Partitioning of Nitrogen Between and Within Leaves Grown under Different Irradiances

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Abstract

The distribution of nitrogen between leaves on individual plants of Phaseolus vulgaris and Cucumis sativus which were grown under different irradiances was examined. For Phaseolus, shading treatments were imposed on individual leaflets when they had reached one-third of full expansion. Adjacent leaflets were either grown under the same irradiance or had different irradiances imposed on them. The nitrogen content of leaves depended on their growth irradiance and not on the growth irradiance of adjacent leaflets, with more nitrogen being found in leaves grown under higher irradiance compared to those grown in shade. For Cucumis, the nitrogen contents of the leaves changed following the imposition of shading treatments. The experiment was repeated four times with different nitrate nutrient treatments, twice in combination with a pretreatment growth irradiance of 40% sunlight. The relative changes in leaf nitrogen content for each irradiance treatment were independent of changes to the leaf nitrogen content of the plant and of the growth irradiance prior to the shading treatments. Again, nitrogen contents were highest in leaves grown at high irradiance.

Acclimation of individual leaves to their irradiance treatment was seen for both Phaseolus and Cucumis. Growth under shade resulted in lower rates of oxygen evolution per unit of chlorophyll, when measured at high irradiance, and increased partitioning of nitrogen into pigment–protein complexes. These two changes working in opposition to each other meant that for Cucumis, the relationship between photosynthetic capacity and nitrogen content was similar between irradiance treatments. For Phaseolus, the increased partitioning of nitrogen into pigment–protein complexes at low irradiance was not as great as the reduction in photosynthetic rate per unit of chlorophyll, so that the photosynthetic rate per unit leaf nitrogen was less for leaves grown under low irradiance compared to those grown under high irradiance. It is shown that acclimation to lower irradiance can increase the potential daily photosynthesis for a given leaf nitrogen content.

Introduction

The photosynthetic capacity of a leaf depends on the nitrogen content and irradiance during growth (Field and Mooney 1986; Evans 1989a). As a plant forms a leaf canopy, a gradient in irradiance is established. Consequently, for a given amount of nitrogen in the leaf canopy, the photosynthetic capacity of the canopy will depend on how the nitrogen is distributed amongst the leaves. Gradients in leaf nitrogen content with respect to the irradiance available to each leaf have been observed in canopies of Prunus persica (DeJong and Doyle 1985), Solidago altissima (Hirose and Werger 1987a), Piper (Walters and Field 1987), Cyamopsis tetragonoloba (Charles-Edwards et al. 1987), Lysimachia vulgaris (Hirose et al. 1988) and Nothofagus solandri (Hollinger 1989),
suggesting that plants are responding to the spatial distribution of irradiance. When grown in controlled environments, leaf nitrogen contents also vary in response to the irradiance during growth (Atriplex patula, Björkman et al. (1972); Diplacus aurantiacus, Gulmon and Chu (1981); Spinacia oleracea, Terashima and Evans (1988); Alocasia macrorrhiza, Sims and Pearcy (1989)).

It is possible to calculate the distribution of nitrogen that maximises the photosynthetic capacity of the leaf canopy if the photosynthetic properties and the irradiance at each leaf position in the canopy are known. Field (1983) proposed that the condition for maximal canopy photosynthesis was that, for a small change in leaf nitrogen content, the increment in daily photosynthesis at one microsite was the same as for any other microsite. Put another way, optimal distribution of nitrogen between leaves in the canopy is when the increase in daily photosynthesis by one leaf due to withdrawing nitrogen from another leaf is cancelled by the reduction in daily photosynthesis of the leaf which lost the nitrogen. At that point, canopy photosynthesis cannot be increased by redistribution of nitrogen between leaves and can be increased only by additional nitrogen in the canopy. When the potential daily canopy photosynthesis of the chaparral shrub Lepechinia calycina was calculated, the increase over a uniform nitrogen distribution was 4% at the highest canopy nitrogen content. Despite this small gain, the observed distribution of nitrogen did show a slight trend towards higher nitrogen content at the high irradiance sites (Field 1983). Hirose and Werger (1987b) showed that the potential gains from distributing nitrogen non-uniformly were sensitive to both the leaf area index, LAI, of the canopy and the nitrogen available. For the Solidago canopy they examined, the potential increase in daily photosynthesis by having a non-uniform nitrogen content was 27% with an LAI of 4.2. The observed distribution of leaf nitrogen was calculated to increase daily photosynthesis by 21% above that for a canopy with uniform nitrogen distribution (Hirose and Werger 1987b).

