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Oxygen sensitivity of C_4 photosynthesis: evidence from gas exchange and chlorophyll fluorescence analyses with different C_4 subtypes

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ABSTRACT

Because photosynthetic rates in C_4 plants are the same at normal levels of O_2 (*c.* 20 kPa) and at *c.* 2 kPa O_2 (a conventional test for evaluating photorespiration in C_3 plants) it has been thought that C_4 photosynthesis is O_2 insensitive. However, we have found a dual effect of O_2 on the net rate of CO_2 assimilation among species representing all three C_4 subtypes from both monocots and dicots. The optimum O_2 partial pressure for C_4 photosynthesis at 30 °C, atmospheric CO_2 level, and half full sunlight (1000 μmol quanta m^{-2} s^{-1}) was about 5–10 kPa. Photosynthesis was inhibited by O_2 below or above the optimum partial pressure. Decreasing CO_2 levels from ambient levels (32·6 Pa) to 9·3 Pa caused a substantial increase in the degree of inhibition of photosynthesis by supra-optimum levels of O_2 and a large decrease in the ratio of quantum yield of CO_2 fixation/quantum yield of photosystem II (PSII) measured by chlorophyll *a* fluorescence. Photosystem II activity, measured from chlorophyll *a* fluorescence analysis, was not inhibited at levels of O_2 that were above the optimum for CO_2 assimilation, which is consistent with a compensating, alternative electron flow as net CO_2 assimilation is inhibited. At suboptimum levels of O_2, however, the inhibition of photosynthesis was paralleled by an inhibition of PSII quantum yield, increased state of reduction of quinone A, and decreased efficiency of open PSII centres. These results with different C_4 types suggest that inhibition of net CO_2 assimilation with increasing O_2 partial pressure above the optimum is associated with photorespiration, and that inhibition below the optimum O_2 may be caused by a reduced supply of ATP to the C_4 cycle as a result of inhibition of its production photochemically.

Key-words: *Sorghum bicolor*; *Flaveria trinervia*; *Eleusine indica*; *Amaranthus edulis*; *Eriochloa borumensis*; C_4 photosynthesis; oxygen sensitivity.

Abbreviations: *A*, net CO_2 assimilation rate; C_a, ambient CO_2 partial pressure; C_i, intercellular CO_2 partial pressure; ϕ_{CO_2}, quantum yield of net CO_2 assimilation; ϕ_{PSII}, quantum yield of PSII reaction centres; Q_A, quinone A; Rubisco, ribulose 1,5-bisphosphate carboxylase/oxygenase; VPD, vapour pressure deficit; Θ, O_2 inhibition index.

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INTRODUCTION

C_4 plants, by a complex biochemical process and with the aid of some modifications in leaf anatomy and ultrastructure, have the ability to concentrate CO_2 at the site of ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) in the bundle-sheath cells to a level that has been estimated to exceed atmospheric CO_2 concentrations by 3–20 times (Jenkins, Furbank & Hatch 1989; Dai, Ku & Edwards 1993, 1995; He & Edwards 1996). Three different C_4 subtypes have been identified on the basis of the biochemistry of CO_2 transfer from the atmosphere to the bundle-sheath cells through the C_4 cycle (Hatch 1992). The exact level of CO_2 in the bundle-sheath cells and the kinetic properties of Rubisco will effect the degree of photorespiration that occurs in the bundle-sheath compartment. Earlier studies on O_2 insensitivity of C_4 photosynthesis, by measuring CO_2 uptake at 21 versus 2 kPa O_2, even under low atmospheric levels of CO_2, led to the conclusion that there is little or no photorespiration in C_4 plants because of the elimination, or strong suppression, of the oxygenase reaction of Rubisco (see Edwards & Walker 1983; Edwards, Ku & Monson 1985). Also, analyses on rates of CO_2 fixation versus the true rate of O_2 evolution under varying CO_2 suggest there is little or no photorespiration in C_4 plants (Furbank & Badger 1982; Badger 1985; Edwards & Baker 1993; Peterson 1994). However, studies with glycine metabolism in *Zea mays* (Marek & Stewart 1983) indicate that, under ambient conditions (*c.* 34 Pa CO_2, 20 kPa O_2 and high light), C_4 plants do photorespire. According to rates of incorporation of $^{18}O_2$ into glycolate, rates of photorespiration (velocity of ribulose 1,5-bisphosphate oxygenase) in maize under ambient conditions were estimated to be *c.* 3% of the net CO_2 assimilation rates in mature leaves and 11% in young seedlings (deVeau & Burris 1989). Models of C_4 photosynthesis also predict levels of photorespiration of this magnitude (Jenkins *et al.* 1989) or higher under limiting CO_2 (He & Edwards 1996); and predict that a measurable increase in the net rate of CO_2 assimilation should occur by decreasing O_2 partial pressure

from 20 to 2 kPa as an inverse function of bundle-sheath diffusive resistance (He & Edwards 1996). Because net CO_2 assimilation rates are essentially the same at these two O_2 partial pressures (Edwards *et al.* 1985; Dai *et al.* 1993), it suggests that some different, inhibitory mechanism may be operating to account for the lower-than-expected photosynthetic activity at low O_2 partial pressures. Indeed, in C_4 *Flaveria* species (Ku *et al.* 1983; Dai, Ku & Edwards 1996) and *Zea mays* (Dai *et al.* 1993), both NADP-ME-type C_4 plants, it was reported that net CO_2 assimilation under both ambient (*c.* 34 Pa) and low CO_2 (*c.* 19 Pa), shows a dual response to O_2, with the optimum O_2 partial pressure for photosynthesis occurring between 5 and 10 kPa. These authors suggest that the progressive decrease in photosynthesis above the optimum O_2 level results from increasing RuBP oxygenase activity, as the magnitude of the decrease is enhanced by lowering the CO_2 level. It is not known, however, if the dual response to O_2 is a general phenomenon among C_4 plants including both monocotyledons (monocots) and dicotyledons (dicots) and the different subtypes, if the magnitude of the response is similar, and what may account for the lower photosynthetic activity at low O_2 partial pressures. In the present study, we report, from simultaneous gas exchange and chlorophyll *a* fluorescence analyses, a dual response of CO_2 assimilation to O_2 in the three different C_4 photosynthetic subtypes including monocots and dicots. In addition, we present new evidence to show that, above the optimal O_2 partial pressure, inhibition of photosynthesis is caused by photorespiration, and that inhibition of photosynthesis below the optimal level is associated with an increased state of reduction of the quinone A pool (Q_A), decreased efficiency of open reaction centres, and decreased activity of PSII.

