Photosynthetic acclimation of plants to growth irradiance: the relative importance of specific leaf area and nitrogen partitioning in maximizing carbon gain

J. R. EVANS & H. POORTER*

Environmental Biology, Research School of Biological Sciences, Australian National University, GPO Box 475, Canberra, ACT 2601, Australia

ABSTRACT
Changes in specific leaf area (SLA, projected leaf area per unit leaf dry mass) and nitrogen partitioning between proteins within leaves occur during the acclimation of plants to their growth irradiance. In this paper, the relative importance of both of these changes in maximizing carbon gain is quantified. Photosynthesis, SLA and nitrogen partitioning within leaves was determined from 10 dicotyledonous C₃ species grown in photon irradiances of 200 and 1000 μmol m⁻² s⁻¹. Photosynthetic rate per unit leaf area measured under the growth irradiance was, on average, three times higher for high-light-grown plants than for those grown under low light, and two times higher when measured near light saturation. However, light-saturated photosynthetic rate per unit leaf dry mass was unaltered by growth irradiance because low-light plants had double the SLA. Nitrogen concentrations per unit leaf mass were constant between the two light treatments, but plants grown in low light partitioned a larger fraction of leaf nitrogen into light harvesting. Leaf absorbance was curvilinearly related to chlorophyll content and independent of SLA. Daily photosynthesis per unit leaf dry mass under low-light conditions was much more responsive to changes in SLA than to nitrogen partitioning. Under high light, sensitivity to nitrogen partitioning increased, but changes in SLA were still more important.

Key-words: Absorptance; photosynthesis; Rubisco; sun/shade acclimation.

INTRODUCTION
Plants can acclimate to their light environment at several integration levels. Firstly, they can change the fraction of biomass invested in leaves, stems and roots. Secondly, they are able to modulate the leaf area per unit biomass invested in leaves, by altering their anatomy. Thirdly, they can change the relative investment of nitrogen between photosynthetic components.

Changes at the whole-plant level to an increase in light are a decreased fraction of biomass allocated to leaves and an increased allocation to roots (Brouwer 1962; Poorter & Nagel 2000). Such a change in allocation maintains a constant transpiration rate per unit root mass (Sims & Pearcy 1994) or may supply the greater demand for nutrients required for faster growth in high light. However, in many instances, biomass allocation to leaves is not particularly sensitive to growth irradiance and is an unimportant factor with respect to the change in plant growth rate (Poorter & Nagel 2000).

The second change, at the leaf level, is in specific leaf area (SLA, the projected leaf area per unit leaf dry mass; see Table 1 for abbreviations). A given amount of biomass can be spread over a small or a large area. Plants grown in high light generally have thick leaves with a low SLA (Björkman 1981), due in part to extra layers of palisade or longer palisade cells (Hanson 1917). This increases the number of chloroplasts and the amount of photosynthetic enzymes and thereby enhances the photosynthetic capacity per unit leaf area. However, by having more biomass in a given area, the increase in photosynthetic capacity of the high-light leaves comes at a cost of having less light capture per unit biomass at lower irradiances. Consequently, growth is stimulated by high light only half as much as photosynthesis per unit area (Poorter & Nagel 2000). Sims, Gebauer & Pearcy (1994) modelled the impact of changing SLA on whole plant relative growth rate and found it to be more important under low- than high-light conditions.

The final level for acclimation, at the cellular level, is the re-allocation of nitrogen between the various pools involved in photosynthesis (Boardman 1977; Björkman 1981; Evans & Seemann 1989). The most important features of high-light-grown leaves in comparison with low-light ones are: (i) less chlorophyll per unit nitrogen; (ii) a higher chlorophyll a : b ratio; (iii) an increased electron transport capacity per unit chlorophyll; (iv) a slightly greater ratio of electron transport capacity to Rubisco activity. In previous papers, it has been shown that nitrogen partitioning within leaves changes with growth irradiance in such a way that it almost maximizes photosynthesis (Evans 1989b, 1989c, 1993a, 1993b; Hikosaka & Terashima...
To our knowledge, no one has quantitatively assessed the relative importance of changes in both SLA and nitrogen partitioning on daily photosynthesis per unit leaf dry mass. In this paper, we studied acclimation to low and high light at both the leaf and cellular level. Photosynthetic characteristics were investigated, along with SLA and allocation of nitrogen within leaves from a range of woody and herbaceous species. These data were evaluated in a model that revealed a far greater sensitivity of daily photosynthesis per unit leaf dry mass to changes in SLA than to nitrogen partitioning.

MATERIALS AND METHODS

Full details of materials and methods are given in Poorter & Evans (1998). In short, 10 plant species were grown from seeds: *Eucalyptus goniocalyx* F. Muell. ex Miq., *Eucalyptus macrocarpha* F. Muell., *Nerium oleander* W., Radyera faragei (F. Muell.) Fryxell & Hashmi, *Datura stramonium* L., *ECHium plantagineum* L., *Nicotiana tabacum* L., *Physalis peruvianum* L. Plantago major L. ssp. pleiosperma and *Raphanus sativus* L. The first four species are woody trees or shrubs whereas the last six are herbaceous plants. The seedlings were placed in a growth cabinet with the following conditions: day: 11 h, photosynthetic photon irradiance either 200 or 1000 ± 30 μmol m⁻² s⁻¹, temperature 25 ± 0.5 °C, relative humidity approximately 70%; and night: 13 h, temperature 20 ± 0.5 °C. The plants were grown hydroponically in a modified Hoagland solution with a nitrate concentration of 2 mM.

