

Ecophysiology

Light alters the allocation of nitrogen to cyanogenic glycosides in *Eucalyptus cladocalyx*

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Abstract. The effect of light on the partitioning of resources between photosynthesis and chemical defence was studied in *Eucalyptus cladocalyx* F. Muell. This species allocates up to 15% of leaf nitrogen to the constitutive cyanogenic glycoside, prunasin, making it an ideal system for studying resource allocation. By controlling the level of leaf nitrogen we were able to test the hypothesis that light limitation would result in the effective reallocation of nitrogen from the defensive to the photosynthetic apparatus. Seedlings were grown in full light or shade and supplied with 1.5 mM or 6 mM nitrogen in a 2 × 2 factorial design. We found that shading effected a decrease in the

concentration of the cyanogenic glycoside, prunasin, and little if any change in the concentration of carbon-based secondary metabolites (total phenolics and condensed tannins). There was also significantly less prunasin, relative to total leaf nitrogen, chlorophyll concentration and carbon assimilation rates, when grown plants were grown in shade, particularly when there was an ample supply of nitrogen. This pattern is likely to be the result of relative changes in the energetic and resource costs of photosynthesis and defensive compounds at different photon flux densities.

Keywords. Phenolics - Herbivore defence - Plant secondary metabolites - Nitrogen - Cyanide

Introduction

Cyanogenesis is a widespread chemical defence mechanism with an estimated 11% of all plant species possessing the trait (Jones 1998). It involves the release of toxic cyanide when tissue is ruptured, which can either deter or kill a herbivore (Nahrstedt 1985; Jones 1988). Because of the effectiveness of cyanogenesis in deterring herbivores, especially against generalist herbivores (Gleadow and Woodrow 2002a), and because of the sizeable amount of nitrogen that can be allocated to cyanogenic compounds, it is of importance to understand inter alia how the capacity for cyanogenesis is regulated by environmental variables. Such an understanding has become even more important with the recent "engineering" of cyanogenesis into an otherwise non-cyanogenic plant (*Arabidopsis thaliana*; Tattersall et al. 2001). This transgenic plant showed greater resistance to a common herbivore of this species and, consequently, it is very likely that we will see attempts to engineer cyanogenesis into a range of crop species.

Our interest in this paper is in the way in which changes in light, and thus photosynthesis, affect the allocation of a limiting resource (nitrogen) between photosynthetic proteins and nitrogen-based defence (cyanogenic glycosides). Previous studies of this issue have yielded conflicting results. Studies of the fern *Pteridium aquilinum* (Cooper-Driver et al. 1977; Schreiner et al. 1984) and white clover (*Trifolium repens*; Vickery et al. 1987), for example, showed that individuals from shady areas were more cyanogenic than those growing in open sites. In contrast, Niedezwied-Siegen and Gierasimiul (2001) found a decrease in cyanogenic glycoside content with shade in flax (*Linum usitatissimum*). It is difficult, however, to interpret any of these results in terms of resource allocation because leaf nitrogen was not measured, and there is increasing evidence that nitrogen supply stimulates cyanogenic glycoside synthesis (e.g. Gleadow et al. 1998; Gleadow and Woodrow 2000). Since nitrogen concentration tends to increase in leaves of shaded plants (Waterman et al. 1984; Hoft et al. 1996), it is possible that an increase in cyanogenic glycoside concentration in the shade will be an indirect response to leaf nitrogen concentration rather than a direct response to reduced photon supply.

Studies of alkaloid production under different light conditions have also produced conflicting results. Hoft et al. (1996), for example, found an increase in the concentration of alkaloids with shading in the evergreen tropical African tree (*Tabernaemontana pachysiphon*). By contrast, Ralphs et al. (1998), in a study of the herb *Delphinium barbeyi*, found a decrease in alkaloid concentration in shade-grown plants. Importantly, nitrogen supply and leaf nitrogen concentration were not controlled in either of these experiments.

