data on a variety of animals. The original foraging data on bees (Fig. 3a) were collected by recording the landing sites of individual bees[15]. We find that, when the nectar concentration is low, the flight-length distribution decays as in equation (1), with $\mu \approx 2$ (Fig. 3b). (The exponent μ is not affected by short flights.) We also find the value $\mu \approx 2$ for the foraging-time distribution of the wandering albatross[6] (Fig. 3b, inset) and deer (Fig. 3c, d) in both wild and fenced areas[16] (foraging times and lengths are assumed to be proportional). The value $2 \leq \mu \leq 2.5$ found for amoebas[4] is also consistent with the predicted Lévy-flight motion.

The above theoretical arguments and numerical simulations suggest that $\mu \approx 2$ is the optimal value for a search in any dimension. This is analogous to the behaviour of random walks whose mean-square displacement is proportional to the number of steps in any dimension[17]. Furthermore, equations (4) and (5) describe the correct scaling properties even in the presence of short-range correlations in the directions and lengths of the flights. Short-range correlations can alter the width of the distribution $P(l)$, but cannot change μ, so our findings remain unchanged. Hence, learning, predator–prey relationships and other short-term memory effects become unimportant in the long-time, long-distance limit. A finite λ ensures that the longest flights are not energetically impossible. Our findings may also be relevant to the study of population dynamics. Specifically, each value of μ is related to a different type of redistribution kernel[18]; for example, $\mu \geq 3$ corresponds to the normal (or similar) distribution, while $\mu = 2$ corresponds to a Cauchy distribution (see also ref. 19). Finally, note that non-destructive foraging is more realistic than destructive foraging because, in nature, 'targets' such as flowers, fish and berries are often found in patches that regenerate. Organisms are often in clusters for reproductive purposes, and sometimes such clusters may have fractal shapes[20]. Thus, the forager can revisit the same food patch many times. We simulated destructive foraging in various patchy and fractal target-site distributions and found results consistent with non-destructive foraging with uniformly distributed target sites. □

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Correspondence should be addressed to G.M.V. (e-mail: gandhi@fis.ufal.br).

Water stress inhibits plant photosynthesis by decreasing coupling factor and ATP

W. Tezara*, V. J. Mitchell†, S. D. Driscoll† & D. W. Lawlor†

** Instituto de Biologia Experimental, Facultad de Ciencias, Universidad Central de Venezuela, Calla Suapore, Colinas de Bello Monte, Apartado 47114, Caracas 1041a, Venezuela*
† Biochemistry and Physiology Department, IACR-Rothamsted, Harpenden, Hertfordshire AL5 2JQ, UK

Water stress substantially alters plant metabolism, decreasing plant growth and photosynthesis[1–4] and profoundly affecting ecosystems and agriculture, and thus human societies[5]. There is controversy over the mechanisms by which stress decreases photosynthetic assimilation of CO_2. Two principal effects are invoked[2,4]: restricted diffusion of CO_2 into the leaf, caused by stomatal closure[6–8], and inhibition of CO_2 metabolism[9–11]. Here we show, in leaves of sunflower (*Helianthus annuus* L.), that stress decreases CO_2 assimilation more than it slows O_2 evolution, and that the effects are not reversed by high concentrations of CO_2[12,13]. Stress decreases the amounts of ATP[9,11] and ribulose bisphosphate found in the leaves, correlating with reduced CO_2 assimilation[11], but the amount and activity of ribulose bisphosphate carboxylase-oxygenase (Rubisco) do not correlate. We show that ATP-synthase (coupling factor) decreases with stress and conclude that photosynthetic assimilation of CO_2 by stressed leaves is not limited by CO_2 diffusion but by inhibition of ribulose biphosphate synthesis, related to lower ATP content resulting from loss of ATP synthase.

