Leaf anatomy enables more equal access to light and CO₂ between chloroplasts

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SUMMARY

The function of a leaf is photosynthesis, which requires the interception of light and access to atmospheric CO₂ while controlling water loss. This paper examines the influence of leaf anatomy on both light capture and CO₂ diffusion. As photosynthetic metabolism is spread between many chloroplasts, a leaf faces the challenge of matching light capture by a given chloroplast with the metabolic capacity of that chloroplast. Chloroplasts nearest the leaf surface receive the greatest irradiance and therefore absorb more light per unit chlorophyll than chloroplasts in the centre of a leaf. Electron transport and carbon fixation capacities per unit of chlorophyll decline with increasing depth in the leaf, to compensate for the decline in light absorbed per unit chlorophyll. Many key photosynthetic protein complexes in chloroplasts have nuclear encoded genetic information. Consequently, all chloroplasts within a given cell have a similar metabolic complement, which limits the potential gradient of photosynthetic capacity per unit chlorophyll across the leaf. A simple model couples light absorption through the leaf (based on the Beer–Lambert law) with the profile of chlorophyll through a leaf and the gradient in photosynthetic capacity. It is validated by comparison with ¹⁴C fixation profiles through spinach leaves obtained in various studies. The model can account for published ¹⁴C fixation profiles obtained with blue, red and green light of different irradiances and white light applied in different combinations to the adaxial and abaxial surfaces of spinach leaves. The model confirms that spongy mesophyll increases the apparent extinction coefficient of chlorophyll compared to palisade tissue. The palisade tissue nearest the surface which receives light facilitates the penetration of light to a greater depth, while spongy mesophyll promotes scattering to enhance light absorption, thus reducing the gradient in light absorbed per unit chlorophyll through a leaf. CO₂ fixation faces a diffusional limitation, which necessitates Rubisco to be spread evenly across the cell walls exposed to intercellular airspace. Mesophyll cell structure reflects the need to have a large cell surface per unit volume exposed to airspaces. The regular array of columnar cells in palisade tissue, or cell lobing in monocot leaves, results in greater exposed surface per unit tissue volume than spongy mesophyll. The exposed surface area per unit leaf area scales with photosynthetic capacity such that the difference in CO₂ partial pressure between substomatal cavities and the sites of carboxylation within chloroplasts is, on average, independent of photosynthetic capacity of the leaf. However, Rubisco specific activity declines as the Rubisco content per unit leaf area increases due to greater internal diffusional limitations.

Key words: light absorption profiles, internal conductance, chloroplast surface area, Rubisco, acclimation to light, CO₂ diffusion.

INTRODUCTION

The function of a leaf is photosynthesis, the capture of light and conversion of that energy into chemical bonds during CO₂ assimilation. While all C₃ leaves share an identical photosynthetic mechanism, an amazing diversity of leaf structures exist in order to achieve the same end. This presumably reflects the conflicting biochemical requirements of light capture and CO₂ uptake versus mechanical requirements for strength and durability. Different environmental niches emphasize different characteristics, so suites of traits may tend to prevail in a particular environment. This paper focuses on the interplay between leaf structure and both light capture and CO₂ fixation.

Chloroplasts are the fundamental units for photosynthesis. Typically, there are about 10 million chloroplasts in each square centimetre of leaf. The composition of chloroplasts is flexible, being particularly responsive to the light environment which alters the relative abundance of many of the protein complexes (Anderson, 1986). Nuclear gene components are present in all of the major complexes,
resulting in coordinated regulation of all chloroplasts in a given cell. However, each cell may contain its own unique complement of chloroplasts. This enables a leaf to fine-tune the deployment of resources, resulting in greater daily photosynthesis per unit of protein than would be achieved in the absence of specialization. Considerable evidence for this specialization has been obtained from studies of chloroplast ultrastructure or biochemical measurements of chloroplasts isolated from paradermal leaf sections (Terashima & Saeki, 1989).

Light capture by a leaf ought to be a complicated process to describe, compared with the absorption of light by pigments in solution. Firstly, pigments form complexes with protein in regular arrays in thylakoid membranes. Secondly, the density of thylakoid membranes varies through a leaf. Thirdly, due to the difference in refractive index between air and water, light is scattered within the leaf by air-cell-wall interfaces. While complicated optical treatments have been attempted (Fukshansky & Remisowski, 1992; Richter & Fukshansky, 1996), an alternative approach has been to measure light directly within intact leaves by inserting fibre-optic microprobes (Vogelmann & Björn, 1984; Vogelmann et al., 1988, 1989; Cui et al., 1991). This method yields profiles of space irradiance that decline rapidly through the initial palisade tissue.

