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Defense chemistry of cyanogenic *Eucalyptus cladocalyx* seedlings is affected by water supply

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Summary Cyanogenesis is a widespread and effective defense mechanism in plants. Published evidence suggests that cyanogenic capacity (i.e., cyanogenic glycoside concentration) is enhanced in response to water stress, although potentially confounding variables preclude a definite conclusion. We used highly cyanogenic *Eucalyptus cladocalyx* var. *nana* F. Muell. seedlings grown with varying amounts of water and nitrogen (N) to determine the relationship between cyanogenic capacity and water stress. We also examined whether variation in cyanogenic capacity affects phenolic biosynthesis because both pathways use phenylalanine as a substrate. Cyanogenic capacity in fully expanded leaves increased 70% in response to moderate water stress when N availability was high but only 30% when growth was N-limited. Absolute cyanogenic capacity also increased with increasing N supply. Total phenolics and condensed tannins decreased with increasing N supply, but these compounds were unaffected by water stress. We conclude that, under the influence of water stress, the enhanced demand for phenylalanine for cyanogenic glycoside biosynthesis can be sustained by enhanced shikimate pathway flux without affecting phenolic metabolism.

Keywords: carbon isotopes, cyanide, cyanogenesis, eucalypt, herbivore defense, phenolics, tannins, water stress.

Introduction

Cyanogenesis is a widespread defense mechanism in plants. It involves the release of cyanide (CN) when tissue is ruptured, which can either deter or kill a herbivore (Nahrstedt 1985, Jones 1988). The capacity for cyanogenesis varies among species, within species, and even within individual populations. In populations of *Turnera ulmifolia* L. for example, it was found that 1-year-old seedlings can release from 0 to 2020 μg CN g^{-1} dry mass (dm) (Schappert and Shore 2000). Although much of the variation in cyanogenic capacity in this and other species is related to genetic differences, it is also influenced by environmental variables (e.g., Dement and Mooney 1974, Stockmal and Oleszek 1997, Gleadow et al. 1998, Schappert and Shore 1999).

There is abundant correlative evidence, especially for cyanogenic agricultural species, that plants become more toxic when subjected to drought (e.g., Robinson 1930, Nelson 1953, Kriedemann 1964, Aikman et al. 1996). However, there have been few controlled experiments testing this relationship. Bokanga et al. (1994) set up a series of irrigated and non-irrigated plots at localities differing in annual rainfall and found that *Manihot esculenta* Cranz. cultivars were consistently more cyanogenic when subjected to drought. Variables such as soil nitrogen (N) were neither controlled nor accounted for in the experiment. Calatayud et al. (1994) found that although the highest concentrations of cyanogenic glycosides in *M. esculenta* were measured in the dry season, the accumulation of the glycosides actually occurred at the end of the wet season before water stress set in.

Woodrow et al. (2002) found that *Eucalyptus cladocalyx* F. Muell. trees growing in dry areas were generally more cyanogenic than those from a comparable high rainfall area; however, both soil and foliage at the more arid site were higher in N—factors known to affect cyanogenic glycoside concentration (Briggs 1990, Gleadow and Woodrow 2000*a*, 2000*b*). Once site differences in leaf N were taken into account, the correlation between water stress and cyanogenic capacity was not statistically significant (Woodrow et al. 2002).

Given the relatively large demand that cyanogenesis makes on plant resources (for example, in *E. cladocalyx*, as much as 15% of leaf N can be allocated to the cyanogenic glycoside, prunasin; Gleadow et al. 1998) and its effectiveness as a defense mechanism (Jones 1988, Bennet and Wallsgrove 1994, Møller and Seigler 1999), we attempted to clarify the relationship between cyanogenic capacity and water stress under controlled conditions. We used *Eucalyptus cladocalyx* var. *nana* F. Muell. because it has a high constitutive capacity for cyanogenesis and has been the subject of a previous field study (Woodrow et al. 2002). Moreover, the release of CN from *E. cladocalyx* is associated with herbivore defense (Pratt 1937, Webber et al. 1985, Gleadow and Woodrow 2000*a*). In addition to water supply, we also varied N supply, because N is an important effector of cyanogenic glycoside synthesis in *E. cladocalyx* (Gleadow and Woodrow 2000*a*). We also measured phenolic compounds to determine if stimulation of cyanogenic glycoside synthesis depresses phenolic synthesis as a result of competition for phenylalanine.