When land plants absorb less water from the environment through their roots than is transpired (evaporated) from their leaves, water stress develops. The relative water content (RWC), water potential (ψ) and turgor of cells are decreased and the concentrations of ions and other solutes in the cells are increased, thereby decreasing the osmotic potential[1–4]. Stomatal pores in the leaf surface progressively close[2,6,13,14], decreasing the conductance to water vapour (g_{H_2O}) and thus slowing transpiration and the rate at which water deficits develop[1–4,6,11,14]. Also, photosynthetic assimilation of CO_2 (A) decreases, often concomitant with, and frequently ascribed to, decreasing conductance to CO_2 (g_{CO_2}) (refs 2–4, 6, 8). However, decreased A is also considered to be caused by inhibition of the photosynthetic carbon reduction (Calvin) cycle[1,2,9,11], although there is uncertainty over which biochemical processes are most sensitive to stress[2,3,6,15,16]. We assessed whether A is controlled by g_{CO_2} or by metabolic factors by measuring CO_2 and O_2 exchange, using large CO_2 concentrations (up to 0.1 mol mol^{-1}, equivalent to 10% volume/volume) to overcome small g_{CO_2} (refs 8, 12–14), and by determining important indicators of photosynthetic biochemistry (for example, ribulose biphosphate (RuBP) and Rubisco of the Calvin cycle)[9,10,16,17]. We considered the role of ATP in particular[2,9,10] because, although inhibition of photophosphorylation has been demonstrated[9,10] but is not widely accepted[3,4,6,18,19], it may explain the decrease in RuBP and A (refs 2, 11).

In the chloroplasts of leaf cells, capture of photons causes electron transport in the thylakoid membranes, evolution of O_2 (refs 2–4)

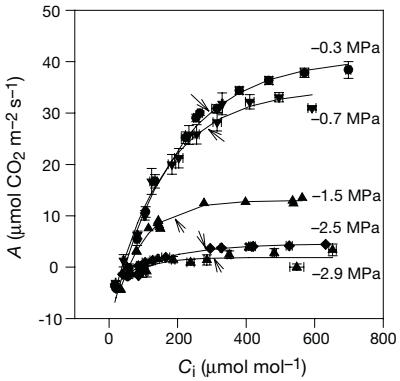

Figure 1 Rate of photosynthetic CO_2 assimilation (A) in sunflower (*H. annuus* L.) as a function of the calculated CO_2 concentration inside the leaf mesophyll (c_i) for leaves of different water potential (ψ, shown against each curve) measured at 800 μmol m^{-2} s^{-1} PAR and 25 °C. The c_i values calculated from the particular photosynthetic rate and stomatal conductance (the operating points) at 360 μmol CO_2 mol^{-1} for each water potential are indicated by arrows on the curves.

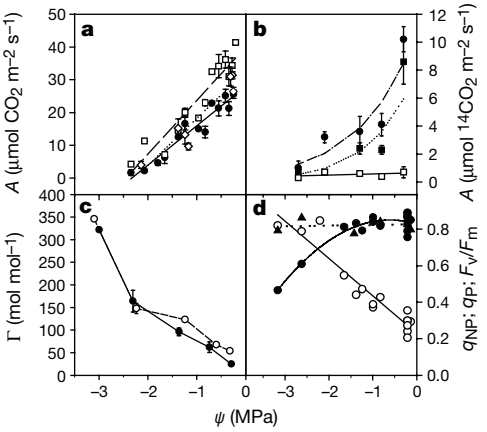

Figure 2 Photosynthetic processes of sunflower (*H. annuus* L.) leaves as a function of leaf water potential (ψ). **a**, Rate of photosynthesis (A) measured at three CO_2 concentrations in the atmosphere: 0.36 mmol mol^{-1} (0.036%) (circles); 1 mmol mol^{-1} (0.1%) (diamonds); 9 mmol mol^{-1} (0.9%) (squares). **b**, A determined from $^{14}CO_2$ supplied to leaf discs under conditions used in the oxygen electrode, at CO_2 concentrations of 10 mmol mol^{-1} (1%) (circles); 50 mmol mol^{-1} (5%) (filled squares); 100 mmol mol^{-1} (10%) (open squares). **c**, Equilibrium CO_2 concentration, Γ, measured at 800 μmol m^{-2} s^{-1} PAR and 25 °C in 0.21 mol mol^{-1} O_2 (21%) (open circles) or 0.02 mol mol^{-1} O_2 (2%) (filled circles). **d**, Photochemical quenching, q_P (filled circles); non-photochemical quenching, q_{NP} (open circles); F_v/F_m (triangles), derived from chlorophyll *a* fluorescence measurements.