MATERIALS AND METHODS

Plant material and growth conditions

Analyses of the dual effect of O_2 on C_4 photosynthesis were performed on the NADP-ME species *Sorghum bicolor* ssp. *arundinaceum* (Desv.) deWet & Harlan. (monocot) and *Flaveria trinervia* (Spreng) C. Mohr (dicot), on the NAD-ME species *Eleusine indica* ssp. *africana* (Kenn.-O'Byrne) S. Phillips (monocot) and *Amaranthus edulis* Speg. (dicot), and on the PEP-CK species *Eriochloa borumensis* Stapf (monocot; no dicot has been identified in this subtype). Plants were grown from seeds in a commercial soil mixture (2:1:1 peat, moss and vermiculite), in a temperature-controlled glasshouse. Growth temperatures were 28–33 °C in the light and 25 °C in the dark, the maximum photosynthetic photon flux density (PPFD) was 1600 μmol quanta m^{-2} s^{-1} with a 12 h photoperiod, and relative humidity of 30–90%.

Gas exchange

Simultaneous gas exchange and Chl *a* fluorescence measurements were performed on newly expanded leaves of 45–60-day-old plants. Gas exchange rates were measured with a Bingham Interspace Bi-6-dp computer-controlled system (Bingham Interspace, Logan, UT, USA) with a ADC225-MK3 infrared gas analyser (ADC Ltd, Hoddesdon, Herts, UK) at 1000 ± 25 μmol quanta m^{-2} s^{-1}, with leaf temperature of 30·1 ± 0·1 °C and leaf-to-air vapour pressure deficit (VPD) of 19·1 ± 0·1 Pa kPa^{-1} in an open-system mode. Gas exchange rates were calculated according to Zeiger, Farquhar & Cowan (1987). The quantum yield of CO_2 fixation (ϕ_{CO_2}) was calculated as the ratio of net photosynthesis to absorbed light, assuming a leaf absorptivity of 85% for C_4 plants (Oberhuber, Dai & Edwards 1993; Oberhuber & Edwards 1993).

Chlorophyll *a* fluorescence

Chl *a* fluorescence was measured with an OS-500 pulse-amplitude-modulated fluorometer (Opti-Sciences, Inc. Tyngsboro, MA, USA). The fluorescence probe was positioned above the cuvette at an angle of ≈45° to the leaf to avoid shading from the probe. PSII yield was expressed as quantum yield of PSII [$\phi_{PSII} = (F'_m - F_s)/F'_m$], calculated according to Genty, Briantais & Baker (1989). The state of reduction of Q_A, or the fraction of closed reaction centres, was calculated as $1 - q_P$ (Dietz, Schreiber & Heber 1985) and the efficiency of the open centres was calculated as $(F'_m - F_o)/F'_m$(Öquist & Chow 1992). In tests with *F. trinervia* and *S. bicolor* no significant difference was found using F_o (minimal fluorescence of open centres in dark-adapted state) versus F'_o (minimal fluorescence with centres open in light-adapted state) in calculating the yield of open centres.

Statistical analysis

All measurements are shown as the mean of four to eight independent replicates at three different ambient CO_2 (C_a) levels: 9·3 Pa, 32·6 Pa and 83·7 Pa on a factorial design. Experiments were performed at a given C_a level by decreasing O_2 from high to low levels. Statistical analysis for each CO_2 level was done by ANOVA and the interaction of CO_2 with O_2 was analysed by a two-way ANOVA. Fisher's LSD at $\alpha = 0{\cdot}05$ is given for each CO_2 level and for the interaction of $CO_2 \times O_2$.

RESULTS

NADP-ME subtype

The dual effect of O_2 on net CO_2 assimilation (A) for the NADP-ME monocot *Sorghum bicolor* at three different CO_2 levels is shown in Fig. 1a. The maximum net CO_2 assimilation rates (A) occurred at *c.* 5 kPa O_2. Above this O_2 partial pressure there is a linear decrease of photosynthetic rate while below the optimum the decrease in A is much more pronounced. The leaf-to-leaf variation results in an O_2 effect of low statistical significance ($P = 0{\cdot}06$). However, when the leaf-to-leaf variance is eliminated by

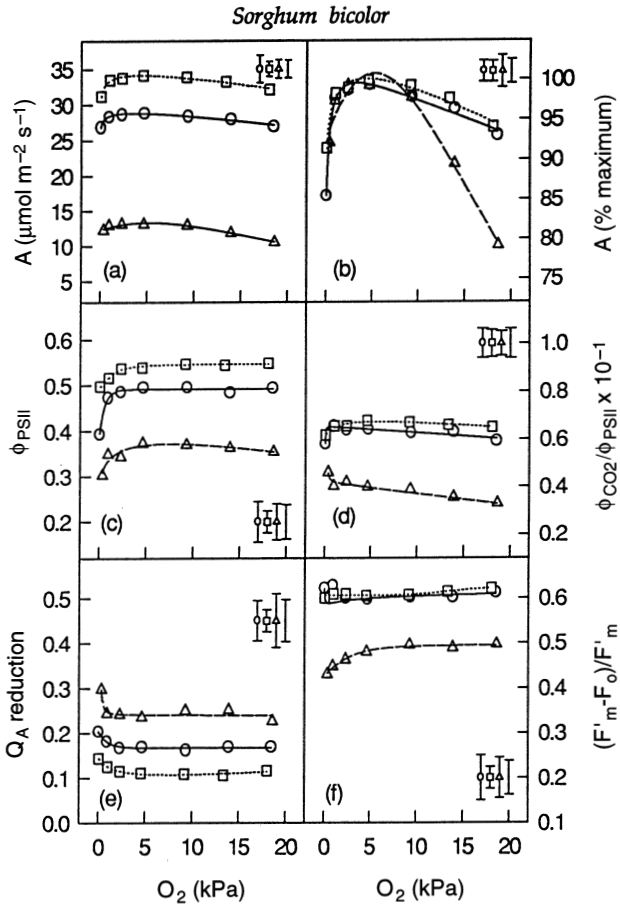

Figure 1. Oxygen responses of (a) *A*, (b) *A* as percentage of the maximum, (c) quantum yield of PSII (ϕ_{PSII}), (d) electron-use efficiency for CO_2 assimilation (ϕ_{CO2}/ϕ_{PSII}), (e) Q_A reduction state, and (f) efficiency of the PSII open centres ($F'_m - F_o$)/F'_m in the monocotyledon *Sorghum bicolor* (NADP-ME). Measurements of O_2 response were performed at C_a values of 9·3 (△), 32·6 (○) and 83·7 Pa (□) with corresponding C_i of 4·2 ± 0·1, 11·4 ± 0·2 and 25·7 ± 1·3 Pa. Error bars with different symbols represent the Fisher least significant differences (LSDs) at $\alpha = 0{\cdot}05$ for the different C_i; error bar without symbols represents the LSD at $\alpha = 0{\cdot}05$ for the $O_2 \times C_i$ interaction.

