Leaf angle responds to nitrogen supply in eucalypt seedlings. Is it a photoprotective mechanism?

DUGALD C. CLOSE¹–⁴ and CHRISTOPHER L. BEADLE¹,⁵

¹ Cooperative Research Centre for Sustainable Production Forestry, Private Bag 12, Hobart 7001, Australia
² Schools of Agricultural and Plant Science and Zoology, University of Tasmania, Private Bag 12, Hobart 7001, Australia
³ Present address: Botanic Gardens and Parks Authority, Science Directorate, Fraser Avenue, West Perth, Perth 6005, Australia
⁴ Corresponding author (dclose@bgpa.wa.gov.au)
⁵ CSIRO Forestry and Forest Products, Private Bag 12, Hobart 7001, Australia

Summary We examined the adjustment of leaf angle (Lₐ) and foliar chlorophyll and xanthophyll chemistry in Eucalyptus nitens (Deane and Maiden) Maiden seedlings maintained in various nitrogen (N)-supply treatments over a 6-month period. Adjustment of Lₐ toward the vertical was greatest under conditions of foliar N deficiency and became incrementally more horizontal with increasing foliar N concentration. Photochemical efficiency (Fᵥ/Fₘ) and quantum yield were lower in seedlings with low foliar N (low-N seedlings) in winter, but not in autumn. Low-N seedlings generally had low area-based chlorophyll concentrations and high xanthophyll-cycle conversion ratios, particularly during months of low temperature. Under mild temperature conditions, high concentrations of zeaxanthin and antheraxanthin were associated with lower electron transport rates (ETR). Incident light, Fᵥ/Fₘ, ETR and total chlorophyll concentration were negatively correlated with Lₐ, with horizontal leaf orientation measured as 0° and vertical leaf orientation as 90°. Xanthophyll conversion ratio was positively correlated with horizontal leaves (He et al. 1996). Adjustments in Lₐ may play a role in photoprotection of E. nitens seedlings by assisting the leaf to balance its utilization and dissipation of energy.

Keywords: acclimation, heliotropic, low temperature, photochemical efficiency, photoinhibition, seasonal, sustained xanthophyll engagement.

Introduction

Leaf angle (Lₐ) can vary between and within species and within individuals and can be viewed as an adaptive characteristic that is habitat or ecosystem dependent. For example, it has been hypothesized that individual species of the South American genus Larrea have contrasting Lₐ that are indicative of the climatic conditions that determine the natural distribution of these species (Ezcurra et al. 1991). In mature Quercus alba L. in eastern Tennessee, USA, Lₐ increased with canopy height: mean Lₐ was 38° in the overstory, 20° below crown closure and 10° in the sub canopy (Hutchison et al. 1986). Quercus coc-cifera L. leaves grown in full sun had 2-fold higher Lₐ than leaves grown in 20% sun and seedlings originating from a forest exhibited greater plasticity in Lₐ adjustment than seedlings from a rocky outcrop (Balaguer et al. 2001). Semideciduous, drought-tolerant Cistus species in Mediterranean south-west Portugal exhibit seasonal adjustment of Lₐ, from a horizontal orientation in spring (< 35°) to a more vertical orientation in summer (> 70°) (Werner et al. 1999). In all of these studies, Lₐ was considered a fixed parameter within a species that is determined by leaf response to a given environment.


Rapid, reversible tropic (termed heliotropic) leaf movements have been studied extensively in Glycine max (L.) Merr. A paraheliotropic movement (orientation of the leaf parallel to the direction of the irradiance source) reduces light interception and can be induced by water deficit (Oosterhuis et al. 1985, Kao and Forseth 1991, 1992, Saitoh et al. 1995). Leaf water potential (Wainwright 1977, Berg and Hsiao 1986, Kao and Forseth 1992, Saitoh et al. 1995), vapor pressure deficit (Reed and Travis 1987), saturation of photosynthetic rate (Prichard and Forseth 1988a, 1988b) and temperature at the leaf base (Fu and Ehleringer 1989) have all been shown to correlate with tropic leaf movement.