and generation of NADPH and the transthylakoid proton gradient (ΔpH) which is responsible for ATP synthesis by ATP synthase (coupling factor, CF_1–CF_0)[10,19,20]. The Calvin cycle uses ATP and NADPH to synthesize RuBP, which reacts with CO_2 in a reaction catalysed by Rubisco. Owing to g_{CO_2}, this uptake of CO_2 decreases its concentration within the leaf (c_i) relative to that in the atmosphere (c_a)[2–4,16,21]: $c_i = c_a - A/g_{CO_2}$ where $g_{CO_2} = g_{H_2O}/1.6$. The factor 1.6 is the ratio of the diffusivities D of H_2O and CO_2 in air ($D_{H_2O}/D_{CO_2} = 1.6$)[7,13]. As g_{CO_2} decreases with stress, A may diminish because of small c_i. We tested this by measuring the relationship between A and c_i using large c_a to overcome limiting g_{CO_2}, and the effects of stress on electron transport, by measuring O_2 evolution and NADPH content[4,18,22,23]. If stress inhibits the Calvin cycle, for example by decreasing Rubisco amounts and activities, then RuBP content will decrease but ATP will increase. If the capacity for ATP synthesis is limiting, indicated by, for example, lower ATP synthase content, then both will decrease[2,16,18,23].

The A/c_i curves (Fig. 1) show that both maximum rate of CO_2 assimilation (A_{max}) and carboxylation efficiency, ϕ ($\phi = A/$μmol CO_2 mol^{-1}), decreased with stress, as observed in wheat[1,2] and sunflower[11]. The operating c_i (Fig. 1) decreased as ψ decreased to about −1.5 MPa, but increased with further stress. This shows some stomatal limitation but greatly impaired metabolism. However, if c_i is incorrectly determined this interpretation is invalid[2–4,14,21,24–26]. Calculation of c_i assumes a uniform and unimodal frequency distribution of g_{CO_2} over the leaf, with no 'patchy stomata'[14,24–26]. We established that there was no heterogeneity of stomatal conductance by exposing the leaf areas on which A was measured to $^{14}CO_2$, as previously shown for sunflower[11] and in other analyses[14]. Calculation of c_i assumes that movement of CO_2 and H_2O across the leaf cuticle, when the stomata are closed, conforms to $D_{H_2O}/D_{CO_2} = 1.6$. However, CO_2 movement may be much restricted[21]. Hence, our calculations of c_i may be erroneous. We recalculated c_i for the most severely stressed leaves using the values of A but assuming different D_{CO_2} values. A 30% decrease in D_{CO_2} progressively reduced c_i, increasing ϕ but not A_{max}. Smaller values of D_{CO_2} led to greatly underestimated c_i and unrealistically increased ϕ, showing that the error is small and does not substantially alter the A/c_i relationships in Fig. 1. This indicates that, despite the very small g_{CO_2}, c_i is not substantially depleted[2,11] and that photosynthetic metabolism is inhibited by water stress.

A may also be limited by the small conductance of the pathway of CO_2 movement between the intercellular spaces and active sites of Rubisco[2,3]. To eliminate these, we measured A with increasing CO_2 concentration[2,4,12,13], but even at c_a = 9 mmol CO_2 mol^{-1} (0.9%) in a normal gas exchange system we observed no stimulation under stress (Fig. 2a). Indeed, A ceased at the same RWC (50%: $\psi \approx -2$ MPa) for all values of c_a. This agrees with the findings of ref. 12 for water-stressed (but not abscisic acid-treated) leaves, but disagrees with those of ref. 8.

