Irradiance and phenotype: comparative eco-development of sun and shade leaves in relation to photosynthetic CO₂ diffusion

Ichiro Terashima¹,*, Yuko T. Hanba², Youshi Tazoe¹, Poonam Vyas¹† and Satoshi Yano¹‡

¹ Department of Biology, Graduate School of Science, Osaka University, 1-1 Machikaneyama-cho, Toyonaka, 560-0043, Japan
² Center for Bioresource Field Science, Kyoto Institute of Technology, 1 Saga-ippongi-cho, Ukyo-ku, Kyoto, 616-8534, Japan

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Abstract

The subject of this paper, sun leaves are thicker and show higher photosynthetic rates than the shade leaves, is approached in two ways. The first seeks to answer the question: why are sun leaves thicker than shade leaves? To do this, CO₂ diffusion within a leaf is examined first. Because affinity of Rubisco for CO₂ is low, the carboxylation of ribulose 1,5-bisphosphate is competitively inhibited by O₂, and the oxygenation of ribulose 1,5-bisphosphate leads to energy-consuming photorespiration, it is essential for C₃ plants to maintain the CO₂ concentration in the chloroplast as high as possible. Since the internal conductance for CO₂ diffusion from the intercellular space to the chloroplast stroma is finite and relatively small, C₃ leaves should have sufficient mesophyll surfaces occupied by chloroplasts to secure the area for CO₂ dissolution and transport. This explains why sun leaves are thicker. The second approach is mechanistic or ‘how-oriented’. Mechanisms are discussed as to how sun leaves become thicker than shade leaves, in particular, the long-distance signal transduction from mature leaves to leaf primordia inducing the periclinal division of the palisade tissue cells. To increase the mesophyll surface area, the leaf can either be thicker or have smaller cells. Issues of cell size are discussed to understand plasticity in leaf thickness.

Why are sun leaves thicker than shade leaves?

The rate of photosynthesis of C₃ leaves strongly depends on the CO₂ concentration in the chloroplast, because affinity for CO₂ of the primary CO₂-fixing enzyme, ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco), is low (von Caemmerer and Quick, 2000). Moreover, the carboxylation of ribulose 1,5-bisphosphate (RuBP) is competitively inhibited by O₂, and the oxygenation leads to the energy-consuming photorespiration processes. For C₃ plants to perform efficient CO₂ fixation in terms of economical use of energy and resources it is essential, therefore, to increase the conductance for CO₂ diffusion from the ambient air to the chloroplasts. Since the thickness of C₃ leaves is one of the important determinants in conductance for CO₂ diffusion and is a key factor to answering the question why sun leaves are thicker than shade leaves, the path of CO₂ diffusion is first traced from the ambient air to the chloroplast stroma.

Rubisco

Rubisco is a hexadecameric enzyme having eight large subunits encoded in the chloroplast DNA and eight small subunits encoded in the nuclear DNA in higher plants and green algae. Each large subunit has one active site that catalyses the following reaction:

\[
\text{RuBP} + \text{CO}_2 + \text{H}_2\text{O} \rightarrow 2 \text{phosphoglycerate (PGA)}
\]

The maximum rate \(k_{\text{cat}}\) at CO₂ saturation at 25 °C is very low and typically 3 mol CO₂ mol active site⁻¹ s⁻¹ (24 mol CO₂ mol enzyme⁻¹ s⁻¹), which is lower than those of other
In the presence of 21% oxygen, the apparent Michaelis constant for CO2, \( K_c \), in the absence of O2 is about 12 \( \mu \)M. This concentration is near the CO2 concentration in the water equilibrated with the atmosphere having a CO2 partial pressure at 37 Pa. Moreover, Rubisco shows high oxygenation activity. Molecular oxygen, O2, competes with CO2 for the same substrate, RuBP, in the active site:

\[
\text{RuBP} + \text{O}_2 \rightarrow \text{PGA} + \text{phosphoglycolate}
\]

In the presence of 21% oxygen, the apparent \( K_c \) increases to about 20 \( \mu \)M due to substantial oxygenation. Because phosphoglycolate is an inhibitor of the triose phosphate isomerase of the Calvin–Benson cycle (Leegood, 1990), plants should detoxify this compound and salvage as much carbon as possible. The photorespiration cycle plays these two roles. By this cycle, 1.5 C is salvaged and 0.5 C is lost as CO2 per oxygenation. The photorespiration pathway cycles back 0.5 PGA (1.5 C) to the Calvin–Benson cycle at the expense of 1 ATP and 0.5 NADPH (=1 ferredoxin). Thus, 1.5 PGA (4.5 C) is produced per oxygenation. For the regeneration of RuBP (5 C), 0.5 C is lacking. If 1/6 triose-phosphate is used for the 0.5 C, then 5 ATP and 3 NADPH are used altogether because the Calvin–Benson cycle requires 3 ATP and 2 NADPH for 1 carboxylation, RuBP regeneration, and 1/3 triose phosphate production. Thus, the energy requirement and carbon loss by photorespiration are huge (for stoichiometry, see Heldt, 1999; von Caemmerer, 2000). Thus, it is of supreme importance for plants to maintain the CO2 concentration at the carboxylation site as high as possible for efficient carboxylation, and suppression of photorespiration as has been repeatedly pointed out (for an early review, see Raven, 1970).