CO$_2$ assimilation within the leaf has been directly measured by paradermal sectioning after $^{13}$C labelling (Nishio et al., 1993). This painstaking work revealed that CO$_2$ fixation peaked about one-third of the way into a spinach leaf. Nishio et al. concluded that 'the carbon fixation gradient did not follow the leaf internal light gradient' and that 'somehow, the light gradient is disconnected from CO$_2$ fixation'. Challenged by these statements, I re-analysed their data (Evans, 1995) using a model based on the work of Terashima & Saeki (1983, 1985), and concluded that the observed $^{13}$C fixation profiles were consistent with light absorption profiles, based on the distribution of Chl through the leaf, and with photosynthetic capacity, based on the profile of Rubisco through the leaf. Subsequently, new $^{14}$C fixation data have become available that enable a more rigorous test of this approach. Thus the first objective of this paper is to examine the recent evidence concerning CO$_2$ diffusion within leaves with respect to leaf structure, and its impact on the performance of Rubisco.

THE SPINACH LEAF

The use of a thick leaf for paradermal sectioning enables finer resolution of the profiles of Chl, Rubisco and light than could be achieved with thin leaves. Spinach leaves are ideal as they are around 600 µm thick, allowing 15 layers to be resolved using 40-µm sections. In addition, there exists a wealth of biochemical knowledge on this species. Spinach leaves are bifacial, having several layers of palisade mesophyll cells beneath the adaxial (upper) surface and spongy mesophyll adjacent to the abaxial (lower) surface. A freeze-fractured view of market spinach obtained by scanning electron microscopy is shown in Fig. 1. By using an uncoated specimen it is possible to visualize the chloroplasts appressed to the cell walls. Ice forms on the frozen specimen during evacuation in the microscope and obscures the view of chloroplasts on some cells. At this magnification, the view is less dramatic than for that of a tobacco leaf (Evans & von Caemmerer, 1996) because the spinach leaf is so much thicker. The profiles of Chl and Rubisco obtained by Nishio et al. (1993) are aligned against the micrograph. Chl content increases steadily, reaching a maximum in the centre of the leaf near the transition from palisade to spongy mesophyll, before declining slightly towards the abaxial surface.

For the modelling that follows, a polynomial function was fitted to the Chl profile which was used to predict Chl content at any depth. Rubisco content shows a narrower peak, reached midway through the palisade tissue before declining to 40% of the maximum near the abaxial surface. In the right-hand panel of Fig. 1, the profile of $^{14}$C fixation following adaxial illumination with white light is shown, along with predicted profiles of light and light absorption.
THE MODEL

A model linking light absorption with \( \text{CO}_2 \) fixation

Light absorption was calculated assuming the Beer–Lambert law, which states that absorbance is given by the product of the extinction coefficient, pigment concentration and path length. The Beer–Lambert law assumes that the system has parallel, monochromatic light and that the pigments are isotropically dispersed. All three of these assumptions are violated in the leaf, yet despite this, calculations based on the Beer–Lambert law can explain much of the detail that can be resolved from work with paradermal sections of both ‘sun’ and ‘shade’ leaves of spinach (Evans, 1995). Predicted light absorption is maximal about 150 \( \mu \text{m} \) into the leaf, despite irradiance declining nearly exponentially through the leaf (Fig. 1). This is because pigment content per layer increases more rapidly than the decline in irradiance.

In order to test the modelling of light absorption through a leaf with profiles of \( ^{14}\text{CO}_2 \) fixation, it is necessary to link the two. This is achieved by incorporating the profile of Rubisco through the leaf which is used to define the light-saturated rate of photosynthesis for each layer. Each layer is assumed to operate along a non-rectangular hyperbolic response that can be described by three parameters: the maximum quantum yield; a curvature factor; and a maximum rate (Evans et al., 1993; Ögren & Evans, 1993). It is assumed that all layers have the same maximum quantum yield and curvature factor. Consequently, it is necessary to specify only the profile of maximum rate, which is assumed to be directly proportional to Rubisco content (see Evans, 1995 for more detailed justification). Three profiles of Rubisco content through spinach leaves have been measured, and show that Rubisco per unit Chl declines linearly with cumulative Chl (Fig. 2). Remarkably, the three data sets show the same relative change, with only minor deviations in the first and last layers sampled. The model therefore calculates the profile of Rubisco with depth from the profile of Chl given in Fig. 1 and the relationship in Fig. 2 (see Rubisco curve, centre panel, Fig. 1). The photosynthetic capacity of a leaf is then simply adjusted by multiplying each layer by a constant.