Materials and methods

Plant material and greenhouse conditions

Forty seedlings of *E. cladocalyx* (2 months old) were transplanted in 1-l pots containing a mixture of sterilized sand:vermiculite (1:1, v/v) and transferred to a greenhouse. Pots were flushed daily with one-quarter strength Hoagland's solution containing either 6 mM N (high-N treatment; HN) or 1.5 mM N (low-N treatment; LN) supplied as nitrate and ammonia (6:1, mole:mole) with sodium as the balancing cation (see Gleadow et al. 1998). Air temperature and relative humidity (measured every 15 min) were 23.9 ± 1.2 °C and 52.0 ± 2.6% (± 1 SD), respectively. The photoperiod during the experimental period (April–June 2000) was about 10 h and mean daytime photosynthetic photon flux density (PPFD; measured every 10 min from 0800 to 1700 h) was 157 ± 104 µmol m^{-2} s^{-1}, with a maximum of 583 µmol m^{-2} s^{-1}. Leaf temperatures were not significantly different in plants from the different treatments (data not shown).

After 2 months, seedlings within each nutrient treatment were assigned randomly to either well-watered control (high-water; HW) or water-stressed (low-water; LW) treatments in a 2 × 2 factorial design (n = 10). Pots were watered to field capacity, allowed to drain, sealed at the bottom with plastic and covered with a 10-mm layer of plastic beads to reduce surface evaporation. Pots were weighed to determine their field water-holding capacity (Li et al. 2000). At noon every second day, HW seedlings were watered to field capacity with either high or low N solutions, whereas LW seedlings were watered with nutrient solution to 25% of field capacity.

Before starting the LW treatments, a leaf disc (1.43 cm^2) was excised from one side of the midrib of the first fully expanded leaf of each plant, frozen in liquid nitrogen, freeze-dried and the "base-line" cyanogenic glycoside concentration determined. Eight weeks after imposing the LW treatments, cyanogenic glycoside concentration was determined on a second leaf disc excised from the other side of the midrib of the same leaves. Previous experiments have shown that cyanogenic capacity of *E. cladocalyx* leaves does not vary within a single leaf, or with wounding (Gleadow and Woodrow 2000*a*). Plants were harvested after 8 weeks. A sample of newly formed leaf tips (about 50 mg) was removed for determination of stable isotope composition ($\delta^{13}C$). Leaves and stems were frozen in liquid nitrogen, freeze-dried and analyzed for total N, total carbon (C) and cyanogenic glycoside concentration. Total phenolic and condensed tannin concentrations were also determined on leaf material. Roots were oven-dried at 60 °C for 4 days, cooled in a desiccator and weighed.

Determination of cyanogenic glycosides

Dried plant material was ground in a cooled IKA Labortechnic A10 microgrinder (Janke and Kunkel, KG, Staufen, Germany) and stored in a desiccator at –20 °C. Cyanogenic glycoside concentration of plant tissue was determined by hydrolysis of cyanogenic glycosides and trapping the resultant HCN in a NaOH well (Brinker and Seigler 1989). Complete hydrolysis was achieved by adding 1 ml of 0.1 mM citrate buffer (pH 5.5) to about 0.02 g of freeze-dried leaf material in a sealed glass vial and incubating for 24 h at 37 °C (see Gleadow and Woodrow 2000*a*). Exogenous β-glucosidase from almond (*Prunus amygdalis* (L.) Benth. and 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 CN (Gleadow et al. 1998). Previous experiments have shown that crude protein extract from *E. cladocalyx* var. *nana* is capable of hydrolyzing prunasin (D-mandeolinitrile β-D-glucoside, Sigma) without the addition of exogenous α-hydroxynitrile lyase (R.M. Gleadow and I.E. Woodrow, unpublished data). Cyanide in the NaOH well was assayed by a modification (Woodrow et al. 2002) of the method of Brinker and Seigler (1989). The amount of CN detected by this method is a measure of the amount of cyanogenic glycoside in the tissue. Cyanide concentration was expressed as mg CN g^{-1} dm, mg CN leaf $^{-1}$ or mg CN leaf N^{-1}.