**Diffusion of CO2 from the ambient air to the intercellular spaces**

In photosynthesizing C3 leaves, which have no biochemical CO2 concentrating mechanisms, the CO2-concentration at the carboxylation site, \( C_c \), is lower than that in the ambient air, \( C_a \), and CO2 diffuses to the chloroplast stroma along the gradient of CO2 concentration (Fig. 1).

The CO2 concentration in the substomatal cavity, \( C_s \), is lower than \( C_a \). \( C_s \) can be estimated by the gas exchange technique (Farquhar and Sharkey, 1982):

\[
C_s = C_a - 1.6P/g_{\text{leaf},w}
\]

where \( P \) is the rate of net photosynthetic CO2 fixation per unit leaf area, \( g_{\text{leaf},w} \) is the leaf conductance for water vapour, and 1.6 is a physical conversion factor from the conductance for water vapour to that for CO2. \( g_{\text{leaf},w} \) is expressed as:

\[
g_{\text{leaf},w} = g_{s,w} + g_{c,w}
\]

where \( g_{s,w} \) is the stomatal conductance for water vapour and \( g_{c,w} \) is cuticular conductance for water vapour. Because CO2 diffuses almost exclusively through stomata and not across the epidermis, the use of \( g_{s,w} \) instead of \( g_{\text{leaf},w} \) is more appropriate. However, due to technical difficulty, \( g_{\text{leaf},w} \) is routinely used for \( g_{s,w} \). When \( g_{\text{c},w} \) is much smaller than \( g_{s,w} \), \( g_{\text{leaf},w} \) can be used for \( g_{s,w} \). In vigorously photosynthesizing C3 leaves with widely open stomata, \( C_s/C_i \) would range from 0.6 to 0.9.

The value of \( g_{s,w} \) is relatively constant, irrespective of \( g_{s,w} \) values, and thereby the contribution of \( g_s \) would be significant when \( g_{s,w} \) is small. Thus, when some stress factor induces the closure of stomata, calculated \( C_s \) using \( g_{\text{leaf},w} \) tends to be overestimated (for a review, see Evans et al., 2004). When stomata tend to close, photosynthesis can be non-uniform over the leaf. This also often causes overestimation of \( C_s \) (for reviews, see Terashima, 1992; Evans et al., 2004).

When the \( C_s \) leaf is vigorously photosynthesizing, the bulk CO2 concentration in the intercellular spaces, \( C_i \), is lower than \( C_s \), due to the resistance to CO2 diffusion in the intercellular spaces, \( r_{\text{las}} \) (Parkhurst, 1994). However, this resistance is usually much smaller than the stomatal resistance, \( r_s \). Except for very thick hypostomatous leaves (Parkhurst and Mott, 1990) or succulent leaves with small intercellular spaces (Maxwell et al., 1997), \( C_i/C_s \) is >0.9 for hypostomatous leaves (Terashima et al., 2001). The resistance to CO2 diffusion in the intercellular spaces in the amphistomatous leaves is one-third to one-quarter of that in the hypostomatous leaves having the same thickness (Parkhurst et al., 1988; Terashima et al., 2001). Thus, it is unlikely that \( r_{\text{las}} \) is a major limiting factor of leaf photosynthesis, particularly in the amphistomatous leaves. From here, therefore, \( C_s \) will not be distinguished from \( C_i \) and only \( C_i \) will be used.
CO₂ concentration at the carboxylation site in the chloroplast stroma, Cᵢ, in C₃ plants is even lower than Cᵢ (Evans and von Caemmerer, 1996; Evans and Loreto, 2000). Two different methods have mainly been used to estimate Cᵢ. One method is based on the comparison of the electron transport rate estimated by the fluorescence method and the gas exchange rate measured simultaneously. This method is simpler but relies on several assumptions. The other one, the concurrent measurement of gas exchange and carbon isotope discrimination, is more complex but gives a more accurate estimation. Cᵢ/Cᵢ, thus obtained for vigorously photosynthesizing C₃ leaves, ranges from 0.5 to 0.75.

From the intercellular spaces to the chloroplast stroma

The data of conductance for CO₂ diffusion from the chloroplast stroma to the intercellular spaces ranges from 0.5 to 0.75.

The flux of inorganic carbon species via the pathway like B (dotted line) in the right panel of Fig. 1 should be negligible compared with that of pathway A (continuous line) because of the large liquid phase resistance. Thus, Sₑ is important as the active area for CO₂ diffusion to the chloroplast stroma (Laisk et al., 1970; Raven, 1970, 1977; Nobel, 1977; Evans and Loreto, 2000; Evans et al., 2004).

The data in Fig. 2 that include plants of various functional types (Table 1) are, however, scattered. Among them, gᵢ values for annual herbs such as crop species are greatest when compared at a given Sₑ and range from 0.2 to 0.5 mol CO₂ m⁻² s⁻¹. On the other hand, those of evergreen broad-leaved trees are much lower and range from 0.03 to 0.2 mol CO₂ m⁻² s⁻¹.

Among the evergreen broad-leaved trees, Citrus spp. showed greater gᵢ than Macadamia integrifolia and the evergreen broad-leaved trees native to Japanese laurel forests (for scientific names and their authorship, see Table 1 and legend to Fig. 3). In mesic deciduous broad-leaved trees, gᵢ values are intermediate between those of annual herbs and evergreen trees. Among them, gᵢ for the tree species that develop leaves successively (Ailuropoda maximowiczii, and Prunus persica) tends to be greater than those for Acer spp. that flush their leaves in the spring. When the data points are grouped into annuals, including wheat and rice, deciduous broad-leaved trees, and evergreen broad-leaved trees, a tendency that gᵢ increases with the increase in Sₑ may be apparent.