The linear decline in Rubisco per unit Chl does not quite match the curve predicted for the absorption of white light per unit Chl (Fig. 2). However, it illustrates the changing composition of chloroplasts required to try and match light capture with carbon metabolism. The mismatch is also evident in Fig. 1 where the \( ^{14}\text{C} \) fixation profile is offset to slightly greater depth relative to the profile of absorbed light. For Rubisco to function efficiently, it requires ready access to intercellular airspace, as discussed below. The large changes in Rubisco per unit Chl mean that chloroplasts near the abaxial surface have to fit in much more Chl per unit of Rubisco than chloroplasts near the adaxial surface. The structural manifestation of this is the change in number of thylakoid membranes per grana stack, which increase from four near the adaxial surface, to seven in the spongy mesophyll (Terashima et al., 1986; Terashima & Evans, 1988). At the same time, thylakoid membranes occupy a greater proportion of the chloroplast volume (Terashima & Evans, 1988, Makovetskii & Manzhulin, 1990).

A test of light capture with monochromatic light

Since this model was published, additional \( ^{14}\text{C} \) fixation profiles using monochromatic light of various irradiances to the adaxial leaf surface have...
and the curves are calculated for blue (B), red (R) and adaxial surface.

The degradation of Rubisco per unit Chl with cumulative Chl from the adaxial surface. Data are from paradermally sectioned spinach leaves obtained by Terasihama & Inoue, 1985 (open triangles) and Nishio et al., 1993 (open squares, sun leaves; closed squares, shade leaves). The regression function is Rubisco = \(34.8 - 0.461 \times \Sigma \text{Chl} \) where \( \Sigma \text{Chl} \) is given by the Chl versus depth function given in Fig. 1. It is converted to photosynthetic capacity by multiplying by the Chl content of the layer and dividing by a scaling factor (5.8). The profile of white light absorption per unit Chl (\(I_{abs} \)) is shown for comparison, calculated as in Fig. 1 (broken curve).

Fig. 2. Function relating Rubisco per unit Chl with cumulative Chl from the adaxial surface. Data are from paradermally sectioned spinach leaves obtained by Terasihama & Inoue, 1985 (open triangles) and Nishio et al., 1993 (open squares, sun leaves; closed squares, shade leaves). The regression function is Rubisco = \(34.8 - 0.461 \times \Sigma \text{Chl} \), where \( \Sigma \text{Chl} \) is given by the Chl versus depth function given in Fig. 1. It is converted to photosynthetic capacity by multiplying by the Chl content of the layer and dividing by a scaling factor (5.8). The profile of white light absorption per unit Chl (\(I_{abs} \)) is shown for comparison, calculated as in Fig. 1 (broken curve).

Fig. 3. Profile of photosynthesis with depth (closed square) for a spinach leaf given 500 \(\mu\text{mol} \) blue quanta \(m^{-2} s^{-1}\) to the adaxial surface. \(^{14}\text{C} \) fixation data are from Sun et al. (1998) and the curves are calculated for blue (B), red (R) and green (G) light using the following values for the extinction coefficient: \(363\), \(2106\), \(900 \text{ m}^2 \text{ (mol Chl)}^{-1}\), respectively.

become available (Sun, 1996; Sun et al., 1998). They provide a robust test of the model, because the absolute response to a variety of lights given to the adaxial surface can be examined for a given leaf. Seven profiles were obtained using sun-type spinach leaves: 500 \(\mu\text{mol} \) quanta \(m^{-2} s^{-1}\) of blue, red or green light; 200 \(\mu\text{mol} \) quanta \(m^{-2} s^{-1}\) of red or green light; and 50 \(\mu\text{mol} \) quanta \(m^{-2} s^{-1}\) of blue or green light. Data were reported as c.p.m. per section, and the mean values used were obtained from four to ten replicates, omitting the error bars which were approx. 30\(\%\) of the mean. The model has two variables: a scaling factor to convert Rubisco content to maximum photosynthetic rate, and an extinction coefficient. Initially, the scaling factor was varied until the photosynthetic rate of the leaf matched the observed irradiance response curve. The photosynthetic capacity of the sun-type spinach leaf was 207 \(\mu\text{mol} \text{ e}^{-} \text{ m}^{-2} \text{ s}^{-1}\), which is comparable to the rate of 240 for spinach measured under slightly higher \(^{14}\text{C} \) conditions (Evans & Terashima, 1988). Having defined the value for the scaling factor, an extinction coefficient was obtained for each colour that best explained the \(^{14}\text{C} \) profile measured under 500 \(\mu\text{mol} \) quanta \(m^{-2} s^{-1}\). Different wavelengths penetrate to different depths in a leaf because both absorption and scattering are wavelength-dependent. Blue light is the most strongly absorbed and green light the most weakly absorbed, with red light intermediate, consistent with the absorption spectrum of chloroplasts.