expressing the rate of CO_2 assimilation as percentage of maximum *A* (Fig. 1b), the O_2 effect is highly significant ($P < 0{\cdot}01$). Furthermore, the effect of O_2 on *A* expressed as a percentage of the maximum CO_2 assimilation rate is dependent on the C_a level ($P < 0{\cdot}01$ for interaction between O_2 and C_a), but not on the absolute magnitude of *A* ($P > 0{\cdot}05$). The C_a level did not change the shape of the O_2 inhibition (as suggested by the non–significant interaction between *A* and C_a) but did change its magnitude (as suggested by the significant interaction of the relative *A* and C_a). At low C_a (9·3 Pa) the inhibition of *A* by atmospheric O_2 (18·6 kPa) compared to the rate at the optimum O_2 level was greater than 20%, while at atmospheric levels of CO_2 (C_a = 32·6 Pa) this inhibition was only *c.* 7% of the maximum *A*. Furthermore, C_a at 83·7 Pa (*c.* 2·5 times normal ambient levels) did not produce a significant decrease of the O_2 inhibition of *A* (Fig. 1b), as compared to the atmospheric levels. The O_2 inhibition index (Θ), which is the percentage inhibition of ***A*** per kPa increase in O_2 (after Dai *et al.* 1995) decreased progressively from 1·48 to 0·45 and 0·32 at C_a of 9·3, 32·6 and 83·7 Pa, respectively (Fig. 2a). There was no effect of O_2 on the quantum yield of electron transport through PSII (ϕ_{PSII}) as O_2 was increased above the optimum at a given C_a value (Fig. 1c). However, when comparing the changes in quantum yield of CO_2 fixation (ϕ_{CO_2}) with the ϕ_{PSII}, there was a linear decrease of ϕ_{CO_2}/ϕ_{PSII} with decreasing O_2, which was most pronounced under low CO_2 (Fig. 1d). This indicates that efficiency of electron use is lower at low O_2 partial pressures, especially in combination with low CO_2. At O_2 partial pressures below the optimum for *A*, there was a considerable decrease in PSII activity (*c.* 20% at 32·6 Pa C_a, Fig. 1c) that was associated with the over-reduction of the quinone A (Q_A) pool (Fig. 1e). Furthermore, at low C_a a reduction of the efficiency of the open centres for electron transport, as measured by ($F'_m - F_o$)/F'_m (Fig. 1f), contributed to the decrease of PSII activity, while at ambient and high CO_2 this effect was not significant.

For the NADP-ME dicot *Flaveria trinervia* (Fig. 3) (see also Ku *et al.* 1983), the optimum O_2 partial pressure for *A* also occurred between 5 and 10 kPa. At ambient levels of O_2 (*c.* 18·6 kPa) the O_2 inhibition of *A*, relative to its maximum, was *c.* 16% at the low C_a (9·3 Pa), 10% at normal atmospheric CO_2 (32·6 Pa) and less than 5% at high ambient CO_2 (83·7 Pa). Consistently, the Θ decreased from 1·12 to 0·72 and 0·26, respectively (Fig. 2a). A smaller leaf-to-leaf variance accounts for the significant effect of O_2 and

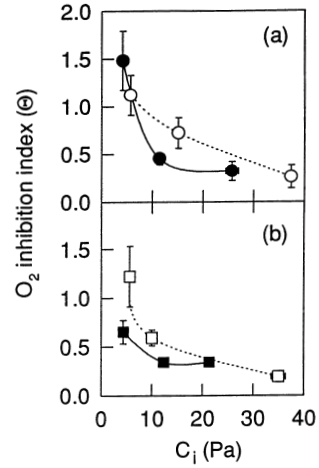

Figure 2. The O_2 inhibition index (Θ) as a function of C_i in (a) the NADP-ME types *Sorghum bicolor* (●) and *Flaveria trinervia* (○) and (b) the NAD-ME types *Eleusine indica* (■) and *Amaranthus edulis* (□). For each species the Θ was calculated from the linear O_2 inhibition of photosynthesis at supraoptimum O_2 pressures measured at C_a of 9·3, 32·6 and 83·7 Pa. Bars are the standard errors. Standard errors are smaller than the size of the symbols whenever error bars are not shown.

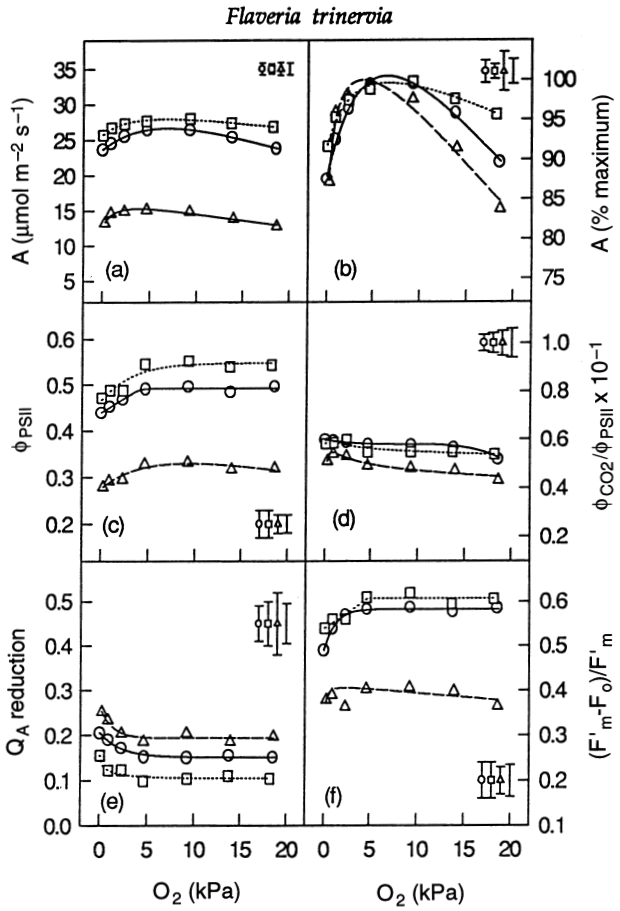

Figure 3. Oxygen responses of (a) *A*, (b) *A* as percentage of the maximum, (c) quantum yield of PSII (ϕ_{PSII}), (d) electron-use efficiency for CO_2 assimilation (ϕ_{CO_2}/ϕ_{PSII}), (e) Q_A reduction state, and (f) efficiency of the PSII open centres ($F'_m - F_o$)/F'_m in the dicotyledon *Flaveria trinervia* (NADP-ME). Measurements of O_2 response were performed at C_a values of 9·3 (△), 32·6 (○) and 83·7 Pa (□)with corresponding C_i of 5·7 ± 0·2, 15·1 ± 0·3 and 37·4 ± 0·5 Pa. Error bars with different symbols represent the Fisher LSDs at $\alpha = 0{\cdot}05$ for the different C_i; error bar without symbols represents the LSD at $\alpha = 0{\cdot}05$ for the $O_2 \times C_i$ interaction.