The plants were measured after they had developed leaves large enough to be adequately measured (3–12 weeks after germination). Firstly, CO₂ exchange was measured at the photon irradiance at which they were grown. Thereafter, photon irradiance was increased to 2000 μmol m⁻² s⁻¹ for high-light-grown plants and 1000 μmol m⁻² s⁻¹ for low-light-grown plants and the relationship between CO₂ assimilation rate (A) and intercellular CO₂ partial pressure (pᵢ) was determined. In the subsequent analysis, we assume these rates were near to light saturation. The reason for measuring the low-light-grown plants at 1000 μmol m⁻² s⁻¹ was to avoid photo-inhibition. After measuring the gas exchange, leaves were removed to determine transmittance and reflectance to photosynthetically active radiation with a Taylor integrating sphere (Taylor 1935) and quantum sensor (Li-Cor, Lincoln, NE, USA). The fraction of light absorbed by the leaf, absorbance, equals 1.0 – reflectance – transmittance. Subsequently, discs were punched out of each leaf, deep-frozen in liquid nitrogen and stored at −80 °C for later analysis of chlorophyll and Rubisco. Fresh and dry mass were determined on some of the discs, as well as the remainder of the leaf. The procedure was carried out independently with four plants from each of two growth cabinets.

Chlorophyll (Chl) was determined spectrophotometrically (Porra, Thompson & Kriedemann 1989). Rubisco content was assessed with a ¹⁴CABP binding method (Mate et al. 1993) assuming a molecular weight of Rubisco of 550 kDa. Soluble protein was measured using the Coo massie Plus assay (Pierce, Rockford, IL, USA) using bovine serum albumin as the standard. Total nitrogen content of the samples was determined with a C–H–N analyser (model 1106; Carlo Erba, Milan, Italy). Nitrate was quantified following Cataldo et al. (1975).

Calculations

The equations of Farquhar & von Caemmerer (1982) were used to fit the A–pᵢ curves. Electron transport rate, J (μmol e⁻ m⁻² s⁻¹), was calculated from data at higher intercellular CO₂ partial pressures as:

\[ J = (A + R)(4pᵢ + 8Γᵢ)/(pᵢ − Γᵢ) \]  

(1)

where Γᵢ is the CO₂ compensation point in the absence of non-photorespiratory mitochondrial CO₂ release (R). R was determined from the A–pᵢ curve near the CO₂ compensation point as the rate at pᵢ = Γᵢ, assuming Γᵢ to be 5–69 Pa for all species at 25 °C (von Caemmerer et al. 1994). Since the A–pᵢ curves were measured close to light saturation, we equate the J derived from the data at high intercellular CO₂ to the electron transport capacity, Jₑₑ (μmol e⁻ m⁻² s⁻¹).

Organic nitrogen was calculated as total nitrogen minus nitrate nitrogen. Thylakoid nitrogen, Nₑₑ (mmol N m⁻²), was derived from Jₑₑ (μmol e⁻ m⁻² s⁻¹), and the chlorophyll content per unit leaf area, χ (μmol m⁻²) (Evans 1989a):

\[ Nₑₑ = 0.079Jₑₑ + 0.0331χ. \]  

(2)

Thylakoid nitrogen was subdivided into two fractions, pigment-protein nitrogen (Nᵢ, mmol m⁻²) and that associated with the electron transport chain and photophosphorylation (Nₑₑ, mmol m⁻²). Growth irradiance alters the relative abundance of the pigment-protein complexes, as evidenced by a change in Chl a : b ratio. Although each species varied slightly in their Chl a : b ratio, we assumed a mean nitrogen to chlorophyll ratio for pigment-protein complexes, η, of 38.5 or 41 mol N (mol Chl)⁻¹ for low- and high-light-grown plants, respectively (Evans & Seemann 1989). The two thylakoid nitrogen fractions were calculated using:

\[ Nᵢ = \frac{χη}{10^{3}}, \]  

(3)

\[ Nₑₑ = Nₑₑ − χη. \]  

(4)

N allocated to Rubisco (Nᵢ) and to total soluble protein (Nₑₑ) was calculated assuming protein is 16% N by mass.

Model

To examine the relative impact that changing specific leaf area and nitrogen partitioning during acclimation to growth irradiance have on net daily CO₂ assimilation per unit organic leaf nitrogen (or dry mass), we derived the following model. Nitrogen concentration per unit leaf dry mass, Nₑₑ, was independent of growth irradiance. The average value for the 10 species was 3.3 mmol g⁻¹. Organic nitrogen
Absorptance, the fraction of I absorbed by the leaf (absorptance, $a$), depends on the chlorophyll content and is calculated as (Evans 1996):

$$a = \chi(\chi + 76).$$

Thylakoid nitrogen ($N_T$) is linearly related to electron transport capacity (Eqn 2). Soluble protein nitrogen, ($N_S$; mmol N m$^{-2}$), also varies in proportion to $J_{\text{max}}$ (Evans 1996):

$$N_S = \nu J_{\text{max}}.$$

where $\nu$ (mmol N s (mmol e$^-$)$^{-1}$) varies between species but is relatively independent of nitrogen content and growth irradiance (see Appendix, the mean value for the 10 species of 0.43 ± 0.03 was used for the modelling). Adding the nitrogen content of thylakoids (Eqn 2) to that of soluble proteins (Eqn 9) and rearranging gives:

$$J_{\text{max}} = (N_S + N_T - 0.0331\chi)/(\nu + 0.079).$$

Substituting Eqs 5, 6 and 7 into Eqn 10 gives:

$$J_{\text{max}} = [(10^3N_{\text{ORG}}^*\sigma)(1 - 33.1\phi_p/\eta) - N_O]/(\nu + 0.079),$$

which defines the electron transport capacity per unit leaf area for any given combination of specific leaf area ($\sigma$) and allocation of nitrogen to pigment-protein complexes ($\phi_p$). The net rate of photosynthetic electron transport was then calculated at the growth irradiance (Ögren & Evans 1993):

$$J = [\phi_p + J_{\text{max}} - ((\phi_p + J_{\text{max}})^2 - 4\phi_p\nu J_{\text{max}}^{0.5})/(2\eta)] - R,$$

$$N_{\text{ORG}} = 10^3N_{\text{ORG}}^*/\sigma.$$
where \( \phi \) is the maximum photon yield in white light \([0.425 \text{ mol } e^{-} (\text{mol photon})^{-1}]\) Evans (1987a), \( I \) is the incident photon irradiance, \( \Theta \) is a curvature factor \((0.78, \text{Ögren & Evans 1993})\) and \( R \) is the mean of the 10 species \((4 \text{ and } 8 \mu \text{mol } e^{-} \text{ m}^{-2} \text{ s}^{-1} \text{ for the low- and high-light treatments, respectively})\). Because plants were grown under constant irradiance, daily photosynthesis was assumed to be proportional to \( J \), with \( R \) doubled to account for leaf respiration through the night (data not shown). The rate was converted to a mass basis by multiplying by the SLA.

The maximum rate of electron transport per unit leaf mass was found by varying specific leaf area \((\sigma)\) and allocation to pigment-protein complexes \((\phi_p)\), using Eqns 7, 8, 11 and 12. All rates were scaled relative to this maximum rate to generate contour plots illustrating the sensitivity to changes in SLA and \( \phi_p \).

RESULTS

In this analysis we focused on the differences between plants grown in low and high light. Measurements of the different species were considered to be a kind of replication, making the results more general because they were based on a range of plant species. We characterize the species-specific distribution of the observed responses by box plots (see legend of Fig. 1 for an explanation). Values for individual species can be found in Appendix 1.

Observations

The rate of photosynthesis per unit leaf area measured under the growth irradiance, differed three-fold between treatments (Fig. 1a, \( P < 0.001 \)) and still differed by two-fold when plants were measured under saturating irradiance (Fig. 1b, \( P < 0.001 \)). Expressed on a leaf dry mass basis, differences in the rate of photosynthesis measured under the growth irradiance were smaller than for those on an area basis, but still significant (Fig. 1c, \( P < 0.001 \)). When determined at saturating irradiance, however, no differences in carbon gain per unit leaf mass were found between the plants of the two treatments (Fig. 1d, \( P > 0.15 \)). Differences in the response between the light-saturated rates of photosynthesis expressed per unit leaf area and per unit leaf mass are caused by a difference in SLA, with leaves formed under low light having on average twice the SLA in comparison with high-light leaves (Fig. 2a, \( P < 0.001 \)).

The absorptance of leaves was similar for plants grown in low and high light (Fig. 2b, \( P > 0.8 \)). Generally, growth under high light increased leaf reflectance slightly (Fig. 2c, \( P < 0.05 \)) and decreased transmittance (Fig. 2d, \( P > 0.1 \)). Absorptance increased with increasing chlorophyll content,
in a way that was well described by a saturating curve (Fig. 3a) found earlier for other data (Evans 1996). Although there was a 2.5-fold variation in chlorophyll content per unit leaf area between the species, absorptance only varied between 0.80 and 0.95. Mean chlorophyll content per unit leaf area was 440 and 510 μmol m⁻² for leaves grown under low and high light, respectively (P < 0.001). We examined the deviation between measured absorptance and that predicted from chlorophyll content to see if it was related to changes in SLA or reflectance. The deviation in absorptance with respect to SLA was small, decreasing from 2.0 to 0.4% for low- and high-light leaves, respectively (Fig. 3b).

For a given chlorophyll content, some species (both species of Eucalyptus, Echium, Plantago and Raphanus) had more reflective leaves than the others, which reduced their absorptance below the value predicted from their chlorophyll content. By contrast, leaves with low reflectance had higher than expected absorptance. The variation in reflectance accounted for nearly all of the deviation in absorptance (Fig. 3c).

Total nitrogen concentration on a dry mass basis was slightly higher for plants grown in low light (Fig. 4a, P < 0.01), as was the nitrate concentration (Fig. 4b, P < 0.001), such that the organic nitrogen concentration per unit dry mass was independent of growth irradiance (Fig. 4c, P > 0.1). Organic nitrogen content per unit leaf area was two-fold higher for the plants grown in high light (Fig. 4d, P < 0.001), due to the difference in SLA. In the subsequent analysis, we express the partitioning of nitrogen with respect to organic nitrogen and ignore the nitrate pool.

Chlorophyll content of leaves expressed per unit organic nitrogen, decreased with increasing irradiance (Fig. 5a, P < 0.001) and there was little variation between species. Photosynthetic acclimation to irradiance during growth affected the relative abundance of the pigment-protein complexes and hence Chl a:b ratio (Fig. 5b, P < 0.001), as well as the electron transport capacity of the thylakoids. The greater Chl a:b ratios in leaves grown under high light relative to the low-light treatment reflects greater numbers of photosystem II reaction centre complexes and fewer chlorophyll b-containing light-harvesting complexes, result-
ing in a greater value for η (see Eqn 3). Concomitantly, there was an increase in electron transport capacity for all species when grown under high versus low light (Fig. 6). Rubisco content was strongly related to electron transport capacity across both light treatments and species. The ratio of Rubisco to electron transport capacity was slightly greater for leaves grown under high light (0.011 versus 0.014 g s (mol e–1)–1 for low- and high-light leaves, respectively).