The main difference among these groups is the thickness of the mesophyll cell wall (δₑ). The δₑ in typical annuals, deciduous broad-leaved species, and evergreen broad-leaved species range from 0.1 to 0.2, 0.2 to 0.3, and 0.3 to 0.5 μm, respectively. Within the deciduous tree species, the flush-type species tend to show greater δₑ than in the successive-type species (Hanba et al., 2001, 2002). Acer rufinerve (100% light) showed a large Sₑ, but a low gᵢ, which is probably explained by its very large δₑ for a deciduous tree species, 0.25 μm for the palisade tissue cells and 0.46 μm for the spongy tissue cells.

A clonal herbaceous perennial, Polygonum cuspidatum (synonymous to Reynoutria japonica Houtt.), is a well-known pioneer plant as the first colonizer of volcanic deserts (Adachi et al., 1996). The plants grown at 2500 m a.s.l. in a volcanic desert on Mt Fuji-san, Japan, had a thick δₑ of 0.35–0.42 μm, while the same species at 10 m a.s.l. had a δₑ of 0.22–0.29 μm. In agreement with the difference in wall thickness, gᵢ for the plants from 2500 m was 0.076 mol m⁻² s⁻¹ while that for the plants from 10 m was 0.2 mol m⁻² s⁻¹ (Kogami et al., 2001; see Fig. 3). When autotetraploidy was artificially induced in Phlox drummon-dii, the leaves became thicker and Sₑ increased. However, at the same time, cell wall thickness increased considerably (from 0.12 to 0.24 μm), which would partly explain the absence of the increase in gᵢ with the increase in Sₑ (P Vyas, unpublished observation; see Fig. 3).
Table 1. Mesophyll surface area, chloroplast surface area and internal conductance of species of various functional types

<table>
<thead>
<tr>
<th>Species</th>
<th>$S_{mc}$ (m² m⁻²)</th>
<th>$S_{c}$ (m² m⁻²)</th>
<th>$g_{i}$ (mol CO₂ m⁻² s⁻¹)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Annuals</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nicotiana tabacum L. (sun)</td>
<td>23.7</td>
<td>21.8</td>
<td>0.470</td>
<td>Evans et al., 1994</td>
</tr>
<tr>
<td>N. tabacum (shade)</td>
<td>17.0</td>
<td>14.5</td>
<td>0.370</td>
<td>Evans et al., 1994</td>
</tr>
<tr>
<td>Triticum aestivum L.</td>
<td>23.5</td>
<td>18.8</td>
<td>0.430</td>
<td>Evans and Vellen, 1996</td>
</tr>
<tr>
<td>Oryza sativa L.</td>
<td>19.0</td>
<td>14.0</td>
<td>0.200</td>
<td>Hanba et al., 2004</td>
</tr>
<tr>
<td>Phaseolus vulgaris L.</td>
<td>14.3</td>
<td>13.0</td>
<td>0.290</td>
<td>Hanba et al., 2003</td>
</tr>
<tr>
<td>P. vulgaris</td>
<td>26.6</td>
<td>21.0</td>
<td>0.143</td>
<td>Hanba et al., 2003</td>
</tr>
<tr>
<td>Phlox drummondii Hook.</td>
<td>14.6</td>
<td>8.5</td>
<td>0.160</td>
<td>P Vyas et al., unpublished</td>
</tr>
</tbody>
</table>

| **Deciduous broadleafed trees** | | | | |
| Acer mono Maxim. (17% sun) | 14.0 | 12.0 | 0.125 | Hanba et al., 2001 |
| A. mono (full sun) | 12.0 | 8.5 | 0.065 | Hanba et al., 2001 |
| Acer palmatum (Thunb.) Steud. | 8.0 | 5.5 | 0.030 | Hanba et al., 2001 |
| Acer palmatum (full sun) | 10.0 | 7.5 | 0.052 | Hanba et al., 2001 |
| Acer rubinerve Sieb. et Zucc. (17% sun) | 8.5 | 5 | 0.047 | Hanba et al., 2001 |
| A. rubinerve (full sun) | 18.0 | 13.5 | 0.047 | Hanba et al., 2001 |
| Alnus japonica (Thunb.) Steud. | 20.0 | 18.0 | 0.110 | Hanba et al., 2001 |
| Populus maximowiczii A. Henry | 78.0 | 55.5 | 0.100 | Hanba et al., 2001 |
| Prunus persica L. Batsch | 30.1 | 21.0 | 0.370 | Svyetser et al., 1995 |
| Pr. persica (shade) | 26.6 | 14.0 | 0.291 | Svyetser et al., 1995 |

