Plant, Cell and Environment (2001) **24,** 755–767

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

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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 C_3 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 absorptance 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

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1996; Hikosaka *et al*. 1998; Niinemets, Kull & Tenhunen 1998).

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 macrorhyncha* F. Muell., *Nerium oleander* W., *Radyera farragei* (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, CO_2 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 CO_2 assimilation rate (A) and intercellular CO_2 partial pressure (p_i) 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, absorptance, 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 ^{14}CABP binding method (Mate *et al*. 1993) assuming a molecular weight of Rubisco of 550 kDa. Soluble protein was measured using the Coomassie 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–p_i curves. Electron transport rate, J (μmol e$^-$ m^{-2} s^{-1}), was calculated from data at higher intercellular CO_2 partial pressures as:

$$J = (A + R)(4p_i + 8\Gamma*)/(p_i - \Gamma*) \quad (1)$$

where $\Gamma*$ is the CO_2 compensation point in the absence of non-photorespiratory mitochondrial CO_2 release (R). R was determined from the A–p_i curve near the CO_2 compensation point as the rate at $p_i = \Gamma*$, assuming $\Gamma*$ to be 3·69 Pa for all species at 25 °C (von Caemmerer *et al*. 1994). Since the A–p_i curves were measured close to light saturation, we equate the J derived from the data at high intercellular CO_2 to the electron transport capacity, J_{max} (μmol e$^-$ m^{-2} s^{-1}).

Organic nitrogen was calculated as total nitrogen minus nitrate nitrogen. Thylakoid nitrogen, N_T (mmol N m^{-2}), was derived from J_{max} (μmol e$^-$ m^{-2} s^{-1}), and the chlorophyll content per unit leaf area, χ (μmol m^{-2}) (Evans 1989a):

$$N_T = 0{\cdot}079J_{max} + 0{\cdot}0331\chi. \quad (2)$$

Thylakoid nitrogen was subdivided into two fractions, pigment-protein nitrogen (N_P, mmol m^{-2}) and that associated with the electron transport chain and photophosphorylation (N_E, 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:

$$N_P = \chi\eta/10^3, \text{ and} \quad (3)$$

$$N_E = N_T - \chi\eta/10^3. \quad (4)$$

N allocated to Rubisco (N_R) and to total soluble protein (N_S) 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 CO_2 assimilation per unit organic leaf nitrogen (or dry mass), we derived the following model. Nitrogen concentration per unit leaf dry mass, N_{ORG}*, was independent of growth irradiance. The average value for the 10 species was 3·3 mmol g^{-1}. Organic nitrogen

Table 1. Abbreviations and symbols used, their units and values (low light, high light)

Symbol	Units	Definition
A	μmol CO_2 m^{-2} s^{-1}	Rate of CO_2 assimilation
Chl		Chlorophyll
I	μmol photons m^{-2} s^{-1}	Incident photosynthetically active photon irradiance
J	μmol e^- m^{-2} s^{-1}	Rate of electron transport
J_{max}	μmol e^- m^{-2} s^{-1}	Electron transport capacity
N	mmol m^{-2}	Nitrogen with subscripts referring to different pools
N_E	mmol m^{-2}	Electron transport plus photophosphorylation
N_{ORG}	mmol m^{-2}	Organic nitrogen
N_{ORG}*	mmol g^{-1}	Organic nitrogen concentration per unit leaf dry mass (3·3)
N_P	mmol m^{-2}	Pigment-protein nitrogen
N_R	mmol m^{-2}	Rubisco nitrogen
N_S	mmol m^{-2}	Total soluble protein nitrogen
N_T	mmol m^{-2}	Thylakoid nitrogen
N_{TOTAL}	mmol m^{-2}	Total nitrogen (organic plus nitrate)
N_O	mmol m^{-2}	Organic nitrogen minus soluble and thylakoid nitrogen (10, 25)
p_i	Pa	Intercellular CO_2 partial pressure
R	μmol e^- m^{-2} s^{-1}	Rate of respiration in the light (4, 8)
SLA, σ	m^2 kg^{-1}	Specific leaf area
Γ*	Pa	CO_2 photocompensation point (3·69)
ϕ	mol e^- (mol photon)$^{-1}$	Maximum photon yield (0·425)
Θ		Curvature factor (0·78)
η	mol N (mol Chl)$^{-1}$	N to Chl ratio of pigment-protein complexes (38·5, 41)
ν	mol N s (mmol e^-)$^{-1}$	Soluble protein N per unit electron transport capacity (0·425)
α		Absorptance, the fraction of I absorbed by the leaf
ϕ_P		Fraction of organic N allocated to pigment-protein complexes
χ	µmol m^{-2}	Chlorophyll content per unit leaf area

content per unit leaf area (N_{ORG}, mmol m^{-2}) is related to SLA (σ, m^2 kg^{-1}) by the following equation:

$$N_{ORG} = 10^3 N_{ORG}^*/\sigma. \quad (5)$$

It can also be defined as the sum of total soluble protein nitrogen, thylakoid nitrogen and the 'rest', N_O:

$$N_{ORG} = N_S + N_T + N_O. \quad (6)$$

We assumed that the N_O content per unit leaf area was constant for a given growth irradiance (10 and 25 mmol N m^{-2} for low- and high-light treatments, respectively, derived from the averages of the 'rest' fraction in the Appendix).