The aim of this study is to quantify the effect of light on the allocation of nitrogen to defence and photosynthesis. To do this, we used *Eucalyptus cladocalyx* var. *nana* F. Muell because it allocates a relatively high proportion of leaf nitrogen to cyanogenic glycosides (up to 15% in leaf tips) and its response to nitrogen is relatively well understood (e.g. Gleadow et al. 1998; Gleadow and Woodrow 2000). In addition, *E. cladocalyx* contains moderately high concentrations of carbon-based defence compounds (phenolics; Gleadow 1999). Our hypothesis is that photosynthetic gain under low light will be maximised by (1) nitrogen reallocation from cyanogenic glycosides to the photosynthetic system, and (2) an increase in less "expensive" carbon-based defence.

Materials and methods

Plant material and growth conditions

Eucalyptus cladocalyx var. *nana* seedlings (6 weeks old) were transplanted into 1.5-l pots containing a 1:1 mixture of sterilised sand and vermiculite and transferred to a glasshouse. Seedlings were randomly assigned to light and nutrient treatments in a 2×2 factorial design ($n=10$). To alter the

supply of nitrogen, pots were flushed once each day with one-quarter strength Hoagland's solution containing either 6 mM or 1.5 mM nitrogen, supplied as nitrate and ammonia (6:1, mole:mole) with sodium as the balancing cation (see Gleadow et al. 1998). A low light environment was created over half the plants by suspending shade cloth 1 m above the plants, which extended 0.5 m beyond the experimental area. Photosynthetic photon flux density (PPFD) was measured at the height of the canopy every 15 min for 8 weeks. Average daytime PPFD (± 1 SE) for the full-light treatment was $440 \pm 293 \mu\text{mol m}^{-2} \text{s}^{-1}$ and $176 \pm 131 \mu\text{mol m}^{-2} \text{s}^{-1}$ under the shade cloth with a natural photoperiod of approximately 12 h. Maximum daily PPFD in the full light was $1041 \pm 7 \mu\text{mol m}^{-2} \text{s}^{-1}$ compared with $236 \pm 76 \mu\text{mol m}^{-2} \text{s}^{-1}$ in the low light treatment. Plants were rotated every week within each light treatment. Air temperature and relative humidity, measured every 15 min with a Spectrum temperature/humidity sensor (Datatronics, Bayswater, Vic. Australia), were not significantly different between light treatments with average temperatures (day/night, ± 1 SE) of $23.9 \pm 0.5 / 23.6 \pm 0.03$ °C and $24.8 \pm 0.08 / 23.8 \pm 0.5$ °C in the full light and shade, respectively. Average relative humidity was $55 \pm 6\%$ in the full light and $52 \pm 1\%$ in the shade. Leaf temperature, measured on representative plants from each treatment with a type T thermocouple every 10 min for 10 days was not significantly different between the four light-nutrient treatments (data not shown).

Plant harvesting and gas exchange

Plants were harvested after 7 weeks and sorted into leaves, stems and roots. Leaves were classified as tips (newly formed leaves) and fully expanded leaves. Leaf and stem material was frozen in liquid nitrogen and freeze dried. Roots were oven-dried at 60 °C. Tissue was ground to a fine powder using an Ultramat 2 Dental Grinder (Southern Dental Industries Ltd., Melbourne, Victoria, Australia; tips) or an IKA Labortechnik A10 Analytical Mill (Janke and Kunkel, Stanfen, Germany) depending on sample size.

The steady state net CO₂ assimilation rate (A_{max}) was measured on five seedlings from each treatment (20 plants) at a saturating PPFD ($800 \mu\text{mol m}^{-2} \text{s}^{-1}$) and a CO₂ mole fraction of $360 \mu\text{mol mol}^{-1}$ in the week prior to harvesting. Leaf temperature was 25 °C, and the ambient relative humidity was 50%. The concentration of Rubisco active sites was calculated from A_{max} using the equations of Woodrow and Berry (1988), and expressed on a dry weight basis using the overall leaf mass per area (LMA) for the plant.