| **Evergreen trees** | | | | |
| Quercus glauca Thunb. ex Murray | 17.0 | 10.8 | 0.066 | Hanba et al., 1999 |
| Q. glauca | 11.2 | 7.8 | 0.076 | Hanba et al., 1999 |
| Q. phyllyraeoides A. Gray | 31.4 | 15.4 | 0.143 | Hanba et al., 1999 |
| Cinnamomum camphora (L.) J. Presl | 19.6 | 14.5 | 0.061 | Hanba et al., 1999 |
| Castanopsis sieboldii (Makino) Hatus. | 20.7 | 16.7 | 0.044 | Hanba et al., 1999 |
| Cas. sieboldii | 22.1 | 17.8 | 0.082 | Hanba et al., 1999 |
| Cas. sieboldii | 25.0 | 25.0 | 0.100 | Miyazawa and Terashima, 2001 |
| Ligustrum lucidum Aiton | 20.3 | 10.5 | 0.067 | Hanba et al., 1999 |
| L. lucidum | 28.0 | 14.6 | 0.113 | Hanba et al., 1999 |
| Camellia japonica L. | 41.6 | 20.5 | 0.119 | Hanba et al., 1999 |
| Cam. japonica | 32.9 | 16.2 | 0.069 | Hanba et al., 1999 |
| Citrus paradisi Macfad | 26.2 | 11.0 | 0.208 | Svyetser et al., 1995 |
| C. paradisi | 24.7 | 11.0 | 0.157 | Svyetser et al., 1995 |
| Citrus limon (L.) Burm.f. | 26.4 | 10.0 | 0.149 | Svyetser et al., 1995 |
| Macadamia integrifolia Maiden et Betch | 24.4 | 15.0 | 0.114 | Svyetser et al., 1995 |
| **CAM** | | | | |
| Kalanchoë daigremontiana Hamet et Perr. | 25.4 | 9.8 | 0.050 | Maxwell et al., 1997 |

$S_{c}$ represents the area for CO₂ dissolution and $\delta_{w}$ represents the path length for CO₂ diffusion in the liquid phase. Thus, it is understandable, that $g_{i}$ is proportional to $S_{c}$ and inversely related to $\delta_{w}$.

The role of aquaporins

In addition to the two physical factors dealt with above, the role of aquaporins in CO₂ diffusion will be highlighted. Changes in $g_{i}$ without marked changes in $S_{c}$ and/or cell $\delta_{w}$ have been reported. For example, Evans and Vellen (1996) followed senescing wheat leaves and observed the drastic decrease in $g_{i}$ without marked changes in $S_{c}$ (Fig. 3). Perhaps $\delta_{w}$ did not increase greatly either. Decreases in $g_{i}$ have been reported for plants under water stress (Flexas et al., 2002) and salt stress (Delfine et al., 1998, 1999).

These studies indicate that CO₂ permeability of the plasma membrane or chloroplast envelope might have changed.

Aquaporins, the most abundant proteins in plant plasma membranes, transport water molecules according to the gradient of the water potential (Maurel, 1997; Kjellborn et al., 1999). However, there are reports indicating that some animal aquaporins transport CO₂ (Cooper and Boron, 1998; Nakhoul et al., 1998; Yang et al., 2000).

Terashima and Ono (2002) estimated $g_{i}$ using the concurrent measurements of gas exchange and fluorescence in the leaves of Vicia faba L. and Phaseolus vulgaris L. before and after the application of HgCl₂, an inhibitor of most of the aquaporins, to the petiole. Because $g_{i}$ and hydraulic conductivity of the mesophyll cells decreased at the same concentration range of HgCl₂, it is proposed that aquaporins are involved in diffusion of CO₂ across the plasma membrane. Application of chloromercuribenzene sulphonate that would not permeate membranes easily gave similar results (I Terashima, Y Tazoe, V Oja, A Laisk, unpublished results). Bernacchi et al. (2002) observed a clear peak at 35–37 °C in the temperature dependence of $g_{i}$ measured by the fluorescence method in tobacco, which suggests the involvement of protein(s) in CO₂ diffusion across the plasma membrane.

Recently, it was shown that the aquaporin 1 of Nicotiana tabacum L., expressed in Xenopus oocytes, transfers CO₂...
In this study, tobacco aquaporin 1 (NtAQP1) was expressed in *Xenopus* oocytes and the CO₂ permeability was monitored as the decrease in pH with a pH micro-electrode. Hanba et al. (2004) attempted to over-express an aquaporin of *Hordeum vulgare* L. (HvPIP2; 1) in rice (*Oryza sativa* L.). By contrast to their original expectation, they obtained plants with varying aquaporin contents. The concurrent measurement of the gas exchange and carbon isotope discrimination of these leaves revealed that $g_i$ clearly increases with the increase in aquaporin abundance.

Besides the abundance of aquaporins, conductance of CO₂ through aquaporins could also be regulated, for example, by pH and/or protein phosphorylation, because water permeability has been reported to be regulated by pH (Tournaire-Roux et al., 2003), and by protein phosphorylation (Maurel, 1997; Kjellbom et al., 1999). These possibilities are to be studied.

**Components of the internal conductance**

The internal conductance (or resistance) can be dissected further into several components (Fig. 4). The resistance to CO₂ diffusion from the cell wall surface to the carboxylation site per unit chloroplast surface area ($R_i$) may be expressed as:

$$R_i = R_w + \frac{1}{R_{aq}} + \frac{1}{R_{bm}} + R_{cytosol} + R_{env} + R_{stroma}$$

where $R_w$, $R_{aq}$, $R_{bm}$, $R_{cytosol}$, $R_{env}$, and $R_{stroma}$ are resistances to CO₂ diffusion across the cell wall, aquaporin, bulk plasma membrane, cytosol, chloroplast envelope, and chloroplast stroma to the carboxylation site expressed on a unit chloroplast surface area, respectively. The corresponding conductances are $G_{aq}$, $G_{bm}$, $G_{cytosol}$, $G_{env}$, and $G_{stroma}$. Note that lower-case letters, $r$ and $g$, are used for the resistance and conductance, respectively, on a leaf area basis.

