Is C₄ photosynthesis less phenotypically plastic than C₃ photosynthesis?*

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Abstract

C₄ photosynthesis is a complex specialization that enhances carbon gain in hot, often arid habitats where photorespiration rates can be high. Certain features unique to C₄ photosynthesis may reduce the potential for phenotypic plasticity and photosynthetic acclimation to environmental change relative to what is possible with C₃ photosynthesis. During acclimation, the structural and physiological integrity of the mesophyll–bundle sheath (M-BS) complex has to be maintained if C₄ photosynthesis is to function efficiently in the new environment. Disruption of the M-BS structure could interfere with metabolic co-ordination between the C₃ and C₄ cycles, decrease metabolite flow rate between the tissues, increase CO₂ leakage from the bundle sheath, and slow enzyme activity. C₄ plants have substantial acclimation potential, but in most cases lag behind the acclimation responses in C₃ plants. For example, some C₄ species are unable to maintain high quantum yields when grown in low-light conditions. Others fail to reduce carboxylase content in shade, leaving substantial over-capacity of Rubisco and PEP carboxylase in place. Shade-tolerant C₄ grasses lack the capacity for maintaining a high state of photosynthetic induction following sunflecks, and thus may be poorly suited to exploit subsequent sunflecks compared with C₃ species. In total, the evidence indicates that C₄ photosynthesis is less phenotypically plastic than C₃ photosynthesis, and this may contribute to the more restricted ecological and geographical distribution of C₄ plants across the Earth.

Key words: Acclimation, C₃, C₄ photosynthesis, phenotypic plasticity, sun–shade, temperature.

Introduction

Phenotypic plasticity refers to the ability of individual organisms to respond to environmental variation by altering their characteristics to compensate for, or acclimate to, variable environmental conditions (Pigliucci, 2001). Organisms considered to be generalists have highly plastic phenotypes and are suited for a wide variety of conditions. They are thus more likely to acclimate and survive in unpredictable environments than less phenotypically plastic species. Organisms with low phenotypic plasticity are less likely to acclimate fully to environmental change, and thus tend to occur in less variable conditions than highly plastic organisms. In part because of a more uniform selection pressure, these organisms are often highly specialized for a restricted set of conditions, and are more fit than generalists within that environment. Increased specialization, however, may result in a loss of plasticity or acclimation potential. As a result, the more evolutionarily derived and specialized species could be more restricted in ecological and geographical distribution.

Within angiosperms, the CO₂-concentrating mechanism of C₄ photosynthesis represents a specialized adaptation derived from C₃ ancestors. C₄ photosynthesis has independently arisen over 45 times in a wide range of advanced angiosperm taxa (Sage, 2004). In almost all known C₄ species, C₄ photosynthesis requires the development of Kranz anatomy (Figs 1, 2; Dengler and Nelson, 1999). Despite the polyphyletic nature of C₄ photosynthesis, certain anatomical features are common to most C₄ plants, including: (i) specialization of two distinct photosynthetic tissue types: bundle sheath (BS) and mesophyll (M) tissue; (ii) the arrangement of BS cells near vascular tissue, with M peripheral to BS cells and adjacent to intercellular spaces; (iii) high vein density and a low ratio of M to BS (often 1:1), resulting in short diffusion pathways for C₄...
metabolites; and (iv) minimal CO₂ leakage from BS cells, reflecting extensive contact between M and BS cells. Within C₃ plants, the photosynthetic assimilation and reduction processes occur in both M and BS tissues, typically within a single photosynthetic cell; however, in C₄ plants, M tissue assimilates CO₂ to form organic acids that diffuse to the BS tissue (Fig. 1). In all cases, the evolution of the C₄ pathway involved the modification of pre-existing biochemistry in C₃ ancestors to enable the concentration of CO₂ into the BS tissue compartment where the CO₂-fixing enzyme Rubisco is localized (Hatch, 1987; Kanai and Edwards, 1999; Sage, 2004).

Together, the modifications of metabolism and anatomy that allow for CO₂ concentration represent a co-ordinated, specialized adaptation that enhances performance of C₄ plants during periods of low atmospheric CO₂ availability and in warm, often dry environments (Ehleringer et al., 1991, 1997). As a derived specialization, however, the metabolic and structural requirements of the C₄ pathway may have decreased the ability of C₄ plants to modify the photosynthetic apparatus in order to acclimate and improve performance in altered environments. This paper explores the possibility that one cost of evolving C₄ photosynthesis is a reduced potential for phenotypic plasticity and acclimation.

Why should C₄ plants have less acclimation potential than C₃ plants?

The success of most plant species requires some ability to acclimate to environmental change, as environmental variation is inevitable (Schlichting, 1986). In addressing acclimation potential between generalists and specialists, or in this paper, C₃ versus C₄ plants, the issue is one of relative degrees of acclimation, and whether barriers exist that might prevent highly plastic acclimation responses in C₄ taxa. Specialist species may have inherent barriers that constrain or even prevent high phenotypic plasticity. The nature of these constraints is not well defined. Examination of C₃ and C₄ acclimation potentials may serve as a useful case study in how specialized adaptations affect phenotypic plasticity.

Conceptually, there are a number of reasons why C₄ plants might not have the same ability to acclimate to low light, temperature variation, or elevated CO₂ as C₃ species. The C₄ pathway requires close integration of distinct photosynthetic processes: PEP carboxylation and regeneration in M tissue with the Calvin cycle in BS tissue (Fig. 1). Failure to co-ordinate M and BS structure and function would reduce photosynthetic capacity and resource use efficiency (Leegood and Walker, 1999). At the structural level, disruption of M to BS tissue arrangements could increase diffusion distances, interfere with diffusion pathways, or enhance pathways for CO₂ leakage from the BS cells. At the metabolic level, ineffective acclimation could lead to a loss of co-ordination between the C₃ and C₄ biochemical cycles. For example, if the C₄ cycle reactions proceed faster than the C₃ cycle following acclimation, too much CO₂ would be pumped into the BS, building up the CO₂ concentration to a point where leakage of CO₂ out of the BS becomes substantial (von Caemmerer and Farbunk, 1999). In effect, the C₄ pump would begin to resemble a futile cycle and lose photosynthetic efficiency. Alternatively, if C₄ cycle activity following acclimation is slow relative to the capacity of Rubisco and the C₃ cycle, then BS CO₂ levels would decline and photorespiration rates increase. To avoid these problems, acclimation of C₄ photosynthesis has to involve co-ordinated changes between the M and BS tissues in order to maintain functional stoichiometries. By contrast, in C₃ species, all photosynthetic cells are functionally equivalent, thereby allowing each cell to acclimate to a new environment in a more autonomous manner than should be possible in a C₄ leaf. The simplicity of the C₃ system relative to the C₄ system, therefore, allows photosynthetic plasticity to be concentrated at the cellular rather than tissue levels, potentially allowing for greater acclimation ability in C₃ leaves.