The chlorophyll content per unit leaf area is related to the fraction of organic nitrogen allocated to pigment-protein complexes, $N_P/N_{ORG} = \phi_P$, by:

$$\chi = 10^6 N_{ORG}^* \phi_P/(\sigma\eta). \quad (7)$$

The fraction of photosynthetically active photon irradiance that is absorbed by the leaf (absorptance, α), depends on the chlorophyll content and is calculated as (Evans 1996):

$$\alpha = \chi/(\chi + 76). \quad (8)$$

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_{max} (Evans 1996):

$$N_S = \nu J_{max}, \quad (9)$$

where ν (mol 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:

$$J_{max} = (N_S + N_T - 0{\cdot}0331\chi)/(\nu + 0{\cdot}079). \quad (10)$$

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

$$J_{max} = [(10^3 N_{ORG}^*/\sigma)(1 - 33{\cdot}1\phi_P/\eta) - N_O]/(\nu + 0{\cdot}079), \quad (11)$$

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

$$J = [\phi\alpha I + J_{max} - [(\phi\alpha I + J_{max})^2 - 4\Theta\phi\alpha I J_{max}]^{0.5}]/(2\Theta) - R, \quad (12)$$

where ϕ is the maximum photon yield in white light [0·425 mol e^-(mol photon)$^{-1}$ Evans 1987a], I is the incident photon irradiance, Θ is a curvature factor (0·78, Ögren & Evans 1993) and R is the mean of the 10 species (4 and 8 μmol e^- m^{-2} s^{-1} 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 (σ) and allocation to pigment-protein complexes (ϕ_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 ϕ_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.

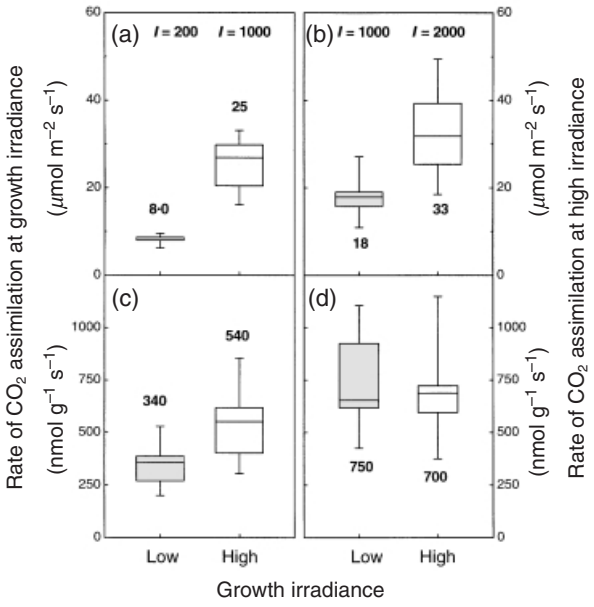

Figure 1. Rates of photosynthesis by leaves on plants grown in low light (photosynthetic photon irradiance of 200 μmol m^{-2} s^{-1}) and high light (1000 μmol m^{-2} s^{-1}) of 10 species expressed as (a) CO_2 assimilation per unit leaf area at growth irradiance; (b) CO_2 assimilation per unit leaf area at saturating irradiance (1000 and 2000 μmol m^{-2} s^{-1} for low- and high-light-grown plants, respectively); (c) CO_2 assimilation per unit leaf dry mass at growth conditions; (d) CO_2 assimilation per unit leaf dry mass at saturating irradiance. Rather than plotting data for each species individually, data distribution is given by box plots. The lower and upper part of the rectangles give the estimated 25th and 75th percentile, the line in the middle indicates the median value and the extent of the lines indicate the lowest and highest values among the 10 species. Bold data printed just above or below the box plots indicate the average values across species.

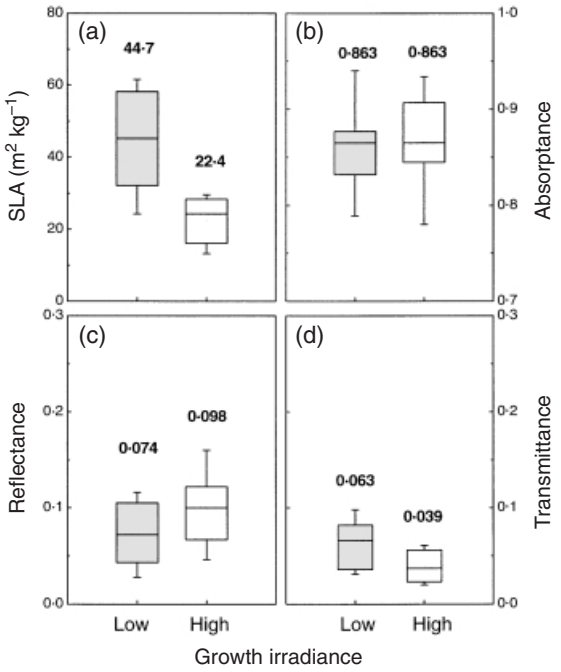

Figure 2. (a) Specific leaf area (projected leaf area per unit leaf dry mass), (b) leaf absorptance, (c) leaf reflectance, (d) leaf transmittance, for 10 species grown at low (200 μmol quanta m^{-2} s^{-1}) and high light (1000 μmol quanta m^{-2} s^{-1}). For more information see the legend of Fig. 1.

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 ($P < 0{\cdot}001$). 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).