Determination of carbon-based secondary metabolites

The concentration of total phenolics was determined using Folin-Ciocalteau reagent, with gallic acid as the standard. Finely ground, freeze-dried leaf samples (50 mg) were extracted in 1 ml of cold 50% acetone by vortexing for 15 s. After centrifuging at 26,000 *g* for 15 s, the supernatant was collected into pre-weighed, chilled tubes. The extraction procedure was repeated a further three times. A 20-µl aliquot of the pooled acetone extract was used in the assay and absorbance measured at 765 nm (see Cork and Krockenberger 1991). Gallic acid was used as a standard and phenolic concentration was expressed in gallic acid equivalents as mg g^{-1} dm. Further aliquots (100 µl) of the 50% acetone extract were analyzed for condensed tannin concentration with (+)-catechin as the standard (Julkunen-Titto 1985). Tannin concentration was expressed in catechin equivalents as mg g^{-1} dm.

Determination of carbon and nitrogen

Total concentrations of C and N in 5 mg samples of oven-dried (roots) or freeze-dried (stems and leaves) material were measured with a Perkin-Elmer 2400 Series II CHNS/O Analyzer (Perkin-Elmer, Norwalk, CT) with high-purity acetanilide as the standard.

Determination of $\delta^{13}C$

Finely ground samples of freeze-dried leaf tips were analyzed for $\delta^{13}C$ with an on-line VG Isochrom mass spectrometer (VG Microtech, Uckfield, U.K.) after combustion in a Carlo Erba 1110 elemental analyzer (ThermoQuest Australia, Rydalmere, NSW, Australia). Stable isotope composition (‰) was expressed relative to the PeeDee Belemnite standard (Farquhar and Richards 1984).

Gas exchange and transpiration

Steady-state stomatal conductance (g_s) and net CO_2 assimilation rate (A_{max}) were measured at saturating PPFD (800 µmol m^{-2} s^{-1}) and ambient CO_2 concentration (360 µmol mol^{-1})

7 weeks after the water regimes were imposed. Measurements were made on a single fully expanded leaf at 25 °C from five replicate plants per treatment with an LI-6400 gas exchange system (Li-Cor, Lincoln, NE). Relative humidity in the chamber for each measurement was 50%.

Statistical analysis

Analyses of variance were carried out with Minitab Release 13 software (Minitab, Pasadena, CA). Most data were normally distributed. Cyanide data, however, required a log transformation to satisfy the assumptions of normality. Treatment means were compared by the least significant difference (LSD) method at the 95% confidence level (Sokal and Rolf 1997).

Results

Morphological and physiological responses

Consistent with other studies, we found that plant biomass was ~30% less ($P < 0.001$) and height was ~15% less ($P < 0.001$) in LN or LW seedlings compared with HN and HW seedlings (Table 1). Partitioning was also altered, with a greater proportion of biomass allocated to the roots in LN and LW plants relative to HN and HW plants. Root/shoot ratios ranged from 0.75 ± 0.08 (± 1 SE) in LW, LN seedlings to 0.31 ± 0.03 in the HW, HN seedlings (Table 1). The lower leaf area ratio (LAR) of LN relative to HN plants ($P < 0.001$) was largely the result of increased root biomass, whereas the low LAR of LW relative to HW plants was associated with smaller leaves and earlier leaf senescence as well as increased root biomass. Leaf weight area ratio (LWAR) was 23% higher in LN plants relative to HN plants ($P < 0.001$, Table 1). Drought had no significant effect on LWAR in LN plants with an overall mean value (± 1 SE) of 75.2 ± 2.1 g m^{-2}. Seedlings from the HN treatment tended to have higher LWAR when water-stressed (53.9 ± 2.2 versus 60.7 ± 1.3 g m^{-2}) ($P = 0.07$).