Photosynthetic acclimation brings the costs of tissue construction and maintenance in line with the probable photosynthetic carbon gain that a new environment can support (Mooney and Gulmon, 1982; Bloom et al., 1985; Sims et al., 1998a; Poorter et al., 2006). Because the photosynthetic unit in C₃ plants is localized within autonomous cells, individual cells can be enlarged or reduced in size and number (in newly developing leaves) without compromising metabolic integrity. In C₄ plants, the requirements to maintain functional relationships between the C₃ and C₄ cycles could constrain the extent...
to which tissue construction and maintenance costs are altered following environmental change. In addition, chloroplasts and other organelles in C4 leaves are spatially localized to either the interior third, or the outer periphery, of BS cells depending on photosynthetic biochemical subtype (Fig. 2; Dengler and Nelson, 1999). Localizing BS chloroplasts in such a manner restricts the total cell volume available to house Rubisco and the enzymes of the carbon reduction cycle. Changing the amount of these enzymes is an important part of the acclimation process in C3 plants (Anderson et al., 1988; Evans and Seemann, 1989; Leegood and Edwards, 1996), but may be constrained in C4 species by restrictions in organelle size and number. Modifications to organelle sizes and numbers in C4 plants may be difficult as it could interfere with ultrastructural arrangements required for an effective C4 pathway. In C3 plants, by contrast, all chlorenchyma cells are functionally equivalent in that all contain Rubisco and carbon reduction cycle enzymes. The C3 leaf is not restricted to packaging Rubisco into the relatively small space located at one end of the BS cells. If Rubisco activity becomes limiting in a C3 leaf, for example, the plant can compensate by increasing Rubisco content per chloroplast, creating more chloroplasts, or producing more cells in new leaves (Oguchi et al., 2005).

Acclimation requires the ability to sense environmental change and transduce it into an effective response. Photosynthetic acclimation is controlled by three general mechanisms: (i) environmental perception by sensory proteins such as phytochrome that activate a signal-transduction pathway, (ii) chloroplast-specific control that is linked to redox state, and (iii) carbohydrate, nutrient, and phytohormone signals that co-ordinate leaf and whole plant responses (Stitt and Krapp, 1999; Malakhov and Bowler, 2001; Lin and Shalitin, 2003; Long et al., 2004; Walters, 2005). In C3 plants, much of the control over the acclimation response is internal to the cell because redox state changes originate within chloroplasts and mitochondria (Anderson et al., 1995; Walters, 2005). Reliance on local command and control is problematic in C4 plants because of the need to co-ordinate M and BS responses; hence, an additional layer of regulatory control is probably required for an effective acclimation response. Furthermore, different promoter systems are required for the development of C4 tissue specialization (Dengler and Taylor, 2000; Matsuoka et al., 2001); therefore, acclimation responses may have to be transduced through additional promoter networks during development.

Alternatively, there may be no barriers associated with the photosynthetic pathway that inherently restrict phenotypic plasticity in C4 plants relative to C3 plants. Instead, low phenotypic plasticity may simply result from specialization for hot, high-light environments in the same manner that many C3 species specialized for these environments have low phenotypic plasticity. Because the main advantage of the C4 pathway occurs in conditions promoting photorespiration, it is probable that many C4 species are specialized for hot, high-light conditions and thus they may not be appropriate for assessing hypotheses regarding varying potential for phenotypic plasticity. There are situations, however, where a high degree of phenotypic plasticity would be advantageous to C4 plants. Numerous C4 species develop dense canopies where self-shading of older leaves is extensive. Acclimation to low light within a canopy is thus required if interior leaves are to maintain high resource-use efficiency and significantly contribute to carbon gain. In addition, a number of C4 species are successful in environments that are atypical for C4 photosynthesis, namely low-light and cooler habitats (Long, 1999; Sage et al., 1999). Although some adaptive specializations may have occurred in C4 species from cooler or low-light habitats, they may also show substantial phenotypic plasticity as most of these species occur in variable environments such as canopy gaps and high elevation (Brown, 1977; Smith and Martin, 1987b; Sage and Sage, 2002). A greater potential for phenotypic plasticity would probably be found in C4 plants from these variable environments.

**Acclimation of C3 and C4 photosynthesis to shade**

Shade acclimation is the best-studied acclimation response of C3 photosynthesis, such that it now serves as a model of

![Fig. 2. Cross-sections of C3 and C4 leaves grown under high-light conditions. Mesophyll and bundle-sheath tissues are indicated by arrows. Note the centripetal arrangement of Rubisco-chloroplasts in the bundle-sheath cells of the C4 species. Leaves were sampled from Flaveria pringlei (C3) and Flaveria trinervia (C4). Scale bars are 100 μm.](http://jxb.oxfordjournals.org)
phenotypic plasticity in classrooms and textbooks. Shade-acclimation demonstrates the range of acclimation responses present in leaves (Table 1; Lambers et al., 1998; Walters, 2005). At one level are the economic-type responses, where the activities of non-limiting processes are modulated to match activities of limiting processes (Bloom et al., 1985). Examples of economic-type responses are where the levels of Rubisco, carbon-reduction-cycle protein, enzymes for sucrose and starch synthesis, and electron-transport machinery are reduced following shading to match light-harvesting capacity (Table 1; Anderson et al., 1988; Evans, 1988; Bailey et al., 2004). At the leaf level, cell size, cell number, and leaf thickness are altered in newly formed leaves to bring construction and maintenance costs in line with the ability of the light environment to support the energetic costs ( Björkman, 1981; Mooney and Gulmon, 1982; Evans and Seemann, 1989). Following shading, pigment levels shift as the levels of photo-protective carotenoids decline, while chlorophyll content increases (Bailey et al., 2004; Horton and Ruban, 2005). Acclimation also involves qualitative adjustments, such as the stacking of thylakoid membranes to enhance light capture and to create additional volume for proton storage (Sharkey et al., 1986). Some acclimation responses are rapid and reversible, such as chloroplast movements, while others are slow and largely irreversible, such as anatomical patterns established when leaves mature in a particular environment. With respect to C3 and C4 acclimation, the structural and economic-type responses are the most relevant for evaluating the potential for phenotypic plasticity. Acclimation responses involving changes in anatomy or enzyme complement are more likely to be linked to the photosynthetic pathway because C3 and C4 species inherently differ in these attributes.