C_a on *A* ($P < 0{\cdot}01$), although there was no significant interaction between O_2 and C_a ($P > 0{\cdot}05$) (Fig. 3a). There was a strong effect of O_2 and C_a on relative *A* ($P < 0{\cdot}01$, Fig. 3b) and a significant interaction between O_2 and C_a ($P < 0{\cdot}01$). Again, the level of C_a did not change the form of the *A* response to O_2, but rather its magnitude. The ϕ_{CO_2}/ϕ_{PSII} ratio decreased with increasing O_2 (Fig. 3d). Below the optimum O_2 level for *A*, there was a reduction of ϕ_{PSII} ($P < 0{\cdot}01$) that can be accounted for by both a decrease in the fraction of open centres (Fig. 3e) and the decrease in their efficiency at *c*. 0 kPa O_2 (but not at a significant level) (Fig. 3f).

Relative to *S. bicolor* (a monocot), *F. trinervia* (a dicot) shows a higher O_2 sensitivity at ambient CO_2 (32·6 Pa) (Fig. 2a). At a given C_i, the Θ of *F. trinervia* was higher than that of sorghum. Furthermore, increasing the C_a level from ambient to about three times ambient level did not decrease the O_2 inhibition of *A* in *Sorghum*, while in *Flaveria* this inhibition was reduced by more than 50% (Fig. 1b and 3b, Fig. 2).

NAD-ME subtype

The dual effect of O_2 on net CO_2 assimilation was also observed in the NAD-ME monocot *Eleusine indica*. Again, because of the leaf-to-leaf variation this effect was not statistically significant ($P = 0{\cdot}06$) (Fig. 4a). However, when the individual leaf response is averaged (Fig. 4b), a statistically significant O_2 effect, as well as its interaction with the C_a level, is observed ($P < 0{\cdot}01$). The C_a level did not change the form of the response of *A* to O_2 but rather its magnitude. At a low C_a (9·3 Pa) and atmospheric O_2, there was 10% inhibition of *A* compared to the maximum *A*

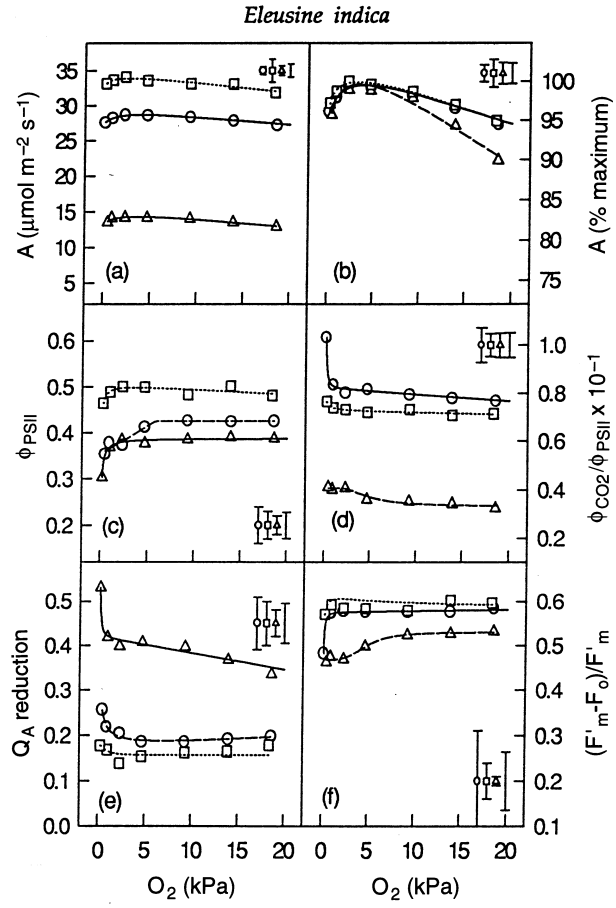

Figure 4. Oxygen responses of (a) *A*, (b) *A* as percentage of the maximum, (c) quantum yield of PSII (ϕ_{PSII}), (d) electron-use efficiency for CO_2 assimilation (ϕ_{CO_2}/ϕ_{PSII}), (e) Q_A reduction state, and (f) efficiency of the PSII open centres ($F'_m - F_o$)/F'_m in the monocot *Eleusine indica* (NAD-ME). Measurements of O_2 response were performed at C_a values of 9·3 (△), 32·6 (○) and 83·7 Pa (□) with corresponding C_i of 4·3 ± 0·1, 12·3 ± 0·3 and 21·3 ± 0·9 Pa. Error bars with different symbols represent the Fisher LSDs at $\alpha = 0{\cdot}05$ for the different C_i; error bar without symbols represents the LSD at $\alpha = 0{\cdot}05$ for the $O_2 \times C_i$ interaction.

observed at 2·3 kPa O_2. Increasing C_a to near-atmospheric levels (32·6 Pa) decreased the inhibition of A to 5%, and a further increase of C_a to 83·7 Pa did not produce a significant reduction in O_2 inhibition. This trend is reflected in the Θ (Fig. 2b). The quantum yield of PSII reaction centres was almost constant until exposure to low O_2 partial pressures, when ϕ_{PSII} decreased ($P < 0{\cdot}05$) (Fig. 4c). This suggests a reduction in PSII activity that can be explained by both the reduction of the proportion of open centres (Fig. 4e) and a decrease in their efficiency in light harvesting (Fig. 4f). In addition, there was a decline in the ϕ_{CO_2}/ϕ_{PSII} (quasi-significant, $P = 0{\cdot}09$) ratio over the entire O_2 range (Fig. 4d). For the NAD-ME dicot. *Amaranthus edulis*, the leaf-to-leaf variation accounts for the lack of significance in the O_2 effect on A (Fig. 5a). However, if the O_2 effect, on an individual leaf basis, is presented as a percentage of the maximum A (Fig. 5b), there is a clear effect of O_2 on A ($P < 0{\cdot}01$). The maximum A occurred at *c.* 5 kPa O_2. At low C_a (9·3 Pa), atmospheric O_2 caused *c.* 20% inhibition of A, while at around ambient CO_2 levels the O_2 inhibition was about 6%. Similar estimates of O_2 inhibition of photosynthesis at ambient CO_2 partial pressures were also reported in studies on the rate of NH_3 production by photorespiration in wildtype *A. edulis* (Lacuesta *et al.* 1997). In the present study, increasing the ambient CO_2 to 83·7 Pa caused a further decrease in the inhibition by O_2. The decline in PSII yield (Fig. 5c) can be explained by a decline in both the proportion of open centres (Fig. 5e) and the light-harvesting efficiency of open centres (Fig. 5f) with decreasing O_2 below the optimum levels. Furthermore, the ratio of ϕ_{CO_2}/ϕ_{PSII} decreased with increasing O_2 (Fig. 5d).