When leaves are grown under different irradiances or are transferred to a different irradiance, acclimation by the photosynthetic system occurs (Anderson 1986). Acclimation reflects changes to the relative balance between leaf proteins. In general, acclimation to low irradiance involves reduction to both electron transport and Calvin cycle capacities and an increase in the ability to capture light. For Spinacia (Terashima and Evans 1988) and Pisum (Evans 1987a), acclimation to lower irradiance resulted in lower photosynthetic capacities for a given leaf nitrogen content. The utilisation of nitrogen within a leaf canopy is thus a function of both competition between leaves and altered partitioning within leaves. In natural leaf canopies, these two effects cannot be separately assessed. A non-uniform distribution of nitrogen within a leaf canopy could also arise from senescence of the older leaves lower in the canopy so that nitrogen can be remobilised to the newly forming leaves at the top of the canopy.

There were two main aims of the present work. Firstly, an attempt was made to assess whether acclimation by the photosynthetic system would benefit the leaf in terms of daily photosynthesis. This approach was first taken by Evans (1989b) and relies on previously established relationships between chlorophyll and leaf nitrogen contents and the nitrogen contents of thylakoid membranes. Essentially, the leaf properties of interest could be accounted for by simply measuring the photosynthetic rate at high irradiance and saturating CO₂, and the chlorophyll, soluble protein and nitrogen contents. Leaf nitrogen contents were deliberately manipulated to clarify the interpretation. Having established the photosynthetic properties of the leaves acclimated to different irradiances, it was possible to calculate whether the changes were beneficial, in terms of daily photosynthesis per unit of nitrogen.

The second aim of the experiments was to examine the interaction between leaves on a plant where the irradiance available to individual leaves could be manipulated. The experiments were designed to avoid the confounding effect of leaf age by randomly imposing the shade treatments between leaves. It was also hoped to determine whether
leaves responded directly to their irradiance environment, or whether the nitrogen content of a leaf was influenced by neighbouring leaves.

Materials and Methods

Seeds of *Phaseolus vulgaris* L. cv. Hawkesbury Wonder (French bean) and *Cucumis sativus* L. cv. Revel (cucumber) were sown in 5 L pots filled with sterilised potting soil. Plants were grown in a glasshouse, watered daily and given complete nutrient solution containing either 12 or 1 mM nitrate (Hewitt and Smith 1975) three times per week.

The cucumber vines were trellised beneath a wire mesh bench so that their leaves lay flat along the bench surface. For experiments I and II, the plants were grown in full sunlight. For experiments III and IV, the plants were grown under 40% of full sunlight (imposed by shadecloth) for 2 weeks prior to the start of the experiment. The plants in experiments I, III and IV were established with 12 mM nitrate and then received 1 mM nitrate from either 2 weeks prior to the start of the experiment (I, III) or from 3 weeks prior to the start of the experiment (IV). In experiment II, the plants received 12 mM nitrate throughout. The plants were trimmed to five leaves per vine and included one or two developing fruit when the first measurements were made. One half of each leaf was harvested for the determination of dark respiration rate, rate of oxygen evolution at 2000 μmol quanta m⁻² s⁻¹, chlorophyll, soluble protein and total nitrogen. Shade treatments were then randomly allocated to the leaves and imposed for 2 weeks by covering the leaves with neutral density filters (Lee Colortran, Andover, U.K.), such that leaves received 100%, 50%, 25% or 13% of full sunlight. The remaining half-leaves were then harvested and the same measurements repeated.

For *Phaseolus*, the first trifoliate leaf was constrained to the horizontal by wire netting and shade treatments were imposed when the leaflets were about one-third of full expansion. The noon irradiances for the three treatments were 820, 220 and 120 μmol quanta m⁻² s⁻¹. Leaflets were either given the same treatment for the whole leaf or were randomly allocated one of the three treatments. Subsequent leaves were removed as they reached one-third full expansion in an attempt to maintain the importance of the first leaf, and to prevent shading. Leaves were sampled 2 weeks after the start of the shade

![Fig. 1](image_url). Soluble protein content versus the electron transport capacity, $J_{\text{max}}$, both expressed per unit of chlorophyll. (A) *Cucumis*, irradiance treatment 1 (○), 2 (△), 3 (▽) and 4 (■). (B) *Phaseolus*, noon irradiance 820 (●), 220 (△) and 120 (■) μmol quanta m⁻² s⁻¹. The lines represent equation (4).
treatments and the same set of measurements that were made on the cucumber leaves were carried out. Plants were given nutrient solution containing 12 mM nitrate in experiment I and 1 mM nitrate in experiment II.

The rates of dark respiration and oxygen evolution at 1500 or 2000 μmol quanta m⁻² s⁻¹ were measured in leaf disc oxygen electrodes (Delieu and Walker (1981); Hansatech, King’s Lynn, U.K.) at 25°C and ~1% CO₂ from a 1 M carbonate-bicarbonate solution at pH 9. The leaf discs were subsequently analysed for total nitrogen by Kjeldahl digestion. Chlorophyll was determined in 80% acetone, using the equation of Graan and Ort (1984). Soluble protein was extracted from leaf discs by grinding in ice-cold buffer (Bicine 50 mM, pH 8, MgCl₂ 5 mM, 1% polyvinylpyrrolidone). The extract was spun in a microcentrifuge for 10 min and the clear supernatant was frozen for later determination of the soluble protein with the bicinchoninic acid reagent (Pierce, Rockford, IL, USA), using bovine serum albumin as the standard.