In the HN treatment, $\delta^{13}C$ of tissue formed under water stress was –26.7 ± 0.6‰ (± 1 SE), compared with –28.1 ± 0.3‰ for tissue from HW plants ($P < 0.001$, Table 2). The corresponding $\delta^{13}C$ values for the LN seedlings showed a similar pattern, but they were slightly less in both cases (–27.6 ± 0.3 and –29.8 ± 0.4‰; Table 2), consistent with increased water stress in the LW treatment. Gas exchange analysis of selected leaves under light-saturated conditions indicated that increases in $\delta^{13}C$ were the result of reduced g_s relative to photosynthetic capacity (Table 2).

Nitrogen concentration

Leaves from HN plants contained about 28.8 ± 1.1 mg N g^{-1} compared with 16.3 ± 0.5 mg N g^{-1} in leaves of LN plants ($P < 0.001$; Table 3). Similar differences between N treatments were observed for stems (12.5 ± 0.1 versus 7.6 ± 0.1 mg N g^{-1}; $P < 0.001$) and roots (11.3 ± 1.2 versus 7.1 ± 0.1 mg N g^{-1}; $P < 0.01$).

Both leaf and root N concentrations were unaffected by water stress ($P = 0.71$ and $P = 0.47$, respectively; Table 3), whereas stems had slightly higher concentrations of N in LW plants that also received a high-N supply ($P = 0.05$, Table 3), however, the carbon/nitrogen (C/N) ratio of stem tissue was unaffected by water supply ($P = 0.17$, Table 3). Leaf and root tissues displayed lower C/N ratios in HN plants, but did not differ between plants from the two water treatments (Table 3).

Cyanogenic capacity

We sampled leaf discs from the same fully expanded leaves before and after the water-stress treatments. In HN plants, cyanogenic glycoside concentration increased from 2.9 ±

Table 2. Net CO_2 assimilation rate (A_{max}) and stomatal conductance (g_s) at light saturation in well-watered (HW) and water-stressed (LW) seedlings of *Eucalyptus cladocalyx* measured 7 weeks after treatments were imposed. Plants were supplied with nitrogen (N) solution containing high (HN; 6 mM) or low (LN; 1.5 mM) N concentrations. Carbon isotope discrimination ($\delta^{13}C$) was measured on leaf tips that emerged after plants had been exposed to the various treatments. All values are means of five replicates ($n = 5$), except for $\delta^{13}C$ ratios ($n = 4$). Means within columns can be compared for significance using the least significant difference (LSD) value ($P < 0.05$).

Treatment	A_{max} (μmol CO_2 m^{-2} s^{-2})	g_s (mmol H_2O m^{-2} s^{-2})	$\delta^{13}C$ (‰)
HN, LW	5.2	0.07	–26.7
HN, HW	11.5	0.18	–28.1
LN, LW	4.2	0.08	–27.6
LN, HW	9.8	0.25	–29.8
$LSD_{0.05}$	1.6	0.04	0.7

Table 1. Growth and biomass partitioning of *Eucalyptus cladocalyx* seedings grown with high nitrogen (N) (HN; 6 mM) or low N (LN; 1.5 mM) supply and either well-watered (HW) or water stressed (LW). Values are means of 10 replicates ($n = 10$). Means within columns can be compared for significance using the least significant difference (LSD) values ($P < 0.05$). Abbreviations: LAR = leaf area ratio; and LWAR = leaf weight to area ratio.

Treatment	Height (mm)	Total dry mass (g)	Root/Shoot	LAR (m^2 g^{-1})	LWAR (g m^{-2})
HN, LW	264	4.8	0.52	7.55×10^{-3}	60.7
HN, HW	323	7.0	0.31	9.93×10^{-3}	53.9
LN, LW	172	3.5	0.75	5.83×10^{-3}	75.7
LN, HW	201	4.9	0.61	6.59×10^{-3}	74.7
$LSD_{0.05}$	23	0.6	0.08	0.63×10^{-3}	3.7

Table 3. Total nitrogen (N) concentration (% dm) and total carbon to nitrogen ratio of fully expanded leaves, stems and roots of *Eucalyptus cladocalyx* seedlings grown with high (HN; 6 mM) or low (LN; 1.5 mM) N supply and under well-watered (HW) or water-stressed conditions (LW). Values are means of 10 replicates ($n = 10$) except data for roots ($n = 4$). Means within columns can be compared for significance using the least significant difference (LSD) value ($P < 0.05$).