As observed in the NADP-ME-type species, the O_2 sensitivity of the dicot *A. edulis* was greater than that of the monocot *E. indica*. In *A. edulis*, Θ further decreased with the increase in C_a from ambient to around three times ambient level, while in the monocot *E. indica* no further reduction of the O_2 inhibition of A was observed by increasing C_a to supra-atmospheric levels (Fig. 3b and 4b, Fig. 2b). These results, taken together, suggest that within each subtype, dicotyledenous C_4 plants may be more sensitive than monocots to O_2 inhibition of photosynthesis.

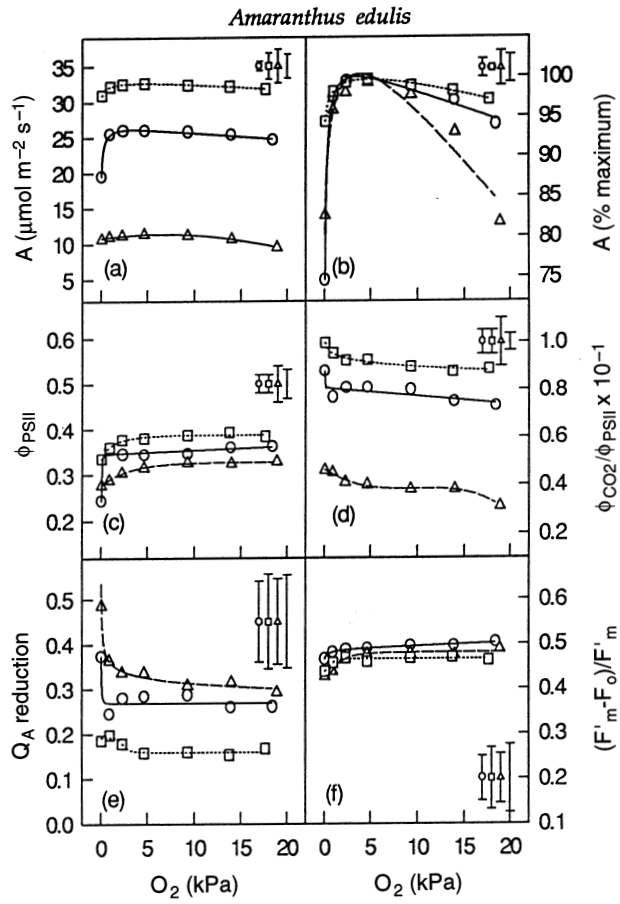

Figure 5. Oxygen responses of (a) A, (b) A as percentage of the maximum, (c) quantum yield of PSII (ϕ_{PSII}), (d) electron-use efficiency for CO_2 assimilation (ϕ_{CO_2}/ϕ_{PSII}), (e) Q_A reduction state, and (f) efficiency of the PSII open centres $(F'_m - F_o)/F'_m$ in the dicot *Amaranthus edulis* (NADP-ME). Measurements of O_2 response were performed at C_a values of 9·3 9·3 (△), 32·6 (○) and 83·7 Pa (□) with corresponding C_i of 5·6 ± 0·3, 10·0 ± 0·4 and 35·0 ± 1·4 Pa. Error bars with different symbols represent the Fisher LSDs at $\alpha = 0{\cdot}05$ for the different C_i; error bar without symbols represents the LSD at $\alpha = 0{\cdot}05$ for the $O_2 \times C_i$ interaction.

PEP-CK subtype

For the PEP-CK subtype *Eriochloa borumensis*, the O_2 effect was statistically significant ($P < 0{\cdot}01$) despite the leaf-to-leaf variation (Fig. 6a). Again, it is not the response pattern of A to O_2, but rather its magnitude, that is dependent on the C_a level ($P < 0{\cdot}001$) (Fig. 6b). At low C_a (9·3 kPa), the inhibition by atmospheric O_2 was *c.* 20% of the maximum A, while at around atmospheric C_a this inhibition was reduced to *c.* 10%. A further increase in the ambient CO_2 to 83·6 Pa reduced the inhibition to less than 3%. A similar trend is also observed for the changes in Θ with respect to C_a; Θ decreased from 1·3 at 9·3 Pa CO_2, 0·53 at 32·6 Pa CO_2 to 0·15 at 83·7 Pa CO_2. The increases in C_a effectively eliminated the inhibition of linear electron flow to CO_2 fixation, as confirmed by the lack of response of ϕ_{CO_2}/ϕ_{PSII} to increasing O_2 (Fig. 6d). At O_2 partial pressures below the optimum (*c.* 5 kPa), the decrease in ϕ_{PSII} (Fig. 6c) can be accounted for by the decrease in the proportion of open centres (Fig. 6e), and by the decrease in the efficiency of the remaining open centres (Fig. 6f).

DISCUSSION

As net CO_2 assimilation rates are essentially the same at *c.* 20 and 2 kPa O_2, C_4 photosynthesis has been previously characterized as being O_2 insensitive (see Edwards & Walker 1983; Edwards *et al.* 1985). However, some evidence for O_2 sensitivity of photosynthesis has been reported in *Flaveria* and maize, NADP-ME-type C_4 plants (Ku *et al.* 1983; Dai *et al.* 1993, 1995, 1996) although the degree of significance and the occurrence of this effect