For the moment, let us neglect $R_{cytosol}$ because the distance between the plasma membrane and chloroplast envelope is very small in many wild plants, and the abundance of inorganic carbon species, mostly HCO$_3^-$ and CO₂, relative to CO₂ ($\Sigma C/CO_2$) may be $>7$ at pH 7 if the carbonic anhydrase activity in the cytosolic is sufficient (Evans et al., 1994; Nobel, 1999; Coleman, 2000). $R_{env}$ is also neglected. In the light, pH in the chloroplast stroma increases and there is carbonic anhydrase (Coleman, 2000). Thus the abundance of HCO$_3^-$ relative to CO₂ would be $>50$ (Nobel, 1999). Because HCO$_3^-$ also diffuses together with CO₂, $R_{stroma}$ would be very small (Raven and Glidewell, 1981; Cowan, 1986).

**Wall**

The wall conductance for the unit chloroplast surface area can be expressed as:

$$G_w = \frac{1}{R_w} = \frac{p \cdot D_C \cdot K_{CO_2}}{\tau \cdot \delta_w}$$

where $p$ is porosity, $D_C$ is the weighed diffusion coefficient of inorganic carbon species in water, $K_{CO_2}$ is the...
partitioning coefficient, the ratio of the concentrations of the inorganic carbon species in the liquid phase to that of CO2 in gas phase (Nobel, 1999). τ is tortuosity, and δw is cell wall thickness.

Nobel (1999) assumed that the p/τ of the mesophyll cell wall is around 0.3. Because Dc at 20 °C is not very different from the diffusion coefficient of CO2 (see below), 1.7 \times 10^{-9} m^2 s^{-1}, he used pDc/τ of 5 \times 10^{-10} m^2 s^{-1}. K_{CO2} used was 1 because apoplast pH is somewhat acidic (Nobel, 1999; Stahlberg and Van Volkenburgh, 1999; but see Raven and Farquhar, 1989) and the inorganic carbon species is almost exclusively CO2 and δw was 0.3 \times 10^{-6} m, then Gw was calculated to be 1.7 \times 10^{-3} m s^{-1}. Assuming S_c is 20 m^2 m^{-2}, the wall conductance on the leaf area basis, g_w, is 0.03 m s^{-1}, which corresponds to 1.2 mol m^{-2} s^{-1}. Based on such calculations, Nobel claimed that wall conductance is very large and thus the cell wall is not limiting photosynthesis. However, the actual p/τ is not known for mesophyll cell walls of higher plants.

Although permeability of CO2 across the cell wall has not been measured, permeability of water across the cell wall was measured. Kamiya et al. (1962) measured water permeability of the cell wall in Nitella flexilis (L.) Ag., a characean plant having giant internodal cells, using the pressurizing method. They found that the cell wall, which is 10 μm thick, showed water permeability of 5 \times 10^{-7} m s^{-1} atm^{-1}. They also found that the permeability is inversely proportional to the wall thickness. Because 1 atm corresponds to the concentration difference of 40.4 mol m^{-3} at 25 °C, p/τ of the cell wall can be calculated from:

\[
\frac{5 \times 10^{-7}}{V_w} = \frac{p \cdot D_{H_2O}}{\tau \cdot \delta_w} \cdot 40.4 \tag{5}
\]

where V_w is the molar volume of water (18 \times 10^{-6} m^3) and D_{H_2O} is the diffusion coefficient of water in water (2.3 \times 10^{-9} m^2 s^{-1} at 25 °C). When δ_w is 10 \times 10^{-6} m, p/τ is calculated to be 3.

It is known that the permeability obtained by the pressurizing technique is greater than that by the diffusion method if the material has bulky holes or pipes (Gutknecht, 1967). The p/τ value of 3, therefore, indicates that there are holes or pipes in the cell wall.

Gutknecht (1967) measured the wall conductance (P_a) in the cell wall of a giant green alga Valonia ventricosa 1. Agardh by the diffusion method using tritiated water and obtained 2.5 \times 10^{-6} m s^{-1}. Because δ_w of these algae would be around 10 μm (not given by Gutknecht, 1967; but see Okuda et al., 1997),

\[
2.5 \times 10^{-6} = (p \cdot D_{H_2O})/(\tau \cdot \delta_w) \tag{6}
\]

p/τ thus obtained is 0.011. If this p/τ value, δ_w of 0.3 μm, and S_c of 20 m^2 m^{-2} are used, then a very low g_w of 0.045 mol m^{-2} s^{-1} is obtained. This value is too low, even when the lowest g_i values in Fig. 2 are considered.

Accurate measurements of wall resistance to diffusion of water and CO2 with higher plants are needed. It should be noted that one cannot use the pressure probe method in the pressurizing mode to determine wall resistance.

Assuming p/τ =0.1, g_i (see the grey lines in Fig. 2) was calculated. If p/τ is less than about 0.1, then wall conductance is an important factor determining internal conductance. Of course, p/τ values would vary considerably, because of the variation in cell wall constituents across species. However, it is proposed that the p/τ value is around 0.1 or lower, because the difference in g_i among leaves having a given S_c, but from different functional groups, is roughly explained by δ_w.