Treatment	Nitrogen (% dm)			Carbon/Nitrogen		
	Leaves	Stem	Roots	Leaves	Stem	Roots
HN, LW	2.85	1.38	1.12	16.7	34.7	34.8
HN, HW	2.93	1.11	1.13	16.5	38.5	37.3
LN, LW	1.62	0.74	0.79	28.3	59.4	52.0
LN, HW	1.63	0.76	0.62	28.7	58.3	52.7
$LSD_{0.05}$	0.23	0.99	0.17	3.6	4.2	6.2

0.3 (± 1 SE) to 4.9 ± 0.9 mg CN g^{-1}, whereas it increased in LN plants from 1.2 ± 0.1 to 1.6 ± 0.4 mg CN g^{-1} ($P = 0.03$, Figure 1). There was no change in the cyanogenic glycoside concentration in the leaves of HW plants over the 8-week experimental period ($P = 0.51$).

Overall, when whole plants were analyzed, N supply had a highly significant ($P < 0.001$) effect on cyanogenic glycoside concentration, but water availability did not ($P = 0.17$). In the HN treatment, cyanogenic glycoside concentration (± 1 SE) increased from 2.4 ± 0.3 to 3.2 ± 0.2 mg CN g^{-1} when the plants were subjected to water stress (Figure 2a). Plants from the LN treatment, however, contained the same amount of CN whether they were water stressed or not (Figure 2a). As a result, the N × water interaction was significant ($P < 0.05$).

A similar pattern was found when the data were analyzed on a leaf, rather than a dry mass, basis. Leaf CN content was much higher in HN plants than in LN plants ($P < 0.001$) (mean of 0.45 ± 0.10 versus 0.11 ± 0.02 mg CN $leaf^{-1}$) (Figure 2b). Moreover, leaves of LW plants contained more CN only in the HN treatment (i.e., the N × water interaction was significant; $P = 0.03$) with a mean CN content of 0.70 ± 0.07 mg $leaf^{-1}$.

The increase in cyanogenic glycoside concentration in response to drought was apparently restricted to fully expanded leaves, which made up most of the total leaf biomass of the seedlings. Emerging leaves that had not expanded showed no significant increase in cyanogenic glycoside concentration

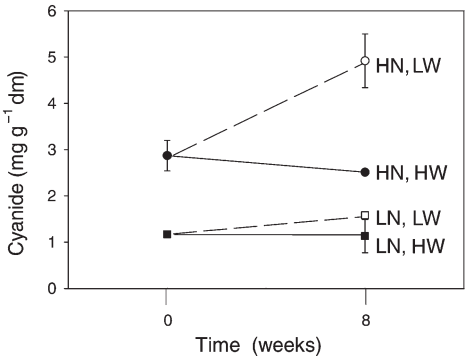

Figure 1. Comparison of the cyanogenic glycoside concentration in leaf discs excised from fully expanded leaves of *Eucalyptus cladocalyx* before and after drought treatment. Plants were grown with 6 mM nitrogen (N) (HN; ●, ○) and 1.5 mM N (LN; ■, □) and were either well watered (HW, closed symbols) or water stressed (LW, open symbols). Cyanogenic glycoside concentration is expressed as CN equivalents. Values are means of five replicates.