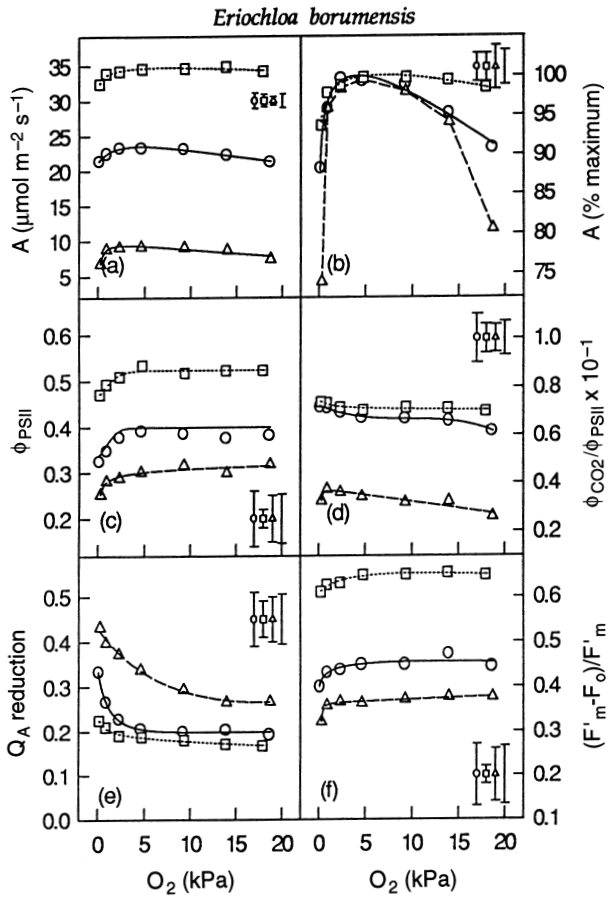

Figure 6. Oxygen responses of (a) *A*, (b) *A* as percentage of the maximum, (c) quantum yield of PSII (ϕ_{PSII}), (d) electron-use efficiency for CO_2 assimilation (ϕ_{CO_2}/ϕ_{PSII}), (e) Q_A reduction state, and (f) efficiency of the PSII open centres $(F'_m - F_o)/F'_m$ in the monocot *Eriochloa borumensis* (PEP-CK). Measurements of O_2 response were performed at C_a values of 9·3 (△), 32·6 (○) and 83·7 Pa (□) with corresponding C_i of 5·5 ± 0·1, 16·5 ± 0·5 and 23·1 ± 1·3 Pa. Error bars with different symbols represent the Fisher LSDs at $\alpha = 0{\cdot}05$ for the different C_i; error bar without symbols represents the LSD at $\alpha = 0{\cdot}05$ for the $O_2 \times C_i$ interaction.

among C_4 subtypes was uncertain. In this study, we show that a dual effect of O_2 on the net CO_2 assimilation activity is present in all three C_4 biochemical subtypes (panel a in Figs 1 & 3–6). When the response of *A* to varying O_2 was measured over a range from near zero to ambient levels, a dual effect of O_2 on *A* is revealed: O_2 stimulates C_4 photosynthesis from 0 to 5–10 kPa while, above this optimum, O_2 is inhibitory for net CO_2 assimilation. Furthermore, this effect is highly significant when the leaf-to-leaf genotypic variation is eliminated by expressing the data on a relative term (panel b in Figs 1 & 3–6).

The progressive decrease of *A* by supraoptimum levels of O_2, especially at low CO_2, provides evidence for inhibition of C_4 photosynthesis by photorespiration. The continuous decline in the PSII electron-use efficiency for CO_2 assimilation (ϕ_{CO_2}/ϕ_{PSII}) from low to high O_2, which is exacerbated under limiting CO_2 (panel d in Figs 1 & 3–6), is consistent with an increase in photorespiration as an alternative electron sink with increasing O_2. The decrease in the ϕ_{CO_2}/ϕ_{PSII} ratio, which occurred over the entire O_2 range, also suggests that in C_4 plants photorespiration increases with increasing O_2, as predicted from modelling O_2 inhibition of C_4 photosynthesis versus O_2 partial pressure (He & Edwards 1996). It is well established that, in C_3 plants, a decrease in CO_2, or an increase in O_2, decreases the ratio ϕ_{CO_2}/ϕ_{PSII} because of an increase in photorespiration (Cornic & Briantais 1991; Krall & Edwards 1992). The linear increase in *A* with decreasing O_2, which is predicted from modelling, is apparently masked by low O_2 inhibition of *A* and by decreased ϕ_{PSII} by a separate process as discussed below. Finally, the decrease in ϕ_{CO_2}/ϕ_{PSII} under low CO_2 (9·3 Pa) in all species suggests there is a substantial increase in photorespiration in bundle-sheath cells because of a decrease in bundle-sheath CO_2 concentration.

Under suboptimum levels of O_2, inhibition of *A* in the C_4 species tested is accompanied by inhibition of ϕ_{PSII} (panel c in Figs 1 & 3–6). Inhibition of ϕ_{PSII} indicates a decrease in PSII activity and whole chain electron flow; analyses indicate that this decrease is due to a reduction of the fraction of open PSII centres (increased reduction of the Q_A pool) (panel e in Figs 1–6) as well as some decrease in efficiency of electron transport for CO_2 assimilation by the remaining open centres (panel f in Figs 1–6). Reduction of ϕ_{PSII} activity, over-reduction of Q_A and decrease in *A* at low levels of O_2 (< 2%) have been observed previously in the C_3 plants *Spinacea oleracea*, *Asarum europaeum* and *Helianthus annuus* by Dietz *et al.* (1985). An inhibition of photosynthesis at low O_2 has also been reported in three photosynthetic microorganisms: *Anacystis nidulans* (Miyachi & Okabe 1976); in a cryptomonad, *Chroomonas* sp. (Suzuki & Ikawa 1984a,b), and in the diatom *Nitzschia rattneri* (Suzuki & Ikawa 1993). The inhibition of photosynthesis in the absence of O_2 in spinach (C_3 species) (Ziem-Hanck & Heber 1980) and *Chroomonas* sp. (Suzuki & Ikawa 1984b) was suggested to result from a deficiency in ATP caused by over-reduction of electron carriers limiting cyclic electron flow.

The inhibition of C_4 photosynthesis under low O_2 may occur because of a deficiency in ATP caused by over-reduction of electron carriers limiting cyclic electron flow, as suggested from studies under anaerobic conditions with other photosynthetic types (Ziem-Hanck & Heber 1980; Suzuki & Ikawa 1984b; Raghavendra, Zu-Hua & Heber 1993), or a deficiency in production of ATP by pseudocyclic electron flow. Cyclic or pseudocyclic photophosphorylation or both are considered to be necessary to meet the ATP demands of the CO_2-concentrating mechanism in C_4 plants (Edwards & Walker 1983; Hatch 1987; Furbank, Jenkins & Hatch 1990).

There is also evidence that the requirement for O_2 is greater in C_4 plants because in C_3 plants the optimum partial pressure of O_2 for maximum photosynthetic rates is lower, as reported by Dai *et al.* (1996) with C_3, C_3–C_4, C_4-like and C_4 *Flaveria* species. Also, the stronger inhibition of photosynthesis by anaerobic conditions during induc-

tion of photosynthesis than under steady-state photosynthesis in spinach (Ziem-Hanck & Heber 1980) may indicate a stronger requirement for cyclic or pseudocyclic photophosphorylation during the induction process for C_3 photosynthesis.

Besides photosynthesis possibly being limited by the supply of ATP under low O_2 as discussed above, the over-reduction of Q_A was most pronounced under low O_2 and low CO_2 (panel e in Figs 1 & 3–6), which indicates that the capacity for down-regulation of PSII is impaired under these conditions. This could occur by inhibition of membrane energization under low O_2 by inhibiting cyclic or pseudocylic electron flow (see Horton, Ruban & Walters 1996; Demmig-Adams, Gilmore & Adams III 1996). A combination of low utilization of energy for CO_2 assimilation under low CO_2, and low membrane energization under low O_2 because of inhibition of cyclic and pseudocyclic electron flow may account for the higher degree of reduction of Q_A. There is also a small decrease in efficiency of open PSII centres under low O_2. The basis for this is unknown although very low O_2 might affect the interconversion of violaxanthin/zeaxanthin (the O_2-dependent epoxidase) or light harvesting complex II state II transitions.