The proportion of soluble protein present as Rubisco varied little, averaging around one-third for most species. Nerium oleander was the lowest at 0.25 and Raphanus sativum the highest at 0.42 (see Appendix). Overall, partitioning of organic nitrogen between the various pools was as follows: pigment-protein complexes decreased when plants were grown under high light (Fig. 7a, P < 0.001), whereas nitrogen in electron transport capacity remained the same (Fig. 7b, P > 0.7), and nitrogen in Rubisco and other soluble proteins increased (Fig. 7c & d, P < 0.001). These four pools accounted for 85% of the organic nitrogen under both low-light and high-light treatments, leaving approximately 15% in other compounds such as nucleic acids and cell wall proteins.

Modelling results

The above data were used in the model to evaluate the consequences of changes in N-allocation or SLA on the carbon gain by leaves.

For a leaf with a given nitrogen content per unit leaf area, there exists a trade-off between light capture (a) and electron transport capacity (Jmax) as allocation to pigment-proteins (fP) is varied. The relationships between these parameters are determined for a leaf by Eqns 7, 8 and 11 presented in the model section. An illustration of the trade-off is given in Fig. 8a for the plants grown under low light. As the proportion of nitrogen allocated to the pigment-protein complexes increased, absorptance increased along a saturating curve. At the same time, Jmax decreased as investment in pigment-protein came at the expense of electron transport and soluble protein. Up to a fP of 0.2, the net result of the increased absorptance was increased daily photosynthesis per unit leaf dry mass. The decrease in Jmax was of no con-

Figure 5. (a) Chlorophyll per unit organic nitrogen and (b) chlorophyll a:b ratio for 10 plant species grown at low (200 μmol m–2 s–1) and high light (1000 μmol m–2 s–1). For more information see the legend of Fig. 1.

Figure 6. Rubisco content per unit leaf area versus electron transport rate per unit leaf area. Rubisco content was quantified in leaf extracts by CABB binding. Electron transport rate under high irradiance and intercellular CO2 partial pressures was calculated from gas exchange measurements (Eqn 1). Each symbol represents the mean (± SE, n = 8) for a species grown at low (●, mean ratio of Rubisco/J = 0.011) or high light (○, mean ratio 0.014). Data for E. macrorhyncha at both growth irradiances and E. goniocalyx at high irradiance are missing due to problems with the quantification of Rubisco.

Figure 7. Relative distribution of organic nitrogen, NORG, between the various pools, for low-light- and high-light-grown plants. NP = N in pigment-protein complexes. NE = N involved in electron transport and photophosphorylation. NR = N in Rubisco. NS = N in total soluble protein. For more information see the legend of Fig. 1.
Photosynthetic acclimation of plants to growth irradiance

sequence, because up to this point, the rate of photosynthesis under the low-light conditions was independent of changes in electron transport capacity. However, for $\phi_p$ greater than 0.2, there was an over-investment in pigment-protein, and daily photosynthesis declined. For each incremental increase in $\phi_p$, the small increase in absorbance was outweighed by the decrease in photosynthetic rate caused by the reduction in $J_{\text{max}}$. The leaf acclimated to low light allocated a greater fraction of nitrogen to pigment-protein complexes, which increased daily photosynthesis per unit leaf mass by about 1% relative to the high-light leaf.

A second trade-off exists between leaf surface area and nitrogen content per unit area, because organic nitrogen per unit leaf dry mass was unaltered by growth irradiance treatment. Electron transport capacity is linearly related to 1/SLA (Eqn 11), so it is appropriate to show the response to the reciprocal of SLA, which is equivalent to the term leaf mass per unit area (Fig. 8b). Although $\phi_p$ was held constant, leaf absorbance again increased along a saturating curve because the nitrogen content per unit leaf area increased linearly as 1/SLA increased. Daily photosynthesis per unit leaf dry mass increased rapidly to a maximum (open circle), then declined with further increases in 1/SLA. Under low-light conditions, the maximum rate of photosynthesis per unit leaf dry mass occurred at a lower value of 1/SLA than under high-light conditions. Acclimation to low light resulted in $\phi_p$ increasing and 1/SLA decreasing, which improved daily photosynthesis per unit leaf dry mass relative to the characteristics of a leaf acclimated to high light (solid versus open squares, Fig. 8). Clearly, for plants acclimated to low light, changes in SLA had a much greater impact on daily photosynthesis per unit leaf dry mass than changes in $\phi_p$.