Chemical analyses

Chlorophyll

Chlorophyll was extracted from ground freeze-dried leaf tissue (0.020 g) with cold 80% (v/v) acetone (1 ml) and vortexing for 30 s. The supernatant was collected after centrifuging the mixture at 10,000 g for 3 min. The pellet was re-extracted a further two times and the supernatant from the three extractions pooled. Previous experiments showed that 98% of chlorophyll is recovered after three extraction volumes (R.M. Gleadow, unpublished data). Absorbance of the extract was measured at 647 nm and 664 nm and the concentration of chlorophyll *a* and chlorophyll *b* calculated using the equations of Jeffrey and Humphrey (1975).

Cyanogenic glycosides

The cyanogenic glycoside concentration was measured by hydrolysing the glycoside and trapping the evolved cyanide in NaOH (Brinker and Seigler 1989). Freeze dried tissue (0.010 g) was incubated with 1 ml of 0.1 M citrate buffer (pH 5.5) in a sealed glass vial containing an open vial containing 1 M NaOH (500 μ l) at 37 °C for 24 h (Gleadow and Woodrow 2000). Exogenous β -glucosidase from

almond (*Prunus amygdalis* (L.) Benth. & Hook.; β -D-glucoside glucohydrolase; EC 3.2.1.21, Sigma)

was added to the buffer (1.12 units ml⁻¹) to ensure complete conversion to cyanide (Gleadow et al. 1998). The amount of cyanide in the NaOH was determined using the method of Brinker and Seigler (1989) as modified by Woodrow et al. (2002). The cyanogenic glycoside concentration, measured by evolved cyanide, will be referred to as "cyanide", and 1 mg of cyanide is equivalent to 11.81 mg of prunasin, the cyanogenic glycoside found in *E. cladocalyx* (Finnemore et al. 1935).

Total phenolics

Finely ground, freeze-dried leaf samples (0.050 g) were extracted using cold 50% (v/v) acetone (Gleadow and Woodrow 2002b). The total phenolic concentration of 20 μ l aliquots was determined using Folin-Ciocalteu's reagent (Cork and Krokenberger 1991), with gallic acid as the standard. Further aliquots (100 μ l) of the 50% acetone extract were analysed for condensed tannin concentration using (+)-catechin as the standard (Julkunen-Titto 1985; Gebauer et al. 1998).

Total nitrogen

Total nitrogen concentration of 0.005 g samples of freeze-dried tissue was determined with a Perkin Elmer 2400 Series II CHNS/O Analyzer (Perkin-Elmer Pty. Ltd., Victoria) with acetanilide (Perkin Elmer no. 0240-1121) as the standard.

Statistical analyses

Statistical analyses were conducted using Minitab Release 13 (Minitab, Pasadena, USA). Data were tested for normality by the Kolmogorov-Smirnov test and Levene's method was used to test for homogeneity of variance (Sokal and Rohlf 1995). Log₁₀ transformations were performed on the cyanide data to satisfy the assumptions of normality. Data were then analysed using ANOVA. Regression lines were calculated using SigmaPlot 2000.

Results

Growth, biomass allocation and photosynthesis

The growth and biomass allocation responses of 3-month old seedlings of *Eucalyptus cladocalyx* to light and nitrogen supply were similar to those found for other tree species (e.g. Wong et al. 1992; Mooney et al. 1995; Hoft et al. 1996; Mutikainen et al. 2000). Plant growth was strongly inhibited by low light when nitrogen supply was high ($P < 0.001$), and also by low compared with high nitrogen ($P < 0.001$; Table 1). The light effect, however, was absent when the nitrogen supply was also restricted. Nitrogen and light also affected the differential allocation of biomass. The root-to-shoot ratio was significantly higher in plants supplied with low nitrogen at both light levels ($P < 0.001$). Plants grown in shade had a lower root to shoot ratio when nitrogen supply was limited ($P < 0.01$), but the ratio was higher when nitrogen supply was also high ($P < 0.05$; Table 1). Consistent with this,

shade- and high nitrogen-grown plants had more leaf area relative to total biomass (i.e. a higher leaf area ratio, LAR) than those grown in full light ($P<0.001$). LMA was also higher in plants grown in the full light compared with shaded plants, particularly when nitrogen supply was limited ($P<0.05$; Table 1).