Oxygen evolution is considered to be evidence for continued CO_2 metabolism under stress[3,4,8]. We measured O_2 evolution in different c_a. At $\psi = -0.6$ MPa in 10 mmol CO_2 mol^{-1}, the rate of O_2 evolution was 29.9 μmol m^{-2} s^{-1}, decreasing by ~45% and 60% at $\psi = -1.5$ and −2.5 MPa, respectively. Oxygen evolution was not stimulated by 50 mmol CO_2 mol^{-1} (5%) compared with 10 mmol CO_2 mol^{-1} at $\psi = -0.6$ MPa, but increased by 28% and 19% at $\psi = -1.5$ and −2.5 MPa, respectively, showing some diffusion limitation. Increasing c_a to 100 mmol CO_2 mol^{-1} (10%) completely inhibited O_2 evolution irrespective of stress. We tested whether CO_2 assimilation (A) continues during O_2 evolution with decreasing ψ by measuring $^{14}CO_2$ assimilation as ^{14}C accumulation in organic compounds at high c_a. A decreased (Fig. 2b) with stress irrespective of CO_2, and 0.1 mol mol^{-1} (10%) CO_2 completely inhibited CO_2 assimilation as well as O_2 evolution, similar to the results of ref. 11 but contrary to those of ref. 8. If O_2 evolution depends on CO_2 influx and assimilation in the leaf[3,4], ^{14}C should have accumulated. However, it did not, despite similar characteristics and pathways of movement of O_2 and CO_2, indicating that O_2 evolution under stress may not be dependent on Calvin cycle function.

Metabolic inhibition of A is also shown by the progressive increase in the equilibrium CO_2 compensation point (Γ), measured on whole, detached pre-stressed leaves, as stress increased (Fig. 2c). This increase occurred at an O_2 concentration of 0.21 mol mol^{-1} (that is, current atmospheric concentration of 21%), as observed for *Triticum aestivum* and *H. annuus*[2,13]. An O_2 concentration (0.02 mol mol^{-1} or 2%) that prevents glycolate pathway photorespiration[2,27] decreased Γ compared with 0.21 mol O_2 mol^{-1} over the range $\psi = -0.3$ to −1.8 MPa, but not at smaller (more negative) ψ, and did not prevent Γ from increasing with stress. This may be related to respiration (by the tricarboxylic acid cycle), which, in darkness at 0.21 mol O_2 mol^{-1}, decreased by about 60% as ψ fell from −0.3 to −3.0 MPa (data not shown), continuing even when A had ceased. This confirms that A decreases more than respiration with increasing stress[2,9]. Thus, under stress, dark respiration determined Γ (which was also increased by photorespiration) but

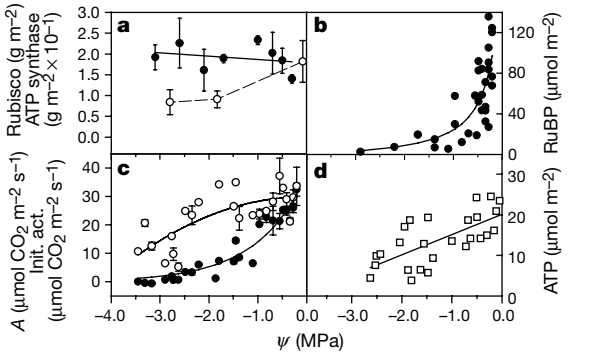

Figure 3 Biochemical composition of sunflower (*H. annuus* L.) leaves as a function of water potential (ψ). **a**, Amounts (m^{-2}) of ribulose bisphosphate carboxylase-oxygenase (filled circles) and ATP synthase (open circles). **b**, Amount of ribulose bisphosphate. **c**, Rate of photosynthetic CO_2 assimilation, *A* (filled circles) and the initial activity (init. act.) of ribulose bisphosphate carboxylase-oxygenase (open circles). **d**, Amount of ATP.

photorespiration ceased at severe stress. Further evidence for a large change in photosynthetic metabolism caused by stress is provided by chlorophyll *a* fluorescence[28], with greatly increasing photochemical quenching (q_{NP}) and decreasing chemical quenching (q_P) (refs 2, 4) but no change in the variable-to-maximum fluorescence ratio (F_v/F_m) (Fig. 2d), showing that the efficiency of open photosystem II reaction centres was not impaired. This suggests that photoinhibition[28,29], often considered to be a primary effect of stress[3,4,28] caused by CO_2 limitation, was not important in our experiments, as seen in sunflower[29] but not in other studies[4].