Table 1. The rates of oxygen evolution and ratios of chlorophyll to leaf nitrogen for cucumber leaves following 2 weeks growth under various shade treatments

<table>
<thead>
<tr>
<th>Irradiance treatment</th>
<th>1 initial</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate of O₂ evolution⁵</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mmol O₂ (mol Chl)⁻¹ s⁻¹)</td>
<td>61±1.5</td>
<td>63±2.6</td>
<td>49±2.6</td>
<td>48±2.5</td>
<td>40±2.1</td>
</tr>
<tr>
<td>Chl/N</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mmol Chl (mol N)⁻¹)</td>
<td>5.2±0.06</td>
<td>4.1±0.1</td>
<td>4.5±0.2</td>
<td>5.0±0.1</td>
<td>5.3±0.2</td>
</tr>
<tr>
<td>Experiments I and II</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate of O₂ evolution⁶</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mmol O₂ (mol Chl)⁻¹ s⁻¹)</td>
<td>60.9±1.1</td>
<td>76±3.6</td>
<td>60±4.3</td>
<td>54±4.0</td>
<td>43±2.4</td>
</tr>
<tr>
<td>Chl/N</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mmol Chl (mol N)⁻¹)</td>
<td>5.4±0.05</td>
<td>4.8±0.3</td>
<td>5.7±0.4</td>
<td>6.1±0.6</td>
<td>7.3±0.2</td>
</tr>
<tr>
<td>Experiments III and IV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

⁵Measured at 1500 μmol quanta m⁻² s⁻¹, ~1% CO₂, 25°C.
⁶Measured at 2000 μmol quanta m⁻² s⁻¹, ~1% CO₂, 25°C.

Calculation of Daily Photosynthesis

The daily photosynthesis was calculated for leaves with different chlorophyll contents and rates of oxygen evolution per unit of chlorophyll. The absorptance of the leaf is given by:

\[
\text{Absorptance} = 0.509 + 1.7\chi - 2.78\chi^2 + 1.99\chi^3 - 0.52\chi^4, \tag{1}
\]

where \(\chi\) is the chlorophyll content (mmol Chl m⁻²) and 0.2<\(\chi\)<1.0, determined with an integrating sphere for leaves of Pisum, Spinacia and Alocasia with a quartz iodine projector light and LiCor quantum sensor (Lambda Instruments Corp., Lincoln, Nebraska, U.S.A.) (Evans, unpublished). The rate of electron transport, \(J\), with respect to irradiance was calculated as

\[
J = (I_2 + J_m - (I_2 + J_m)^2 - 4\Theta I_2 J_m))/2\Theta, \tag{2}
\]

where \(I_2 = I_2\text{absorptance}(1-f)/2\), \(I_2\) is the useful light absorbed by photosystem 2 (μmol quanta m⁻² s⁻¹), \(I_i\) is the incident irradiance and \(f\) is to correct for the spectral quality of light (~0.15, Evans 1987b), \(J_m\) is the electron transport capacity of the leaf (μmol e⁻ m⁻² s⁻¹), \(J_m = J_{\text{max}}\). Chl, \(J_{\text{max}}\) is the electron transport capacity [mmol e⁻ (mol Chl)⁻¹ s⁻¹] multiplied by the chlorophyll content of the leaf (mmol Chl m⁻²) and \(\Theta\) is a convexity term (0.69, Evans and Terashima 1987). The rate of oxygen evolution is simply \(J/4\) as there are 4 electrons per oxygen.
Thylakoid nitrogen is mainly present in the pigment–protein complexes and the nitrogen content per unit of chlorophyll is relatively independent of the growth irradiance. The second major nitrogen-containing fraction comprises the coupling factor and cytochrome b/f complex (Evans 1987a; Evans and Seemann 1989). The electron transport capacity per unit of chlorophyll is proportional to both coupling factor activity and cytochrome b/f content. Since the coupling factor and cytochrome f contents change in parallel with growth irradiance, the nitrogen cost of the thylakoids per unit of chlorophyll increases as the cytochrome f content increases. That is, the nitrogen cost of the thylakoids, $T$ [mol N (mol Chl)$^{-1}$], is related to the electron transport capacity, $J_{\text{max}}$ [mmol e$^{-}$ (mol Chl)$^{-1}$ s$^{-1}$] (Evans 1987a, 1989a):

$$T = 0.063 J_{\text{max}} + 33.$$  \hspace{1cm} (3)

This equation was derived empirically and appears to hold across several diverse species. To utilise the electron transport capacity, it is necessary to have a matching Calvin cycle capacity. This is evident from the strong relationship between soluble protein content and $J_{\text{max}}$ (Fig. 1), where the nitrogen cost of soluble protein, $S$ [mol N (mol Chl)$^{-1}$] is also related to $J_{\text{max}}$:
where $a = 0.37$, $b = 14.9$ for *Phaseolus* and $a = 0.40$, $b = 0$ for *Cucumis* (Fig. 1).