Table 1. Growth and biomass partitioning in *Eucalyptus cladocalyx* seedlings grown in full sunlight or shade (40% full light), and supplied with 1.5 mM or 6 mM nitrogen. Least significant differences (LSD) can be used to compare significant differences between means within the row at the 95% probability level

	Full light		Shade		LSD _{0.05}
	1.5 mM N	6 mM N	1.5 mM N	6 mM N	
Height (cm)	14.68	19.59	14.40	16.33	1.11
Biomass (g)	2.15	4.25	0.98	1.25	0.47
Root:shoot	0.59	0.31	0.44	0.39	0.06
LMA (g m ⁻²) ^a	75.3	50.6	43.5	43.5	4.9
LAR (m ² g ⁻¹) ^b	0.68×10^{-2}	1.16×10^{-2}	1.09×10^{-2}	1.32×10^{-2}	0.042×10^{-2}

^aLMA was measured on fully expanded leaves

^bLAR is the area of fully expanded leaves as a proportion of total plant biomass

Similar to the growth responses, A_{\max} was reduced by some 24% in shade at high nitrogen ($P<0.05$), but there was no shade effect at low nitrogen. We also estimated the concentration of Rubisco active sites using the A_{\max} data, the measured intercellular CO₂ concentrations and respiration rates, and the assumption that Rubisco was 90% active under light saturation (Woodrow and Berry 1988; Table 2). The active site concentrations were approximately proportional to the A_{\max} values, indicating a reduction in Rubisco concentration of some 32% in shade under high nitrogen. Leaf chlorophyll concentration (mg m⁻²), however, increased in shade under both nitrogen treatments (Table 2; $P<0.0001$), and it was also sensitive to nitrogen supply, decreasing markedly at low nitrogen ($P<0.001$). The relative changes were similar when chlorophyll was expressed on a dry weight basis (data not shown). Combining both light treatments, plants grown at high nitrogen had a higher proportion of chlorophyll *a*, compared with chlorophyll *b* ($P<0.001$). The effect of light was only significant at high nitrogen, with chlorophyll *b* concentration enhanced in shade-grown plants (i.e. nitrogen × light interaction was significant, $P<0.001$).

Table 2. Maximum carbon assimilation rate (A_{\max}), and the concentrations of active sites of Rubisco and chlorophyll in fully expanded leaves of *Eucalyptus cladocalyx* seedlings grown in full sunlight or shaded (40% full light) and supplied with 1.5 mM or 6 mM nitrogen. Least significant differences (LSD_{0.05}) can be used to compare significant differences between means within the row at the 95% probability level

	Full light		Shade		LSD _{0.05}
	1.5 mM N	6 mM N	1.5 mM N	6 mM N	
A_{\max}^a ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	7.83	12.18	8.37	9.24	1.02
Rubisco active sites ^b ($\mu\text{mol m}^{-2}$)	15.70	23.02	16.60	18.07	n.a.
Chlorophyll (g m^{-2})	0.59	1.85	1.30	2.47	0.26
Chlorophyll <i>a: b</i>	2.82	3.33	2.76	2.94	0.24

^a Assimilation measurements were made at saturating PPFD ($800 \mu\text{mol m}^{-2} \text{s}^{-1}$) and $25 \text{ }^\circ\text{C}$ ($n=5$)

^b The concentration of Rubisco was calculated from the assimilation data using the equations of Woodrow and Berry (1988)

Chemical composition

Nitrogen

By controlling the supply of nitrogen to the plants, we attempted to minimise the effect of light on leaf nitrogen concentration. Nevertheless, we found that in the low nitrogen treatment, leaf nitrogen was significantly higher in shade compared to full sunlight ($P<0.01$), whereas in the high nitrogen treatment leaf nitrogen was slightly lower ($P<0.05$; Fig. 1a). Also, leaf nitrogen was significantly higher in plants grown at high compared with low nitrogen ($P<0.001$). Combining both light treatments, the mean leaf nitrogen concentration (± 1 SE) was $37.10 \pm 0.89 \text{ mg g}^{-1}$ dry wt in seedlings supplied with high nitrogen compared with $18.36 \pm 0.74 \text{ mg g}^{-1}$ dry wt for those supplied with low nitrogen.