Table 1. General characteristics of sun and shade leaves in C3 plants (after Björkman, 1981; Lambers et al., 1998)

<table>
<thead>
<tr>
<th>Structural characteristics</th>
<th>Sun grown</th>
<th>Shade grown</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf thickness</td>
<td>Thick</td>
<td>Thin</td>
</tr>
<tr>
<td>Palisade parenchyma</td>
<td>Multilayered</td>
<td>1–2 cell layered</td>
</tr>
<tr>
<td>Chloroplasts per area</td>
<td>Many</td>
<td>Few</td>
</tr>
<tr>
<td>Thylakoids per granum</td>
<td>Few</td>
<td>Many</td>
</tr>
<tr>
<td>Intervenel distance</td>
<td>Low</td>
<td>High</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Biochemical characteristics</th>
<th>Sun grown</th>
<th>Shade grown</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorophyll/l per chloroplast</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Chlorophyll/l per area</td>
<td>Similar</td>
<td>Similar</td>
</tr>
<tr>
<td>Chlorophyll/ a/b ratio</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Light-harvesting complex per area</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Antennae size per photosystem</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Electron-transport protein</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Rubisco per area</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Nitrogen per area</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Xanthophylls per area</td>
<td>High</td>
<td>Low</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Physiological characteristics</th>
<th>Sun grown</th>
<th>Shade grown</th>
</tr>
</thead>
<tbody>
<tr>
<td>Photosynthetic capacity per area</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Dark respiration rate</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Light-compensation point</td>
<td>High</td>
<td>Low</td>
</tr>
</tbody>
</table>

Using the well-described responses of C3 plants to shade as a reference (Table 1), it can be evaluated whether C4 plants have the same potential for shade acclimation as C3 photosynthesis. Two parameters of particular value in evaluating structural and biochemical acclimation are leaf thickness and Rubisco activity, respectively. A common acclimation response to shading is the thinning of leaves; hence, the relative degree of leaf thinning can be compared to examine whether there are inherent differences between the photosynthetic pathways that might be associated with anatomical requirements to maintain the M-BS stoichiometry. Second, Rubisco contents decline markedly during shade acclimation in C3 plants, on the basis of leaf area, chlorophyll, and leaf nitrogen content (Evans, 1988; Evans and Seemann, 1989). Because Rubisco activity is non-limiting in shaded C4 leaves, significant reductions in Rubisco content should also occur during shade acclimation if resource use efficiency is to be maintained (von Caemmerer and Furbank, 1999).

No consistent differences are apparent in the ability of C3 and C4 species to reduce leaf thickness following shading. Comparisons of leaves produced at high light and low light generally show that leaf thickness declines by 30–50% in both C3 and C4 species (Louwerse and Zweerde, 1977; Ward and Woolhouse, 1986a). In a direct comparison of Phaseolus vulgaris (C3) and Zea mays (C4), the reduction in leaf thickness from high light to low light was 34% for the C3 plants and 32% in maize (Louwerse and Vanderzweerde, 1977). Exceptions have been noted, for example, in Amaranthus retroflexus, a sun-adapted C4 plant with extensive self-shading, there is little difference in the thickness of leaves from high- and low-light-grown plants (Tazoe et al., 2005).

Rubisco content in C4 plants does not appear to be as responsive to changes in light availability as in C3 plants, particularly in terms of the percentage of leaf nitrogen invested in Rubisco. In numerous C3 species, Rubisco content or activity is reduced by over 55% in shaded compared to high-light grown leaves (Table 2). By contrast, the degree of reduction is generally less in C4 species, being 10–54% when Rubisco content or activity is expressed on a chlorophyll basis (Table 2). In terms of the percentage of nitrogen allocated to Rubisco, there is modest (about 15%) reduction in Rubisco content per unit nitrogen in Amaranthus retroflexus between high- and low-light-grown plants (Tazoe et al., 2005). The fraction of nitrogen in Rubisco increases in maize and Paspalum leaves grown in low light (50 μmol photons m\(^{-2}\) s\(^{-1}\)) compared with high-light (1000 μmol photons m\(^{-2}\) s\(^{-1}\); this observation is based on Rubisco; protein ratios derived from Ward and Woolhouse, 1986b). These results indicate that C4 plants have a low ability to reduce their allocation of nitrogen to Rubisco following shading, in contrast to the typical C3 response where the allocation of leaf nitrogen to Rubisco declines substantially (>50%) in low compared...
to high-light-grown leaves (Seemann et al., 1987; Evans and Seemann, 1989).