Another possible basis for inhibition of photosynthesis at very low O_2 might be that CO_2 assimilation has some dependence on mitochondrial respiration. Mitochondrial respiration may supply ATP for conversion of triose-P to sucrose in the cytosol (Kromer & Heldt 1991). If low O_2 were to cause inhibition of mitochondrial respiration, this could inhibit sucrose synthesis, which in turn could cause a feedback inhibition of the rate of photosynthesis. However, the increase in reduction of Q_A under low O_2 is prominent under low CO_2, where the demand for ATP for synthesis of sucrose is limited. There is also a requirement for mitochondrial respiration in bundle-sheath cells of PEP-CK-type C_4 species to supply ATP for the decarboxylation of oxaloacetate by PEP carboxykinase (Hatch, Agostino & Burnell 1988). However, there is no noticeable difference in the change in the reduction state of Q_A with varying O_2 between this (Fig. 6) and other subtypes. It may be that the O_2 generated from PSII activity by bundle-sheath chloroplasts can meet the mitochondrial needs for O_2 even under very low atmospheric levels of O_2.

The optimum O_2 level for CO_2 assimilation in C_4 plants occurs between 2 and 10 kPa, being generally lower at low C_a than at atmospheric or high C_a. The lower O_2 requirement at low C_a might reflect a lower ATP demand by the CO_2-concentrating mechanism at lower intercellular CO_2 partial pressure or a greater relative contribution of ATP from whole-chain electron transport to NADP.

Within NADP-ME or NAD-ME subtype, the dicot species tend to have a higher inhibition of photosynthesis by supraoptimum levels of O_2 than do the monocot species under limiting C_a values (over the range 9·3–33 Pa) (Fig. 2). This suggests that under a given limiting level of CO_2 the dicots have a lower bundle-sheath CO_2 concentration, and a higher level of photorespiration. In the monocots, there is little effect of increasing CO_2 above ambient levels on the inhibition of A by supraoptimum levels of O_2 or on the O_2 inhibition index, while in the dicots changing CO_2 from ambient (32·6 Pa) to high CO_2 (83·7 Pa) substantially reduces the O_2 inhibitory effect. Perhaps, at atmospheric or lower levels of CO_2, monocots are more efficient than dicots at keeping high CO_2 at the carboxylation site (the bundle-sheath cells). Modelling of C_4 photosynthesis indicates that inhibition of photosynthesis by O_2 is dependent on C_a, bundle-sheath resistance to CO_2 diffusion and the capacity of the C_4 cycle relative to that of the C_3 cycle, all of which can affect the bundle-sheath CO_2 level (He & Edwards 1996). The higher O_2 sensitivity in the malic-enzyme-type C_4 dicots may reflect a difference in the supply of CO_2 to the C_4 cycle or the capacity of the CO_2-concentrating mechanism, or a higher degree of bundle-sheath leakiness in the dicots. It has been suggested that C_4 dicots may have higher bundle-sheath leakage than monocots because of a higher relative apoplastic surface area of bundle sheath cells and lack of suberized lamella in the walls of the bundle-sheath cells (Ehleringer & Pearcy 1983). On average, NADP-ME dicots were reported to have a lower maximum quantum yield for CO_2 fixation than NADP-ME monocots; as were NAD-ME dicots compared with NAD-ME monocots (Ehleringer & Pearcy 1983). Higher leakage from bundle-sheath cells could lower the quantum yield of CO_2 fixation as a result of increased utilization of ATP through overcycling in the C_4 pathway. However, there is no agreement on estimates of leakiness between monocots and dicots as these vary depending on the method used. For example, Henderson, von Caemmerer & Farquhar (1992), using long-term ^{13}C discrimination analysis, estimated higher bundle-sheath leakiness for dicots than for monocots, and high leakiness of C_4 dicot *Flaveria* species was reported by Hatch, Agostino & Jenkins (1995). However, in a short-term, online analysis of ^{13}C discrimination, the estimated leakiness of both monocots and dicots was the same (Henderson *et al.* 1992). Therefore further comparative studies will be needed to determine the basis for the apparent difference between monocots and dicots in O_2 inhibition.

In summary, the dual effect of O_2 on photosynthesis is observed in all three C_4 subtypes; it is observed in both C_4 monocots and dicots and it occurs whether or not O_2 is generated in bundle-sheath chloroplasts by PSII activity (the bundle-sheath chloroplasts of NADP-ME subtypes are known to have deficient PSII activity whereas the other subgroups have normal PSII activity in bundle-sheath chloroplasts; see Edwards & Walker 1983). Within each of the two malic-enzyme C_4 subtypes, monocots may be more efficient than dicots in maintaining a higher CO_2 level in the bundle-sheath cells and thus photorespire less. A common feature of C_4 plants may be a requirement for a low level of O_2 for function of cyclic/pseudocyclic electron flow, to meet the extra requirement for ATP for operating the C_4 pathway, and a low level of inhibition of photosynthesis by atmospheric levels of O_2 through photorespiration.

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REFERENCES

Badger M.R. (1985) Photosynthetic oxygen exchange. *Annual Review of Plant Physiology* **36**, 27–53.

Cornic G. & Briantais J.M. (1991) Partitioning of photosynthetic electron flow between CO_2 and O_2 reduction in a C_3 leaf (*Phaseolus vulgaris* L.) at different CO_2 concentrations and during drought stress. *Planta* **183**, 178–184.

Dai Z., Ku M.S.B. & Edwards G.E. (1993) C_4 Photosynthesis: The CO_2-concentrating mechanism and photorespiration. *Plant Physiology* **103**, 83–90.

Dai Z., Ku M.S.B. & Edwards G.E. (1995) C_4 photosynthesis: the effects of leaf development on the CO_2-concentrating mechanism and photorespiration in maize. *Plant Physiology* **107**, 815–825.

Dai Z., Ku M.S.B. & Edwards G.E. (1996) Oxygen sensitivity of photosynthesis and photorespiration in different photosynthetic types in the genus *Flaveria*. *Planta* **198**, 563–571.

Demmig-Adams B., Gilmore A.M. & Adams W.W. III (1996) In vivo functions of carotenoids in higher plants. *Federation of American Societies for Experimental Biology Journal* **10**, 403–412.

deVeau E.J. & Burris J.E. (1989) Photorespiratory rates in wheat and maize as determined by ^{18}O-labelling. *Plant Physiology* **90**, 500–511.

Dietz K.-J., Schreiber U. & Heber U. (1985) The relationship between the redox state of Q_A and photosynthesis in leaves at various carbon-dioxide, oxygen and light regimes. *Planta* **166**, 219–226.