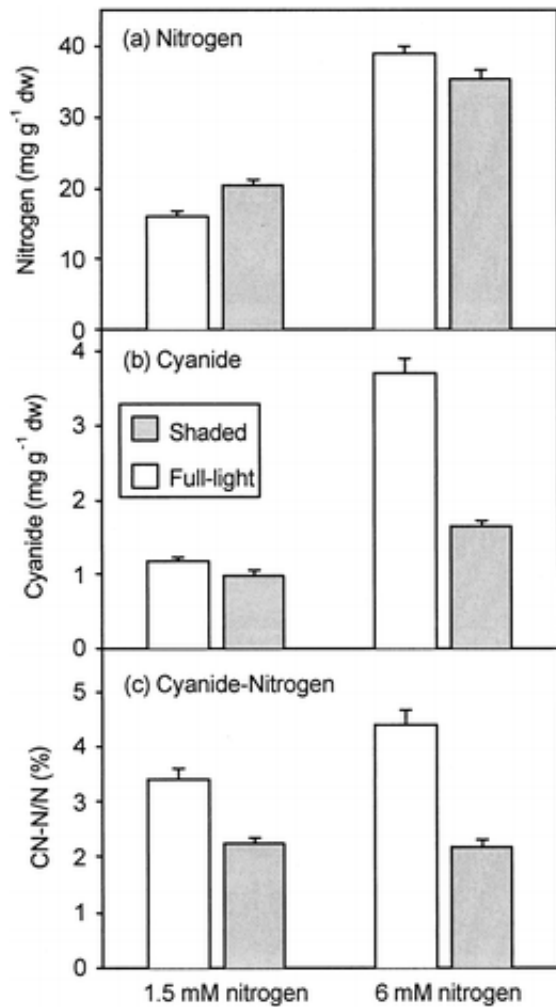


Fig. 1. Concentration of total leaf nitrogen (a), concentration of prunasin, measured as evolved cyanide (b), and the proportion of nitrogen allocation to cyanide (c) in fully expanded leaves of *Eucalyptus cladocalyx* seedlings grown in full sunlight (*open*) or 40% full light (*shaded*) and supplied with 1.5 mM or 6 mM nitrogen. Values are the mean of 10 measurements (± 1 SE)

Cyanogenic glycosides

Both light and nitrogen supply affected cyanogenic glycoside concentration in fully expanded leaves (Fig. 1b). For plants grown in full light, the mean prunasin concentration (measured as evolved cyanide ± 1 SE) was 3.70 ± 0.21 mg CN g⁻¹ dry wt and 1.18 ± 0.07 mg CN g⁻¹ dry wt for the high and low nitrogen treatments, respectively ($P < 0.001$). Shaded plants, by comparison, had consistently lower concentrations of cyanogenic glycosides, with values of 1.64 ± 0.08 mg CN g⁻¹ dry wt and 0.99 ± 0.06 mg CN g⁻¹ dry wt, ($P < 0.001$) for the high and low nitrogen treatments, respectively. Because of the smaller response at low nitrogen, the nitrogen \times light interaction for leaf cyanide was significant ($P < 0.001$).

Importantly, there was a significant shift in the proportion of nitrogen allocated to cyanide (CN-N/N) in shaded plants compared to plants grown in full sunlight ($P < 0.001$). The CN-N/N value was $4.41 \pm 0.24\%$ in plants from the full-light, high-nitrogen treatment, but only $2.17 \pm 0.14\%$ in plants

grown in the shade and at the same nitrogen level (Fig. 1c). When plants were grown with limited nitrogen, the effect of shade was less pronounced, but still highly significant ($P < 0.01$; Fig. 1c) with CN-N/N values of 3.42 ± 0.20 and 2.25 ± 0.11 in the full- and low-light treatments, respectively. We further explored this relationship between cyanogenic glycoside concentration and nitrogen using regression analysis. We found strong correlations between leaf nitrogen and $\log_{10}([\text{CN}])$ for both the shade ($r^2 = 0.68$; $P < 0.001$; slope = 0.013) and the full-sunlight treatments ($r^2 = 0.90$; $P < 0.001$; slope = 0.021) (Fig. 3a). This analysis shows that shading effects a marked reduction in the response of cyanogenic glycoside production to leaf nitrogen.