Tree seedlings employ multiple mechanisms for photoprotection, which is necessitated either by inherently low rates of photosynthesis (Krause et al. 1995, Dodd et al. 1998), or by low rates of photosynthesis resulting from such factors as cold air stratification (Jordan and Smith 1994) or drought because
of low root occupancy of the soil profile (Bursett 1990). Demonstrated mechanisms of photoprotection in *Eucalyptus nitens* (Deane and Maiden) Maiden, the species investigated in this paper, include degradation of chlorophylls, increased xanthophyll:chlorophyll ratios (Close et al. 2001b), sustained xanthophyll cycle engagement (Close et al. 2003a) and increased production of the purported antioxidants, flavonol and galloyl-glucose (Close et al. 2001a, 2003b).

Kao and Forseth (1991) demonstrated that nitrogen (N) deficiency induced more vertical leaf growth in *G. max*. Close et al. (2000, 2001a, 2001b) have shown that N deficiency induced increased photoinhibition in *Eucalyptus* seedlings.

It has been suggested that differences in leaf angle of *Eucalyptus* species contribute to their adaptation to different environments (King 1997). However, active adjustment of leaf angle in response to environmental stress has not been reported in non-heliotropic plant species like eucalypts. There is a need to investigate the relationships between leaf form and function, as related to photosynthetic performance, to further understand the mechanisms that affect leaf angle (Smith et al. 1997). The objective of this experiment was to investigate adjustment of leaf angle as a potential contributor to photoprotection in eucalypt seedlings through manipulation of N supply to seedlings.

**Materials and methods**

**Plant material and treatments**

*Eucalyptus nitens* seedlings studied here had previously been used in an earlier experiment designed to assess the effect of N application on susceptibility to herbivore browsing (Close et al. 2003b). Seedlings of *E. nitens* were raised from a Highlands Seed Orchard seedlot (Forestry Tasmania improved seed) germinated in 115-cm³ plug trays at Forestry Tasmania’s tree nursery, Perth, Tasmania (139°40′ E, 41°34′ S). The potting mix was saturated with Aquasol every 4 days (solution concentration 1 g l⁻¹) for 3.5 months. In late February 2003, seedling trays were randomly allocated to one of five nutrient treatments: (1) non-fertilized (NNF); (2) fertilized every 14 days (N F14); (3) fertilized every 7 days (N F7); (4) fertilized every 4 days (N F4); and (5) fertilized every 2 days (N F2) until September 2003. When fertilized, each seedling received about 1.25 mg of commercial fertilizer (N, P, K 20, 2.2, 0.78; solution concentration 1 g l⁻¹) for 14 days before the sampling dates were 9.1, 8.6, and 5.8 °C, respectively (measured at an Australian Bureau of Meteorology weather station 42.89° S, 147.32° E located 2 km from the nursery).

**Chlorophyll fluorescence**

Predawn photochemical efficiency (ΦPSII / ΦPSII), solar noon incident photosynthetic photon flux (PPF) and steady-state fluorescence parameters, namely solar noon light-adapted photochemical quantum yield (ΦPSIIa’ – ΦPSIIa) and electron transport rate (ETR; ΦPSII × PPF × leaf absorbance × 0.5) were measured with a PAM-2000 fluorometer and 2030-B leaf-clip holder (Heinz Walz GmbH, Effeltrich, Germany) on clear days. For the calculations of ETR, leaf absorbance values were estimated from spectrophotometric measurements as 0.78, 0.81, 0.83, 0.86 and 0.88 for leaves in the N F14, N F14, N F2, N F7, N F4 and N F2 treatments, respectively (Close and Beadle 2005). Care was taken not to alter the leaf angle during measurement of the steady-state fluorescence parameters, so that the PPF and chlorophyll fluorescence values were indicative of those of undisturbed leaves. Single measurements were made on each leaf of the most recently expanded leaf pair of the tagged seedlings within each plot. Blocks and treatments were measured in a randomized order.

**Pigment analysis**

Two leaf discs were punched from each leaf of the most recently expanded leaf pair of the four seedlings sampled per block. Four discs (out of eight per block) were randomly selected for analysis. Chlorophyll and carotenoid extraction and analyses were carried out as described by Close et al. (2001a).

**Statistical analysis**

We used analysis of variance in the general linear model procedure (PROC GLM; SAS Institute Inc.) to test for effects of fertilizer (nutrient treatment) and time (sampling period) on seedling height, leaf angle, chlorophyll and carotenoid pigments and chlorophyll fluorescence parameters. Linear regressions were conducted with the statistical analysis software in SigmaPlot.

