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Oxygen sensitivity of photosynthesis and photorespiration in different photosynthetic types in the genus *Flaveria*

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Abstract. Two major indicators were used to access the degree of photorespiration in various photosynthetic types of *Flaveria* species (C_3, C_3–C_4, C_4-like, and C_4): the O_2 inhibition of photosynthesis measured above the O_2 partial pressure which gives a maximum rate, and O_2- and light-dependent whole-chain electron flow measured at the CO_2 compensation point (Γ). The optimum level of O_2 for maximum photosynthetic rates under atmospheric levels of CO_2 (34 Pa) was lower in C_3 and C_3–C_4 species (ca. 2 kPa) than in C_4-like and C_4 species (ca. 9 kPa). Increasing O_2 partial pressures from the optimum for photosynthesis up to normal atmospheric levels (ca. 20 kPa) caused an inhibition of photosynthesis which was more severe under lower CO_2. This inhibition was calculated as the O_2 inhibition index (Θ_A, the percentage inhibition of photosynthesis per kPa increase in O_2). From measurements of 18 *Flaveria* species at atmospheric CO_2, the Θ_A values decreased from C_3 (1.9–2.1) to C_3–C_4 (1.2–1.6), C_4-like (0.6–0.8) and C_4 species (0.3–0.4), indicating a progressive decrease in apparent photorespiration in this series. With increasing irradiance at Γ under atmospheric levels of O_2, and increasing O_2 partial pressure at 300 μmol quanta $\cdot m^{-2} \cdot s^{-1}$, there was a similar increase in the rate of O_2 evolution associated with whole-chain electron flow (J_{O_2}, calculated from chlorophyll fluorescence analysis) in the C_3 and C_3–C_4 species compared to a much lower rate in the C_4-like and C_4 species. The results indicate that there is substantial O_2-dependent electron flow in C_3 and C_3–C_4 species, reflecting a high level of photorespiration compared to that in C_4-like and C_4 species. Consistent with these results, there was a significant decrease in Γ from C_3 (6–6.2 Pa) to C_3–C_4 (1.0–3.0 Pa), to C_4-like and C_4 species (0.3–0.8 Pa), indicating a progressive decrease in apparent photorespiration. However, C_3 and C_3–C_4 species examined had high intrinsic levels of photorespiration with the latter maintaining low apparent rates of photorespiration and lower Γ values, primarily by refixing photorespired CO_2. The C_4-like and C_4 *Flaveria* species had low, but measurable, levels of photorespiration via selective localization of ribulose-1,5-bisphosphate carboxylase in bundle sheath cells and operation of a CO_2 pump via the C_4 pathway.

Key words: Chlorophyll fluorescence – *Flaveria* – Oxygen – Photosynthesis – Photorespiration

Introduction

Analyses of physiological and biochemical aspects of photosynthesis and leaf anatomy indicate that the genus *Flaveria* contains species which represent a progression of photosynthetic types from C_3, to C_3–C_4 intermediates and C_4-like, to C_4 with complete development of the Kranz syndrome (Edwards and Ku 1987; Ku et al. 1991; Brown and Bouton 1993). Compared to the typical C_3 or C_4 plants, which have distinct types of leaf anatomy and photosynthetic biochemistry, the C_3–C_4 intermediates have a gradation in photosynthetic characteristics between those of C_3 and C_4 plants. They are classified as photosynthetic intermediate or C_4-like plants on the basis of physiological and biochemical criteria, i.e. CO_2 compensation point (Γ), carboxylation efficiency (CE), O_2 sensitivity of photosynthesis, the extent of development of Kranz-like features in the bundle-sheath cells, and the activity and compartmentation of key photosynthetic and photorespiratory enzymes (Ku et al. 1991; Morgan et al. 1993). In C_4 species, low Γ, high CE and the apparent lack