The two trade-offs shown in Fig. 8 define a surface that can be represented as a contour plot of daily photosynthesis per unit leaf dry mass for each growth irradiance relative to the value with the optimum 1/SLA and $\phi_p$ (Fig. 9, open circles). The average (mean of the 10 species) values of 1/SLA and $\phi_p$ for leaves from the low- and high-light treatment are shown as solid and open squares, respectively. The dashed arrow joining the squares shows the change observed with acclimation. Profiles of the surface through the open circle in Fig. 9a cut vertically or horizontally were shown in Fig. 8a & b, respectively. The maximum rate of photosynthesis for the low-light environment occurred with values of 1/SLA of 15 g m$^{-2}$ and $\phi_p$ of 0.18, whereas for the high-light environment, the values were 45 and 0.08, respectively. Leaves acclimated to low light had greater daily photosynthesis per unit leaf dry mass under the low-light conditions than leaves acclimated to high light and vice versa. Under low-light conditions, the mean 1/SLA and $\phi_p$ values for leaves acclimated to low light enabled 87% of the maximum rate to be achieved, in comparison with only 53% using 1/SLA and $\phi_p$ values for leaves acclimated to high light (Fig. 9a). If the plant acclimated to high light could change either 1/SLA or $\phi_p$ to the value for the low-light plant, it would increase relative photosynthesis far more by changing 1/SLA (29% gain, horizontal arrow) than by changing $\phi_p$ (2% gain, vertical arrow). Under high-light conditions, the mean 1/SLA and $\phi_p$ values for leaves acclimated to high light enabled 98% of the maximum to be achieved, compared with 71% using 1/SLA and $\phi_p$ values for leaves acclimated to low light (Fig. 9b). Changing 1/SLA from the low- to the high-light value increased photosynthesis by 22% (horizontal arrow) whereas changing $\phi_p$ increased photosynthesis by 10% (vertical arrow). Changing $\phi_p$ was more important under high than low light, but under either light was less important than changing SLA.

**DISCUSSION**

The changes in parameters that characterize acclimation to growth irradiance were quantitatively similar between and within the groups of woody trees/shrubs and herbaceous species. This allowed us to conclude that our results
revealed general principles which were incorporated into a model. Acclimation to low light resulted in a doubling of SLA and halving of organic nitrogen content per unit leaf area. When expressed per unit leaf dry mass, both organic nitrogen concentration and photosynthetic capacity were unchanged. However, nitrogen allocation within the leaf changed. For leaves acclimated to low light, less nitrogen was allocated to soluble proteins and nitrogen in pigment-protein complexes increased from 13 to 21% of organic leaf nitrogen content per unit leaf area. For leaves acclimated to low light, less nitrogen was allocated to soluble proteins and nitrogen in pigment-protein complexes increased from 13 to 21% of organic leaf nitrogen content per unit leaf area. Similar conclusions can be drawn from data of other studies (Lee & Graham 1986; Lee et al. 1990; Thompson, Huang & Kriedemann 1992; Poorter, Oberbauer & Clark 1995; Ishida et al. 1999; Poorter et al. 2000).

These results may seem at variance with studies in which the leaf structure has been shown to increase light absorption by leaves, mainly by increasing the path length of light by scattering (Terashima & Saeki 1983; De Lucia et al. 1996). Scattering occurs at air–cell wall interfaces, especially in the spongy mesophyll. Leaf porosity and mesophyll structure can therefore influence scattering, but neither relate to SLA. The conclusion that leaf absorptance is independent of SLA is crucial if one wishes to consider the consequence of altering SLA when calculating the trade-off between absorptance and photosynthetic capacity.

**Relationship between $J_{\text{max}}$ and N**

The amount of thylakoid nitrogen per unit chlorophyll has been measured for several species and from leaves acclimated to different irradiances (Evans 1987b; Terashima & Evans 1988; Evans 1989a; Hikosaka & Terashima 1996). Rather than measuring thylakoid nitrogen directly, we relied on the linear relationship between electron transport capacity and thylakoid nitrogen per unit chlorophyll (Evans 1989a).

The ratio of electron transport capacity to Rubisco activity has been found to be relatively constant when nitrogen content per unit leaf area varies (von Caemmerer & Farquhar 1981; Evans 1983), or across growth irradiance treatments (Sims & Pearcy 1989; Thompson et al. 1992; Wullschleger 1993; Evans 1996). It has also been observed...
Photosynthetic acclimation of plants to growth irradiance 763

that soluble protein varies in direct proportion with electron transport capacity (see Appendix, Evans 1989b, c, 1993b). Soluble protein nitrogen content per unit electron transport capacity ranged from 0·29 to 0·70 across the species, with a mean of 0·43 mol N s (mmol e–)–1 (see Appendix), similar to the mean value of other studies [0·35 mol N s (mmol e–)–1; Evans 1996]. Rather than using just the nitrogen in the Calvin cycle, we chose to use the total soluble protein nitrogen pool when calculating the amount of nitrogen needed per unit electron transport capacity.

We found that the ratio of Rubisco content to electron transport activity was slightly less for leaves grown under low versus high light (Fig. 6). This was not included in our calculations because we were not comparing the absolute rates of photosynthesis between the two light treatments. Carboxylase activity calculated from A–p, curves per unit Rubisco declined with increasing Rubisco content across all the species examined here (Poorter & Evans 1998). This effect has been explicitly incorporated into the model of Hikosaka & Terashima (1995). However, in the optimization performed herein, we have ignored this complication, regarding it as of secondary importance.

Photosynthetic capacity is linearly related to nitrogen content per unit leaf area (Hirose & Werger 1987; Pons et al. 1989; Evans 1993b; Anten, Schieving & Werger 1995), declining to zero at a certain nitrogen content, equivalent to the term N0 in Eqn 11. The nitrogen content where photosynthesis equals zero also coincides with the nitrogen content where Rubisco declines to zero (Evans 1983; Makino, Mae & Ohira 1984, 1988; Sage, Pearcy & Seemann 1987; Sims & Pearcy 1989; Harley et al. 1992). However, whether this residual nitrogen present in the leaf equals the non-photosynthetic nitrogen associated with epidermal and vascular tissue, cell walls and nucleic acids, is not clear. It is also not known whether the non-photosynthetic nitrogen changes with respect to leaf age or SLA. For our model simulations, we assigned a fixed amount of non-photosynthetic nitrogen per unit leaf area, N0, that was the observed mean value for that light treatment. The model therefore predicts electron transport rates most accurately near the observed mean leaf nitrogen content for a given light treatment, whereas the influence of N0 becomes more important as SLA increases.

