%DM}$ status of *E. nitens* seedlings ranging from deficient through to above-adequate. Furthermore, variation in nutrient availability at the whole-plant scale differentially affects the distribution of $N_{\%DM}$, $NDF_{\%DM}$, gallotannins (and flavonols), and cineole, and the percent protein precipitable phenols across leaves of different ages. This distribution is likely to differentially influence the capacity of leaves within plants to acclimate to abiotic stresses and/or to influence their susceptibility to herbivores. We are currently investigating some of these potential effects.

5. Experimental

5.1. Plant material and treatments

Seedlings of *Eucalyptus nitens* (Deane and Maiden) Maiden were raised from Hastings Seed Orchard seedlot (Forestry Tasmania improved seed) in 115 cm^3 plugs at Forestry Tasmania's tree nursery, Perth, Tasmania (41°34′S, 147°11′E). The potting mix was saturated with Aquasol® every 4 days (N:P:K 23:4:18; solution concentration 1 g L^{-1}) for 4 months. On 5 March 2001, seedlings were moved to an outdoor growing area. Seedling trays were randomly allocated to one of four nutrient-application treatments: (1) non-fertilised (Treatment A); (2) fertilised every 14 days (Treatment B); (3) fertilised every 7 days (Treatment C); and (4) fertilised every 2 days (Treatment D) for a further 3–4 months. Each seedling received approximately 1.25 mg Peters Excel® (N:P:K 20:2.2:6.6; solution concentration 1 g L^{-1}) when fertilised. Each treatment was replicated twice and randomly allocated within two blocks orientated east–west.

5.2. Chemical analyses of seedlings

Twelve seedlings (eight per block) per treatment were randomly sampled across blocks daily, as part of another study (described in Close et al., 2003) and stored at −20 °C before chemical analyses. Seedlings were then randomly allocated to three replicate samples ($n = 3$) within each treatment, each replicate consisting of four seedlings, for each of the following analyses: nitrogen (% DM; $N_{\%DM}$), fibre (neutral detergent fibre; $NDF_{\%DM}$), flavonols and gallotannins, which are both phenols, and cineole. Cineole represents 40–50% of the total essential oils in *E. nitens* (31 essential oils have been identified in *E. nitens*, see Li and Madden, 1995 for details), changes of which have been shown to be proportional to changes in total essential oils (Close et al., 2003). Gallotannins were measured by a chemical method (HPLC–MS) and with a functional assay (protein precipitation). These three secondary constituents were chosen for analysis because they may be important in protecting plants against abiotic stresses such as photoinhibition and/or in affecting herbivory (Grace and Logan, 2000; Close et al., 2003; Boyle et al., 2000).

Leaves of seedlings were separated from the stems and allocated to three fractions based on age; young (not yet fully expanded, typically at nodes 1 and 2), mature (fully expanded, leaves typically at nodes 3 and 4) and old leaves (smaller, thicker leaves fully expanded during an earlier, lower temperature period and typically at nodes 5 and 6). The use of multiple seedlings within each replicate sample was necessary to provide sufficient leaf material for chemical analyses.

Leaves were dried to constant weight at 65 °C, finely ground and analysed for total nitrogen as described by Lowther (1980). Neutral detergent fibre was analysed in an ANKOM^{220} fibre analyser (ANKOM Technology, Fairport, NY) and expressed as % total dry matter (DM) as described in Close et al. (2003). Acid detergent and acid lignin results are not presented here due to strong correlations found between these fractions and neutral detergent fibre in *E nitens* in a previous study (Close et al., 2004). For measurement of flavonols and gallotannins leaves were frozen fresh at the time of sampling, and were subsequently extracted in acidified methanol, analysed by HPLC and expressed as mg g DM^{-1} as described by Close et al. (2001). Essential oils were extracted from leaves that were frozen fresh at sampling, in dichloromethane with heptadecane as an internal standard. Cineole was analysed by combined gas chromatography–mass spectrometry (GC–MS) and expressed as % total DM as described in McArthur et al. (2003). The method of Hagerman and Butler (1978) was optimised for the measurement of protein precipitable phenols (expressed as % of total phenols) in *E. nitens* extracts. The acid butanol assay (Porter et al., 1986) was used to evaluate anthocyanins and proanthocyanins in methanol extracts of stressed or unstressed *E. nitens* leaf tissue. Samples were spectrophotometrically evaluated immediately after adding the Porter reagent, and again after heating the mixtures (80 °C, 50 min). As expected, the control sample of purified *Sorghum* procyanidin released cyanidin ($l_{\max}$ 550 nm) only after heating. Extracts of *E. nitens* yielded pelargonidin ($l_{\max}$ 535 nm) upon addition of the Porter reagent, with no additional pigment production upon heating. We conclude that *E. nitens* contains a pelargonidin-derived anthocyanin, but no proanthocyanidins, similar to the conclusions of Hillis (1966).