Carbon-based secondary metabolites

The concentration of total phenolics and condensed tannins in expanded leaves of *E. cladocalyx* was also influenced by light and nitrogen supply (Fig. 2a). Plants grown in the shade had a higher leaf phenolic concentration ($P < 0.05$), but only when supplied with high nitrogen (i.e. the light \times nitrogen interaction was significant; $P < 0.01$). The mean phenolic concentration (± 1 SE) in leaves of seedlings from the high nitrogen treatment was $13.98 \pm 1.82 \text{ mg g}^{-1}$ compared with $24.21 \pm 1.47 \text{ mg g}^{-1}$ in the full-light and shade-grown plants, respectively. Leaves of plants supplied with low nitrogen did not differ significantly between light treatments; both contained approximately 40 mg g^{-1} . Decreased nitrogen supply alone effected an increase in leaf phenolic concentration whether the plants were grown in full light or shade ($P < 0.001$). In plants grown in full light, for example, mean total phenolic concentration was 54% higher in plants supplied with low nitrogen (Fig. 2a).

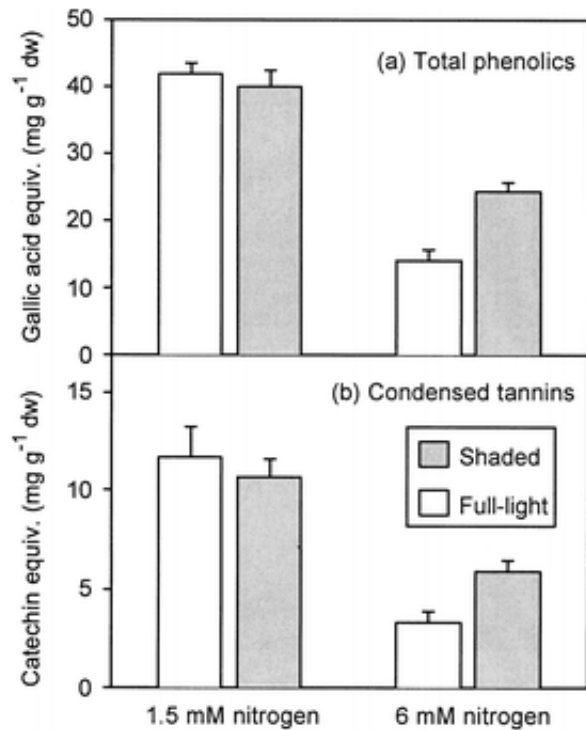


Fig. 2. Total phenolic (a), and condensed tannin (b) concentrations of fully expanded leaves of *E. cladocalyx* seedlings grown in full sunlight (*open*) or 40% full light (*shaded*) and supplied with 1.5 mM or 6 mM nitrogen. Values are the mean of 10 measurements (± 1 SE)

The variation in the concentration of condensed tannins was similar to the differences in total phenolics (of which they are a component) with an overall mean (± 1 SE) of 11.12 ± 1.22 mg g⁻¹ and 4.70 ± 0.58 mg g⁻¹, for plants from the high and low nitrogen treatments, respectively (Fig. 2b; $P < 0.001$). While the concentration of condensed tannins tended to be higher in leaves of shaded plants (8.28 ± 0.77 mg g⁻¹), compared those grown in full light (7.53 ± 1.06 mg g⁻¹), the difference was not significant ($P = 0.07$).

Because nitrogen varied somewhat between the sun and shade treatments (Fig. 1a), we conducted a regression analysis of the relationship between the leaf nitrogen and phenolic concentration (Fig. 3b). We found strong negative correlations between leaf nitrogen and phenolic concentration for both the shade ($r^2 = 0.85$; $P < 0.001$; slope = -1.13 g mg⁻¹) and the full sunlight ($r^2 = 0.93$; $P < 0.001$; slope = -1.23 g mg⁻¹) treatments (Fig. 3b). This analysis shows that shading does not have a significant effect on leaf phenolic concentration and its relationship to leaf nitrogen. Moreover, the apparent increase in phenolics with shading under the high nitrogen treatment can be largely accounted for by the decrease in leaf nitrogen (Fig. 1a).