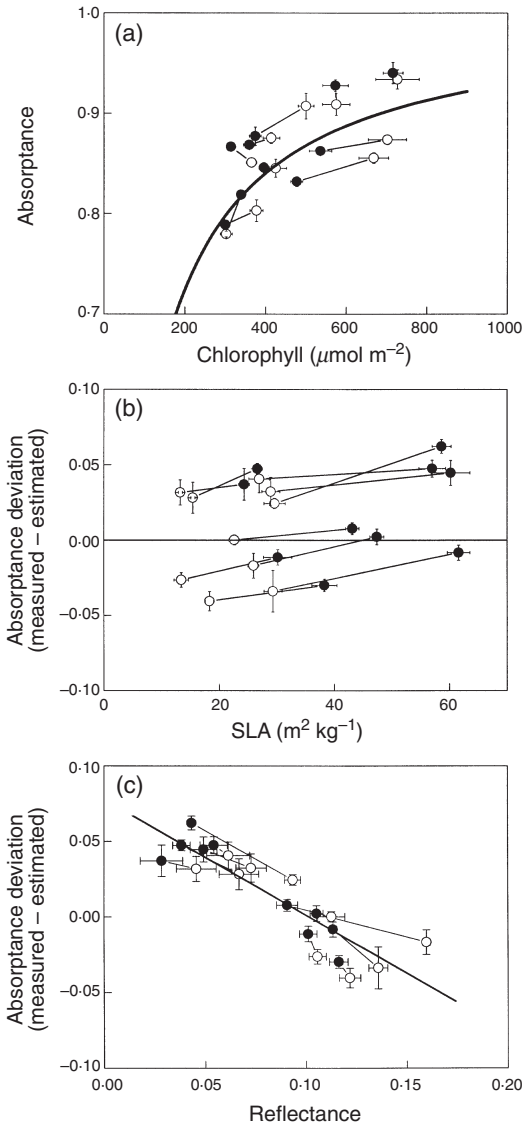

Figure 3. (a) Leaf absorptance versus chlorophyll content. The solid curve is given by Eqn 8. The deviation in absorptance (observed absorptance – absorptance calculated from the chlorophyll content, Eqn 8) with respect to (b) SLA or (c) reflectance ($y = 0{\cdot}078 - 0{\cdot}77x$, $r^2 = 0{\cdot}78$). The mean for each species (± SE, $n = 8$), for the low-light-grown plants (solid) is joined to that of the high-light-grown plants (open) by a line.

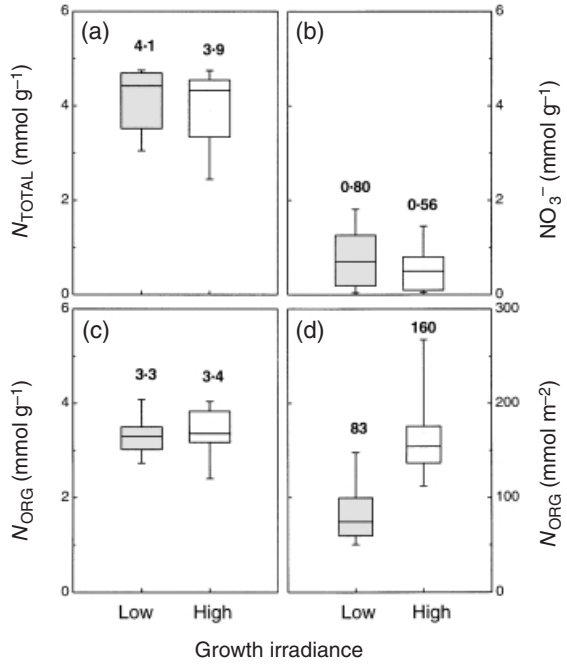

Figure 4. (a) Total N concentration, (b) nitrate concentration and (c) organic nitrogen concentration, all expressed as mmol g^{-1} dry mass and (d) organic nitrogen content per unit leaf area for 10 species grown at low (200 μmol m^{-2} s^{-1}) and high light (1000 μmol m^{-2} s^{-1}). For more information see the legend of Fig. 1.

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, $P < 0{\cdot}01$), as was the nitrate concentration (Fig. 4b, $P < 0{\cdot}001$), such that the organic nitrogen concentration per unit dry mass was independent of growth irradiance (Fig. 4c, $P > 0{\cdot}1$). Organic nitrogen content per unit leaf area was two-fold higher for the plants grown in high light (Fig. 4d, $P < 0{\cdot}001$), 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, $P < 0{\cdot}001$) 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, $P < 0{\cdot}001$), 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-

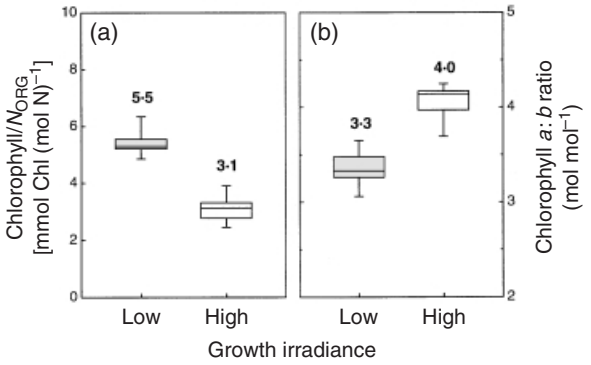

Figure 5. (a) Chlorophyll per unit organic nitrogen and (b) chlorophyll *a* : *b* ratio for 10 plant species grown at low (200 μmol m^{-2} s^{-1}) and high light (1000 μmol m^{-2} s^{-1}). For more information see the legend of Fig. 1.

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 (μmol e$^-$)$^{-1}$ 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, $P < 0{\cdot}001$), whereas nitrogen in electron transport capacity remained the same (Fig. 7b, $P > 0{\cdot}7$), and nitrogen in Rubisco and other soluble proteins increased (Fig. 7c & d, $P < 0{\cdot}001$). 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.

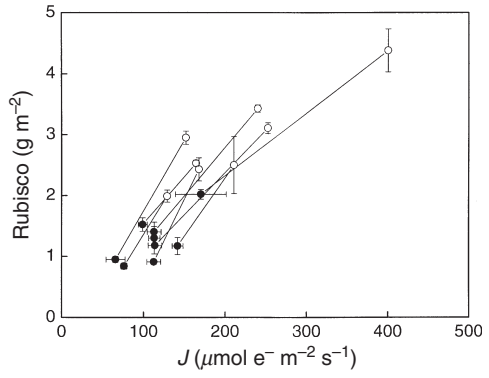

Figure 6. Rubisco content per unit leaf area versus electron transport rate per unit leaf area. Rubisco content was quantified in leaf extracts by CABP binding. Electron transport rate under high irradiance and intercellular CO_2 partial pressures was calculated from gas exchange measurements (Eqn 1). 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.