**Results**

**Nitrogen concentration**

Area-based leaf N concentration increased significantly (P < 0.0001) from the non-fertilized treatment (N F0) to the highest N application treatment (N F2), although the difference in leaf N concentration between the N F4 and N F7 treatments...
was not significant (Figure 1). Leaf N concentration was 0.75, 1.03, 1.38, 1.56 and 2.01% dry matter in the N_{NF}, N_{F14}, N_{F7}, N_{F4} and N_{F2} treatments, respectively. There was no effect of time ($P = 0.3970$) or interaction between time and fertilizer treatment ($P = 0.6470$) on leaf N concentration.

**Leaf angle**

Mean seedling $L_0$ was $39 \pm 1.5^\circ$ just before the start of fertilizer treatment on March 31. There was a significant effect of fertilizer treatment on $L_0$ ($P < 0.0001$). On April 23, $L_0$ was around $57^\circ$ in N_{NF} and gradually decreased to $28^\circ$ in N_{F2} (Figure 2a). Differences in $L_0$ between treatments N_{F14} through to N_{F2} decreased with time ($P < 0.01$) and on September 3 there were no significant treatment differences in $L_0$. There was no significant interaction between the effects of time and fertilizer on $L_0$.

**Photosynthetic photon flux and chlorophyll fluorescence**

Incident PPF at midday was $743 \pm 42 \mu\text{mol m}^{-2}\text{s}^{-1}$ before fertilizer treatment on March 31. Incident PPF on leaves at midday on April 23 was lowest in N_{NF} ($\sim 240 \mu\text{mol m}^{-2}\text{s}^{-1}$) and increased incrementally to around $600 \mu\text{mol m}^{-2}\text{s}^{-1}$ in N_{F2} (fertilizer effect $P < 0.0001$; Figure 2b). Results for April 23, June 11 and September 3 were generally similar and there was no significant effect of time on incident PPF. Photochemical quantum yield was $0.25 \pm 0.02$ and ETR was $65 \pm 5 \mu\text{mol m}^{-2}\text{s}^{-1}$ on March 31. There was no effect of fertilizer treatment on $\phi_{PSII}$ ($P = 0.1823$; Table 1), although $\phi_{PSII}$ of leaves in the N_{F14}, N_{F7} and N_{F4} treatments was higher on September 3 than at other times (time effect $F_{2,36} = 4.50$; $P < 0.05$). In contrast, ETR increased incrementally in April from around $23 \mu\text{mol m}^{-2}\text{s}^{-1}$ in N_{NF} to $50 \mu\text{mol m}^{-2}\text{s}^{-1}$ in N_{F2} ($P < 0.0001$; Figure 2c). Photochemical efficiency was $0.74 \pm 0.01$ on March 31. There was an overall effect of fertilizer treatment on $F_v/F_m$ ($P < 0.05$; Table 1), although there were only significant differences in June when $F_v/F_m$ of leaves in the N_{NF} treatment was lower than in the other treatments.

**Pigment chemistry**

There was a significant interaction between fertilizer and time on area-based total chlorophyll concentration ($P < 0.05$; Table 1). Total chlorophyll concentration was higher in N_{F2} than other treatments on April 23, higher in N_{F4} and N_{F7} than other treatments on September 3, but incrementally increased from N_{NF} to N_{F3} on June 11. There was a significant fertilizer x time interaction on xanthophyll-cycle conversion ratio ($P < 0.05$). The conversion ratio decreased incrementally from around $0.64$ in the N_{NF} treatment to $0.19$ in the N_{F2} treatment on April 23. On June 11, the conversion ratio was high in the N_{NF} treatment but not in the other treatments and on September 3 it was high in the N_{NF} and N_{F14} treatments but not in the other treatments.
Table 1. Photochemical quantum yield (\(\phi_{psii}\)), xanthophyll cycle conversion ratio \(((A + Z)/(V + A + Z))\) measured under clear conditions, predawn photochemical efficiency (\(F_{v}/F_{m}\)) and foliar total chlorophyll of \(E.\ nitens\) seedlings that were either non-fertilized (NF) or fertilized every 14 (NF14), 7 (NF7), 4 (NF4) or 2 (NF2) days from early April until September 2003 and measured in April, June and September 2003. Measurement of \(\phi_{psii}\) was made while taking care not to alter leaf angle. Values are least squares means ± standard error (SE). Abbreviation: N = nitrogen.