An expensive component of a leaf is the vascular tissue, both from the greater investment costs (lignin is one of the most energetically-expensive molecules in plants), and because non-photosynthetic vascular tissue replaces photosynthetic cells, thereby reducing the light-absorbing capacity of the leaf. In low light, the rate of transpiration is reduced, and with it, the need for an extensive vascular network. Consequently, vein density can decline in shaded C₃ plants, allowing M cells to occupy a greater proportion of the leaf area (Wylie, 1939, 1951; Bjorkman et al., 1972; Boardman, 1977; Jurick et al., 1982). By contrast, C₄ plants must maintain a high vein density and tight vein spacing because of the requirement for close proximity of M and vein-associated BS cells. C₄ grasses have, on average, interveinal distance (IVD) values that are less than half that of C₃ grasses (Crookston and Moss, 1974; Morgan and Brown, 1979; Hattersley and Watson, 1975; Kawamitsu et al., 1985; Dengler et al., 1994). The average C₄ grass IVD is 120 μm, whereas the average IVD of C₃ grasses is 280 μm (values estimated from Dengler et al., 1994; Ogle, 2003). Veins in C₄ grasses are spaced 50–200 μm apart, while in C₃ plants they are 200–400 μm apart (Ogle, 2003). The lower IVD values in C₄ grasses are correlated with decreases in M:BS tissue volume ratios compared with C₃ (Lee, 1985). Leaf thickness declined proportionally more in shade-acclimation responses seen in natural situations (Powell, 1978). Leaf thickness, vein density, IVD, M tissue area, BS tissue area, and M:BS ratios were compared between F. australasica (C₃) and F. robusta (C₄) plants grown in a growth chamber at either 500 μmol photons m⁻² s⁻¹ or 100 μmol photons m⁻² s⁻¹. Far red-to-red ratios were also lowered to mimic natural shading using plastic filters, thereby inducing the full range of shade acclimation responses seen in natural situations (Lee, 1985). Leaf thickness declined proportionally more in the C₃ than in the C₄ species (Figs 4, 5a). In C₃ F. robusta, vein density and IVD changed little while significant changes occurred in the C₄ F. australasica (Fig. 5b, c).

Table 2. The ratio of Rubisco activity or content in plants grown in high versus low light conditions

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Rubisco in low versus high-light-grown leaves</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₃ species</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atriplex patula</td>
<td>0.33</td>
<td>Evans, 1988</td>
</tr>
<tr>
<td>Alocasia macrorhiza</td>
<td>0.21</td>
<td>Seemann et al., 1987</td>
</tr>
<tr>
<td>Orzsa sativa</td>
<td>0.46</td>
<td>Evans, 1988</td>
</tr>
<tr>
<td>Phaseolus vulgaris</td>
<td>0.29</td>
<td>Seemann et al., 1997</td>
</tr>
<tr>
<td>Solanum dalcamara</td>
<td>0.26</td>
<td>Osmond, 1983</td>
</tr>
<tr>
<td>Spinacea oleracea</td>
<td>0.43</td>
<td>Evans, 1988</td>
</tr>
<tr>
<td>Average for C₃ species</td>
<td>0.34</td>
<td></td>
</tr>
<tr>
<td>C₄ species</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amaranthus retroflexus</td>
<td>0.46</td>
<td>Tazoe et al., 2005</td>
</tr>
<tr>
<td>Microstegium vimineum</td>
<td>0.81</td>
<td>Winter et al., 1982</td>
</tr>
<tr>
<td>Paspalum conjugatum</td>
<td>0.66</td>
<td>Ward and Woolhouse, 1986a, b</td>
</tr>
<tr>
<td>Zea mays</td>
<td>0.90</td>
<td>Ward and Woolhouse, 1986a, b</td>
</tr>
<tr>
<td>Average for the C₄ species</td>
<td>0.71</td>
<td></td>
</tr>
</tbody>
</table>

In low-light environments, there is an energetic cost associated with widely-spaced veins in C₄ plants, as indicated by surveys showing the quantum yield is lower in species with greater IVD (Fig. 3; Ogle, 2003). By contrast, quantum yield is independent of vein spacing in C₃ plants (Fig. 3). Carbon-isotope discrimination increases in shaded C₄ plants with wider vein spacing, indicating greater leakage of CO₂ out of the bundle sheath (Ogle, 2003). The increase in CO₂ leakage is probably responsible for the decline in the quantum yield of the C₄ species with greater vein spacing (Ogle, 2003). Reducing vein density as a means of shade-acclimation is not a restriction for C₃ leaves, but could be for C₄ leaves as it can compromise the efficiency of the C₄ apparatus.

Direct tests of growth light intensity on vein spacing of closely-related C₃ and C₄ species are not apparent in the literature, so a study was established to examine shade responses of leaf anatomy and vein pattern in C₃ and C₄ species of the dicot genus Flaveria. Flaveria species are valuable for comparing the effect of C₄ evolution on various characteristics in plants, because the C₃ species is ancestral to the derived C₄ species (McKown et al., 2005) and the weedy C₃ and C₄ species in this genus occur in roughly similar habitats (Powell, 1978). Leaf thickness, vein density, IVD, M tissue area, BS tissue area, and M:BS ratios were compared between F. australasica (C₃) and F. robusta (C₄) plants grown in a growth chamber at either 500 μmol photons m⁻² s⁻¹ or 100 μmol photons m⁻² s⁻¹. Far red-to-red ratios were also lowered to mimic natural shading using plastic filters, thereby inducing the full range of shade acclimation responses seen in natural situations (Lee, 1985). Leaf thickness declined proportionally more in the C₃ than in the C₄ species (Figs 4, 5a). In C₃ F. robusta, vein density and IVD changed little while significant changes occurred in the C₄ F. australasica (Fig. 5b, c).
Fig. 4. Leaf cross-sections of *Flaveria robusta* (C₃) and *Flaveria australasica* (C₄) grown in illuminated (500 μmol m⁻² s⁻¹ at a red:far red ratio of 1.55) and shaded (100 μmol m⁻² s⁻¹ at a red:far red ratio of 0.5) treatments in a plant growth chamber. (A) C₃ *F. robusta* grown in full light conditions. (B) *F. robusta* grown under reduced light and red:far red conditions. (C) C₄ *F. australasica* in full-light conditions. (D) *F. australasica* grown under reduced light and red:far red conditions. Scale bars are 50 μm.