Abbreviations and symbols: A = CO_2 assimilation rate; CE = carboxylation efficiency; C_i = intercellular CO_2 partial pressure; I_a = absorbed PPFD; J_{O_2} = oxygen evolution from PSII; PPFD = photosynthetic photon flux density (μmol $\cdot m^{-2} \cdot s^{-1}$); Rubisco = ribulose-1,5-bisphosphate carboxylase/oxygenase; RuBP = ribulose-1,5-bisphosphate; VPD = water-vapor pressure difference between the leaf and atmospheric air; Γ = CO_2 compensation point; Φ_{CO_2} = quantum yield of CO_2 assimilation; Φ_{PSII} = quantum yield of photosystem II; Θ_A = O_2 inhibition index for photosynthesis (percentage inhibition of photosynthesis per kPa increase in O_2)

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of O_2 sensitivity of photosynthesis result from the high ribulose-1,5-bisphosphate (RuBP) carboxylase activity and low RuBP oxygenase activity in bundle-sheath cells due to the CO_2-concentrating mechanism of the C_4 cycle. The C_4-like *Flaveria* species have well-differentiated Kranz anatomy and high activities of C_4 enzymes, but lack a complete differential compartmentation of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and C_4 enzymes between mesophyll and bundle-sheath cells. In C_3–C_4 intermediates, Monson et al. (1984) proposed two mechanisms to account for the low apparent photorespiration. Results supporting one mechanism indicate that CO_2 assimilation occurs via Rubisco in mesophyll cells, with refixation of photorespired CO_2 in bundle-sheath cells where glycine decarboxylase is located (Rawsthorne 1992). In these species, reduced photorespiration occurs without the operation of a C_4 cycle. In other intermediates the operation of a limited C_4 cycle may contribute to the further reduction of photorespiration (Edwards and Ku 1987; Rawsthorne 1992).

Despite the identification of photosynthetic intermediates and C_4-like species, and low apparent photorespiration, the actual degree of photorespiration in these groups relative to C_3 and C_4 species is unclear. For example, low Γ values are not necessarily an indicator of low photorespiration since photorespired CO_2 may be refixed. One of the indicators of the extent of photorespiration in plants is the inhibition of photosynthesis by 21% O_2 versus 2% O_2, as shown in various studies including those on *Flaveria* species (Byrd and Brown 1989; Ku et al. 1991). However, recently a dual effect of O_2 was found in the C_4 plant maize (enhancement up to a certain level of O_2 followed by inhibition at a higher level of O_2; Dai et al. 1993, 1995). In mature leaves the optimum partial pressure for photosynthesis of the C_4 plant maize is ca. 9 kPa compared to 1–2 kPa for the C_3 plant wheat (Dai et al. 1993). Previous analyses of O_2 inhibition of photosynthesis in *Flaveria* species did not account for a requirement for O_2 which differs among photosynthetic groups as shown in this study. The magnitude of photorespiration based on O_2 inhibition of photosynthesis by 21% versus 2% O_2 will be underestimated if O_2 levels above 2% also have a stimulatory effect on photosynthesis.

Another recent measure of the magnitude of photorespiration in plants has been obtained by combining in-situ analysis of chlorophyll fluorescence to estimate whole-chain electron flow (associated with O_2 evolution from PSII, J_{O_2}) versus net rates of CO_2 fixation (A) by infrared gas analysis. For example, the increase in the ratio of whole-chain electron flow to CO_2 fixation with decreasing CO_2 in C_3 plants is reflecting an increase in partitioning of energy into photorespiration (see Krall and Edwards 1992). In a previous study on representative *Flaveria* species a progressive enhancement of PSII activity (from chlorophyll fluorescence analysis) was observed from C_4, to C_4-like, to intermediate to C_3 species on increasing the O_2 from 2–21 kPa under atmospheric levels of CO_2; and similarly the PSII activity per CO_2 fixed progressively increased from C_4 to the C_3 species under limiting intercellular CO_2 partial pressure (C_i) at 21 kPa O_2 (Krall et al. 1991). Likewise, with increasing O_2 there was little effect on quantum yield of PSII values in the C_4 species *Panicum antidotale*, a substantial enhancement with the C_3 species *P. bisulcatum* and less effect on the C_3–C_4 intermediate species *P. milioides* (Peterson 1994). These results show an increase in the partitioning of electrons from whole-chain electron flow into photorespiration in the progression from C_4 to intermediate to C_3 species.