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 (α) and electron transport capacity (J_{max}) as allocation to pigment-proteins (ϕ_P) 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, J_{max} decreased as investment in pigment-protein came at the expense of electron transport and soluble protein. Up to a ϕ_P of 0·2, the net result of the increased absorptance was increased daily photosynthesis per unit leaf dry mass. The decrease in J_{max} was of no con-

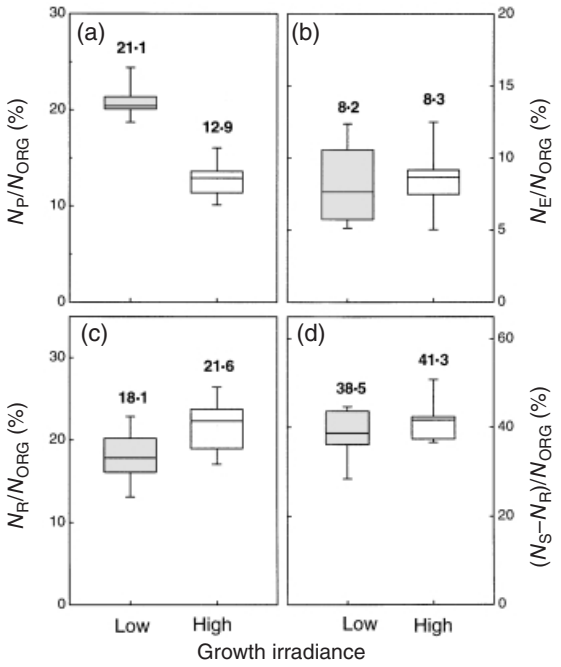

Figure 7. Relative distribution of organic nitrogen, N_{ORG}, between the various pools, for low-light- and high-light-grown plants. N_P = N in pigment-protein complexes. N_E = N involved in electron transport and photophosphorylation. N_R = N in Rubisco. N_S = N in total soluble protein. For more information see the legend of Fig. 1.

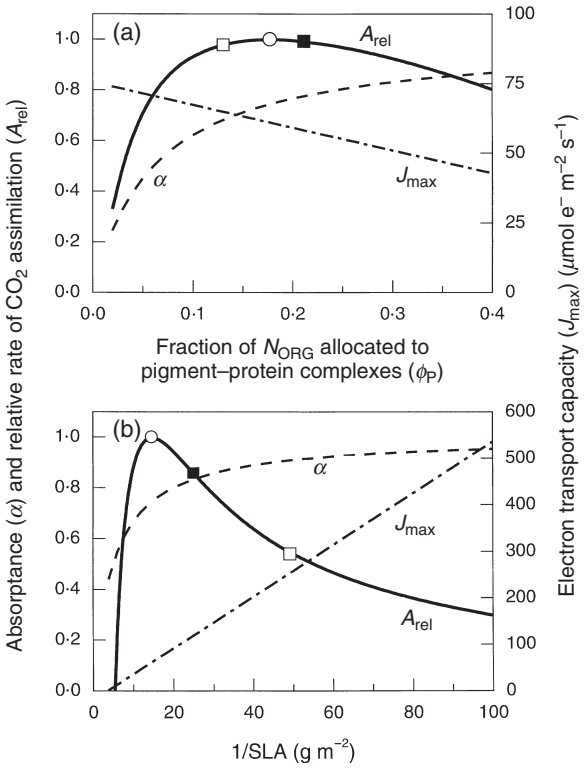

Figure 8. The dependence of leaf absorptance, α, electron transport capacity, J_{max}, and the relative daily photosynthesis per unit leaf dry mass, A_{rel}, under the low-light conditions on (a) the allocation of nitrogen to pigment-protein complexes, ϕ_P, and (b) specific leaf area and nitrogen content per unit leaf area. The relative daily photosynthesis curves were calculated using the optimum value of 1/SLA (a, 15 g m^{-2}) or ϕ_P (b, 0·18), a photosynthetic photon irradiance of 200 μmol m^{-2} s^{-1} and a constant organic nitrogen concentration of 3·3 mmol g^{-1}, using Eqns 7, 8, 11 and 12. Rates were normalized by dividing by the maximum rate possible under the growth conditions when both SLA and ϕ_P were free to vary (○). The rates calculated using the mean characteristics of the 10 species are shown for low light (■) and high light (□).

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 ϕ_P greater than 0·2, there was an over-investment in pigment-protein, and daily photosynthesis declined. For each incremental increase in ϕ_P, the small increase in absorptance was outweighed by the decrease in photosynthetic rate caused by the reduction in J_{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 ϕ_P was held constant, leaf absorptance 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 ϕ_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 ϕ_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 ϕ_P (Fig. 9, open circles). The average (mean of the 10 species) values of 1/SLA and ϕ_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 ϕ_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 ϕ_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 ϕ_P values for leaves acclimated to high light (Fig. 9a). If the plant acclimated to high light could change either 1/SLA or ϕ_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 ϕ_P (2% gain, vertical arrow). Under high-light conditions, the mean 1/SLA and ϕ_P values for leaves acclimated to high light enabled 98% of the maximum to be achieved, compared with 71% using 1/SLA and ϕ_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 ϕ_P increased photosynthesis by 10% (vertical arrow). Changing ϕ_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