Fig. 5. Quantitative anatomical data from *Flaveria robusta* (C₃) and *Flaveria australasica* (C₄) grown under illuminated (500 μmol m⁻² s⁻¹, red:far red ratio of 1.55) and shaded (100 μmol m⁻² s⁻¹, red:far red ratio of 0.5) conditions in a plant growth chamber. All leaves sampled were at maturity, and had been initiated and fully developed under their respective light treatments. Measurements were conducted on cross-sectioned leaves and leaf clearings. Lower case letters indicate statistical groupings based on one-way ANOVA and Tukey’s tests. Bars indicate standard errors. (A) Mean leaf thickness (μm); (B) vein density (mm mm⁻²); (C) interveinal distance (μm); (D) mesophyll tissue area in cross-section, including intercellular space area (μm²); (E) bundle sheath area in cross-section (μm²); (F) mesophyll area to bundle sheath area ratio.
In the C4 species, the reduction in vein density was approximately 20%. Mesophyll and BS tissue areas in leaf cross-sections decreased in both species, but the change in cross-sectional area (which indicates changes in tissue volume) was greater in the C4 _F. robusta_ (Fig. 5d, e). Intercellular spaces (ICS) increased and BS exposure to ICS doubled from 4.6% to 10.5% (P < 0.0001) in the shaded C4 _F. australasica_ plants (data not shown), indicating there is a greater potential for CO2 leakage out of the BS directly into the intercellular air spaces. This hypothesis is supported by carbon isotope measurements. _Flaveria australasica_ plants grown in the shade had a lower carbon isotope ratio (–16.0 per mil) than plants grown in bright light (–15.2 per mil; P < 0.05; the plant were grown as described in the legend of Fig. 5). The more negative isotopic values for the shade-grown C4 leaves indicate a greater degree of CO2 leakage from the bundle sheath (von Cammerer et al., 2005). When the mean IVD values from the C4 _Flaveria_ species are plotted on the quantum yield versus IVD relationship developed for grasses (Fig. 3), the predicted reduction in quantum yield is modest (about 9%); however, the importance of this decline is substantial, since the quantum yield advantages of C4 over C3 dicots at 30 °C (0.061 mol CO2 mol−1 photons for C4 versus 0.052 mol mol−1 for C3 dicots; Ehleringer and Pearcy, 1983) would be halved. Quantum yield differences between C3 and C4 species are important for understanding the relative performance of C3 versus C4 photosynthesis in low-light and variable thermal environments (Ehleringer and Pearcy, 1983; Ehleringer et al., 1997).

_Flaveria_, _Zea mays_, and _Amaranthus_ are sun-adapted plants; they may acclimate to low light (as during self-shading), but would not be able to complete their life cycle in the shade of a forest canopy (Björkman, 1981). Sun-adapted plants are generally considered specialists for open environments and, as such, comparisons involving only sun-plants may not show the full range of acclimation that could be seen in a true generalist species. Hence, generalist species should also be considered if the potential of C4 plants to acclimate to low light is to be fully evaluated. Many C4 species persist in heavily shaded environments (Brown, 1977; Pearcy and Calkin, 1983; Long, 1999) indicating they either possess a substantial capacity for shade acclimation, or they are truly shade-adapted (Horton and Neufeld, 1998; Sage and Pearcy, 2000). Based on floristic descriptions, it does not appear that the majority of these species are shade specialists, as they also occur along canopy openings such as paths or tree-fall gaps, and most can also occur in partially open habitats. For example, in the _Flora of the Guianas_ (Judsonwicz, 1990; the Guianas occur in the wet-tropics of northern South America), no C4 grass species is described as being localized in forest interiors, while 34% of the C3 grass flora is described as such (Sage, 2000). One per cent of the C4 grass flora of the Guianas is described as occurring along forest margins. These particular species represent the sun–shade generalists, and would be the best group to examine shade acclimation potentials in the C3 flora.

In contrast to sun-adapted C4 species (such as _Flaveria_), shade-tolerant C4 grasses are able to maintain close vein spacing under shaded conditions, for example, in _Microstegium vimineum_ (Winter et al., 1982), _Muhlenbergia frondosa_, _M. sobolifera_, _M. schreberi_ (Smith and Martin, 1987a), _Paspalum conjugatum_ (Ward and Woolhouse, 1986a, b), and _Rottboellia exaltata_ (Paul and Patterson, 1980). Instead of increasing, IVD decreases in _Muhlenbergia frondosa_ and _Rottboellia exaltata_ as M and BS cells do not expand to normal size. Reductions in the size of BS and M cells have also been observed in _Paspalum conjugatum_ relative to _Zea mays_ grown in shade (Ward and Woolhouse, 1986a, b). Ogle (2003) suggested that surviving shade conditions with sufficient quantum yield involves maintaining a threshold IVD lower than that observed in most C4 species. The shade-adapted species of _Microstegium_, _Muhlenbergia_, and _Paspalum_ have much lower IVD values than the average reported for C4 grasses, respectively, 72 μm (Winter et al., 1982), 73 μm (Smith and Martin, 1987a), and 78 μm (Kawamitsu et al., 1985).

A particularly interesting case of maintaining low IVD is observed in the shade-tolerant dicot, _Chamaesyce herbstdii_ (formerly _Euphorbia forbesii_) from the Hawaiian Islands (Herbst, 1972; Pearcy, 1983). _Chamaesyce herbstdii_ is a small-to-medium stature tree that is scattered in the understory of mesic Hawaiian forests (Koutnik and Huft, 1990). During normal leaf development in shaded _C. herbstdii_, a number of ‘disjunct’ veins arise, consisting of isolated xylem tracheids (Herbst, 1972). There is no physical connection between the vein ‘islands’ and the rest of the leaf venation, yet normal BS develops around these disjunct veins. C4 dicots are generally not shade-tolerant, so this unique solution to the problem of maintaining close vein spacing and M:BS ratios exemplifies the challenge posed by the C4 pathway during low-light acclimation. In a direct comparison of physiological acclimation to shade in _C. herbstdii_ with the co-occurring understory C3 tree, _Claoxylon sandwicense_, grown under identical high- and low-light conditions, Pearcy and Fraceschi (1986) observed that the shade-grown C3 species reduced the dark respiration and electron-transport rates to a greater relative degree than shade-grown _Chamaesyce herbstdii_ (Table 3). Leaf chlorophyll content declined little in _Chamaesyce herbstdii_, while it rose in _Claoxylon sandwicense_. Increased chlorophyll content is indicative of a greater ability to harvest photons in low light (Evans, 1988; Evans and Seemann, 1989). The result of these changes is that the C3 species in low-light
environments has a lower light-compensation point than the C₄ species, indicating a greater tolerance for shaded conditions (Table 3).