For a given irradiance, the profile of \(^{14}\text{C} \) fixation depends on the extinction coefficient (Fig. 3). Fixation peaks in blue light just above 200 \(\mu\text{m} \) and at slightly greater depth in red or green light. More importantly, the fraction of photosynthesis that occurs in the first 300 \(\mu\text{m} \) declines from 81\(\%\) for blue to 67\(\%\) for red and 57\(\%\) for green light. The curve predicted for blue light matches the \(^{14}\text{C} \) fixation data very closely from 200 \(\mu\text{m} \) onwards, but underestimates the peak rate around 180 \(\mu\text{m} \). The peak rate could be better matched by increasing the photosynthetic capacity of those layers in the leaf. However, the rate predicted for red and green light would then be poorer. It should be remembered that the error associated with each mean data point was about 30\(\%\), so the predicted profile for blue light falls well within the envelope of uncertainty.

The predicted profiles for all three colours, each at different irradiances, are shown in Fig. 4 along with the light profiles. The model predictions match the \(^{14}\text{C} \) profiles very well, accounting for changes due to colour as well as irradiance. Due to the strong absorption of blue light, only 10\(\%\) penetrates deeper than 300 \(\mu\text{m} \), yet it results in 19\(\%\) of the photosynthesis. This is because light captured by the first few layers exceeds the capacity of the chloroplasts to convert it into carbohydrates, and is lost as heat. By contrast, 23\(\%\) of red light is absorbed in the deeper layers, contributing 33\(\%\) of photosynthesis, while for green light 43\(\%\) of the absorbed light occurs in the deeper layers, accounting for 43\(\%\) of photosynthesis. The match between green light absorption and \(^{14}\text{C} \) fixation occurs because the profile of green light absorption is similar to the profile of Rubisco.

The only noticeable deviation of the model from the \(^{14}\text{C} \) fixation data occurs in the first few layers at intermediate irradiance, where the predicted photosynthetic rate exceeds the observed rate. This has two possible causes. Firstly, because chloroplasts only line the periphery of a cell (e.g. Evans et al.,
Access to light and CO\textsubscript{2} between chloroplasts

Fig. 4. Profile of relative irradiance and photosynthesis with depth for a spinach leaf given various adaxial light treatments. Irradiance profiles were calculated for blue, red and green light using the following values for the extinction coefficient: 3636, 2106, 900 m\textsuperscript{2} (mol Chl)\textsuperscript{−1}, respectively, and the profile of Chl shown in Fig. 1. Irradiances were: squares, 500; triangles, 200; circles, 50 μmol quanta m\textsuperscript{−2} s\textsuperscript{−1}; data are from Sun et al. (1998), with curves calculated from the model.

1994), collimated light entering the palisade tissue will generally travel some distance before encountering a chloroplast. From paradermal sections, it can be seen that chloroplasts occupy only about 20% of the plane. Consequently, the model is likely to overestimate the amount of monochromatic light absorbed in this region. While the model could be modified to incorporate a variable to deal with this, it would detract from the present simplicity, and in white light there is little evidence of any consistent error. Alternatively, the deviation could be used to estimate the fraction of light that bypasses chloroplasts in the initial layers. The addition of another variable improves the fit of the model for the first 200 μm under monochromatic light but not with white light. Secondly, measurement errors for the first section are greatest as it contains a variable proportion of epidermal tissue. This leads to the greatest uncertainty in the Chl and Rubisco contents where they have the largest impact.

**DISCUSSION**

**Effect of mesophyll structure on light capture**

The values for the extinction coefficients cannot be directly equated to those obtained for pigments in solution, because the pigments are not distributed uniformly through the tissue, and scattering of light increases with depth leading to path lengthening. The consequence of path lengthening is evident in the work of Terashima & Saeki (1983), who showed that the apparent extinction coefficient for red and green light was greater in spongy tissue.