We conclude that water stress does not inhibit *A* by decreasing CO_2 diffusion (occurring even in elevated CO_2) or by preventing O_2 production and electron transport, but by inhibition of the Calvin cycle.

What in the Calvin cycle is impaired by water stress? Amounts of Rubisco[2,11,15] per unit leaf area remained approximately constant (Fig. 3a), despite leaf shrinkage, whereas RuBP content (Fig. 3b) was severely decreased by very mild stress, confirming earlier results[11]. Rubisco activity was decreased only by relatively severe stress (Fig. 3c) and did correlate with the decrease in *A* (Fig. 3c), although the decrease in *A* did correlate with the decrease in RuBP as shown[11]. If Rubisco were inhibited, then RuBP would accumulate relative to the decrease in *A*, even though the Calvin cycle is autocatalytic and decreased *A* would decrease synthesis of RuBP[2,16]. The 3-phosphoglyceric acid (3-PGA) content per unit leaf area was unaffected by water deficit (data not shown). However, this is expected, as respiration, which we have confirmed is relatively less affected by stress than *A*, produces 3-PGA, thus obscuring Calvin cycle function[16].

ATP content decreased with increasing stress (Fig. 3d), indicating that stress inhibits ATP synthesis rather than the Calvin cycle *per se*[2,9,10] (disagreeing with ref. 18). If the Calvin cycle is inhibited then ATP content should rise, as in transgenic plants with much decreased phosphoribulokinase activity[23]. Decreased ATP indicates that ATP synthesis may be impaired, although increased ATP consumption cannot be excluded[2]. As respiration continues in stressed leaves, although at a smaller rate than in unstressed leaves, it could possibly maintain a relatively large ATP content. However, if the capacity for phosphorylation by dark respiration in leaves is smaller than that for photophosphorylation, decreased ATP is more likely to be caused by inhibition of photophosphorylation[9,10]. We measured ATP synthase to assess whether loss of this enzyme was related to decreased ATP content. The amount of ATP synthase per m^2 decreased progressively with stress (Fig. 3a). The ATP content per m^2 correlates strongly with the ratio of ATP synthase to Rubisco (ATP $= -4.07 + 1.91$ (% ATP synthase/Rubisco); $r^2 = 0.99$). We conclude that decreased ATP content is related to a decrease in ATP synthase with increasing stress. Decreasing ATP synthase in leaves by transformation with antisense had very similar effects to water stress, with increased non-photochemical and decreased photochemical quenching, explained by decreased ATP synthesis[17].

ATP synthase is composed of CF_0, embedded in the thylakoid membrane, attached to CF_1, which projects into the chloroplast stroma[20]. ATP is synthesized by physical rotation of components that alters the conformation of the active enzyme sites, driven by movement of protons through the complex as a consequence of the ΔpH between thylakoid lumen and stroma[2,4,20]. Catalytic activity of ATP synthase must depend on very stringent spatial arrangements within and between the subunits. Ionic concentration affects ATP synthase activity and binding between CF_1 and CF_0 *in vivo*[20]. Large magnesium concentrations decrease photophosphorylation of chloroplasts isolated from stressed leaves[9,10], providing a mechanism for the effects of decreasing ψ and RWC on RuBP content and CO_2 assimilation[11]. We calculate that magnesium concentrations in the chloroplasts of leaves used in our experiment increased twofold between −0.3 and −3 MPa (95% to 50% RWC). As it is the amount of CF_1 that decreases, binding between CF_1 and CF_0 is probably affected, although concentrated Mg^{2+} may also impair catalysis: these aspects require further examination. Differences between ATP synthase from chloroplasts and that from mitochondria, and in their environments[20], may underlie the different responses of photosynthesis and respiration to stress. ATP synthesis depends on ΔpH, which is large under stress, judging from the greatly increased q_{NP} (refs 2, 20, 28), and also in leaves with decreased ATP synthase content[17]. Loss of CF_1 must block proton flux out of the thylakoid, causing a large ΔpH (and large non-photochemical quenching), so lack of energy does not decrease ATP.