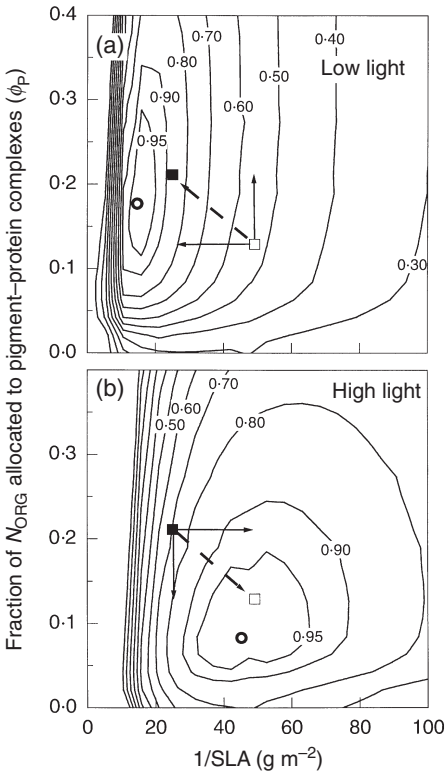

Figure 9. Contour plots of the relative rate of photosynthesis per unit leaf dry mass under (a) low light (200 μmol m^{-2} s^{-1}) and (b) high light (1000 μmol m^{-2} s^{-1}) conditions, as dependent on 1/SLA and the fraction of organic nitrogen partitioned to pigment-protein complexes, ϕ_P. Contour plots are derived from the model described in Materials and Methods. The observed mean values of 1/SLA and ϕ_P for plants grown in high light (□) and low light (■) are shown as well as the calculated maximum (○). Leaf material had a constant organic nitrogen concentration of 3·3 mmol g^{-1}, for other details, see the model. The profiles shown in Fig. 8 were vertical (Fig. 8a) or horizontal (Fig. 8b) slices through the contour surface passing through the open circle in (a). Solid arrows from the squares show the potential benefits from acclimation when only one parameter varies. Dashed arrows show the combined changes observed with acclimation.

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 (Fig. 7). Previous studies have examined either the consequences of re-allocation of nitrogen within the leaf (Evans 1989b, c, 1993a, b; Hikosaka & Terashima 1996; Hikosaka *et al*. 1998; Niinemets *et al*. 1998), or of changing SLA or nitrogen content (Evans 1993b; Hirose & Werger 1987; Sims *et al*. 1994) on optimizing photosynthesis under different growth irradiances. We have been able to combine these two factors in a model that enables quantitative comparison of the effects of changing SLA and nitrogen partitioning (Eqn 11). We will first discuss the key assumptions in the model. We then examine the impact of either changing allocation between proteins within the leaf, or changing SLA and thus nitrogen content per unit leaf area. Finally, we consider the relative importance of these two parameters.

Light capture

Chlorophyll plays a key role in determining the absorptance of a leaf (Gabrielsen 1948). By combining data from several different species with a range of chlorophyll contents, a general hyperbolic function relating absorptance to chlorophyll content per unit leaf area was obtained (Evans 1996). The data presented here for 10 species are well described by the same function (Fig. 3a), with minor deviations due to slight differences in reflectance (Fig. 3c). If leaf structural material absorbed light, one would expect the deviation in absorptance to increase as SLA decreased. As this was not the case (Fig. 3b), this suggests that SLA does not affect the whole leaf efficiency of light capture by the pigments. We therefore conclude that in the absence of structures altering leaf reflectance (e.g. waxes and hairs), leaf absorptance is simply related to the chlorophyll 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_{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

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_i 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 N_O 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, N_O, 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 N_O 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 (ϕ_P = 0·21) achieved 99% of the low-light optimum (ϕ_P = 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 (ϕ_P = 0·13). Under high-light conditions, for a leaf with a 1/SLA of 45 g m^{-2}, the optimum ϕ_P was 0·08. Leaves achieved 98 and 92% of the optimum with ϕ_P 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 modelling, 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 of 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 N_O, 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 & Werger 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 ϕ_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 ϕ_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 ϕ_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 absorptance 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_{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 ϕ_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 sunflecks. 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

decade makes. This investigation was supported by the Netherlands Organization for Scientific Research.

REFERENCES

Anten N.P.R., Schieving F. & Werger M.J.A. (1995) Patterns of light and nitrogen distribution in relation to whole canopy carbon gain in C_3 and C_4 mono- and dicotyledonous species. *Oecologia* **101**, 504–513.

Boardman N.K. (1977) Comparative photosynthesis of sun and shade plants. *Annual Review of Plant Physiology* **28**, 355–377.

Björkman O. (1981) Responses to different quantum flux densities. In *Physiological Plant Ecology I. Responses to the Physical Environment* (eds O.L. Lange, P.S. Nobel, C.B. Osmond & H. Ziegler). Encyclopedia of Plant Physiology, New Series, Vol. 12A, pp. 57–107. Springer-Verlag, Berlin.

Brouwer R. (1962) Distribution of dry matter in the plant. *Netherlands Journal of Agricultural Sciences* **10**, 361–376.

von Caemmerer S. & Farquhar G.D. (1981) Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta* **153**, 376–387.

von Caemmerer S., Evans J.R., Hudson G.S. & Andrews T.J. (1994) The kinetics of ribulose-1,5-bisphosphate carboxylase/oxygenase in vivo inferred from measurements of photosynthesis in leaves of transgenic tobacco. *Planta* **195**, 88–97.

Cataldo D.A., Haroon M., Schrader L.E. & Youngs V. (1975) Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. *Communications in Soil Science and Plant Analysis* **6**, 71–80.

Coley P.D. (1983) Herbivory and defensive characteristics of tree species in a lowland tropical forest. *Ecological Monographs* **53**, 209–233.

De Lucia E.H., Nelson K., Vogelmann T.C. & Smith W.K. (1996) Contribution of intercellular reflectance to photosynthesis in shade leaves. *Plant, Cell & Environment* **19**, 159–170.

Ellsworth D.S. & Reich P.B. (1993) Canopy structure and vertical patterns of photosynthesis and related leaf traits in a deciduous forest. *Oecologia* **96**, 169–178.