Providing light to the abaxial surface of the leaf during \textsuperscript{14}C labelling provides two useful tests for the model. Firstly, it uncouples the light absorption profile from the Rubisco profile; and secondly, it shows whether mesophyll structure alters the apparent extinction coefficient. In a separate experiment from that described above, Sun (1996) carried out \textsuperscript{14}C labelling of sun leaves of spinach that were given white light to either or both surfaces. In modelling this data set, the same leaf parameters were used apart from one change: as the layers were aligned slightly differently, it became evident that the photosynthetic rate for the first adaxial layer was overestimated, and consequently the Rubisco content for this layer was halved.

As white light was used, an extinction coefficient was fitted to the data with adaxial light of 800 μmol quanta m\textsuperscript{−2} s\textsuperscript{−1}. The value 1350 m\textsuperscript{2} mol\textsuperscript{−1} is slightly less than that derived in the previous paper for white light (1500 m\textsuperscript{2} mol\textsuperscript{−1}; Evans, 1995). A poor fit to data...
obtained with abaxial light was observed using 1350 m$^2$ mol$^{-1}$ (Fig. 5). The first six layers in from the abaxial surface fitted well because they reflect light-saturated capacity, i.e. Rubisco content. At deeper layers, the model overestimated the photosynthetic rate because too much light was reaching these layers. Good agreement between the model and $^{14}$C fixation data could be restored if a larger extinction coefficient of 2340 m$^2$ mol$^{-1}$ was used. Choosing an even higher extinction coefficient (3150 m$^2$ mol$^{-1}$) resulted in too much light absorption in the first eight layers, leaving too little for photosynthesis in the adaxial half of the leaf.

The requirement by the model for a greater extinction coefficient for abaxial light is consistent with expectations. Spongy tissue is known to scatter light more than the regular array of palisade cells. By increasing the path length of light, absorption per unit Chl increases in spongy tissue, which is equivalent to a greater apparent extinction coefficient. Terashima & Saeki (1983) observed a 47% increase in extinction coefficient for 680 nm light, and an 88% increase for 550 nm light, for spongy versus palisade tissue in a *Camellia* leaf. Infiltrating the leaf with oil, which had a refractive index comparable to that of the cells, reduced the apparent extinction coefficient for spongy and palisade tissue by 39 and 26%, respectively, for 680 nm light. Bornman et al. (1991) also showed that light does not penetrate as far through spongy tissue compared to palisade tissue in a *Medicago sativa* leaf. Spongy tissue therefore enhances the light capture per unit of Chl by scattering light. By contrast, palisade tissue minimizes light scattering and allows much of the light to bypass chloroplasts by guiding it down the centre of palisade cells. This enables light to penetrate further into the leaf, thereby spreading light capture more evenly between chloroplasts. This was shown by Vogelmann & Martin (1993), who compared *Thermopsis montana*, a legume with columnar palisade tissue, with that of the monocot *Smilacina stellata*, without palisade. Collimated green light was able to penetrate considerably further through the leaf with palisade mesophyll compared to the monocot leaf, whereas there was little difference when diffuse light was used. It is the combination of better light penetration with a greater cell surface area per unit of mesophyll volume that makes palisade tissue a more efficient structure in terms of photosynthesis than spongy mesophyll immediately adjacent to the adaxial surface. Conversely, spongy tissue adjacent to the abaxial surface increases the efficiency of capturing the small amount of remaining light for a given investment in pigment–protein complexes.

The $^{14}$C fixation profiles with adaxial or abaxial white light also included a case where light was given to both surfaces (Fig. 6). The model predicts that receiving light simultaneously on both surfaces would increase photosynthesis only in the central part of the leaf. The initial profiles from either surface change little, either because such a small amount of additional light reaches the opposite side (when 200 μmol quanta m$^{-2}$ s$^{-1}$ are given), or because
these layers are already light-saturated (when 800 μmol quanta m$^{-2}$ s$^{-1}$ are given). The model predicts lower photosynthetic rates under 200 μmol quanta m$^{-2}$ s$^{-1}$ at some depths than the observed $^{14}$C fixation data. However, this is probably a reflection on the uncertainty of the $^{14}$C profiles. Despite relatively small error bars, only three to four replicate leaves were used to obtain the low light data. At 600 μm depth, 200 μmol quanta m$^{-2}$ s$^{-1}$ to the abaxial surface resulted in 40% more $^{14}$C fixation than 800 μmol quanta m$^{-2}$ s$^{-1}$, which is highly unlikely.