The decrease in *A* correlates with decreased ATP and ATP synthase. However, ATP content with severe stress was still 30% of that without stress, so *A* might be expected to continue. This ATP may not be in the chloroplast, or the concentration may be insufficient for RuBP synthesis[2,11,16,30]. A RuBP concentration below ~3–5 mol mol^{-1} of Rubisco active sites, required for A_{max} in unstressed plants, would decrease *A* (ref. 11) and may increase concentrations of inhibitors that decrease catalytic activity[30]. We show that at ~2, 1.5 and 0.5 mol RuBP mol^{-1} sites, *A* decreased by 25, 80 and almost 100%, consistent with earlier analysis of water-stressed[11] and phosphate-deficient[22] plants.

Inhibition of ATP synthesis and large q_{NP} are consistent with continued O_2 evolution and electron transport[28]. Our measurements show that the reduction state of the pyridine nucleotides in stressed leaves did not alter significantly between $\psi = -0.5$ and −2 MPa, the ratio NADH+NADPH/NAD+NADP remaining constant at 0.14 (data not shown). Such conditions are damaging to photosynthetic and cellular metabolism[1–4], with increased production of active oxygen species which damage lipids, proteins and so on[2,4,28]. In water-stressed leaves, particularly under high solar radiation, decreased *A* removes the primary sink for reductant and energy[2,4,18], although photorespiration dissipates energy[2–4,27] (however, this is disputed[7]) until *A* and any internal recycling of CO_2 ceases. Energy dissipation in the chloroplast, for example, by the violaxanthin cycle, may not be sufficient and damage, such as photoinhibition, may ensue[2–4,28]. Inhibition of ATP synthesis has been rejected (based on indirect assessment of CF_1 activity) in favour of reduced g_{CO_2} as a cause of decreased *A* in slowly but severely water-stressed field-grown sunflowers[6,8,19]. Possibly, in the field, adjustment of the root/shoot ratio and g_{H_2O} may precede or be concomitant with the metabolic effects of stress, thus ameliorating or avoiding its impacts[1]. Metabolic effects have been ascribed to damage caused by rapid stressing[4,6,16], but our studies were on relatively slowly stressed plants and resulted, after several days of severe stress, in irreversible damage and death. The loss of capacity to synthesize ATP, in conjunction with the reductant status, may explain many aspects of metabolism under stress[2]. We suggest, as an example, that inadequate ATP impairs synthesis of proteins[31], thus endangering cell

functions[2]. However, it may stimulate accumulation of 'heat-shock' (chaperone) proteins, because phosphorylation represses heat-shock factor, which prevents expression of heat-shock proteins[32].

We conclude that water stress inhibits photosynthesis through decreased RuBP supply caused by low ATP content, itself a result of decreased ATP synthase, confirming that ATP synthesis is sensitive to cellular dehydration[9,10]. Decreased ATP content has important consequences for cellular metabolism and is relevant to unravelling the effects of water stress[2,13,18]. □

Methods

Growth conditions

Sunflower (*Helianthus annuus* L. cultivar Avanti) plants were grown at 25/18 °C day/night temperature, ~70% relative humidity and 360 μmol CO_2 mol^{-1}, under 400 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR) (day length 14 h) in 5 l of soil-based compost and were given ample nutrition and water. The third pair of leaves, attached to the plant, was used for all measurements for two weeks after full expansion.