Edwards G.E. & Walker G.A. (1983) *C_3, C_4: Mechanisms, and Cellular and Environmental Regulation, of Photosynthesis*. Blackwell Scientific, Oxford.

Edwards G., Ku M.S.B. & Monson R.K. (1985) C_4 photosynthesis and its regulation. In *Photosynthetic Mechanisms and the Environment* (ed. J. Barber & N. R Baker), pp. 287–328. Elsevier Science, Amsterdam.

Edwards G.E. & Baker N.R. (1993) Can CO_2 assimilation in maize leaves be predicted accurately from chlorophyll fluorescence analysis? *Photosynthesis Research* **37**, 89–102.

Ehleringer J. & Pearcy R.W. (1983) Variation in quantum yield for CO_2 uptake among C_3 and C_4 plants. *Plant Physiology* **73**, 555–559.

Furbank R.T. & Badger M.R. (1982) Photosynthetic oxygen exchange in attached leaves of C_4 monocotyledons. *Australian Journal of Plant Physiology* **9**, 553–558.

Furbank R.T., Jenkins C.L.D. & Hatch M.D. (1990) C_4 photosynthesis: quantum requirement, C_4 acid overcycling and Q-cycle involvement. *Australian Journal of Plant Physiology* **17**, 1–7.

Genty B., Briantais J.-M. & Baker N.R. (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochemistry and Biophysics Acta* **990**, 87–92.

Hatch M.D. (1987) C_4 photosynthesis: a unique blend of modified biochemistry, anatomy and ultrastructure. *Biochemistry and Biophysics Acta* **895**, 81–106.

Hatch M.D. (1992) I can't believe my luck. *Photosynthesis Research* **33**, 1–14.

Hatch M.D., Agostino A. & Burnell J.N. (1988) Photosynthesis in phospho*enol*pyruvate carboxykinase-type C_4 plants. *Archives of Biochemistry and Biophysics* **261**, 357–367.

Hatch M.D., Agostino A. & Jenkins C.L.D. (1995) Measurement of the leakage of CO_2 from the bundle sheath cells of leaves during C_4 photosynthesis. *Plant Physiology* **108**, 173–181.

He D. & Edwards G.E. (1996) Estimation of diffusive resistance of bundle sheath cells to CO_2 from modeling of C_4 photosynthesis. *Photosynthesis Research* **49**, 195–208.

Henderson S.A., von Caemmerer S. & Farquhar G.D. (1992) Short-term measurements of carbon isotope discrimination in several C_4 species. *Australian Journal of Plant Physiology* **19**, 263–85.

Horton P., Ruban A.V. & Walters R.G. (1996) Regulation of light harvesting in green plants. *Annual Review of Plant Physiology and Plant Molecular Biology* **47**, 655–684.

Jenkins C.L.D., Furbank R.T. & Hatch M.D. (1989) Mechanism of C_4 photosynthesis. A model describing the inorganic carbon pool in bundle sheath cells. *Plant Physiology* **91**, 1372–1381.

Krall J. & Edwards G.E. (1992) Quantum yield of photosystem II and of CO_2 fixation in higher plants. *Physiologia Plantarum* **86**, 180–187.

Krömer S. & Heldt H.W. (1991) On the role of the mitochondrial oxidative phosphorylation in photosynthesis metabolism as studied by the effect of oligomycin on photosynthesis in protoplasts and leaves of barley (*Hordeum vulgare*). *Plant Physiology* **95**, 1270–1276.

Ku M.S.B., Monson R.K., Littlejohn R.O. Jr., Nakamoto H., Fisher D.B. & Edwards G.E. (1983) Photosynthetic characteristics of C_3-C_4 intermediate *Flaveria* species. I. Leaf anatomy, photosynthetic responses to O_2 and CO_2, and activities of key enzymes in the C_3 and C_4 pathways. *Plant Physiology* **71**, 944–948.

Lacuesta M., Dever L.V., Muñoz-Rueda A. & Lea P.J. (1997) A study of photorespiratory ammonia in the C_4 plant *Amaranthus edulis*, using mutants with altered photosynthetic capacities. *Physiologia Plantarum* **99**, 447–455.

Marek L.F. & Stewart C.R. (1983) Photorespiratory glycine metabolism in corn leaf discs. *Plant Physiology* **73**, 118–120.

Miyachi S. & Okabe K. (1976) Oxygen enhancement of photosynthesis in *Anacystis nidulans* cells. *Plant Cell Physiology* **17**, 973–986.

Oberhuber W. & Edwards G.E. (1993) Temperature dependence of the linkage of quantum yield of photosystem II to CO_2 fixation in C_4 and C_3 plants. *Plant Physiology* **101**, 507–512.

Oberhuber W., Dai Z.-Y., Edwards G.E (1993) Light dependence of quantum yields of photosystem II and CO_2 fixation in C_3 and C_4 plants. *Photosynthesis Research* **35**, 265–274.

Öquist G. & Chow W.S. (1992) On the relationship between the quantum yield of photosystem II electron transport, as determined by chlorophyll fluorescence and the quantum yield of CO_2-dependent O_2 evolution. *Photosynthesis Research* **33**, 51–62.

Peterson R.B. (1994) Regulation of electron transport in photosystem I and II in C_3, C_3-C_4, and C_4 species of *Panicum* in response to changing irradiance and O_2 levels. *Plant Physiology* **105**, 349–356.

Raghavendra A.S., Zu-Hua Y. & Heber U. (1993) Light-dependent pH changes in leaves of the C_4 plants. Comparison of the pH responses to carbon dioxide and oxygen with that of C_3 plants. *Planta* **189**, 278–287.

Suzuki K. & Ikawa T. (1993) Oxygen enhancement of photosynthetic $^{14}CO_2$ fixation in a freshwater diatom *Nitzschia ruttneri*. *Japanese Journal of Phycology* **41**, 19–28.

Suzuki K. & Ikawa T. (1984a) Effect of oxygen on photosynthetic $^{14}CO_2$ fixation in *Chroomonas* sp. (Cryptophyta).I. Some characteristics of the oxygen effect. *Plant Cell Physiology* **25**, 367–375.

Suzuki K. & Ikawa T. (1984b) Effect of oxygen on photosynthetic $^{14}CO_2$ fixation in *Chroomonas* sp. (Cryptophyta) II. Effects of inhibitors, uncouplers and an artificial electron mediator on the inhibition of $^{14}CO_2$ fixation by anaerobiosis. *Plant Cell Physiology* **25**, 377–384.

Zeiger E., Farquhar G.D. & Cowan I.R. (1987) *Stomatal Function*. Stanford University Press, Stanford.

Ziem-Hanck U. & Heber U. (1980) Oxygen requirement of CO_2 assimilation. *Biochemistry and Biophysics Acta* **591**, 266–274.

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