Effect of specific leaf area on light absorption

The model describes light capture based on the Beer–Lambert law. It is sensitive to wavelength and mesophyll structure. Unfortunately, the only data available for testing the model are from spinach leaves, which have the classical dicotyledonous bifacial anatomy. In the work of Nishio et al. (1993), $^{14}$C fixation profiles were obtained with spinach leaves that had been grown under sun conditions (800 μmol quanta m$^{-2}$ s$^{-1}$) or shade conditions (200 μmol quanta m$^{-2}$ s$^{-1}$, with an R:FR ratio of 0.25). This resulted in sun-type leaves having 15% more Chl per unit leaf area and being one third thicker than shade-type spinach leaves. The increased leaf thickness was mainly due to more palisade tissue, which had five rather than three cell layers. The palisade tissue of sun leaves also had narrower cells than that of shade leaves (26 versus 40 μm; Cui et al., 1991), resulting in a 40% increase in palisade mesophyll surface area per unit cell volume. Despite these differences, the model could describe $^{14}$C fixation profiles in white light supplied to the adaxial surface, using the same apparent extinction coefficient of 1500 m$^{-2}$ mol$^{-1}$ for both leaf types (Evans, 1995). This suggests that the distribution of Chl through the leaf tissue is of the greatest importance in defining light capture.

While spongy mesophyll tissue does alter the apparent extinction coefficient, it is not yet possible to assess the impact of other mesophyll structures, apart from their impact on absorbance of the intact leaf. Absorbance is the fraction of light falling onto a leaf that is not reflected or transmitted. Absorbance is strongly related to Chl content by a hyperbolic function. Does the underlying mesophyll structure contribute directly to variation in leaf absorbance? This was addressed by examining leaves obtained from a diverse range of species (Evans, 1998a). The deviation of leaf absorbance from that predicted from Chl content was examined as a function of specific leaf area, which varied for a given species as a result of growth light environment. Changing specific leaf area had no effect on absorbance after accounting for any change in Chl content. How much change in absorbance would we expect if a leaf had only palisade tissue? If we examine a spinach leaf with an apparent extinction coefficient of 1350 m$^{-2}$ mol$^{-1}$, 15% of light that enters the leaf reaches the lower surface. Adding a different extinction coefficient for spongy tissue of 2340 reduces the light reaching the lower surface to 7%. The 8% difference ought to be detectable if a bifacial leaf were compared to a leaf which had only palisade tissue. Undoubtedly this analysis is too crude because, in reality, light becomes progressively more scattered, even by palisade tissue which would reduce the immediate impact of the transition to spongy tissue. No indication of an abrupt increase in light absorption was evident in the $^{14}$C fixation profiles at the palisade–spongy boundary in spinach leaves (Fig. 4). However, an increase was evident in direct optical measurements of Camellia leaves (Terashima & Saeki, 1983). The fact that leaf absorbance can be well described simply by the Chl content, regardless of specific leaf area, suggests that internal leaf structure may play a role in altering the profile of light capture through a leaf, but it does not alter the absolute amount captured by a leaf.

Matching light capture to photosynthetic capacity

The patterns of $^{14}$C fixation through the leaf are the consequence of interactions between light capture and photosynthetic capacity. Ideally, the profile of light capture matches that of photosynthetic capacity, as this results in the best use of protein invested in photosynthesis: it corresponds to each chloroplast operating at the point of its light response function, where an increase in incident light results in the same increase in photosynthesis for any chloroplast. This cannot be achieved exactly, for several reasons. Firstly, all chloroplasts in a given cell share a common nucleus which dictates the composition of all the chloroplasts in that cell. A considerable light gradient could exist along the length of a palisade cell which therefore could not be matched by different chloroplast properties along the cell. Secondly, light of different wavelengths will be absorbed in different profiles and it is only possible to match one profile. Giving different monochromatic lights to a leaf leads to a dramatic mismatch between light absorption and photosynthetic capacity. This is shown in Fig. 7, where the rate of electron transport per absorbed quanta is shown as a function of depth for red, blue and green light of 500 μmol quanta m$^{-2}$ s$^{-1}$. The assumption of a constant extinction coefficient which overestimates monochromatic light absorption in the first 200 μm results in uncertainty for the initial layers, but including this would still result in differences between profiles remaining for the different wavelengths. Blue light is absorbed most strongly, leading to light saturation near the adaxial surface which lowers the quantum yield. Since little blue light penetrates to the abaxial surface, these
Quantum yield profiles in white light of different irradiances are shown in the lower panel of Fig. 7. Profiles of quantum yield of electron transport with depth through a sun spinach leaf calculated from the model with adaxial illumination. Upper panel calculated for blue, red and green light of 500 mol quanta m\(^{-2}\) s\(^{-1}\); lower panel calculated for white light of different irradiances as shown.