For a given leaf nitrogen content, the chlorophyll content of the leaf can vary as a function of $J_{\text{max}}$ (Evans 1989b), and the daily photosynthesis can be calculated using a sinusoidal variation in irradiance. The consequences of using a sinusoidal versus other daily functions for irradiance will be addressed elsewhere. The potential daily photosynthesis refers to the oxygen evolution in the absence of any stomatal limitation or photorespiration. To calculate the effect of altered partitioning between $J_{\text{max}}$ and Chl/N, daily photosynthesis was calculated for various $J_{\text{max}}$ ranging from 150 to 650 mmol e$^-$ (mol Chl)$^{-1}$s$^{-1}$. The chlorophyll content of the leaf was calculated for each $J_{\text{max}}$ by dividing the fixed nitrogen content available for photosynthesis by $S + T$ calculated from equations (3) and (4). Knowing the chlorophyll content of the leaf, the absorptance and irradiance response curves are then defined by equations (1) and (2).
Results

Acclimation and Nitrogen Partitioning within Leaves

Following the imposition of the shade treatments on the cucumber vines, the rate of oxygen evolution expressed on the basis of chlorophyll changed (Table 1). For experiments I and II, where the plants were initially grown under full irradiance, shading reduced the rate by up to 30% while the full irradiance treatment remained unchanged. For experiments III and IV, where the plants were initially grown under shade, the rate increased or decreased by up to 25% for the full irradiance or maximum shade treatments, respectively, and remained unchanged where the shade treatment was maintained at a similar level. The acclimation is shown for the two extreme shade treatments (Fig. 2). When measured initially (solid triangles), the rate of oxygen evolution was

![Graph showing the relationship between soluble protein content and leaf nitrogen content for cucumber. Full light (○), 13% sunlight (■). Soluble protein (g m⁻²) = 0.0585N-1.52, r² = 0.86.]

60·9 mmol O₂ (mol Chl)⁻¹ s⁻¹ (slope of the dashed line). Following 2 weeks at full irradiance, the rate increased to 76 mmol O₂ (mol Chl)⁻¹ s⁻¹ whereas, for the lowest light treatment, the rate decreased to 43 mmol O₂ (mol Chl)⁻¹ s⁻¹. For both treatments the rates per unit of chlorophyll were independent of the chlorophyll contents of the leaves (Fig. 2).

The converse change was seen in the ratio of chlorophyll to total leaf nitrogen which increased with acclimation to lower irradiance (Table 1). However, unlike the photosynthetic rate, the ratio altered with leaf age (compare the initial ratio with that a fortnight later for the same irradiance treatment). The chlorophyll contents were proportional to the total leaf nitrogen contents, but had a non-zero intercept (Fig. 3).
The fraction of total leaf nitrogen present in soluble protein was independent of the irradiance treatment. Soluble protein was proportional to total leaf nitrogen with a non-zero intercept of 25 mmol N m\(^{-2}\) (Fig. 4). About 50% of the nitrogen was present in soluble protein for leaves containing 100 mmol N m\(^{-2}\). For simplicity, the intercept that was clearly evident from the regression in Fig. 4 was also used in Fig. 3.

**Fig. 5.** (A) Rate of oxygen evolution, \(H\), versus leaf nitrogen content for cucumber leaf discs at 2000 \(\mu\)mol quanta m\(^{-2}\) s\(^{-1}\) and \(-1\% CO_2\). Full light (○) or 13% sunlight (■), \(H = 0.458N - 11.4, \quad r^2 = 0.90\). (B) Rate of oxygen evolution predicted from the rates of electron transport per unit of chlorophyll and ratios of chlorophyll to leaf nitrogen. Full light treatment (—), 325 mmol e\(^-\) (mol Chl\(^{-1}\) s\(^{-1}\), 6.2 mmol Chl (mol N\(^{-1}\)), where N = leaf N – 25 (mmol m\(^{-2}\)). 13% sunlight treatment (---), 184 mmol e\(^-\) (mol Chl\(^{-1}\) s\(^{-1}\), 10.4 mmol Chl (mol N\(^{-1}\)), where N = leaf N – 25 (mmol m\(^{-2}\)).