The shade-tolerant C₄ grass *Microstegium vimineum* is a summer-active species that occurs in gaps and understoreys in deciduous forests (Horton and Neufeld, 1998). In the eastern US, *M. vimineum* is a serious invasive species that can displace native C₃ herbs in shaded habitats within the forest. Shade-acclimation of *M. vimineum* has not been directly compared with that of C₃ species, but a number of patterns stand out that indicate it has less potential to acclimate to low-light environments than similar C₃ species. Winter et al. (1982) observed typical patterns of shade acclimation in terms of leaf thickness and leaf area responses, but not carboxylating enzymes (Table 4). Rubisco activity rose slightly from high to low light, while PEPCase activity was unchanged. The failure of carboxylating enzymes to adjust to low-light conditions is a sign of limited acclimation potential in this shade-tolerant grass.

Horton and Neufeld (1998) characterized the ability of *Microstegium vimineum* to utilize sunflecks (short episodes of high light that shine through small canopy gaps). Most sunflecks last between a few seconds to a few minutes, and often represent the major source of photons in the understorey. The ability to capture and store light energy in a sunfleck is an important component of shade-tolerance (Sharkey et al., 1986; Pearcy et al., 1996, 1997). Utilization of sunflecks is related to the ability of a leaf to keep its photosynthetic apparatus in an active, induced state, ready to use the photonic energy in a sunfleck. Normally, photosynthetic enzymes deactivate following shading and, once deactivated, several minutes are required for reactivation (Sharkey et al., 1986; Sage et al., 1993; Pearcy et al., 1996). In the deactivated state, the photosynthetic apparatus cannot use most of the photons in a light fleck. If induction requires more than a minute or so, most sunflecks would not be exploited. Shade-adapted C₃ plants are able to maintain the leaf in a partially induced state for the better part of an hour after the last sunfleck has passed. In *Alocasia macrorrhiza*, an understorey C₃ species from northern Australia, the half-time for relaxation of the induction state of photosynthesis is 30 min (Chazdon and Pearcy, 1986). By contrast, it is just 2.4 min in the C₄ *Microstegium vimineum* (Horton and Neufeld, 1998).

This result indicates that shade-tolerant C₄ plants do not maintain the ability to exploit sunflecks for more than a few minutes after the last sunfleck has passed, while in understorey C₃ species, the photosynthetic apparatus remains primed and ready for action for a considerably longer period. Failure of C₄ plants to maintain leaves in an induced state as well as shade-adapted C₃ plants could be associated with the additional requirement to maintain high activation of the C₄ cycle in addition to the C₃ cycle. High activation of the C₄ cycle would entail maintaining high gradients between the metabolite pools of the M and BS compartments, as well as maintaining activated forms of PEP carboxylase, pyruvate-phosphate dikinase and other C₄ cycle enzymes.

The sun-plant *Zea mays* also uses sunflecks less efficiently than C₃ plants such as soybean and *Alocasia*, particularly short duration sunflecks (<10 s; Krall and Pearcy, 1993). In C₃ plants, the photosynthesis rate increases as lightfleck duration falls below 10 s, while in maize it decreases. Much of the stored energy in short-duration lightflecks is apparently not used in C₄ plants due to a breakdown in the co-ordinated metabolism of the C₃ and C₄ cycles. Krall and Pearcy (1993) propose that the decline in maize photosynthesis during short duration sunflecks results from a burst of CO₂ leaving the BS cells. This is caused by the C₄-cycle reactions moving CO₂ into the BS faster than the deactivated C₃-cycle reactions can utilize it. The inability to maintain a high activation state of the C₃ cycle in maize appears to create conditions favouring the futile cycling of CO₂ during short-duration sunflecks (Krall and Pearcy, 1993).

### Temperature acclimation

Research on temperature acclimation has emphasized responses to thermal extremes. Responses to thermal extremes do not obviously vary between photosynthetic pathways, so there is little reason to expect acclimation to extreme temperatures to be inherently different between

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**Table 3.** Selected photosynthetic properties in C₄ Chamaesyce herbstii and C₃ Claoxylon sandwicense grown at high light (HL, 1200 μmol photon m⁻² s⁻¹) or low light (LL, 55 μmol photons m⁻² s⁻¹) (Pearcy and Franceschi, 1986)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>C. herbstii (C₄)</th>
<th>C. sandwicense (C₃)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dark respiration rate (μmol m⁻² s⁻¹)</td>
<td>1.5</td>
<td>1.1</td>
</tr>
<tr>
<td>Light-compensation point (μmol m⁻² s⁻¹)</td>
<td>41</td>
<td>31</td>
</tr>
<tr>
<td>Leaf chlorophyll content (mg m⁻²)</td>
<td>630</td>
<td>350</td>
</tr>
<tr>
<td>Electron transport rate (μmol O₂ mg⁻¹ chl hr⁻¹)</td>
<td>185</td>
<td>337</td>
</tr>
</tbody>
</table>

**Table 4.** Selected parameters from the shade-tolerant C₄ grass *Microstegium vimineum* grown at high- or low-light conditions (Winter et al., 1982)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Full sun</th>
<th>5% of full sun</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area per leaf (cm²)</td>
<td>3.9</td>
<td>8.2</td>
</tr>
<tr>
<td>Leaf thickness (μm)</td>
<td>122</td>
<td>66</td>
</tr>
<tr>
<td>Rubisco activity (μmol m⁻² s⁻¹)</td>
<td>6.4</td>
<td>7.6</td>
</tr>
<tr>
<td>PEPCase activity (μmol m⁻² s⁻¹)</td>
<td>37.1</td>
<td>37.8</td>
</tr>
</tbody>
</table>
ecologically similar C₃ and C₄ plants. There have been hypotheses that C₄ species are more prone to chilling injury because C₄-cycle enzymes can be cold-labile (Long, 1983; Potvin et al., 1986). Recent studies show that the C₄-cycle enzymes from cold-tolerant C₄ plants are stable in chilling conditions (Simon and Hatch, 1994; Matsuba et al., 1997; Long, 1999; Pittermann and Sage, 2000), demonstrating that chilling sensitivity is not an inherent feature of the C₄ pathway. In contrast to responses to thermal extremes, there may be inherent differences in the acclimation response of C₃ and C₄ photosynthesis to non-stressful temperature variation. C₄ plants exhibit a different pattern of biochemical limitation across a range of temperatures than C₃ species (von Caemmerer, 2000; Sage, 2002; Kubien et al., 2003) which should alter the nature of the acclimation response between the two pathways.