<table>
<thead>
<tr>
<th></th>
<th>NF</th>
<th>NF14</th>
<th>NF7</th>
<th>NF4</th>
<th>NF2</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\phi_{psii})</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>April</td>
<td>0.35</td>
<td>0.34</td>
<td>0.32</td>
<td>0.33</td>
<td>0.29</td>
<td>0.06</td>
</tr>
<tr>
<td>June</td>
<td>0.19</td>
<td>0.36</td>
<td>0.25</td>
<td>0.28</td>
<td>0.25</td>
<td>0.06</td>
</tr>
<tr>
<td>September</td>
<td>0.32</td>
<td>0.43</td>
<td>0.48</td>
<td>0.40</td>
<td>0.27</td>
<td>0.06</td>
</tr>
<tr>
<td>((A + Z)/(V + A + Z))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>April</td>
<td>0.64</td>
<td>0.39</td>
<td>0.41</td>
<td>0.41</td>
<td>0.19</td>
<td>0.08</td>
</tr>
<tr>
<td>June</td>
<td>0.62</td>
<td>0.02</td>
<td>0.02</td>
<td>0.04</td>
<td>0.07</td>
<td>0.08</td>
</tr>
<tr>
<td>September</td>
<td>0.39</td>
<td>0.49</td>
<td>0.11</td>
<td>0.24</td>
<td>0.10</td>
<td>0.08</td>
</tr>
<tr>
<td>(F_{v}/F_{m})</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>April</td>
<td>0.71</td>
<td>0.71</td>
<td>0.70</td>
<td>0.71</td>
<td>0.71</td>
<td>0.04</td>
</tr>
<tr>
<td>June</td>
<td>0.55</td>
<td>0.67</td>
<td>0.69</td>
<td>0.74</td>
<td>0.75</td>
<td>0.04</td>
</tr>
<tr>
<td>September</td>
<td>0.61</td>
<td>0.71</td>
<td>0.65</td>
<td>0.68</td>
<td>0.68</td>
<td>0.04</td>
</tr>
<tr>
<td>Total chlorophyll ((\mu\text{mol m}^{-2}))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>April</td>
<td>414.8</td>
<td>308.6</td>
<td>399.7</td>
<td>435.0</td>
<td>771.1</td>
<td>102.3</td>
</tr>
<tr>
<td>June</td>
<td>162.8</td>
<td>304.1</td>
<td>461.0</td>
<td>713.3</td>
<td>1504.1</td>
<td>102.3</td>
</tr>
<tr>
<td>September</td>
<td>280.2</td>
<td>256.0</td>
<td>268.8</td>
<td>780.4</td>
<td>642.4</td>
<td>102.3</td>
</tr>
</tbody>
</table>

**Leaf angle correlations**

Linear regression analysis showed that PPF incident on leaves at midday and ETR decreased linearly with increasing \(L_{0}\) across treatments on April 23, June 11 and September 3, 2003, and that \(F_{v}/F_{m}\) decreased linearly with increasing \(L_{0}\) on June 11 (Table 2). Area-based total chlorophyll concentration decreased linearly and xanthophyll cycle conversion ratio \(((A + Z)/(V + A + Z))\) increased linearly with increasing \(L_{0}\) in all treatments on April 23, June 11 and September 3, 2003.

**Discussion**

We obtained evidence that adjustments in \(L_{0}\) within 4 weeks of nutrient application treatments may play a photoprotective role in \(E.\ nitens\) seedlings. These adjustments were linked to N-deficit-induced photoinhibition that led to a regulated decrease in the chlorophyll pool and to increased xanthophyll-cycle conversion ratios.

The direction of the adjustments in \(L_{0}\) in response to the N application treatments was consistent with the anticipated degree of photoinhibition; i.e., \(L_{0}\) increased and leaves became more vertical with increasing N deficit and vice versa. Thus, under conditions of excess light absorption, the adjustment in \(L_{0}\) generally led to decreased irradiance at the leaf surface, whereas under conditions of limiting light absorption, the adjustment led to increased irradiance at the leaf surface. These findings indicate that non-heliotropic plants may actively adjust \(L_{0}\) in response to photoinhibition independently of any effect of drought. We also observed that \(L_{0}\) decrease became more horizontal, with plant age, independent of N treatment. The reason why the adjustment in \(L_{0}\) was unaccompanied by a measurable increase in irradiance at the leaf surface may be a consequence of increased self-shading with plant growth during the experimental period. Similarly, self-shading may explain the discrepancy between higher \(L_{0}\) in NF plants than in NF14 plants, although irradiance at the leaf surface was similar in these treatments.