Ku et al. (1991) have shown that the responses of Γ to increasing O_2, and to increasing photosynthetic photon flux density (PPFD), are very different between C_3 and intermediate *Flaveria* species, which could be due to differences in the true rate of photorespiration or in the degree of refixation of photorespired CO_2. In this study, a detailed profile of the O_2 dependence of photosynthesis was obtained, and a measure of the rate of whole-chain electron flow to O_2 determined at Γ under varying PPFDs and varying partial pressures of O_2 to evaluate the extent of photorespiration in *Flaveria* photosynthetic types.

Materials and methods

Plant material and growth conditions. Eighteen *Flaveria* species were used: *F. angustifolia* (Cav.) Pers. (C_3–C_4), *F. anomala* B. Robinson (C_3–C_4), *F. australasica* Hook (C_4), *F. bidentis* (L.) Kuntze (C_4), *F. brownii* A.M. Powell (C_4-like), *F. chloraefolia* A. Gray (C_3–C_4), *F. cronquistii* A.M. Powell (C_3), *F. floridana* J.R. Johnston (C_3–C_4), *F. linearis* Lag. (C_3–C_4), *F. oppositifolia* (DC.) Rydb. (C_3–C_4), *F. palmeri* J.R. Johnston (C_4), *F. pringlei* Gandoger (C_3), *F. pubescens* Rydb. (C_3–C_4), *F. ramosissima* Klatt (C_3–C_4), *F. robusta* Rose (C_3), *F. sonorensis* A.M. Powell (C_3–C_4), *F. trinervia* (Spreng.) C. Mohr (C_4), and *F. vaginata* B.L. Robinson and Greenman (C_4-like). The identification of photosynthetic types is based on leaf anatomy, Γ, sensitivity of photosynthesis to O_2, activity and compartmentation of photosynthetic enzymes, and initial photosynthetic products (Ku et al. 1991).

Plants were grown either from seeds or from vegetative cuttings in a compost:sand:perlite mixture (2:1:1, by vol.) in 4-L plastic pots, and cultivated in a greenhouse under natural light. The maximum illumination on a clear day during the summer months was about 1750 μmol quanta $\cdot m^{-2} \cdot s^{-1}$. Supplementary light from metal-halide lamps provided a PPFD of 350 μmol $\cdot m^{-2} \cdot s^{-1}$ on cloudy days. The temperature was controlled at 25 ± 1 °C day and 18 ± 0.5 °C night, and the relative humidity was maintained at 60 ± 5%. Plants were fertilized twice per week with commercial fertilizer (N:P:K, 20:20:20) and supplemented with micronutrients. Young, fully expanded leaves (usually the third or fourth leaves from the apex) from plants grown during the summer months (June to September) were used for various experiments.

Gas-exchange measurements. Rates of CO_2 assimilation (A) were measured on the third or fourth intact leaves from the apex using an Analytical Development Co. (ADC) infrared gas analyzer (IRGA) (225-MK3; PP Systems, Haverhill, Mass., USA) and a Bingham Interspace (Hyde Park, Utah, USA) Model BI-6-dp Computer Controller System or BI-2-dp Mini Cuvette Manual Controller System (Dai et al. 1992). These were operated as open systems where a given partial pressure of CO_2 is passed through the sample cell (in line with the leaf enclosed in a cuvette) and the reference cell; the rate of CO_2 removal by photosynthesis was compensated for by a controlled rate of injection of CO_2 from a high CO_2 source. The leaf cuvette contained a dewpoint sensor for measuring humidity and a copper-constantan thermocouple for monitoring leaf temperature. Both A and the partial pressure of CO_2 in the intercellular air space in the leaf, C_i, were directly calculated from gas-exchange measurements according to von Caemmerer and Farquhar (1981).