5.3. Statistical analyses

We used the general linear model procedure (PROC GLM, (SAS Institute Inc., 1989)) to test for effects of fertiliser (nutrient-application) and leaf age on seedling chemical constituents. A term for block could not be included in the statistical models as seedlings were sampled across blocks for replicates in the chemical analyses (described earlier). Any variation associated with block was therefore incorporated into the error term. This still enabled us to adequately test for the effects of interest, but may have reduced the power of the test if variation between blocks was high.

Acknowledgements

We thank Dr. Karen Barry for access to tetragalloylglucose standard data. AE Hagerman's visiting fellowship was funded by the Cooperative Research Centre for Sustainable Production Forestry and DC Close was funded by an Australian Research Council postdoctoral fellowship.

References

Bernays, E.A., Cooper Driver, G., Bilgener, E.D., 1989. Herbivores and plant tannins. Advanced Ecological Research 19, 263–302.

Boland, D.J., Brooker, M.I.H., Turnbull, J.W., 1980. Eucalyptus Seed. CSIRO, Canberra, Australia.

Boyle, R., McLean, S., Davies, N.W., 2000. Biotransformation of 1,8-cineole in the brushtail possum (*Trichosurus vulpecula*). Xenobiotica 30, 915–932.

Bryant, J.P., Chapin III, F.S., Klein, D.R., 1983. Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. Oikos 40, 357–368.

Bryant, J.P., Chapin, F.S. III, Reichardt, P.B., Clausen, T.P., 1987. Response of winter chemical defense in Alaska paper birch and green alder to manipulation of plant carbon/nutrient balance. Oecologia 72, 510–514.

Burchard, P., Bilger, W., Weissenböck, G., 2000. Contribution of hydrocinnamates and flavonoids to epidermal shielding of UV-A and UV-B radiation in developing rye primary leaves as assesssed by ultraviolet-induced chlorophyll fouirescence measurements. Plant Cell and Environment 23, 1373–1380.

Chesterfield, E.A., McCormick, M.J., Hepworth, G., 1991. The effect of low root temperature on the growth of mountain forest eucalypts in relation to the ecology of *Eucalyptus nitens*. Proceedings of the Royal Society of Victoria 103, 67–76.

Close, D.C., Davies, N.W., Beadle, C.L., 2001. Temporal variation of tannins (galloylglucoses), flavonols and anthocyanins in leaves of *Eucalyptus nitens* seedlings: implications for light attenuation and antioxidant activities. Australian Journal of Plant Physiology 28, 269–278.

Close, D.C., McArthur, C., Paterson, S., Fitzgerald, H., Walsh, A., Kincade, T., 2003. Photoinhibition: a link between effects of the environment on eucalypt leaf chemistry and herbivory. Ecology 84, 2952–2966.

Close, D.C., Battaglia, M., Davidson, N.J., Beadle, C.L., 2004. Within canopy gradients of nitrogen and photosynthetic activity of *Eucalyptus nitens* and *Eucalyptus globulus* in response to nitrogen nutrition. Australian Journal of Botany 52, 133–140.

Dell, B., Malajczuk, N., Grove, T.S., 1995. Nutrient Disorders in Plantation Eucalypts. The Australian Centre for International Agricultural Research, Canberra, Australia.

Field, C., 1983. Allocating leaf nitrogen for the maximization of carbon gain: leaf age as a control on the allocation program. Oecologia 56, 341–347.

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

Grace, S.C., Logan, B.A., 2000. Energy dissipation and radical scavenging by the plant phenylpropanoid pathway. Philosophical Transactions of the Royal Society of London. Series B. 355, 1499–1510.

Hagerman, A.E., Butler, L.G., 1978. Protein precipitation method for the quantitative determination of tannins. Journal of Agricultural and Food Chemistry 26, 809–812.

Hagerman, A.E., Riedl, K.M., Jones, G.A., 1998. High molecular weight plant polyphenolics (tannins) as biological antioxidants. Journal of Agricultural and Food Chemistry 46, 1887–1892.

Harper, J.L., 1989. The value of a leaf. Oecologia 80, 53–58.

Hartley, S.E., Nelson, K., Gorman, M., 1995. The effect of fertilizer and shading on plant chemical composition and palatability to Orkney voles, *Microtus arvalis orcadensis*. Oikos 72, 79–87.