The resistance to CO2 diffusion in the liquid phase is 10^4 of that in the gas phase. If p/τ is 0.1, then the wall thickness of 0.3 μm will correspond to an air layer 30 mm thick. Thus, wall resistance would be an important limiting factor of photosynthesis.

**Plasma membrane**

When HgCl2 was applied to the leaflets of Phaseolus vulgaris L., g_i decreased by up to 60% depending on the concentration of HgCl2 (Terashima and Ono, 2002). Some aquaporins are known to be insensitive to HgCl2 (Kjellbom et al., 1999) and, therefore, a part of the residual conductance could be attributed to such insensitive aquaporins. As already mentioned above, the tobacco aquaporin (NtAQPI) has been shown to transport CO2 across the plasma membrane of Xenopus oocytes (Uehlein et al., 2003).

Hanba et al. (2004) have reported that rice leaves expressing various amounts of barley aquaporin (HvPIP2;1) showed differences in g_i, the range of which corresponds to 50% of the g_i in the control plants. g_i in tobacco plants expressing antisense aquaporin 1 was lower than that of the control plants by 40%, while the plants over-expressing aquaporin 1 showed g_i greater than that in the control plants by 30% (Flexas et al., 2004; International Photosynthesis Congress, 2004; Montreal; J Flexas, personal communication).

There are >10 different aquaporin species that are expressed in the plasma membrane of plants (Kjellbom et al., 1999). It is not known how many of these are responsible for the transport of CO2. Thus, CO2 transport activity in these aquaporins should be examined on a one by one basis. Then, the contribution of aquaporins to the overall conductance of the plasma membrane should be studied.

At this stage, only a rough estimation can be made. Let us assume that the CO2 diffusion through aquaporins in a P. vulgaris leaf is completely suppressed by HgCl2 and g_i decreased from 0.3 mol m^{-2} s^{-1} to 0.12 mol m^{-2} s^{-1}. If p/τ, cell wall thickness, and S_c are 0.1, 0.1 μm, and 13 m^2 m^{-2}, respectively, then g_w will be 0.9 mol m^{-2} s^{-1}. If it is further assumed that internal resistance is the sum of...
Why are sun leaves thicker than shade leaves?

The light-saturated rate of leaf photosynthesis per unit area \( P_{\text{max}} \) in \( C_3 \) plants strongly depends on leaf nitrogen content and photosynthetic components such as Rubisco, cytochrome \( f \), \( \text{H}^+\text{-ATPase} \), and reaction centres. \( P_{\text{max}} \) is also strongly correlated with structural parameters such as leaf thickness, leaf mass per area, mesophyll surface area \( S_{\text{mes}} \), and chloroplast surface area \( S_c \). Importance of \( S_c \) as an area for \( \text{CO}_2 \) dissolution and for the \( \text{CO}_2 \) diffusion pathway has been already discussed.

Let us consider the drawdown of \( \text{CO}_2 \) concentration from the intercellular spaces to the stroma \( (\Delta C=C_i-C_e) \) for a unit chloroplast surface area (Fig. 5). The drawdown is proportional to the flux of \( \text{CO}_2 \) across the cell wall, plasma membrane, cytosol, and chloroplast envelope per unit chloroplast surface area and to the internal resistance, \( R_i \). With the increase in the amount of Rubisco per unit chloroplast surface area, the \( \text{CO}_2 \) flux increases. However, the photosynthetic rate per Rubisco decreases because \( C_c \) decreases. From the viewpoint of efficiency of Rubisco use (or nitrogen use), thicker leaves with greater \( S_c \) would be advantageous because the amount of Rubisco per \( S_c \) becomes smaller and thereby \( C_c \) would increase. On the other hand, the construction and maintenance costs of thick leaves are expensive. Also, the drawdown of \( \text{CO}_2 \) concentration during the diffusion in the intercellular spaces, \( C_c-C_i \), becomes greater with the increase in leaf thickness, which thus causes a decrease in the bulk \( C_i \). In the latter two aspects, thick leaves are not at all advantageous.

Effects of various aspects of mesophyll structure, in particular mesophyll thickness on photosynthesis, were evaluated using a one-dimensional model of \( \text{CO}_2 \) diffusion in the \( C_3 \) leaf (Terashima et al., 2001). In this model, mesophyll is composed of columnar cells, the lateral surfaces of which are fully occupied by chloroplasts. When mesophyll thickness was increased, keeping the Rubisco content per leaf area constant, \( S_c \) increases and Rubisco content per \( S_c \) decreases. The model leaves are either hypostomatous or amphistomatous. The main results can be summarized as follows:

(i) When mesophyll thickness was increased keeping the Rubisco content per leaf area constant, the rate of photosynthesis per leaf area increased at first due to the increase in \( C_c \), attained the peak value, and then gradually decreased. The gradual decrease was due to the gradual decrease in \( C_i \) due to the increased \( r_{\text{las}} \). The thickness that gives the peak value was identical for the leaves with various Rubisco contents per leaf area.

(ii) The thickness that gives the maximum photosynthetic rate for the amphistomatous leaves was greater than that for the hypostomatous leaves because \( r_{\text{las}} \) in the former were one-quarter to one-third of that in the latter leaves.

(iii) The mesophyll thickness that realizes a given photosynthetic rate per unit mesophyll thickness increased with the increase in the Rubisco content per leaf area.