Thermal acclimation to low temperature in C₃ plants often involves an enhancement of the photosynthetic rate below the thermal optimum (Slattery, 1977; Berry and Raison, 1981; Mawson and Cummings, 1989; Savitch et al., 1997; Strand et al., 1999; Yamasaki et al., 2002; Yamori et al., 2005). In C₄ plants, early acclimation studies observed an enhancement in photosynthesis at cooler temperatures in desert species grown in moderate conditions (Pearcy, 1977; Berry and Raison, 1981). However, these studies often compared plants grown near 20 °C with species grown under hot (>40 °C) conditions, so that the thermal acclimation observed may have been more a case of heat acclimation than low-temperature acclimation. Recent studies of C₄ performance below 20 °C indicate little change in the photosynthetic rate of cold-tolerant C₄ plants upon growth in cool conditions (Matsuba et al., 1997; Pittermann and Sage, 2001; Cavaco et al., 2003; Naidu et al., 2003; Naidu and Long, 2004; Kubien and Sage, 2004a).

C₄ photosynthesis is well recognized to be inhibited by low temperatures to a greater degree than C₃ photosynthesis (Berry and Raison, 1981). Three leading hypotheses have been proposed to explain poor photosynthetic performance at low temperature in C₄ leaves. First, the activity of the C₄-cycle enzymes PEPCase and PPDK decline due to a cold-induced lability of these enzymes (Long, 1983, 1999). This hypothesis may explain poor photosynthetic performance in species from warm regions, but C₄ species that are naturally cold-tolerant do not show declines in PPDK or PEPCase activity with prolonged cold exposure (Simon and Hatch, 1994; Usami et al., 1995; Matsuba et al., 1997; Pitterman and Sage, 2000). Hence, this limitation is not the obvious problem that necessarily prevents the C₄ pathway from performing in cool climates. Second, the maximum quantum yield of C₄ photosynthesis is less than that of C₃ species in cooler environments, due to the additional energy cost of running the C₄ pump (Ehleringer and Pearcy, 1983). This is proposed to be an inherent limitation on C₄ plants in the cold (Ehleringer et al., 1997), but this constraint would mainly be an issue in low-light environments. At high light, where most C₄ species are found (including most of the cold-tolerant C₄ species), the quantum yield differences are not directly relevant, because there is an excess of photons, and much of the absorbed light energy is given off as heat (Kubien and Sage, 2004b). Therefore, quantum yield differences can contribute, but are not the main cause of poor C₄ photosynthetic performance in low temperature conditions (Sage and Kubien, 2003).

In cold-tolerant C₄ species, Rubisco capacity becomes limiting at low temperature and imposes a ceiling on photosynthetic rate below 20 °C (Pearcy, 1977; Pittermann and Sage, 2000; Sage, 2002; Kubien et al., 2003). Rubisco capacity in vitro and gross photosynthesis become the same in a variety of C₄ species below 20 °C which should be the case if Rubisco controls the rate of CO₂ assimilation in C₄ plants (Fig. 6). Fluorescence and gas exchange measurements show that the ratio of \( \Phi_{\text{PSII}}/\Phi_{\text{CO}_2} \) increases at low temperature where Rubisco capacity and the gross photosynthesis rate are equivalent (Fig. 7; Kubien et al., 2003, Kubien and Sage, 2004a). \( \Phi_{\text{PSII}}/\Phi_{\text{CO}_2} \) should rise with increasing leakiness of CO₂, because leakage of CO₂ does not affect the photochemical yield of PSII, but does reduce

Fig. 6. The response of the maximum Rubisco activity (\( V_{\text{cmax}} \)) determined in vitro as a function of assay temperature, and the gross photosynthesis rate (\( A^* \)) measured with gas exchange in the C₄ grass *Muhlenbergia glomerata.* (A) *M. glomerata* grown at 26 °C during the day and 22 °C at night in a plant growth chamber. (B) *M. glomerata* grown at 14/10 °C day/night temperature. Plants were grown at 800 \( \mu \text{mol} \) photons m \(^{-2} \) s \(^{-1} \). Note: photosynthesis and Rubisco activities are the same below 20 °C, but not at the thermal optimum. Rubisco contents for each treatment are not statistically different. From Kubien and Sage (2004a); with kind permission of Blackwell Publishing.
In the chilling-sensitive species *Zea mays* and *Zoysia japonica*, Rubisco contents decline substantially with prolonged exposure to chilling conditions, along with the photochemical capacity and the activity of C₄-cycle enzymes (Matsuba et al., 1997; Naidu et al., 2003; Naidu and Long, 2004).

In summary, there is little evidence that C₃ species compensate for low-temperature exposure by building up Rubisco content to overcome a strong limitation caused by low Rubisco activity. Instead, cold-tolerant C₄ plants are able to maintain Rubisco content and photosynthetic capacity, in contrast to cold-sensitive C₄ species where numerous components of the photosynthetic apparatus degrade with prolonged exposure to cool conditions. Cold-tolerant C₄ grasses have a pronounced ability to acclimate to chilling conditions qualitatively, as indicated by carotenoid changes that show they have well-developed mechanisms that protect against photoinhibition at low temperature (Kubien and Sage, 2004a).

In contrast to C₄ species, C₃ species show substantial acclimation to low temperature that involves increases in enzyme content. In C₃ plants, the ability to regenerate Pᵢ for photophosphorylation becomes a major limitation at low temperature (Sharkey, 1985; Sage and Sharkey, 1987; Falk et al., 1996; Strand et al., 1999). Acclimation to low temperature involves a partial, if not complete, removal of the Pᵢ-regeneration limitation. This is brought about by increasing enzyme capacity for starch and sucrose synthesis relative to Rubisco capacity and the capacity for RuBP regeneration, or a change in the internal Pᵢ status in leaves which improves Pᵢ regeneration in low-temperature conditions (Leegood and Edwards, 1996; Stitt and Hurry, 2002; Hendrickson et al., 2004). Improving Pᵢ-regeneration capacity often increases photosynthetic capacity at low temperature (Savitch et al., 1997; Strand et al., 1999). The limitation that dominates the rate of photosynthesis after acclimation increases the Pᵢ-regeneration capacity is unclear. At lower CO₂ levels than at present, Rubisco capacity can become limiting at cooler temperatures, and hence acclimation may involve an increase in Rubisco content (Sage, 2002). Consistently, Rubisco levels often increase at low temperature, and this is associated with increased rates of CO₂ assimilation in cold-acclimated leaves (Strand et al., 1999; Yamori et al., 2005). Electron-transport capacity also increases at low temperature, such that limitations caused by a deficient electron transport capacity are alleviated (Mawson and Cummings, 1989; Savitch et al., 1997). This limitation could be particularly important in CO₂-enriched atmospheres when the capacity for RuBP regeneration is the primary limitation.