**Table 2.** The rates of oxygen evolution, ratios of chlorophyll to leaf nitrogen and leaf nitrogen contents of *Phaseolus* leaflets following 2 weeks growth under different irradiances

<table>
<thead>
<tr>
<th>Noon irradiance ((\mu)mol quanta m(^{-2}) s(^{-1}))</th>
<th>820</th>
<th>220</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate of oxygen evolution(^a) (mmol O(_2) (mol Chl(^{-1}) s(^{-1}))</td>
<td>94 ± 6</td>
<td>67 ± 2</td>
<td>57 ± 2</td>
</tr>
<tr>
<td>Chl / N (mmol Chl (mol N(^{-1}))</td>
<td>3.9 ± 0.1</td>
<td>5.3 ± 0.1</td>
<td>5.8 ± 0.1</td>
</tr>
<tr>
<td>Nitrogen content(^b) (mmol N m(^{-2}))</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Expt I</td>
<td>s</td>
<td>108 ± 3</td>
<td>79 ± 3</td>
</tr>
<tr>
<td></td>
<td>d</td>
<td>106 ± 5</td>
<td>79 ± 3</td>
</tr>
<tr>
<td>Expt II</td>
<td>s</td>
<td>75 ± 2</td>
<td>62 ± 2</td>
</tr>
<tr>
<td></td>
<td>d</td>
<td>84 ± 6</td>
<td>65 ± 5</td>
</tr>
</tbody>
</table>

\(^{a}\)Measured at 2000 \(\mu\)mol quanta m\(^{-2}\) s\(^{-1}\), \(-1\% CO_2\), 25°C.

\(^{b}\)s, all leaflets given the same shade treatment; d, each leaflet given a different shade treatment.

Acclimation by the photosynthetic system is masked when photosynthetic rate as a function of total leaf nitrogen is examined (Fig. 5A). The relationship between photosynthetic rate and total leaf nitrogen predicted for cucumber leaves from Figs 2 and 3 is shown in Fig. 5B. The lower rate of oxygen evolution per unit of chlorophyll
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Fig. 6. Soluble protein content versus leaf nitrogen content for Phaseolus. Noon irradiances were 820 (●), 220 (△) and 120 μmol quanta m⁻² s⁻¹ (■).

Fig. 7. (A) Rate of oxygen evolution versus leaf nitrogen content for Phaseolus leaf discs measured at 2000 μmol quanta m⁻² s⁻¹ and ~1% CO₂. Noon irradiances for the three treatments were 820 (●), 220 (△) and 120 (■) μmol quanta m⁻² s⁻¹. (B) Rates of oxygen evolution predicted from the rates of electron transport per unit of chlorophyll and ratios of chlorophyll to leaf nitrogen. Noon irradiance of 820 μmol quanta m⁻² s⁻¹, 408 mmol e⁻ (mol Chl)⁻¹ s⁻¹, 3·9 mmol Chl (mol N)⁻¹ (—), 220, 286, 5·3 (—), 120, 235, 5·8 (-----).
by leaves acclimated to low irradiance is almost completely compensated by their increased ratio of chlorophyll to nitrogen.

In most respects the pattern was similar for *Phaseolus*. The rate of oxygen evolution declined from 94 to 57 mmol O$_2$ (mol Chl)$^{-1}$ s$^{-1}$ for the high and low irradiance treatments, respectively (Table 2). This was accompanied by an increase in the ratio of chlorophyll to total leaf nitrogen, but the relative increase was less than the decrease in the rate of oxygen evolution. Soluble protein as a function of total leaf nitrogen also depended on the irradiance treatment (Fig. 6). The increasing ratio of chlorophyll to nitrogen in leaves grown under shade was insufficient to offset the decline in the rate of oxygen evolution so that the photosynthetic rate per unit leaf nitrogen was less for leaves in the lower irradiance treatments (Fig. 7A). When the relationships were predicted from Table 2, the 13% difference between the high and low irradiance treatments is more clearly seen (Fig. 7B).

### Table 3. The proportions of total leaf nitrogen present in thylakoid and soluble protein for *Cucumis* and *Phaseolus* leaves grown at different irradiances

The calculations refer to a standard leaf nitrogen content of 80 mmol N m$^{-2}$. For *Cucumis* noon irradiances were 1000, 550, 260 and 150 µmol quanta m$^{-2}$ s$^{-1}$ for treatments 1-4, respectively. The noon irradiances (µmol quanta m$^{-2}$ s$^{-1}$) are given for *Phaseolus*.