Differences in adjustment of \(L_{0}\) had large effects on incident PPF on leaves, with consequences for light energy utilization and dissipation, consistent with results of photosynthetic gas exchange responses to artificial alterations in \(L_{0}\) within a species (Liu et al. 2003) and with comparisons of photosynthetic gas exchange of species with inherently different \(L_{0}\) (Ishida et al. 2001). Adjustments between the lowest and highest values of \(L_{0}\) were associated with a 2- to 5-fold difference in ETR, and ETR was negatively related to \(L_{0}\). In addition, predawn \(F_{v}/F_{m}\) decreased with increasing \(L_{0}\) on June 11 and September 3, when mean temperatures before sampling were 0.5 and 3.3 °C lower, respectively, than on April 23. In the most nutrient-deficient treatment (NF), \(F_{v}/F_{m}\) was lower than optimal (i.e., < 0.7; see Björkman and Demmig 1987) on June 11 and September 3, indicating that the increase in \(L_{0}\) did not completely alleviate photoinhibition of seedlings in the N-deficient treatments. The high values of predawn \((A + Z)/(V + A + Z)\) in the NF treatment are indicative of sustained xanthophyll engagement that allows plants to retain zeaxanthin overnight and thus obtain protection from photodamage on cold mornings (Ottander and Öquist 1991, Huner et al. 1993, Ottander et al. 1995). High values of predawn \((A + Z)/(V + A + Z)\) were
associated with depressed $F_l/F_m$ on June 11 and September 3, but not on April 23. Relatively high values of A and Z were observed in seedlings in all fertilizer treatments on April 23 and were associated with high $F_l/F_m$. Barker et al. (2002) also found high accumulations of A and Z in *Yucca schidigera* Roezl ex Ortg. and *Y. brevifolia* Engelm. that were associated with low predawn $F_l/F_m$ in winter but not in summer. Because A and Z would not be engaged in energy dissipation before sunrise on a warm summer morning, these authors speculated that high nocturnal temperatures disengaged A and Z from a state primed for energy dissipation. A similar response may have occurred in *E. nitens* seedlings under the relatively mild conditions that preceded the April 23 sampling. N-deficit-induced effects may be reflected in the higher values of θ in NNF seedlings than in seedlings in the other treatments in April.

Area-based chlorophyll concentrations were correlated with $L_a$. Lower specific leaf areas developed in plants of lower nutrient status. However, we would not expect the relative differences in chlorophyll concentration to alter if we expressed chlorophyll on a mass basis. Light absorption is proportional to chlorophyll concentration (Baker 1994, Evans and Poorter 2001) and chlorophyll synthesis and degradation are regulated to balance light absorption with its utilization and dissipation (Mattoo et al. 1999). The xanthophyll-cycle conversion state and its correlation with $L_a$ are consistent with more photoinhibited seedlings having a higher $L_a$ to reduce incident PPF. The role of the xanthophyll cycle in photoprotection is a ubiquitous plant process (Müller et al. 2001) and is highly active in eucalypt seedlings (Close et al. 2001a, 2003a).

Our results indicate that $L_a$ plays an important role at the whole-plant level in regulating incident light in *E. nitens* seedlings. Adjustment of $L_a$ may assist the leaf to balance its capacity to utilize and dissipate light energy under a given set of conditions of nutrient limitation.

**Acknowledgments**

Thanks to Dr. Clare McArthur for assistance in the inception of this project and for advice on trial design, Dr. Rod Seppelt of the Antarctic Division for the use of the PAM-2000, Mr. Hugh Fitzgerald for managing the fertilizer treatment, Dr. Noel Davies for the HPLC analyses, Ann Wilkinson for conducting the nitrogen analyses and Drs. Mark Hovenden and Phil Brown for reviewing the manuscript. D.C. Close gratefully acknowledges a post-doctoral fellowship of the Australian Research Council.

**References**