Obviously, neither (i) nor (ii) explains the strong relationship between \( P_{\text{max}} \) and \( S_c \). Thus (iii) or constraints of this kind explain the strong relationships between \( P_{\text{max}} \) and leaf morphological parameters such as mesophyll thickness and \( S_c \). In other words, leaf thickness is determined as a compromise between the increase in chloroplast surface area for \( \text{CO}_2 \) dissolution and the decrease in the construction and maintenance costs of the leaf. If such the economical optimum is strongly favoured, \( S_i/S_{\text{mes}} \) can be expected to be very high. Moreover, thickness of chloroplasts or Rubisco/\( S_c \) would not vary much. Both are roughly true in nature (see below). Thus, this would be the answer to the question: why are sun leaves thicker than shade leaves?
Because C₄ plants have CO₂ concentration mechanisms, and the CO₂ fixation enzyme is located in the cytosol of mesophyll cells, the arguments above are exclusively for C₃ plants. The changes in leaf thickness or mesophyll cell surface area have not been intensively studied for C₄ plants. It was found recently that the thickness of the leaves of *Amaranthus cruentus* L., an NAD-ME-type C₄ dicotyledonous plant, did not respond to growth in the light (Fig. 6; Tazoe et al., 2005). For responses of C₄ photosynthesis to growth photosynthetic photon flux densities (PPFD), see the review by Sage (2006).

**How do C₃ sun leaves have greater $S_c$ than shade leaves?**

In many deciduous broad-leaved tree species, sun leaves have a greater $S_c$ than shade leaves. In sun leaves, the height of the palisade tissue is greater than in shade leaves. In some cases, thickening of the palisade tissue is accompanied by periclinal cell division as well as cell elongation of the palisade tissue cells.

Some ecotypes of Japanese beech (*Fagus crenata* Blume), including those on the Pacific side, differentiate sun leaves with thick palisade tissue comprising two cell layers (T Koike, personal communication; Fig. 7). In such ecotypes, not only the number of leaves (Kozlowski and Clausen, 1966) but also the number of cell layers in the palisade tissue of these leaves is determined in the winter buds, by early winter of the year prior to leaf unfolding (Eschrich et al., 1989). When the sun-exposed branches with young expanding leaves of *F. crenata* were shaded by shade cloths, the resultant leaves showed intermediate characteristics: they had the palisade tissue that comprised two cell layers but the height of the palisade tissue and $P_{\text{max}}$ were lower than those in the fully exposed sun leaves (Uemura et al., 2000; A Uemura and A Ishida, personal communication). These strongly indicate that several different signals are used for the determination of characteristics of sun leaves, including multi-layered palisade tissue, greater cell height, larger $S_c$, higher contents of photosynthetic enzymes, and higher stomatal frequency and conductance. At least, the current-year PPFD and previous-year PPFD play different roles (Kimura et al., 1998; Uemura et al., 2000).

Using *Chenopodium album* L., an annual herb, Yano and Terashima (2001) established a more sophisticated experimental system and examined the differentiation processes of sun and shade leaves. The plants were shaded in various ways and the effects of these shade treatments on the properties of the developing leaves were examined. When mature leaves were exposed to high light, the developing leaves, irrespective of their light environments, formed palisade tissue with two cell layers. On the other hand, when mature leaves were shaded, palisade tissue with one cell layer was formed. These results clearly showed that the light environment of mature leaves determined the number of cell layers in the palisade tissue of new leaves. There must be a signal transduction system that conveys the signal(s) from the mature leaves to the developing leaves (Yano and Terashima, 2001). Yano and Terashima (2004) also conducted a detailed developmental study of sun and shade leaves of *C. album*. Whether the plants were grown under typical sun or shade conditions, the number of cells in the palisade tissue per leaf was almost identical. Moreover, in sun leaves, anticlinal and periclinal divisions occur almost synchronously. Thus, the signal from the mature leaves regulates the direction of cell division. In the future sun leaves, the signal probably induces periclinal division in addition to anticlinal division, while the signal from the shaded mature leaves only allows the cells to divide anticlinally (Yano and Terashima, 2004).

Yano and Terashima (2001, 2004) hypothesized that the signal is the abundance of photosynthates; when the photosynthates from mature leaves are abundant, leaves would develop into sun leaves. Currently, the effects of the sucrose content of the agar on leaf development in *Arabidopsis thaliana* (L.) Heynh. are being examined. There is a clear
indication that the number of cell layers in the palisade tissue increases with increasing sucrose content (S Yano, H Tsukaya, personal communication). Stomatal frequency and morphology of epidermal cells in young leaves are also regulated by the environment of the mature leaves (Lake et al., 2001; Thomas et al., 2003; Coupe et al., 2006), although the signal transduction mechanisms involved may be different from those that regulate the direction of cell division. For the palisade tissue cells to elongate, the local light environment of the developing leaves appears to be essential, although detailed studies have not been conducted.

On the other hand, chloroplast properties are mostly determined by the local light environment of the developing leaves (Terashima and Hikosaka, 1995; Yano and Terashima, 2001). It is known that there is a gradient in light environment within a leaf (Terashima and Saecki, 1983, 1985) and that chloroplasts within the leaf acclimate to their respective light environments (Terashima and Inoue, 1985a, b). When the leaves were inverted or irradiated from the bottom after their unfolding, the properties of chloroplasts changed and acclimated to their new light environments (Terashima and Takenaka, 1986; Terashima et al., 1986). Thus, acclimation of chloroplast properties to their local light environments is very plastic.