Water stress treatments

Control plants were fully watered; plants were stressed by replacing part of the water transpired to allow stress to develop over 12 days. Stresses were achieved at different rates by differential watering to ensure that measurements were made on leaves of similar age[11]. The decrease in ψ was ~0.3 MPa per day in the most severe stress. We measured leaf water potential (ψ) with a Scholander pressure chamber[11] on the leaf remaining after freeze-clamping[11,22,23], then leaf discs were removed for measurement of RWC[11]. The correlation between ψ and RWC was $\psi = 5.05 + 0.053\text{RWC}$ ($r^2 = 0.90$). Preliminary studies showed no significant differences between tissue used for gas exchange and the remaining leaf.

Gas exchange measurements

Carbon dioxide exchange and water loss were measured and used to calculate photosynthetic rate, stomatal conductance and sub-stomatal CO_2 concentration (c_i)[11,21–23]. All measurements were made in triplicate on 10 cm^2 of intact leaf under 800 μmol m^{-2} s^{-1} PAR, at 25 °C and 1 kPa vapour pressure, in a multi-chamber gas-exchange system, using intact leaves attached to three different plants[11,22,23]. Measurements on intact leaves at large c_a were made with a Ciras-1 gas analyser (PPSystems).

Stomatal heterogeneity

After gas exchange measurements, leaves were exposed to $^{14}CO_2$ for 10 s and freeze-clamped, followed by autoradiography[11,14,26].

Assimilation of CO_2 under oxygen electrode conditions

We measured ^{14}C accumulation in organic compounds on discs removed from illuminated control and wilted leaves and exposed for 10 min to $^{14}CO_2$ of known specific radioactivity with CO_2 concentrations of up to 0.1 mol mol^{-1} (10% by volume) in 5 ml glass vials with rubber seals. Conditions were the same as in the oxygen electrode. Leaves were immediately killed and ^{14}C measured after combustion of the sample.

Equilibrium CO_2 compensation points

Detached leaves were measured in a sealed glass chamber with internal air circulation coupled with an infrared gas analyser (Mark III, ADC); the system had a CO_2 leakage rate less than 0.5 μmol mol^{-1} h^{-1} at 800 μmol m^{-2} s^{-1} PAR at 25 °C.

Oxygen evolution

Leaf discs were measured in an oxygen electrode (Hansatech) at 400 μmol m^{-2} s^{-1} PAR, CO_2 concentrations up to 0.1 mol CO_2 mol^{-1} (refs 3, 4) and 25 °C.

Fluorescence

Chlorophyll *a* fluorescence was measured with a modulated meter (Hansatech), concomitant with gas exchange, for calculation of quenching parameters and F_v/F_m (refs 3, 4, 7, 22, 28).

Biochemical analyses

Leaf areas in the gas exchange chambers were freeze-clamped at liquid nitrogen temperature, reaching 0 °C within 0.05 s and −20 °C in 0.1 s, immediately after gas exchange measurements[11,22,23,33]. ATP, RuBP, 3-PGA and pyridine nucleotides were measured on 10 cm^2 leaf extracted in 5% (v/v) perchloric acid as described[11,16,18,22,23]. We measured Rubisco content and activity as described[11,33]. The amounts of ATP synthase α- and β-subunits were measured[33]: frozen leaf tissue was homogenized in a 50 mM Bicine buffer, pH 7.6, 20 mM EDTA, 1 mM $MgCl_2$ and 50 μM mercaptoethanol. Material precipitated by centrifugation at 10,000*g* contained ATP synthase, which was solubilized in a 250 mM Tris buffer, pH 7.6 containing 250 mM dithiothreitol, 10% (w/v) SDS and 10% (w/v) glycerol at an SDS to protein ratio of 4:1. The denatured ATP synthase was separated by electrophoresis and the separated proteins blotted onto PVDF membrane, followed by blocking. The blot was developed using primary polyclonal antibody to the α- and β-subunits of ATP synthase (supplied by J. C. Gray). ATP synthase prepared from sunflower leaves was used as standard to quantify the protein from the experiments by visualizing the luminescence onto radiographic film and scanning. The mass ratio of total ATP synthase to the two subunits was taken as 1.61 (ref. 33).

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Correspondence and requests for materials should be addressed to D.W.L. (e-mail: david.lawlor@bbsrc.ac.uk).