Hartley, S.E., Iason, G.R., Duncan, A.J., Hitchcock, D., 1997. Feeding behaviour of Red Deer (*Cervus elaphus*) offered Sitka Spruce saplings (*Picea sitchensis*) grown under different light and nutrient regimes. Functional Ecology 11, 348–357.

Haukioja, E., Ossipov, V., Koricheva, J., Honkanen, T., Larsson, S., Lempa, K., 1998. Biosynthetic origin of carbon-based secondary compounds: cause of variable responses of woody plants to fertilization. Chemoecology 8, 133–139.

Hillis, W.E., 1966. Variation in polyphenol composition within species of *Eucalyptus* l'herit. Phytochemistry 5, 541–556.

Hirose, T., Werger, M.J.A., Van Rheenen, J.W.A., 1989. Canopy development and leaf nitrogen distribution in a stand of *Carex acutiformis*. Ecology 70, 1610–1618.

Iason, G.R., Hester, A.J., 1993. The response of heather (*Calluna vulgaris*) to shade and nutrients – predictions of the carbon–nutrient balance hypothesis. Journal of Ecology 81, 75–80.

Iason, G.R., Duncan, A.J., Hartley, S.E., 1996. Feeding behaviour of red deer (*Cervus elaphus*) on Sitka spruce (*Picea sitchensis*): the role of carbon–nutrient balance. Forest Ecology and Management 88, 121–129.

Kouki, M., Manetas, Y., 2002. Toughness is less important than chemical composition of *Arbutus* leaves in food selection by *Poecilimon* species. New Phytologist 154, 399–407.

Krause, G.H., Virgo, A., Winter, K., 1995. High susceptibility to photoinhibition of young leaves of tropical forest trees. Planta 197, 583–591.

Li, H., Madden, J.L., 1995. Analysis of leaf oils from a *Eucalyptus* species trial. Biochemical Systematics and Ecology 23, 167–177.

Lowther, J.R., 1980. Use of a single sulphuric acid–hydrogen peroxide digest for the analysis of *Pinus radiata* needles. Communications in Soil Science and Plant Analysis 11, 178–188.

McArthur, C., Marsh, N., Close, D.C., Walsh, A., Paterson, S., Fitzgerald, H., Davies, N.W., 2003. Nursery conditions affect seedling chemistry, morphology and herbivore preferences for *Eucalyptus nitens*. Forest Ecology and Management 176, 585–594.

McKey, D., 1974. Adaptive patterns in alkaloid physiology. American Naturalist 108, 305–320.

Miliauskas, G., Venskutonis, P.R., Van Beek, T.A., 2004. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chemistry 85, 231–237.

Porter, L.J., Hrstich, L.N., Chan, B.G., 1986. The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. Phytochemistry 25, 223–230.

Robbins, C.T., Hanley, T.A., Hagerman, A.E., Hjeljord, O., Baker, D.L., Schwartz, C.C., Mautz, W.W., 1987. Role of tannins in defending plants against ruminants: reduction in protein availability. Ecology 68, 98–107.

SAS Institute Inc., 1989. SAS/STAT User's Guide, Version 6, fourth ed. SAS Institute, Cary, NC, USA.

Saur, E., Nambiar, E.K.S., Fife, D.N., 2000. Foliar nutrient retranslocation in *Eucalyptus globulus*. Tree Physiology 20, 1105–1112.

Stefano, J., 2003. Mammalian browsing in the Mt Cole State Forest: defining a critical browsing level and assessing the effect of multiple browsing events. Australian Forestry 66, 287–293.

van Dam, N.M., Verpoorte, R., van der Meuden, E., 1994. Extreme differences in Pyrrolizidine alkaloid levels between leaves of *Cynoglossum officinale*. Phytochemistry 37, 1013–1016.

van Dam, N.M., De Jong, T.J., Iwasa, Y., Kubo, T., 1996. Optimal distribution of defences: are plants smart investors?. Functional Ecology 10, 128–136.

Wallace, S.K., Eigenbrode, S.D., 2002. Changes in the glucosinolate–myrosinase defense system in *Brassica juncea* cotyledons during seedling development. Journal of Chemical Ecology 28, 243–256.

Warren, C.R., Adams, M.A., Chen, Z-L., 2000. Is photosynthesis related to concentrations of nitrogen and Rubisco in leaves of Australian native plants. Australian Journal of Plant Physiology 27, 407–416.

Wiggins, N.L., 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, 1423–1441.

Wilkens, R.T., Spoerke, J.M., Stamp, N.E., 1996. Differential responses of growth and two soluble phenolics of tomato to resource availability. Ecology 77, 247–258.