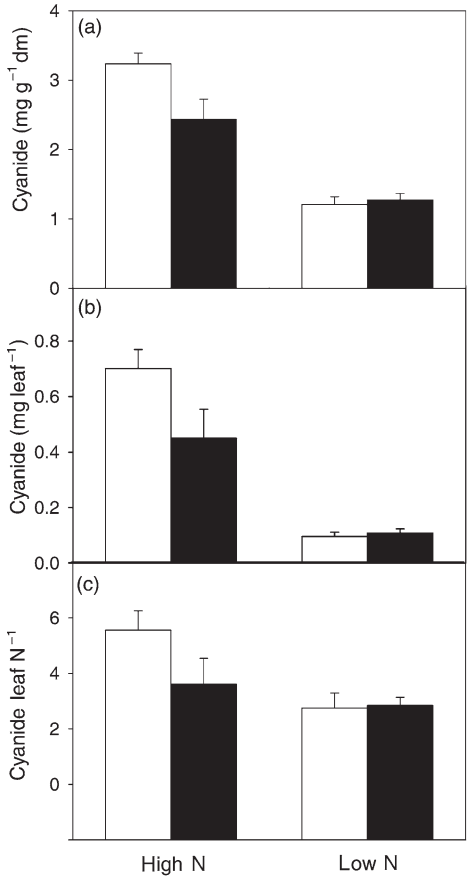

Figure 2. Cyanogenic glycoside and nitrogen (N) concentration of leaves of *Eucalyptus cladocalyx* seedlings 8 weeks after watering treatments were imposed. Plants were grown with high (6 mM) or low (1.5 mM) N supply and were either well watered (solid bars) or water stressed (open bars). (a) Cyanogenic glycoside concentration, measured as CN equivalents per mass; (b) Cyanogenic glycoside content of individual fully expanded leaves, measured as CN equivalents per leaf; and (c) Amount of N allocated to cyanide as a proportion of the total leaf N. Values are means (+ SE) for pooled fully expanded leaves on each seedling ($n = 10$).

with water stress ($P = 0.08$). Rather, the trend was in the opposite direction, with a slight decrease in concentration from 6.5 ± 0.4 to 5.7 ± 0.7 mg CN g^{-1} in HN plants and 5.7 ± 0.6 to 4.2 ± 0.3 mg CN g^{-1} in LN plants. Stems were similarly unaffected by the LW treatment and had mean cyanogenic glycoside concentrations of 1.1 ± 0.1 and 0.6 ± 0.1 mg g^{-1} in the HN and LN treatments, respectively. Roots contain no cyanogenic glycosides (cf. Gleadow and Woodrow 2000*a*).

Increased leaf CN concentration in response to water stress was not the result of differences in leaf N concentration, which did not differ significantly between HW and LW plants (Table 3). Moreover, among treated leaves, only leaves in the HN, LW treatment had a significantly ($P < 0.01$) higher CN to leaf N ratio (Figure 2c).

Carbon-based secondary metabolites

Nitrogen supply had a marked effect on both the total phenolic ($P < 0.001$) and condensed tannin ($P < 0.001$) concentrations in leaves, but water stress had no effect ($P = 0.58$; Figure 3). Mean total phenolic concentration increased about twofold in the HN treatment (39.8 ± 2.6 versus 94.9 ± 2.9 mg g^{-1}; Figure 3a), whereas the condensed tannin concentration increased almost fivefold (8.3 ± 9.1 versus 40.0 ± 16.6 mg g^{-1}; Figure 3b).

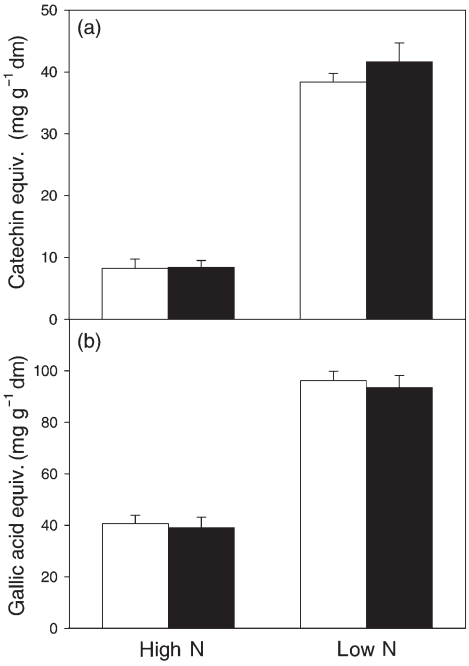

Figure 3. Concentration of carbon-based secondary metabolites in leaves of well-watered (solid bars) and water-stressed (open bars) seedlings of *Eucalyptus cladocalyx* grown with high (6 mM) or low (1.5 mM) nitrogen (N) supply. (a) Total phenolics measured as gallic acid equivalents, and (b) condensed tannins, measured as catechin equivalents. Values are means (+ SE) of 10 replicates.