Changing N allocation between protein pools

Acclimation to low growth irradiance primarily involves reallocation of nitrogen from soluble protein into pigment-protein complexes (Fig. 7). The relative change in the soluble protein nitrogen pool (which includes Rubisco), is rather small because it is five times larger than the pigment-protein pool under high-light growth conditions. This is evident in the data of Niinemets et al. (1998) that was collected on four deciduous tree species, in which allocation of nitrogen to Rubisco and bioenergetics was relatively independent of the light environment of the leaf, whereas allocation to pigment-protein complexes was dramatically higher in leaves collected from low irradiance sites.

There are now a number of species for which acclimation to growth irradiance has been analysed in terms of nitrogen allocation within the leaf (Evans 1989c, 1993b; Hikosaka & Terashima 1996). The analyses reveal that acclimation improves potential daily photosynthesis and in most cases the acclimated leaf characteristics fell remarkably close to the model optimum, achieving greater than 98% of the optimal case for a given SLA. Had high-light characteristics been retained under low-light growth conditions, then the leaf would generally have only achieved around 80% of the optimal case. For the analysis performed here using the average characteristics of 10 species, the fraction of nitrogen allocated to pigment-protein complexes under low light (ϕϕϕϕ = 0·21) achieved 99% of the low-light optimum (ϕϕϕϕ = 0·18) for a leaf with a value of 1/SLA of 15 g m–2. Leaves would have achieved 98% of the optimum with the allocation by leaves acclimated to high light (ϕϕϕϕ = 0·13). Under high-light conditions, for a leaf with a 1/SLA of 45 g m–2, the optimum ϕϕϕϕ was 0·08. Leaves achieved 98 and 92% of the optimum with ϕϕϕϕ values of 0·13 and 0·21, respectively. Acclimation thus improved potential photosynthetic gain for a given amount of leaf nitrogen, but the penalty for retaining the opposite allocation to pigment-protein complexes was not as large as in the previous studies.

Changing SLA

Nitrogen concentration per unit leaf dry mass did not differ between the two light treatments (Fig. 4c). Consequently, when SLA changes, this alters the nitrogen content per unit leaf area. At the same time, a change in SLA alters the amount of light that can be intercepted per unit leaf dry mass. Consider the case of two blocks of leaf tissue that can be arranged on top of one another (low SLA) or beside one another (high SLA). If both leaves have the same chlorophyll content per unit leaf area (Fig. 3), then the high SLA leaf has approximately half the Rubisco content per unit leaf area but can absorb twice the number of photons in comparison with the low SLA leaf. This would clearly be advantageous under low-light conditions. For our modeling, we assumed that there was no limit to the area available to intercept light (i.e. no self-shading).

Several models have been developed to calculate the leaf nitrogen content that results in the maximum rate of photosynthesis per unit leaf nitrogen (Hirose & Werger 1987; Pons et al. 1989; Evans 1993b; Anten et al. 1995; Hikosaka & Terashima 1995; Schieving & Poorter 1999). These have generally been based on empirically determined relationships between A and nitrogen content per unit leaf area. Because of the non-zero intercept N0, there exists a nitrogen content where photosynthetic rate per unit nitrogen is maximized for a given irradiance (e.g. Hirose & Werger 1987). The greater the irradiance, the greater the nitrogen content that is needed to reach the maximum photosynthetic rate per unit nitrogen.

If non-photosynthetic nitrogen was a constant fraction of leaf nitrogen, then regardless of the light environment, the maximum photosynthetic rate per unit dry mass would

be achieved by an infinitely thin, high SLA leaf. This is unlikely to be the case because among other things, there must be a finite amount of nitrogen in epidermal and vascular tissue. The difference between the carbon gain of leaves with high or low SLA ranged from 29 to 22% under low- and high-light conditions, respectively.

When leaves are analysed with respect to different growth irradiances, it is generally the case that the nitrogen concentration per unit dry mass is rather constant, although there are considerable changes in SLA (Hollinger 1989; Ellsworth & Reich 1993; Hirose & Weger 1994; Niinemets & Kull 1998; Niinemets et al. 1998; Ishida et al. 1999; Figs 2a & 5c). Why does SLA change rather than nitrogen concentration? A case has been made that for Rubisco to operate efficiently in terms of gaining access to CO\(_2\), it needs to be spread thinly across the surface of cell walls exposed to intercellular airspaces (Evans 1998). It has been shown for several species that chloroplasts cover most of the available mesophyll surface area exposed to intercellular airspace (Evans & Loreto 2000). Therefore, any increase in nitrogen concentration for a leaf with a given SLA would lead to an increase in chloroplast thickness. This would reduce the apparent activity of Rubisco by reducing the diffusion of CO\(_2\) to the catalytic sites (Evans 1999). Conversely, when a lower photosynthetic capacity is beneficial, this can be placed into a structure with greater SLA, leading to improvements in both photosynthesis per unit nitrogen and dry mass.