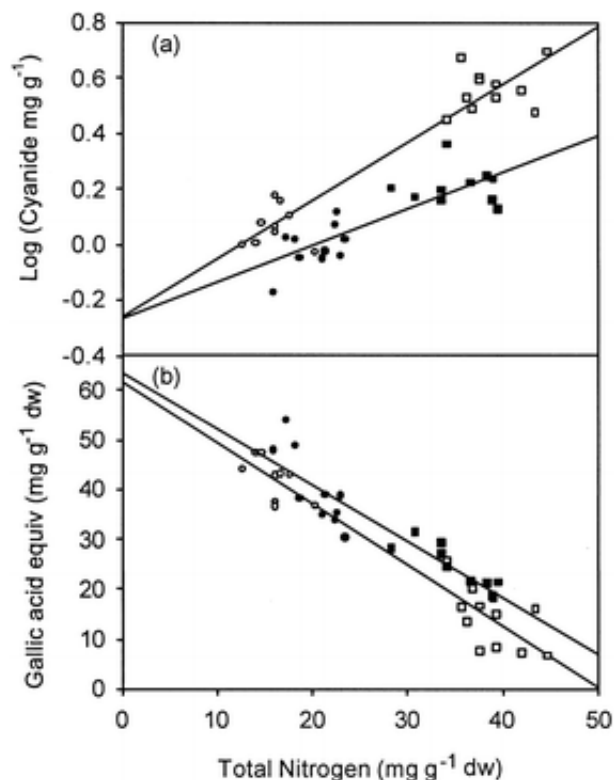


Fig. 3. Concentrations of cyanide (a) and phenolics (b) as a function of total nitrogen concentration in fully expanded leaves of *E. cladocalyx* seedlings grown in full light (*open*) or shade (*closed*). A range of leaf nitrogen concentrations was achieved by supplying plants with 6 mM (*squares*) or 1.5 mM (*circles*) nitrogen. Data within each light treatment were pooled. Each point represents one plant. The regression equations were: $\log_{10}([\text{CN}]) = -0.259 + 0.021[\text{N}]$ (full light; $r^2 = 0.90$); $\log_{10}([\text{CN}]) = -0.269 + 0.013[\text{N}]$ (shade; $r^2 = 0.60$); $[\text{Phenolics}] = 61.6 - 1.23[\text{N}]$ (full light; $r^2 = 0.92$); and $[\text{Phenolics}] = 63.6 - 1.13[\text{N}]$ (shade; $r^2 = 0.84$)

Discussion

This study compares for the first time the changes in carbon- and nitrogen-based secondary metabolites in a single species (*Eucalyptus cladocalyx*) in response to light, while controlling for nitrogen. We found that shading effected a decrease in the concentration of the cyanogenic glycoside, prunasin. In addition, there was a small increase in the concentration of carbon-based secondary metabolites (total phenolics and condensed tannins), but only when nitrogen supply was high. The carbon-based compounds were, however, strongly and negatively correlated with leaf nitrogen. We found previously (Gleadow and Woodrow 2000) that leaf nitrogen concentration was positively correlated with prunasin concentration in *E. cladocalyx*. We measured a similar relationship here, and showed that light intensity changed the slope of the leaf nitrogen - prunasin relationship (Fig. 3). Consistent with this finding, we found that the proportion of leaf nitrogen allocated to prunasin decreased significantly when the plants were grown in shade, although this effect was moderated to some extent when nitrogen supply was limited.

Studies of other plants with appreciable quantities of cyanogenic glycosides and alkaloids have also detected a decrease in the concentration of these compounds in shaded plants (Ralphs et al. 1998; Niedeziwied-Siegien and Gierasimiul 2001). However, it is evident from our results that the

relationship between these changes in concentration and light intensity cannot be appropriately quantified until the total nitrogen pool is measured. In other words, changes in the proportion of nitrogen allocated to the defence chemical need to be measured. The same comment can be made about studies showing an increase in nitrogen-based defence in shade (Cooper-Driver et al. 1977; Schreiner et al. 1984; Vickery et al. 1987).