<table>
<thead>
<tr>
<th>Irradiance treatment:</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>820</th>
<th>220</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) $J_{\text{max}}$ (mmol e$^-$ (mol Chl)$^{-1}$ s$^{-1}$)</td>
<td>326</td>
<td>255</td>
<td>225</td>
<td>180</td>
<td>405</td>
<td>283</td>
<td>235</td>
</tr>
<tr>
<td>(2) $J_{\text{max}}$/N (mmol e$^-$ (mol N)$^{-1}$ s$^{-1}$)</td>
<td>1.46</td>
<td>1.42</td>
<td>1.42</td>
<td>1.40</td>
<td>1.59</td>
<td>1.50</td>
<td>1.37</td>
</tr>
<tr>
<td>(3) T/N (thylakoid N/total N (equation 3))</td>
<td>0.24</td>
<td>0.27</td>
<td>0.30</td>
<td>0.35</td>
<td>0.23</td>
<td>0.27</td>
<td>0.28</td>
</tr>
<tr>
<td>(4) S/N (soluble protein N/total N (equation 4))</td>
<td>0.59</td>
<td>0.57</td>
<td>0.57</td>
<td>0.56</td>
<td>0.52</td>
<td>0.47</td>
<td>0.42</td>
</tr>
<tr>
<td>(5) R (soluble + thylakoid N)/total N</td>
<td>0.83</td>
<td>0.84</td>
<td>0.87</td>
<td>0.91</td>
<td>0.76</td>
<td>0.74</td>
<td>0.70</td>
</tr>
</tbody>
</table>

The strategies for nitrogen partitioning into thylakoid and soluble protein for both *Cucumis* and *Phaseolus* are compared in Table 3. Acclimation to lower irradiance reduced the electron transport capacity per unit of chlorophyll ($J_{\text{max}}$), while increasing the proportion of chlorophyll (Tables 1, 2). Reduced electron transport capacity is associated with less thylakoid nitrogen per unit of chlorophyll (equation 3), so that the change in the proportion of thylakoid nitrogen to total leaf nitrogen is considerably less than the ratio of chlorophyll to nitrogen (Table 3). As mentioned above (Fig. 5), the reduced electron transport capacity per unit of chlorophyll is compensated by the increased ratio of chlorophyll to nitrogen. Consequently, $J_{\text{max}}$/N is similar between irradiance treatments in *Cucumis*. However, for this to occur, the leaves from the low irradiance treatment need to devote one-third of their total leaf nitrogen in the thylakoids as opposed to only one-quarter in leaves from the high irradiance treatment. For *Phaseolus*, $J_{\text{max}}$/N declines at the lower irradiance treatments because the increase in the proportion of nitrogen partitioned to the thylakoids was insufficient to compensate for the reduction in $J_{\text{max}}$. Since the soluble protein content was proportional to the electron transport capacity (Fig. 1, equation 4), the soluble protein as a proportion of total leaf nitrogen ($S$/N) was unaffected by the growth irradiance treatment for *Cucumis*, but declined for *Phaseolus* leaves (Table 3). Consequently, the sum of thylakoid plus soluble protein as a proportion of total leaf nitrogen increased from 0.83 to 0.91 in *Cucumis* leaves grown under sunlight and shade, respectively, but decreased from 0.76 to 0.70 in *Phaseolus* leaves grown under sunlight and shade, respectively (Table 3).
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Distribution of Nitrogen between Leaves

To overcome the variation between leaves along the cucumber vine, caused to some extent by different leaf age, the nitrogen content of each leaf was measured before and after the irradiance treatment. For the first two experiments, all leaves were grown in full irradiance, whereas for the last two experiments the leaves were grown for 2 weeks in 40% sunlight. The total nitrogen in the leaf canopy was deliberately varied at the start of each experiment and remained unchanged over the fortnight (I, III), increased (II) or decreased (IV) (Table 4), depending on the nitrate treatment. However, for a given irradiance treatment, the relative changes in leaf nitrogen were similar in all four experiments. Nitrogen was preferentially allocated to leaves under high irradiance and remobilised from leaves placed under the most shade. The relative nitrogen content was slightly reduced for older leaves. For the highest irradiance treatment, the relative nitrogen content for the two oldest leaves was $1.11 \pm 0.06$ (8) versus $1.21 \pm 0.04$ (14) for the two youngest leaves. For the lowest irradiance treatment, the ratios were $0.83 \pm 0.02 (12)$ versus $0.92 \pm 0.03 (11)$, respectively.