The effect of O_2 on photosynthesis was measured at different C_i partial pressures using a computer-controlled system (Dai et al.

1993). With this system A and C_i were continuously displayed during the experiment. A constant C_i (within 5% of the desired level) was maintained under varying levels of O_2 by controlling the external partial pressure of CO_2 and its flow rate. Different O_2 and CO_2 partial pressures were obtained by mixing N_2 gas, CO_2-free air (79% N_2 and 21% O_2), and 1000 Pa CO_2 balanced in N_2, through a BI-6-dp computerized controller. Depending on the desired C_i, the reference and span gases were prepared with a partial pressure difference of about 2 Pa. Light was provided by a 1000-W metal-halide lamp, leaf temperature was 30 °C, and the water-vapor pressure difference between the leaf and atmospheric air (VPD) was 5–10 mbar.

The degree of O_2 inhibition of photosynthesis, defined as the O_2 inhibition index (Θ_A), was calculated as the percentage inhibition of photosynthesis per kPa increase in O_2 around the leaf analyzed above the optimum partial pressure of O_2 (Dai et al. 1995) according to the equations below.

For C_4 and C_4-like species, measurements were made at 18.6 kPa O_2 versus 9.3 kPa O_2:

$$\Theta_A = \frac{\left(\frac{A_{9.3\,kPa\,O_2} - A_{18.6\,kPa\,O_2}}{A_{9.3\,kPa\,O_2}}\right) \times 100}{(18.6\,kPa\,O_2 - 9.3\,kPa\,O_2)}.$$

For C_3 and intermediate species, measurements were made at 18.6 kPa O_2 versus 2 kPa O_2:

$$\Theta_A = \frac{\left(\frac{A_{2\,kPa\,O_2} - A_{18.6\,kPa\,O_2}}{A_{2\,kPa\,O_2}}\right) \times 100}{(18.6\,kPa\,O_2 - 2\,kPa\,O_2)}.$$

Photosynthetic CO_2 compensation point (Γ) and carboxylation efficiency (CE). Two methods were employed to determine Γ. In the first method, Γ was measured in a closed, modified Bingham Interspace leaf chamber (volume 200 mL), which contained a dewpoint sensor and a copper-constantan thermocouple to monitor humidity and leaf temperature, coupled to an ADC IRGA (225-MK3) operating in an absolute mode. Different partial pressures of CO_2 and O_2 gases were prepared with an ADC gas mixer or an air compressor unit (PURUS manufactured by Bauer, Scuba Air Compressor Service, Dallas, Tex., USA) through controlling the pressure of ambient air, compressed air, pure O_2, or pure N_2. The partial pressures of O_2 and CO_2 were determined with a Hansatech oxygen electrode (Hansatech Instruments, Kings Lynn, Norfolk, UK) and ADC IRGA, respectively. A commercial CO_2 standard was used to calibrate the IRGA. Leaf temperature was maintained at 30 °C during measurements through a Bingham Interspace Model BI-2-dp minicuvette manual controller system. The PPFD inside the cuvette was ca. 1300 $\mu mol \cdot m^{-2} \cdot s^{-1}$, provided by a 1000-W metal-halide lamp. Lower PPFDs were obtained by inserting layers of cheesecloth between the light source and the cuvette. Irradiance was measured using a Lambda Li-85 quantum sensor (Li-Cor, Lincoln, Neb., USA). The leaf cuvette was first flushed with the desired gas mixture at 400 $mL \cdot min^{-1}$ for 5 min before it was completely sealed after which the air was circulated in a closed loop by a pump inside the IRGA. After reaching steady state (taking about 30–40 min) the partial pressure of CO_2 around the leaf was taken as Γ.

The value of Γ was also determined at 30°C, 19.5 kPa O_2 and 1200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD by measuring CO_2 assimilation in response to low partial pressure of CO_2 (0–10 Pa for C_3 and C_3–C_4 species and 0–5 Pa for C_4-like and C_4 species) and extrapolating the initial CO_2 response curve through the x-axis. Generally, these two methods gave very similar values.