Our results show that as leaf nitrogen rises- which can result from increases in soil nitrogen, for example (Gleadow and Woodrow 2002b)- the proportion of the "extra" nitrogen allocated to cyanogenic glycosides is reduced under shade conditions. This reduction effectively makes nitrogen available for other functions which, as we and others have found, may largely involve the synthesis of increased amounts of elements of the photosynthetic electron transport system, especially the light-harvesting chlorophyll-protein complexes (Evans 1989; Pons and Percy 1994). Consistent with this, we measured an increase in total chlorophyll and the chlorophyll *b*: chlorophyll *a* ratio in the seedlings with high concentrations of leaf nitrogen (chlorophyll *b* is only associated with the light-harvesting complexes; Table 2). Some of the nitrogen required for increasing the ability of the leaf to "harvest" light may also effectively come from other photosynthetic proteins such as Rubisco. From changes in the rate of photosynthesis (A_{\max}) we estimate that there was a reduction in the amount of Rubisco of some 32% in shade under high nitrogen. Nevertheless, we did not detect a significant change in Rubisco concentration under low nitrogen. It is noteworthy that, overall, the low nitrogen treatment was limiting for growth in full light, but not in the shade (Table 2).

Changes in carbon-based defence in relation to leaf nitrogen mirrored the changes in cyanogenic glycoside concentration. We found that as leaf nitrogen increased, both the total phenolic and condensed tannin concentrations declined (Fig. 3). This finding is in line with the results of a meta-analysis of 147 studies involving carbon-based defence compounds in a variety of woody species (Koricheva et al. 1998). These authors reported overwhelming evidence that high nitrogen fertilisers led to a decrease in foliar concentrations of a range of carbon-based secondary compounds. Nevertheless, our finding of little if any change in carbon-based defence under shade (after correcting for changes in leaf nitrogen; Fig. 3b) is at odds with the findings of several other studies of both total phenolics and condensed tannins (e.g. Waterman et al. 1984, Hartley et al. 1995; Lawler et al. 1997; Koricheva et al. 1998). These studies found a decrease in carbon-based compounds, which they attributed to the lower overall energy and carbon supply from photosynthesis in shaded plants.

The changes in defence chemistry in *E. cladocalyx* under shade are, however, logical if the relative "costs" of carbon- and nitrogen-based compounds are considered. While the biosynthetic "cost" of phenolics and cyanogenic glycosides may be similar - Gershenzon (1994) estimated that the average "cost" of both types of compound to be about 2.1 g glucose g⁻¹ - the higher turnover rates of cyanogenic glycosides (Gershenzon 1994) makes them more "expensive" to maintain. Turnover of prunasin in eucalypts has not been measured, but Adewusi (1990) calculated the rate of turnover of dhurrin (a cyanogenic glycoside) in *Sorghum bicolor* seedlings to be 17.4 nmol per hour. At this rate, one milligram of cyanogenic glycoside would be turned over every 7.5 days. In a leaf with a lifetime of 2 years (a typical age for adult *Eucalyptus* leaves), approximately 97.3 mg of cyanogenic glycoside would be synthesised to maintain a constant concentration and require 200 mg of glucose, making these compounds more energetically expensive than phenolics. *E. cladocalyx* is unusual in that it contains significant concentrations of both phenolics and cyanogenic glycosides. On an economic basis, then, it is not surprising that the concentration of the more "expensive" nitrogen-based defence compounds was reduced relative to carbon-based defence compounds in shaded plants.

In summary, when *E. cladocalyx* seedlings are shaded the requirement of the photosynthetic system for nitrogen increases. As a result the cost of cyanide apparently becomes too high, and nitrogen is diverted away from defence to the primary metabolism. Just how readily the cyanide can be

remobilised is unknown, but the rates of turnover of cyanogenic glycosides in *Sorghum* are certainly high enough to allow for significant alterations in allocation over a period of days or weeks (Bough and Gander 1971; Adewusi 1990). This, together with the relatively high energetic cost of maintaining cyanogenic glycosides, alters the economics of defence, so that it is of benefit to plants to rely more on carbon-based and less on nitrogen-based defence compounds under conditions of low light.

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