Table 4. Leaf nitrogen contents of cucumber leaves following 2 weeks growth under various shade treatments

<table>
<thead>
<tr>
<th>Expt</th>
<th>Average N content (mmol N m$^{-2}$)</th>
<th>Relative N content$^A$</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td>1</td>
</tr>
<tr>
<td>I</td>
<td>69</td>
<td>73</td>
<td>$1.15 \pm 0.02$</td>
</tr>
<tr>
<td>II</td>
<td>95</td>
<td>129</td>
<td>$1.28 \pm 0.04$</td>
</tr>
<tr>
<td>III</td>
<td>94</td>
<td>99</td>
<td>$1.14 \pm 0.07$</td>
</tr>
<tr>
<td>IV</td>
<td>78</td>
<td>63</td>
<td>$1.16 \pm 0.07$</td>
</tr>
<tr>
<td>mean</td>
<td>78</td>
<td>63</td>
<td>$1.18 \pm 0.03$</td>
</tr>
</tbody>
</table>

A$^A$(mean of $N_{\text{final}}/N_{\text{initial}}$) x $\bar{N}_{\text{initial}}/\bar{N}_{\text{final}}$

Phaseolus plants allocated more nitrogen to leaflets grown under high irradiance (Table 2). For experiment I, plants received 12 mM nitrate while, in experiment II, plants were given 1 mM nitrate in their nutrient solution. This treatment reduced the nitrogen contents of leaves under full irradiance but did not affect the leaves grown under the most shade (Table 2). In contrast to Cucumis, the nitrogen contents of Phaseolus leaflets were independent of the irradiance treatment given to adjacent leaflets.

Discussion

Nitrogen Partitioning within the Leaf

Acclimation of the photosynthetic system to the irradiance during growth occurs in many species. However, the net result differs between species. For example, with Spinacia (Terashima and Evans 1988) and Pisum (Leong and Anderson 1984; Evans 1987a), acclimation to low irradiance involved considerable reduction in the rate of electron transport per unit of chlorophyll and was associated with a reduction in the relative amounts of cytochrome f and coupling factor. Since there was little increase in the ratio of chlorophyll to total leaf nitrogen, the photosynthetic capacity (i.e.
Rate of oxygen evolution ($\mu$mol O$_2$ m$^{-2}$ s$^{-1}$)

$\frac{\text{mmol e}^-}{\text{mol Chl}^{-1} \text{s}^{-1}}$

Fig. 8. The trade-off between $J_{\text{max}}$ and leaf absorptance for a Phaseolus leaf containing 80 mmol N m$^{-2}$ and (soluble + thylakoid N)/total N = 0.7 (---). Also shown is the trade-off between the rate of oxygen evolution at 2000 $\mu$mol quanta m$^{-2}$ s$^{-1}$ and leaf absorptance (---).

Fig. 9. Calculated daily potential photosynthesis for a given leaf nitrogen content when nitrogen allocation between electron transport capacity and light absorption is varied. The curves have been calculated for the different shade treatments using a sinusoidal variation in irradiance and other equations presented in the Methods. Total leaf nitrogen was taken as 80 mmol N m$^{-2}$, with the fraction as soluble and thylakoid N being 0.85 for Cucumis and 0.7 for Phaseolus (see Table 3). The closed circles represent the $J_{\text{max}}$ observed for leaves from the various treatments. The daily potential photosynthesis for each curve was 0.35, 0.35, 0.84 and 1.12 mol O$_2$ m$^{-2}$ day$^{-1}$ for the Cucumis and 0.29, 0.46 and 0.91 mol O$_2$ m$^{-2}$ day$^{-1}$ for the Phaseolus.
measured under saturating CO₂ and high irradiance) per unit of nitrogen varied considerably between leaves grown at different irradiances (Terashima and Evans 1988; Evans 1988). At the other extreme are *Cucumis* (Fig. 5) and *Alocasia* and *Colocasia* (Sims and Pearcy 1989), where the ratio of chlorophyll to nitrogen increases to offset the declining rate of electron transport per unit of chlorophyll. The net effect is that the relationship between photosynthetic capacity and total leaf nitrogen is independent of the irradiance during growth.

If acclimation results in no change to the relationship between photosynthetic capacity and total leaf nitrogen, does the plant benefit from acclimation? This can be analysed by examining the potential daily photosynthesis for leaves with varying electron transport capacities per unit of chlorophyll and different ratios of chlorophyll to nitrogen (Evans 1989b). Essentially, there is a trade-off between light absorption and photosynthetic capacity (Fig. 8). Increasing the electron transport capacity per unit of chlorophyll requires more nitrogen per unit of chlorophyll so that, for a given amount of nitrogen, this results in less chlorophyll and hence a lower leaf absorptance. The potential daily photosynthesis can be calculated for different combinations of chlorophyll content and electron transport capacity, for a given amount of nitrogen, using the equations presented in the Methods. The calculations were made for both *Cucumis* and *Phaseolus* leaves using the daily noon irradiances imposed by the experimental shade treatments and the observed relationships between electron transport capacity and soluble protein content (Fig. 9). For a given noon irradiance, there is an optimum for the balance between electron transport capacity and the ratio of chlorophyll to leaf nitrogen. Acclimation by *Phaseolus* leaves maintained the daily potential photosynthesis within 1% of the maximum and, except for the intermediate irradiance treatment, resulted in the acclimated leaves being superior to leaves acclimated to the other irradiances. For *Cucumis*, acclimation was effective at the lowest irradiances where leaves were within 1% of the maximum. Although leaves grown under higher irradiance did have lower ratios of chlorophyll to nitrogen and higher rates of electron transport per unit of chlorophyll, the changes were small relative to the shift in the optima. Consequently, the leaf acclimated to the highest irradiance should outperform those acclimated to noon irradiances of 550 and 260 μmol quanta m⁻² s⁻¹ under their growth conditions.