The relative importance of changes in SLA and nitrogen allocation

By combining \(\phi_P\) and SLA into the same equation, we have been able to assess the relative importance of both parameters. Whereas previous studies have shown that changes to \(\phi_P\) due to acclimation to growth irradiance improve photosynthesis per unit leaf nitrogen, we have shown that changes in SLA have a greater impact under both low- and high-light conditions. Niinemets & Tenhunen (1997) took a different approach, combining data from different experiments and linking all of the parameters to leaf dry mass per unit area via empirical regressions through data collected on leaves at different canopy positions. They concluded that in low light, nitrogen re-allocation within the leaf was at least as important as changes to SLA. By contrast, we suggest that under low light, changes to \(\phi_P\) increased relative daily photosynthesis per unit leaf dry mass by only 2% in comparison with a 29% increase by changing SLA (Fig. 8). It was under high light that re-allocation of nitrogen had a greater impact, enabling a 10% increase compared to a 22% increase by changing SLA (Fig. 8,9).

The reason for this is that light capture by a leaf is very efficient at low chlorophyll contents and to increase absorbance requires a lot of additional pigment-protein complexes (Fig. 3a). More light can be captured by spreading a given amount of pigment-protein complexes over a greater area than by concentrating it in a given area. Under high light, increasing daily photosynthesis requires greater amounts of nitrogen allocated to electron transport capacity and the soluble proteins. Nitrogen per unit leaf area is increased by decreasing SLA. As none of the additional nitrogen needs to be allocated to pigment-protein to maintain absorptance, all of the additional nitrogen can be allocated towards increasing \(J_{\text{max}}\). Thus under high light, the two parameters interact and daily photosynthesis increases by more if the increase in 1/SLA is accompanied by a decrease in \(\phi_P\).

Limitations of the model

The optimum value for 1/SLA predicted by the model for the low-light condition was less than that observed (18 versus 25 g m\(^{-2}\)). This suggests that other factors left out of the model are also important. These factors include the need for support, herbivory, leaf longevity, leaf overlap and sun-flecks. An analysis of 15 temperate woody species revealed that allocation to the leaf lamina fell with increasing leaf length whereas vascular tissue increased (Givnish 1984). As vascular tissue must contain some nitrogen, an increasing proportion of leaf nitrogen would be associated with vascular tissue as SLA increased. Coping with herbivory or physical damage by wind, for example, may be another important factor. It has been shown that increased leaf toughness and lower nitrogen concentrations were associated with lower rates of herbivory (Coley 1983). Increased toughness can be achieved through a higher fibre content, but is generally related to lower SLA. Lower SLA is also associated with greater longevity (Reich, Walters & Ellsworth 1997) and if photosynthesis over the lifetime of a leaf were important, this would favour a lower SLA. The assumption of an unlimited area available for light interception is also questionable. As SLA increases, the probability of leaf overlap occurring may increase. All of these factors would increase the optimum value of 1/SLA under low light, bringing it closer to our observed mean value. Another feature of natural environments is that irradiance fluctuates diurnally and much of the light within canopies occurs as sunflecks (Pearcy 1990) and factoring this in could favour lower SLA values.

CONCLUSIONS

Across a range of species, acclimation to a low-light environment is characterized by increased allocation of leaf organic nitrogen to pigment-proteins, as well as by increased SLA. Increasing SLA was found to be far more important in maximizing carbon gain per unit leaf mass than re-allocating nitrogen between leaf pools, especially under low light.

ACKNOWLEDGMENTS

We thank Thijs Pons and Susanne von Caemmerer for critical reading of this manuscript. What a difference a
REFERENCES


Received 4 January 2001; received in revised form 20 April 2001; accepted for publication 20 April 2001
### APPENDIX

**Table A1.** Average values for specific leaf area (SLA), organic N content ($N_{\text{ORG}}$), chlorophyll content (Chl), electron transport capacity ($I_{\text{max}}$), the fraction of leaf organic nitrogen invested in the various components, as well as the fraction of soluble protein in Rubisco and the amount of soluble protein N per unit electron transport capacity ($v$), measured for 10 species grown at low light and high light. The first four species are woody, the next six are herbaceous. Mean values ±SE, $n = 8$. Assays for soluble protein and Rubisco were not successful for three of the four Eucalyptus cases.