Evans J.R. (1983) Nitrogen and photosynthesis in the flag leaf of wheat (*Triticum aestivum* L.). *Plant Physiology* **72**, 297–302.

Evans J.R. (1987a) The dependence of quantum yield on wavelength and growth irradiance. *Australian Journal of Plant Physiology* **14**, 69–79.

Evans J.R. (1987b) The relationship between electron transport components and photosynthetic capacity in pea leaves grown at different irradiances. *Australian Journal of Plant Physiology* **14**, 157–170.

Evans J.R. (1989a) Photosynthesis and nitrogen relationships in leaves of C_3 plants. *Oecologia* **78**, 9–19.

Evans J.R. (1989b) Photosynthesis – the dependence on nitrogen partitioning. In *Causes and Consequences of Variation in Growth Rate and Productivity of Higher Plants* (eds H. Lambers, M.L. Cambridge, H. Konings & T.L. Pons), pp. 159–174. SPB Academic Publishing, The Hague, The Netherlands.

Evans J.R. (1989c) Partitioning of nitrogen between and within leaves grown under different irradiances. *Australian Journal of Plant Physiology* **16**, 533–548.

Evans J.R. (1993a) Photosynthetic acclimation and nitrogen partitioning within a lucerne canopy. I. Canopy characteristics. *Australian Journal of Plant Physiology* **20**, 55–67.

Evans J.R. (1993b) Photosynthetic acclimation and nitrogen partitioning within a lucerne canopy. II. Stability through time and comparison with a theoretical optimum. *Australian Journal of Plant Physiology* **20**, 69–82.

Evans J.R. (1996) Developmental constraints on photosynthesis: Effects of light and nutrition. In *Photosythesis and the Environment* (ed. N.R. Baker), pp. 281–304. Kluwer, Dordrecht, The Netherlands.

Evans J.R. (1998) Photosynthetic characteristics of fast- and slow-growing species. In *Inherent Variation in Plant Growth. Physiological Mechanisms and Ecological Consequences* (eds H. Lambers, H. Poorter & M.M.I.Van Vuuren), pp. 101–119. Backhuys Publishers, Leiden, The Netherlands.

Evans J.R. (1999) Leaf anatomy enables more equal access to light and CO_2 between chloroplasts. *New Phytologist* **143**, 93–104.

Evans J.R. & Loreto F. (2000) Acquisition and diffusion of CO_2 in higher plant leaves. In *Photosynthesis: Physiology and Metabolism* (eds R.C. Leegood, T.D. Sharkey & S. von Caemmerer), pp. 321–351. Kluwer, Dordrecht, The Netherlands.

Evans J.R. & Seemann J.R. (1989) The allocation of protein nitrogen in the photosynthetic apparatus: costs, consequences, and control. In *Photosynthesis* (ed. W.R. Briggs), pp. 183–205. A.R. Liss, New York.

Farquhar G.D. & von Caemmerer S. (1982) Modelling of photosynthetic responses to environmental conditions. In *Physiological Plant Ecology II. Water Relations and Carbon Assimilation* (eds O.L. Lange, P.S. Nobel, C.B. Osmond & H. Ziegler) Encyclopedia of Plant Physiology, New Series, **Vol. 12b**, pp. 549–587. Springer Verlag, Berlin.

Gabrielsen E.K. (1948) Effects of different chlorophyll concentrations on photosynthesis in foliage leaves. *Physiologia Plantarum* **1**, 5–37.

Givnish T.J. (1984) Leaf and canopy adaptations in tropical trees. In *Physiological Ecology of Plants of the Wet Tropics* (eds E. Medina, H.A. Mooney & C. Vazquez-Yanes), pp. 51–84. Dr W. Junk, The Hague, The Netherlands.

Hanson H.C. (1917) Leaf-structure as related to environment. *American Journal of Botany* **4**, 533–560.

Harley P.C., Thomas R.B., Reynolds J.F. & Strain B.R. (1992) Modelling photosynthesis of cotton grown in elevated CO_2. *Plant, Cell and Environment* **15**, 271–282.

Hikosaka K. & Terashima I. (1995) A model of the acclimation of photosynthesis in leaves of C_3 plants to sun and shade with respect to nitrogen use. *Plant, Cell and Environment* **18**, 605–618.

Hikosaka K. & Terashima I. (1996) Nitrogen partitioning among photosynthetic components and its consequence in sun and shade plants. *Functional Ecology* **10**, 335–343.

Hikosaka K., Hanba Y.T., Hirose T. & Terashima I. (1998) Photosynthetic nitrogen-use efficiency in leaves of woody and herbaceous species. *Functional Ecology* **12**, 896–905.

Hirose T. & Werger M.J.A. (1987) Maximizing daily canopy photosynthesis with respect to the leaf nitrogen allocation pattern in the canopy. *Oecologia* **72**, 520–526.

Hirose T. & Werger M.J.A. (1994) Photosynthetic capacity and nitrogen partitioning among species in the canopy of a herbaceous plant community. *Oecologia* **100**, 203–212.

Hollinger D.Y. (1989) Canopy organisation and foliage photosynthetic capacity in a broad-leaved evergreen montane forest. *Functional Ecology* **3**, 53–62.

Ishida A., Uemura A., Koike N., Matsumoto Y. & Hoe A.L. (1999) Interactive effects of leaf age and self-shading on leaf structure, photosynthetic capacity and chlorophyll fluorescence in the rain forest tree, *Dryobalanops aromatica*. *Tree Physiology* **19**, 741–747.

Lee D.W. & Graham R. (1986) Leaf optical properties of rainforest sun and extreme shade plants. *American Journal of Botany* **73**, 1100–1108.

Lee D.W., Bone R.A., Tarsis S.L. & Storch D. (1990) Correlates of leaf optical properties in tropical forest sun and extreme-shade plants. *American Journal of Botany* **77**, 370–380.