It is possible that 2 weeks was insufficient to allow complete acclimation or that the ability to re-acclimate declines with leaf age. Where the time-course of acclimation has been examined, it usually took 3–5 days to reach half the maximal change (Medicago, Pearce and Lee 1969; Phaseolus, Caemmerer and Farquhar 1984; Pisum, Grahl and Wild 1975; Chow and Anderson 1987; Lycopersicon, Withers et al. 1984; Davies et al. 1986), although for *Solanum* acclimation was still occurring after 2 weeks (Ferrar and Osmond 1986). For *Cucumis* in experiments III and IV, leaves were shaded for 2 weeks prior to the initial measurement. Following a further 2 weeks under similar shade, the rate of oxygen evolution per unit of chlorophyll remained the same. This suggests that, while 2 weeks was sufficiently long for *Cucumis* to fully acclimate, some constraints exist in the flexibility of the system. If the comparison is restricted to just the two extreme irradiance treatments, considerable advantage was gained through acclimation by the cucumber leaves.

The ability to alter the ratio between chlorophyll and nitrogen varies between species. *Phaseolus* and *Cucumis* could vary the ratio 1.5-fold, whereas *Alocasia* (Seemann et al. 1987), *Colocasia* (Sims and Pearcy 1989) and the rainforest trees *Agathis* (Langenheim et al. 1984), *Argyrodroendon* (Pearcy 1987) and *Flindersia* (Thompson et al. 1988), can vary the ratio by 2.5–3-fold. At the other extreme, *Pisum* (Evans 1987a) and *Spinacia* (Terashima and Evans 1988) are unable to vary the ratio by more than 20%. In the natural stand of *Solidago* which Hirose and Werger (1987a) examined, a gradient in the
ratio of chlorophyll to nitrogen has also been found (Hirose, personal communication), indicating that acclimation does occur within leaf canopies.

**Distribution of Nitrogen between Leaves**

For both *Cucumis* and *Phaseolus*, the nitrogen content of the leaves responded to the irradiance treatment, increasing at higher irradiances. Although *Phaseolus* leaves from high and low irradiance treatments varied by relatively more than the *Cucumis* leaves, this may have been related to leaf development. Inversion of leaves that were expanding resulted in greater acclimation than when fully expanded leaves were inverted (Terashima and Takenaka 1986; Terashima et al. 1986). For the experiments reported here, the irradiance treatments for *Phaseolus* were imposed during leaf expansion, whereas with *Cucumis* the treatments were applied to mature leaves. For leaves in a canopy, it would be necessary to be able to re-acclimate after full expansion when new leaves are produced above.

The relationships between the rate of electron transport, chlorophyll and nitrogen can be used to calculate the potential daily photosynthesis available to the plant, for any distribution of nitrogen between its leaves. The optimal solution can be obtained by differentiation of the equation relating the rate of electron transport to irradiance. The solution requires that the electron transport capacity of the leaf is proportional to the irradiance at the leaf (Oja and Laisk 1976; Evans 1983, 1989b; Terashima and Saeki 1985; Zhang 1988; Farquhar 1989). For this example, the optimal nitrogen distribution was calculated using the observed ratio of chlorophyll to total leaf nitrogen and the rates of electron transport per unit of chlorophyll. The potential daily photosynthesis was also calculated using the observed distribution of nitrogen and a uniform leaf nitrogen content. For *Cucumis*, the observed distribution gained 90·8% and the uniform 86·4% of that gained by the optimal distribution. For *Phaseolus*, the observed and uniform distributions gained 96·7% and 89·4%, respectively.

The two species differed in their response to the treatment of adjacent leaves. The nitrogen content of *Phaseolus* leaflets was independent of the irradiance treatment of adjacent leaflets (Table 2). This may have been related to the vigorous growth of the seedling which was producing many more leaves. The plant would not normally be constrained to distribute nitrogen between so few leaves. By contrast, *Cucumis*, which did not produce new leaves during the experiment, did remobilise nitrogen from leaves given shade treatments and could increase the nitrogen content of the leaves in high irradiance (Table 4). Since the irradiance treatments were allocated at random along the vine, leaf age was not responsible. Interestingly, the reallocation of nitrogen between leaves was independent of the initial state of the leaves and also independent of whether there was a net increase or decrease of nitrogen in the leaves as a whole over the experimental period.

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**References**


Partitioning of Nitrogen under Different Irradiances


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