Layers have maximal quantum yield. On the other hand, the absorption profile of green light nearly matches the Rubisco profile, which results in the quantum yield being stable through the leaf. Red light is intermediate between blue and green. It would be intriguing to grow leaves under different monochromatic lights and observe chloroplast ultrastructure to see if there were more dramatic gradients in thylakoid number per grana in leaves from blue versus green light.

Quantum yield profiles in white light of different irradiances are shown in the lower panel of Fig. 7. Quantum yield declines as irradiance increases, and the adaxial layers are relatively more light saturated than the abaxial layers. This should mean that estimates of photosynthetic electron transport based on Chl fluorescence, elicited by a measuring beam that does not penetrate very deeply into a leaf, underestimate the whole leaf rate (Evans et al., 1993). The kink in all the profiles between the first and second layers reflects the uncertainty in the model associated with the Rubisco content and light absorption in the first layer. The fact that the profiles are not flat indicates that photosynthetic capacity and light absorption do not covary exactly. The resulting light–response curve of leaf photosynthesis thus has a curvature factor of 0.69 compared to that defined for the chloroplast of 0.86, and the integral of the curve achieves 95.6\% of that of an ideal leaf.

It should be remembered that the underlying photosynthetic biochemistry is flexible and able to acclimate to changing circumstances. When eucalypt leaves were restrained to a particular orientation and subsequently re-oriented, light–response curves provided evidence for a re-alignment of the biochemistry (Ogren & Evans, 1993). The change took place over about 7 days for both bifacial and isolateral leaf types.

**Restrictions to CO\(_2\) diffusion**

To survive on land with a limited water supply requires that plants regulate the loss of water in exchange for CO\(_2\) uptake. This is achieved by restricting gaseous diffusion into and out of the leaf through stomata. Stomatal morphology and density vary considerably between species, and stomatal responses to light, humidity and CO\(_2\) have been widely studied by conventional gas-exchange techniques. The CO\(_2\) partial pressure inside the leaf has routinely been calculated in the process. Leaf photosynthesis can be predicted if Rubisco activity and CO\(_2\) partial pressure at the sites of carboxylation are known (Farquhar & von Caemmerer, 1982). It is frequently assumed that the CO\(_2\) partial pressure at the sites of carboxylation is sufficiently close to that calculated in the intercellular airspaces for the two to be equated. However, several techniques are now available that have enabled the CO\(_2\) partial pressure at the sites of carboxylation to be calculated (Evans & von Caemmerer, 1996). They reveal that the drawdown in CO\(_2\) partial pressure inside actively photosynthesizing leaves is nearly as great as the drawdown through stomata. For ease of argument, standard atmospheric pressure is assumed to enable the pressure unit to be omitted from internal conductance. Otherwise, because dissolution of CO\(_2\) into liquid is pressure-dependent, the units of atmospheric pressure are usually needed (see Harley et al., 1992).

Assembling the growing number of published measurements is quite revealing. It is well known that photosynthetic capacity is strongly correlated with stomatal conductance between species (Körner et al., 1979; Wong et al., 1979; Yoshie, 1986). A similar relationship exists between photosynthetic capacity and internal conductance (from substomatal cavities to the sites of carboxylation; Fig. 8). The data have been separated into two groups: mesophytic leaves which are short-lived, herbaceous or deciduous species; and sclerophytic leaves which are evergreen or have a low specific leaf area. As the fluorescence method becomes unreliable when internal conductances exceed 0.3 mol m\(^{-2}\) s\(^{-1}\), only data collected using the isotopic method were used.
mean values for simulation measured under high irradiance. The mol respectively. While leaves of woody species tend to conductance is used (Fig. 9). While this calculation of CO from substomatal cavities to the sites of carboxylation, the drawdown in CO$_2$ across stomata, because the diffusivity of CO$_2$ is 2.3 times greater in helox than in air. Parkhurst & Mott (1990) found that helox increased photosynthetic rates of amphistomatous and hypostomatous leaves by 2 and 12%, respectively. Genty et al. (1998) combined helox with Chl fluorescence imaging to separate the gaseous and liquid diffusion pathways. They measured both amphistomatous Populus koreana × trichocarpa cv. Peace and heterobaric hypostomatous Rosa rubiginosa leaves. Switching from air to helox made no measurable difference to the CO$_2$-response curves.
suggesting that for these two contrasting leaf types the limitation imposed by gaseous diffusion inside leaves was substantially less than that in the liquid phase. Sun (1996), working with spinach leaves, also found that the pattern of $^{14}$C fixation was independent of which leaf surface the label was supplied to, consistent with a negligible gaseous diffusion limitation inside these leaves.