Makino A., Mae T. & Ohira K. (1984) Relation between nitrogen and ribulose-1,5-bisphosphate carboxylase in rice leaves from emergence through senescence. *Plant and Cell Physiology* **25**, 429–437.

Makino A., Mae T. & Ohira K. (1988) Differences between wheat and rice in the enzymic properties of ribulose-1,5-bisphosphate carboxylase/oxygenase and the relationship to photosynthetic gas exchange. *Planta* **174**, 30–38.

Mate C.J., Hudson G.S., von Caemmerer S., Evans J.R. & Andrews T.J. (1993) Reduction of Ribulose bisphosphate carboxylase activase levels in tobacco (*Nicotiana tabacum*) by antisense RNA reduces ribulose bisphosphate carboxylase carbamylation and impairs photosynthesis. *Plant Physiology* **102**, 1119–1128.

Niinemets Ü. & Kull O. (1998) Stoichiometry of foliar carbon constituents varies along light gradients in temperate woody canopies: implications for foliage morphological plasticity. *Tree Physiology* **18**, 467–479.

Niinemets Ü. & Tenhunen J.D. (1997) A model separating leaf structural and physiological effects on carbon gain along light gradients for the shade tolerant species *Acer saccharum*. *Plant, Cell and Environment* **20**, 845–866.

Niinemets Ü., Kull O. & Tenhunen J.D. (1998) An analysis of light effects on foliar morphology, physiology, and light interception in temperate deciduous woody species of contrasting shade tolerance. *Tree Physiology* **18**, 681–696.

Ögren E. & Evans J.R. (1993) Photosynthetic light response curves. 1. The influence of CO_2 partial pressure and leaf inversion. *Planta* **189**, 182–190.

Pearcy R.W. (1990) Sunflecks and photosynthesis in plant canopies. *Annual Review of Plant Physiology and Plant Molecular Biology* **41**, 421–453.

Pons T.L., Scheiving F., Hirose T. & Werger M.J.A. (1989) Optimization of leaf nitrogen allocation for canopy photosynthesis in *Lysimachia vulgaris*. In *Causes and Consequences of Variation in Growth Rate and Productivity of Higher Plants* (eds H. Lambers, M.L. Cambridge, H. Konings & T.L. Pons), pp. 175–186. SPB Academic Publishing, The Hague, The Netherlands.

Poorter H. & Evans J.R. (1998) Photosynthetic nitrogen-use efficiency of species that differ inherently in specific leaf area. *Oecologia* **116**, 26–37.

Poorter H. & Nagel O.W. (2000) The role of biomass allocation in the growth response of plants to different levels of light, CO_2, nutrients and water: a quantitative review. *Australian Journal of Plant Physiology* **27**, 595–607.

Poorter L., Kwant R., Hernandez R., Medina E. & Werger M.J.A. (2000) Leaf optical properties in Venezuelan cloud forest trees. *Tree Physiology* **20**, 519–526.

Poorter L., Oberbauer S.F. & Clark D.B. (1995) Leaf optical properties along a vertical gradient in a tropical rainforest canopy in Costa Rica. *American Journal of Botany* **82**, 1257–1263.

Porra R.J., Thompson W.A. & Kriedemann P.E. (1989) Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls *a* and *b* extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. *Biochimica et Biophysica Acta* **975**, 384–394.

Reich P.B., Walters M.B. & Ellsworth D.S. (1997) From tropics to tundra: global convergence in plant functioning. *Proceedings of the National Academy of Sciences USA* **94**, 13730–13734.

Sage R., Pearcy R.W. & Seemann J.R. (1987) The nitrogen use efficiency of C_3 and C_4 plants. III. Leaf nitrogen effects on the activity of carboxylating enzymes in *Chenopodium album* (L.) and *Amaranthus retroflexus* (L.). *Plant Physiology* **85**, 355–359.

Schieving F. & Poorter H. (1999) Carbon gain in a multispecies canopy: the role of specific leaf area and photosynthetic nitrogen-use efficiency in the tragedy of the commons. *New Phytologist* **143**, 201–211.

Sims D.A. & Pearcy R.W. (1989) Photosynthetic characteristics of a tropical forest understorey herb, *Alocasia macrorrhiza*, and a related crop species, *Colocasia esculenta* grown in contrasting light environments. *Oecologia* **79**, 53–59.

Sims D.A. & Pearcy R.W. (1994) Scaling sun and shade photosynthetic acclimation of *Alocasia macrorrhiza* to whole-plant performance – I. Carbon balance and allocation at different daily photon flux densities. *Plant, Cell and Environment* **17**, 881–887.

Sims D.A., Gebauer R.L.E. & Pearcy R.W. (1994) Scaling sun and shade photosynthetic acclimation of *Alocasia macrorrhiza* to whole-plant performance – II. Simulation of carbon balance and growth at different photon flux densities. *Plant, Cell and Environment* **17**, 889–900.

Taylor A.H. (1935) Errors in reflectometry. *Journal of the Optical Society of America* **25**, 51–56.

Terashima I. & Evans J.R. (1988) Effects of light and nitrogen nutrition on the organization of the photosynthetic apparatus in spinach. *Plant and Cell Physiology* **29**, 143–155.

Terashima I. & Saeki T. (1983) Light environment within a leaf I. Optical properties of paradermal sections of *Camellia* leaves with special reference to differences in the optical properties of palisade and spongy tissues. *Plant and Cell Physiology* **24**, 1493–1501.

Thompson W.A., Huang L.K. & Kriedemann P.E. (1992) Photosynthetic response to light and nutrients in sun-tolerant and shade-tolerant rainforest trees. II. Leaf gas exchange and component processes of photosynthesis. *Australian Journal of Plant Physiology* **19**, 19–42.

Wullschleger S.D. (1993) Biochemical limitations to carbon assimilation in C_3 plants – A retrospective analysis of the A/C_i curves from 109 species. *Journal of Experimental Botany* **44**, 907–920.