<table>
<thead>
<tr>
<th>Species</th>
<th>SLA (m² kg⁻¹)</th>
<th>$N_{\text{ORG}}$ (mmol m⁻²)</th>
<th>Chl (µmol m⁻²)</th>
<th>$I_{\text{max}}$ (µmol e⁻ m⁻² s⁻¹)</th>
<th>Percentage of $N_{\text{ORG}}$ in:</th>
<th>Rubisco/ soluble protein (%)</th>
<th>$v$ [mol N (mol e⁻)⁻¹]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Low light</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Eucalyptus goniocalyx</em></td>
<td>38.3 ± 2.2</td>
<td>88.3 ± 3</td>
<td>477 ± 15</td>
<td>113 ± 7</td>
<td>21.0 ± 0.7</td>
<td>7.0 ± 0.7</td>
<td>13.1 ± 0.4</td>
</tr>
<tr>
<td><em>Eucalyptus macrophyllina</em></td>
<td>30.1 ± 2.5</td>
<td>108.11</td>
<td>536 ± 28</td>
<td>102 ± 8</td>
<td>20.1 ± 1.8</td>
<td>5.6 ± 1.2</td>
<td></td>
</tr>
<tr>
<td><em>Nerium oleander</em></td>
<td>26.6 ± 0.9</td>
<td>103 ± 5</td>
<td>573 ± 32</td>
<td>99 ± 5</td>
<td>21.5 ± 1.1</td>
<td>5.1 ± 0.7</td>
<td>17.8 ± 0.9</td>
</tr>
<tr>
<td><em>Radyera faragai</em></td>
<td>24.3 ± 0.6</td>
<td>103 ± 5</td>
<td>716 ± 25</td>
<td>170 ± 31</td>
<td>18.7 ± 0.7</td>
<td>5.7 ± 1.2</td>
<td>14.7 ± 0.7</td>
</tr>
<tr>
<td><em>Datura stramonium</em></td>
<td>57.0 ± 2.3</td>
<td>72.3 ± 3</td>
<td>374 ± 15</td>
<td>113 ± 9</td>
<td>20.0 ± 0.7</td>
<td>9.6 ± 0.6</td>
<td>22.6 ± 1.3</td>
</tr>
<tr>
<td><em>Echinus plantagineum</em></td>
<td>43.1 ± 1.2</td>
<td>76 ± 3</td>
<td>396 ± 13</td>
<td>142 ± 7</td>
<td>20.1 ± 0.8</td>
<td>11.7 ± 0.4</td>
<td>17.3 ± 1.9</td>
</tr>
<tr>
<td><em>Nicotiana tabacum</em></td>
<td>58.6 ± 1.7</td>
<td>50 ± 2</td>
<td>314 ± 7</td>
<td>76 ± 4</td>
<td>24.3 ± 1.1</td>
<td>8.2 ± 0.4</td>
<td>18.5 ± 0.2</td>
</tr>
<tr>
<td><em>Phiosalis peruvianum</em></td>
<td>60.2 ± 3.6</td>
<td>57.3 ± 3</td>
<td>359 ± 14</td>
<td>66 ± 12</td>
<td>24.4 ± 0.5</td>
<td>5.8 ± 1.3</td>
<td>20.2 ± 2.1</td>
</tr>
<tr>
<td><em>Plantago major</em></td>
<td>47.4 ± 1.2</td>
<td>63.1 ± 3</td>
<td>339 ± 7</td>
<td>112 ± 8</td>
<td>20.7 ± 0.8</td>
<td>10.9 ± 1.1</td>
<td>16.1 ± 0.9</td>
</tr>
<tr>
<td><em>Raphanus sativus</em></td>
<td>61.6 ± 2.0</td>
<td>58 ± 3</td>
<td>300 ± 7</td>
<td>114 ± 8</td>
<td>20.1 ± 0.8</td>
<td>12.4 ± 1.1</td>
<td>22.8 ± 1.7</td>
</tr>
<tr>
<td><strong>High light</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Eucalyptus goniocalyx</em></td>
<td>18.3 ± 0.7</td>
<td>573 ± 27</td>
<td>668 ± 37</td>
<td>253 ± 20</td>
<td>15.8 ± 0.9</td>
<td>8.7 ± 0.4</td>
<td></td>
</tr>
<tr>
<td><em>Eucalyptus macrophyllina</em></td>
<td>13.4 ± 0.4</td>
<td>182 ± 10</td>
<td>702 ± 47</td>
<td>233 ± 19</td>
<td>16.0 ± 1.1</td>
<td>7.5 ± 0.6</td>
<td></td>
</tr>
<tr>
<td><em>Nerium oleander</em></td>
<td>15.4 ± 0.4</td>
<td>176 ± 7</td>
<td>575 ± 34</td>
<td>164 ± 6</td>
<td>13.5 ± 0.9</td>
<td>5.0 ± 0.4</td>
<td>17.1 ± 0.6</td>
</tr>
<tr>
<td><em>Radyera faragai</em></td>
<td>13.2 ± 0.3</td>
<td>267 ± 8</td>
<td>727 ± 54</td>
<td>401 ± 66</td>
<td>11.1 ± 0.6</td>
<td>8.9 ± 2.1</td>
<td>17.6 ± 1.5</td>
</tr>
<tr>
<td><em>Datura stramonium</em></td>
<td>26.9 ± 1.8</td>
<td>153 ± 7</td>
<td>500 ± 19</td>
<td>240 ± 27</td>
<td>13.5 ± 0.6</td>
<td>9.3 ± 1.1</td>
<td>24.4 ± 0.9</td>
</tr>
<tr>
<td><em>Echinus plantagineum</em></td>
<td>22.6 ± 0.9</td>
<td>156 ± 8</td>
<td>425 ± 27</td>
<td>211 ± 15</td>
<td>11.3 ± 0.8</td>
<td>9.3 ± 0.5</td>
<td>19.5 ± 0.9</td>
</tr>
<tr>
<td><em>Nicotiana tabacum</em></td>
<td>29.6 ± 1.9</td>
<td>112 ± 8</td>
<td>365 ± 13</td>
<td>129 ± 13</td>
<td>13.6 ± 0.7</td>
<td>7.5 ± 1.2</td>
<td>22.8 ± 1.6</td>
</tr>
<tr>
<td><em>Phiosalis peruvianum</em></td>
<td>28.9 ± 1.6</td>
<td>138 ± 5</td>
<td>414 ± 21</td>
<td>152 ± 21</td>
<td>12.3 ± 0.4</td>
<td>6.0 ± 1.0</td>
<td>25.5 ± 0.4</td>
</tr>
<tr>
<td><em>Plantago major</em></td>
<td>25.9 ± 0.9</td>
<td>123 ± 4</td>
<td>302 ± 15</td>
<td>168 ± 6</td>
<td>10.1 ± 0.5</td>
<td>8.6 ± 0.6</td>
<td>21.8 ± 1.6</td>
</tr>
<tr>
<td><em>Raphanus sativus</em></td>
<td>29.3 ± 1.6</td>
<td>136 ± 7</td>
<td>377 ± 16</td>
<td>253 ± 16</td>
<td>11.5 ± 0.8</td>
<td>12.5 ± 0.7</td>
<td>26.4 ± 1.2</td>
</tr>
</tbody>
</table>