Diffusion in the liquid phase is restricted by the permeability of membranes and the thickness of cell wall, cytoplasm and chloroplasts. These restrictions are likely to be shared by all chloroplasts in a given leaf, so that the liquid phase conductance will be proportional to the surface area of chloroplasts exposed to intercellular airspace per unit leaf area. The maximum value that can be reached for a given leaf is set by the mesophyll anatomy, which defines the surface area of mesophyll cells exposed to intercellular airspace per unit leaf area. A causal correlation between internal conductance and exposed chloroplast surface area was put forward by von Caemmerer & Evans (1991). To test this, wild-type and transgenic tobacco having reduced amounts of Rubisco were examined in order to separate the effects of photosynthetic capacity from chloroplast surface area. The results clearly showed that internal conductance was related to exposed chloroplast surface area (Evans et al., 1994). Remarkably, the relationship between internal conductance and exposed chloroplast surface area found for tobacco also fits the data available for three other species: wheat, peach and citrus (Syvertsen et al., 1995; Evans & Vellen, 1996; Evans, 1998b; Evans & Loreto, 1999). The slope yields a value of 24 mmol CO$_2$ (mol chloroplast)$^{-1}$ s$^{-1}$ for the liquid phase conductance per unit of chloroplast surface area exposed to intercellular airspace.

**Relationship between internal conductance and Rubisco**

To link CO$_2$ fixation to diffusion restrictions, we need to know how Rubisco content is related to chloroplast surface area. Data are available for both wheat and tobacco, although only in tobacco were all the measurements made on the same material. Both internal conductance and exposed chloroplast surface area show hyperbolic relationships with Rubisco content (Fig. 10). For a given Rubisco content, tobacco has greater internal conductance and more chloroplast surface area than wheat. The concentration of Rubisco in chloroplasts is also lower in tobacco (0.9–1.2 mM sites; Evans et al., 1994) compared to wheat (1.5–2.2 mM sites; Evans, 1983b). Fig. 10 implies that internal conductance per unit exposed chloroplast surface area is greater in wheat than tobacco. However, the wheat data are from two different experiments. Internal conductance per unit of exposed chloroplast surface area in young, fully expanded wheat leaves (Evans & Vellen, 1996) was similar to that of tobacco (Evans et al., 1994; see above). The hyperbolic relationship with chloroplast surface area indicates that increasing Rubisco content per unit leaf area is initially achieved by chloroplasts progressively covering more of the available exposed cell surface, but eventually when all the exposed surface is occupied, the chloroplasts have to become thicker and/or the Rubisco concentration must increase. Because internal conductance cannot increase in direct proportion to Rubisco content, leaves with greater Rubisco content face greater internal diffusion resistances. This can be seen for wheat leaves where Rubisco activity calculated from the slope of CO$_2$ response curves near the CO$_2$ compensation point is related to the extractable Rubisco content (Fig. 11). Plants were grown either in summer or winter, but the curvature is apparent only when very high Rubisco contents were achieved. Two model lines are shown. The straight broken line represents the relationship expected for Rubisco with a catalytic rate constant of 5 mol CO$_2$ (mol sites)$^{-1}$ s$^{-1}$. The solid
Subsequently, better resolution enabled the profile of chloroplasts and total water versus depth to be obtained (McCain et al., 1993). This suggests that the spatial picture within the leaf is more complex than that revealed by the stereological average. Data are needed on Rubisco profiles in relation to Chl for species other than spinach, in order to test the generality of predictions based on the model spinach leaf in terms of both light capture and CO\textsubscript{2} diffusion limitations.

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