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_{ORG}), chlorophyll content (Chl), electron transport capacity (J_{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 (ν), 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.

Species	SLA (m^2 kg^{-1})	N_{ORG} (mmol m^{-2})	Chl (μmol m^{-2})	J_{max} (μmol e^- m^{-2} s^{-1})	Percentage of N_{ORG} in:					Rubisco/ soluble protein (%)	ν [mol N s (mmol e^-)$^{-1}$]
					N_P	N_E	Rubisco	Other soluble	Rest		
Low light											
Eucalyptus goniocalyx	38·3 ± 2·2	88 ± 3	477 ±15	113 ± 7	21·0 ± 0·7	7·0 ± 0·7	13·1 ± 0·4	32·2 ± 1·4	26·7	33·1 ± 3·4	0·36 ± 0·02
Eucalyptus macrorhyncha	30·1 ± 2·5	108 ± 11	536 ± 28	102 ± 8	20·1 ± 1·8	5·6 ± 1·2					
Nerium oleander	26·6 ± 0·9	103 ± 5	573 ± 32	99 ± 5	21·5 ± 1·1	5·1 ± 0·7	17·8 ± 0·9	44·4 ± 5·0	11·2	29·3 ± 3·3	0·63 ± 0·09
Radyera farragei	24·3 ± 0·9	148 ± 6	716 ± 25	170 ± 31	18·7 ± 0·4	5·7 ± 1·2	14·7 ± 0·7	28·4 ± 1·8	32·5	34·2 ± 2·1	0·43 ± 0·05
Datura stramonium	57·0 ± 2·3	72 ± 3	374 ± 15	113 ± 9	20·0 ± 0·7	9·6 ± 0·6	22·6 ± 1·3	44·7 ± 5·7	3·1	34·2 ± 3·4	0·43 ± 0·06
Echium plantagineum	43·1 ± 1·2	76 ± 3	396 ± 13	142 ± 7	20·1 ± 0·8	11·7 ± 0·4	17·3 ± 1·9	36·1 ± 4·2	14·8	32·7 ± 3·6	0·29 ± 0·02
Nicotiana tabacum	58·6 ± 1·7	50 ± 2	314 ± 7	76 ± 4	24·3 ± 1·1	8·2 ± 0·4	18·5 ± 0·2	38·7 ± 4·7	10·2	33·0 ± 2·8	0·39 ± 0·04
Physalis peruvianum	60·2 ± 3·3	57 ± 3	359 ± 14	66 ± 12	24·4 ± 0·5	5·8 ± 1·3	20·2 ± 2·1	37·0 ± 3·9	12·6	35·3 ± 1·8	0·55 ± 0·15
Plantago major	47·4 ± 1·2	63 ± 1	339 ± 7	112 ± 8	20·7 ± 0·8	10·9 ± 1·1	16·1 ± 0·9	43·7 ± 4·5	8·7	27·1 ± 1·0	0·35 ± 0·06
Raphanus sativus	61·6 ± 2·0	58 ± 3	300 ± 7	114 ± 8	20·1 ± 0·8	12·4 ± 1·1	22·8 ± 1·7	40·9 ± 9·3	3·8	38·0 ± 4·7	0·34 ± 0·07
High light											
Eucalyptus goniocalyx	18·3 ± 0·7	175 ± 7	668 ± 37	253 ± 20	15·8 ± 0·9	8·7 ± 0·4					
Eucalyptus macrorhyncha	13·4 ± 1·3	182 ± 10	702 ± 47	233 ± 19	16·0 ± 1·1	7·5 ± 0·6					
Nerium oleander	15·4 ± 0·4	176 ± 7	575 ± 34	164 ± 6	13·5 ± 0·9	5·0 ± 0·4	17·1 ± 0·6	50·8 ± 1·9	13·6	25·1 ± 0·2	0·70 ± 0·01
Radyera farragei	13·2 ± 0·3	267 ± 8	727 ± 54	401 ± 66	11·1 ± 0·6	8·9 ± 2·1	17·6 ± 1·5	42·2 ± 2·0	20·2	29·2 ± 0·9	0·47 ± 0·10
Datura stramonium	26·9 ± 1·8	153 ± 7	500 ± 19	240 ± 27	13·5 ± 0·6	9·3 ± 1·1	24·4 ± 0·9	37·4 ± 0·5	15·4	39·5 ± 0·7	0·43 ± 0·04
Echium plantagineum	22·6 ± 0·9	156 ± 8	425 ± 27	211 ± 15	11·3 ± 0·8	9·3 ± 0·5	19·5 ± 2·9	37·4 ± 2·5	22·5	33·7 ± 3·3	0·39 ± 0·04
Nicotiana tabacum	29·6 ± 1·9	112 ± 8	365 ± 13	129 ± 13	13·6 ± 0·7	7·5 ± 1·2	22·8 ± 1·6	42·8 ± 1·6	13·3	34·6 ± 1·7	0·52 ± 0·05
Physalis peruvianum	28·9 ± 1·6	138 ± 5	414 ± 21	152 ± 21	12·3 ± 0·4	6·0 ± 1·0	23·5 ± 0·4	41·3 ± 4·9	16·8	36·8 ± 2·7	0·63 ± 0·05
Plantago major	25·9 ± 0·9	123 ± 4	302 ± 15	168 ± 6	10·1 ± 0·5	8·6 ± 0·6	21·8 ± 1·6	42·0 ± 2·2	17·5	34·1 ± 0·9	0·48 ± 0·03
Raphanus sativus	29·3 ± 1·6	136 ± 7	377 ± 16	253 ± 16	11·5 ± 0·8	12·5 ± 0·7	26·4 ± 1·2	36·6 ± 3·1	12·9	42·2 ± 2·8	0·34 ± 0·03

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