After maturation, leaves of some species respond to changes in the light environment. When grown with sufficient nutrients, most of the mesophyll cell surfaces facing the intercellular spaces are occupied by chloroplasts. However, it was shown that, for the shade leaves of *Chenopodium album*, unoccupied spaces are indispensable for re-acclimating to brighter light environments (Oguchi et al., 2003). When the shade leaves of *C. album* were exposed to a higher light for growth, an increase in $S_C$ was observed. This increase in $S_C$ was not accompanied by an increase in mesophyll surface area. Oguchi et al. (2005) examined shade leaves of three deciduous tree species, *Betula ermanii* Cam., *Fagus crenata* Blume, and *Acer rufinerve* Sieb. et Zucc. In *F. crenata* mesophyll surfaces were fully occupied by chloroplasts and did not increase $P_{\text{max}}$ when the plants were exposed to a higher growth PPFD. On the other hand, shade leaves of *B. ermanii* that

![Fig. 7. Sun and shade leaves of *Fagus crenata* Blume (A–C) and *Fagus japonica* Maxim. (D–F). (A) and (D) were from fully exposed shoots; (B) and (E) were from the shoots that had been exposed but were shaded from early spring until sampling in mid-summer; (C) and (F) were from the lower shaded shoots. Note that the palisade tissues of *F. japonica* had only one cell layer irrespective of the light conditions (Uemura et al., 2000). Original micrographs of I Terashima.](https://academic.oup.com/jxb/article-abstract/57/2/343/489948)
had unoccupied mesophyll surfaces increased $P_{\text{max}}$ in response to the exposure. Shade leaves of *A. rafiferve* elongated palisade tissue cells and increased $P_{\text{max}}$ in response to the increase in growth PPFD.

In *Hedera helix* L., the mature palisade tissue cells divided periclinally in response to the increase in growth PPFD (Bauer and Thoni, 1988). Such division of the palisade tissue cells has not been described for other species. However, for the evergreen leaves of extended longevity, such plastic adjustment may be important. Recently, mechanisms of the sun and shade leaf differentiation were reviewed elsewhere (Terashima et al., 2005).

### Plasticity of leaf structure: importance of cell size

In this review, attention was first drawn to the importance of having sufficient $S_c$ for efficient photosynthesis. Mechanisms responsible for the regulation of $S_c$ are also discussed. These arguments are based on an assumption that cell diameter would not change. However, in nature, the size of mesophyll cells varies greatly and the leaf can increase $S_c$ and decrease resistance to CO$_2$ diffusion in the intercellular spaces by decreasing cell size (Terashima et al., 2001, 2005; Miyazawa et al., 2003). The leaf with smaller cells is also mechanically tougher (Terashima et al., 2001). Actually, mesophyll cell size differs considerably across species (Terashima et al., 2001). However, leaves do not have very small cells. This could be because leaves exhibiting considerable rates of leaf area expansion, adequate heat capacitance, high efficiency of resource use, etc. have been favoured by natural selection (Terashima et al., 2001). The merits or demerits of having large and small mesophyll cells are summarized in Table 2. If the leaves have large $S_c$ with large cells, as is found in annual herbs, the leaves should be thick. Such leaves would expand quicker, keep their shape by turgor, and have stomata on both leaf surfaces. The leaves with extended longevity tend to have small cells (Terashima et al., 2001). We mentioned previously that alpine plants tend to have smaller mesophyll cells (Terashima et al., 2005). However, their cells are not necessarily smaller than those of related lowland species (Körner, 1999). It is also worth mentioning that the mesophyll cell sizes of bonsai plants are comparable with those of plants grown normally (Körner et al., 1989). Although such leaves with small cells are mechanically tougher, area expansion would be slower. Moreover, the volume ratio of chloroplasts/nucleus in small cells might be smaller. Because N and P are important constituents of nucleic acids or proteins in nuclei as well, the cost, which may be called ‘information cost,’ would be much greater in leaves of small cells. If this informational cost is large, the nitrogen use efficiency of photosynthetic production would decrease. If this is very important, cell size would be greater in leaves under low availability of P and/or N. There are other features such as water relationships with respect to cell size. Comprehensive studies approaching the diversity in leaf thickness based on causes and consequences of cell size should be made.

### Table 2. Possible effects of cell size on leaf characteristics

<table>
<thead>
<tr>
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<th>Small cells</th>
<th>Large cells</th>
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<tbody>
<tr>
<td>Leaf thickness for the same $S_c$</td>
<td>Thin</td>
<td>Thick</td>
</tr>
<tr>
<td>Mechanical strength</td>
<td>Strong</td>
<td>Weak</td>
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<tr>
<td>Maintenance of leaf morphology</td>
<td>Cell walls (armour)</td>
<td>Turgor (balloon)</td>
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<tr>
<td>Longevity</td>
<td>Long</td>
<td>Short</td>
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<tr>
<td>Heat capacity</td>
<td>Small</td>
<td>Large</td>
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<tr>
<td>Intercellular resistance to CO$_2$ diffusion</td>
<td>Small</td>
<td>Large</td>
</tr>
<tr>
<td>Stomatal occurrence</td>
<td>Hypostomatous</td>
<td>Amphistomatous</td>
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<tr>
<td>Area expansion</td>
<td>Slow</td>
<td>Fast</td>
</tr>
<tr>
<td>Informational cost (nuclear N and P/chloroplast N and P)</td>
<td>High</td>
<td>Low</td>
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### References