Discussion

Cyanogenic capacity of *E. cladocalyx* leaves was influenced markedly by water status, independently of changes in leaf N or any other identifiable variable. In the HN treatment, cyanogenic glycoside concentration of individual leaves increased by about 70% in response to water stress, whereas in the LN treatment the concentration increased by only 30% (Figure 1). Consistent with other studies (Gleadow and Woodrow 2000*a*), we found no effect of sampling on either leaf N (data not shown) or cyanogenic glycoside concentration within water regimes. Moreover, leaf aging can be ruled out as a significant variable because previous work has shown that it is associated with a decrease in both N and cyanogenic glycoside content (Gleadow et al. 1998).

The effect of water stress was also measured by harvesting whole plants before and after imposing the water-stress regime. No stimulation of cyanogenesis was measured in plants in the LN treatment, and only a 30% increase was found in plants in the HN treatment. The difference between these results and those obtained in the single leaf experiments may be explained by the inclusion of older, relatively shaded, leaves in the bulk sample. Such leaves have a relatively low leaf N and cyanogenic glycoside content, and it is likely that because of the low N content, cyanogenesis is less affected by water stress. Moreover, leaves that expanded during the water-stress treatment were also included in the sampled material. These leaves may not have responded to water stress in the same way as leaves that expanded before the imposition of water stress.

The effect of drought may also have been moderated by morphological adaptations of the seedlings to treatments. Plants from the LN treatment had a higher root/shoot ratio (Table 2), which may have alleviated the water stress. This is consistent with the significantly lower $\delta^{13}C$ for LN plants in both water regimes (Table 2), and has been noted previously (Schulze et al. 1998, Livingston et al. 1999).

It is difficult to make quantitative comparisons between our results and other studies that found enhanced cyanogenesis in response to water stress (e.g., Nelson 1953, Majak et al. 1980, Calatayud et al. 1994) because variables such as the degree of water stress and foliar N were not measured in those studies. We note that the $\delta^{13}C$ values in our LW treatment are comparable with those measured for plants growing under semi-arid conditions (e.g., Ehleringer 1993, Schulze et al. 1998), including *E. cladocalyx* growing on the Eyre Peninsula (South Australia) (Woodrow et al. 2002). In the latter case, plants had mean $\delta^{13}C$ and cyanogenic capacities of –26.8‰ and 0.45 mg CN g^{-1}, respectively. These values were significantly higher than those measured at a wetter site (–28.6‰ and 0.28 mg CN g^{-1}, respectively); however, the authors concluded that most of the increase in cyanogenic capacity could be accounted for by higher foliar N rather than water stress. Variation in N between water supply treatments did not occur in our study, and therefore N could not have contributed to the observed variation in cyanogenic capacity.

Water stress may influence many plant secondary metabolites (Gershenzon 1984), although few experimental studies have examined more than one biosynthetic pathway in the same plant (Waring and Cobb 1992, Blodgett and Stanosz 1998). In our study, the concentrations of phenolics and con-

densed tannins were similar in HW and LW plants, in both N regimes. This contrasts with the strong negative relationship that we observed between N and both total phenolic and condensed tannin concentrations, which is consistent with many other studies, including studies of trees (e.g., Gershenzon 1984, Cipollini et al. 1993, Bennett and Wallsgrove 1994, Lawler et al. 1997, Keinänen et al. 1999; Figure 2). Given the positive response of cyanogenic capacity to N, we speculate that the cyanogenic glycoside and phenolic biosynthetic pathways compete for a limited phenylalanine pool (the cyanogenic glycoside in *E. cladocalyx*, prunasin, is synthesized from phenylalanine). However, the absence of a significant water-stress effect on the phenolic biosynthetic pathway indicates that regulation in response to this variable is complex and may involve alterations in the capacity of the shikimate pathway, which synthesizes phenylalanine.

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