Limitations controlling photosynthesis at elevated temperature remain unclear. Rubisco activase is reported to dissociate above the thermal optimum in both C₃ and C₄ species, creating a limitation on photosynthesis from a low activation state of Rubisco (Crafts-Brandner and Salvucci, 2003).
Acclimation to elevated temperature in C₃ plants involves stabilization of Rubisco activase, in part by the increased presence of a longer, more heat-stable isofrom of activase (Law et al., 2001; Portis, 2003). Similar mechanisms appear to be present in maize, as acclimation to elevated temperature is associated with expression of a larger isofrom of Rubisco and partial recovery of the Rubisco activation state (Crafts-Brandner and Salvucci, 2002). Electron-transport capacity can also become limiting for photosynthesis at elevated temperature in numerous C₃ species adapted to warm climates (Bukhov et al., 1999; Schrader et al., 2004; Wise et al., 2004; Sharkey, 2005; Cen and Sage, 2005). The relative importance of limitations in electron transport capacity versus activation state remain uncertain. In C₄ species, the uncertain nature of the limiting processes at elevated temperature is a large part of the overall problem in understanding acclimation of C₄ plants to heat. In addition to the Rubisco activase and activation state limitations, photosynthesis may be limited by electron transport, PEP carboxylation, and PEP regeneration at elevated temperature (Sage, 2002; Kubien et al., 2003). Without a clear picture of the limitations on C₄ photosynthesis at elevated temperature, it is difficult to assess how C₄ leaves acclimate to heat in terms of the biochemical reactions that determine photosynthetic capacity.

In summary, the limited amount of work on low-temperature acclimation in C₄ photosynthesis shows there is little enhancement of Rubisco capacity, as should be the case if a widespread limitation in Rubisco capacity is to be overcome. C₃ species do show substantial acclimation, and this is often explained by increases in P₇ regeneration capacity and Rubisco content. The difference in thermal acclimation between C₃ and C₄ species is consistent with the hypothesis that the relatively low volume of leaves devoted to Rubisco-containing chloroplasts restricts the ability of C₄ species to compensate for low temperature by increasing Rubisco content. By contrast, C₃ species lack this restriction, and appear to have a greater ability to pack in extra enzyme as needed. Thus, there is evidence indicating that C₃ species may be constrained by their unique structural requirements to have a lower potential to acclimate to cooler temperatures than C₃ leaves. This could have consequences for the overall performance of C₄ species in environments where cool temperatures are common throughout the growing season.

**Acclimation to elevated atmospheric CO₂ partial pressure**

Acclimation of photosynthesis to atmospheric CO₂ variation deserves brief mention, largely because C₃ and C₄ plants respond differently to increases in atmospheric CO₂ content, although neither C₃ nor C₄ species show acclimation responses that are directly linked to CO₂ level. Instead, the CO₂ effect on the photosynthetic biochemistry is largely mediated by carbohydrate accumulation in leaves under conditions where carbon sinks in the plant are also experiencing high carbon supply (Sims et al., 1998b; Long et al., 2004). C₃ species show a greater degree of acclimation to elevated CO₂ partial pressure than C₄ species, largely because C₃ photosynthesis is stimulated more by rising CO₂, and hence the degree to which carbohydrate supply becomes excessive is potentially greater in the C₃ species (Sage, 1994). In C₃ species, there is a general decline in photosynthetic enzyme content with prolonged exposure to high CO₂; Rubisco is preferentially reduced during early phases of acclimation, but most photosynthetic genes are switched off after long-term exposure, particularly when sink limitations are substantial (Sage and Coleman, 2001; Long et al., 2004). In C₄ plants, acclimation is often negligible due to the lack of a strong response to increased CO₂ partial pressure that is common in C₄ plants (Sage and Kubien, 2003). C₄ photosynthesis is CO₂-saturated, or almost CO₂-saturated at current atmospheric CO₂ levels, so a strong response is not expected. This feature of the C₄ photosynthetic pathway largely explains the relative lack of acclimation to rising CO₂ level in C₄ plants. However, certain C₄ species show a slight to modest short-term stimulation of photosynthesis by increased CO₂ availability, particularly under certain environmental conditions such as higher temperature and reduced mineral nutrition that increase the CO₂ saturation point of photosynthesis (Ziska et al., 1999; Ghannoum et al., 2000; Sage and Kubien, 2003). Where CO₂ stimulates photosynthesis, CO₂ acclimation can be observed, typically as a slight reduction in photosynthesis at both high and low levels of CO₂ (Tissue et al., 1995; LeCain and Morgan, 1998; Watling et al., 2000; Sage and Kubien, 2003). Acclimation may preferentially reduce the C₄ cycle, as indicated by a reduction of PEPCase but not Rubisco content in *Sorghum bicolor*, *Zea mays*, and *Flaveria* species grown at elevated CO₂ (Watling et al., 2000; Gascoigne-Owens et al., 2002; Snowdon et al., 2002).

**The consequence of reduced phenotypic plasticity in C₄ plants**

Compared with C₃ species, C₄ plants have a restricted ecological and biogeographical distribution (Sage et al., 1999). C₄ species are absent from polar biomes, rare if not absent in cool temperate to boreal biomes, rare in alpine and montane elevations at all latitudes, and uncommon in forest understoys. C₄ photosynthesis is absent from certain plant life-forms, notably, canopy-forming forest trees. It is uncommon in short-stature trees, and in most shrub species, such that the woodland vegetation of the planet is almost exclusively C₃ plants. The only regions where C₄ photosynthesis is common in woody vegetation are in desert...
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