Carboxylation efficiency (CE) was calculated from the initial slope of photosynthetic response to increasing C_i (0–10 Pa for C_3 and C_3–C_4 species and 0–5 Pa for C_4-like and C_4 species) at 30 °C, 19.5 kPa O_2 and a PPFD of 1200 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

Measurement of the quantum yield for PSII (Φ_{PSII}). For determination of Φ_{PSII}, fluorescence measurements were made with a PAM fluorometer (Walz model 101; Heinz Walz, Effeltrich, Germany) simultaneously with measurements of Γ at 30 °C and varying O_2 partial pressures or varying PPFDs. During the experiment, the steady-state fluorescence, F_s, was monitored. Saturating light pulses (800 ms duration) were given during steady-state photosynthesis at 300-s intervals to determine the maximum fluorescence, F'_m. The quantum yield of PSII-dependent electron transport was calculated as $(F'_m - F_s)/F'_m$ according to the method of Genty et al. (1989).

The true rate of O_2 evolution (J_{O_2}) was calculated as $[(\Phi_{PSII} \cdot I_a \cdot f)/4]$ (see Edwards and Baker 1993), where I_a is the absorbed light, f is the partitioning factor between PSI and PSII, and division by 4 is based on 4 e^- transported per O_2 evolved. The value of I_a was calculated from the PPFD and light absorptance of individual species; f, the fraction of light absorbed by PSII, was taken as 0.5.

Determining leaf absorptance of PPFD. Light absorption by the individual leaves used in gas-exchange experiments was determined with an integrating sphere (10 cm; diameter; Labsphere, North Sutton, N.H., USA). The light source was a Schott's Lamp and the detector was a Li-Cor quantum sensor, with modification of the meter to provide sensitivity over a scale of 0–0.3 μmol quanta $\cdot m^{-2} \cdot s^{-1}$. The light entering the sphere was measured with and without the leaf covering the port to determine transmittance. The light reflected from the leaf was measured by placing the leaf over a port on the opposite site of the sphere from the light source and by comparison with a 10% reflectance calibration standard from Labsphere. The PPFDs used for reflectance and transmittance measurements were 10 and 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively.

Replications. Duplicate measurements were performed for each species in the various experiments which gave similar results. Only one set data is presented. For many species, two to three similar leaves from different plants were enclosed in the leaf cuvette for each measurement.

Results

A dual effect of O_2 on photosynthesis in species of the genus Flaveria. Under atmospheric level of CO_2 (34 Pa), a PPFD of 1200 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and 30 °C there were significant differences in O_2 response of photosynthesis among the representative C_3 (*F. pringlei*), C_3–C_4 (*F. floridana*), C_4-like (*F. vaginata*), and C_4 (*F. bidentis*) *Flaveria* species (Fig. 1). In the C_3 and C_3–C_4 species, photosynthesis was enhanced only slightly as O_2 increased from 0 kPa up to 2 kPa. However, in the C_4-like and C_4 species photosynthesis was low in the absence of O_2 and increased markedly with increasing O_2 from 0 up to 9 kPa. While the normal atmospheric level of O_2 is ca. 20 kPa, the optimum partial pressure of O_2 for photosynthesis was about 9 kPa for the C_4 and C_4-like species, and about 2 kPa for the C_3–C_4 and C_3 species (Fig. 1).

Under a low external CO_2 partial pressure (ca. 8 Pa) there was a much greater stimulation of photosynthesis from zero up to the optimum level of O_2 in the C_3 (*F. pringlei*) and C_3–C_4 (*F. floridana*) species than at normal partial pressure of CO_2 (comparing % of A_{max} at each CO_2 level); although the optimum O_2 partial pressure remained about the same (2–3 kPa). At 8 Pa CO_2, the optimum O_2 level for maximum rates of photosynthesis was similar (1–3 kPa) between the photosynthetic groups. By comparison, as noted above, under atmospheric CO_2 (34 Pa) the C_4 and C_4-like species required a much higher O_2 (ca. 9 kPa) for maximum photosynthesis.

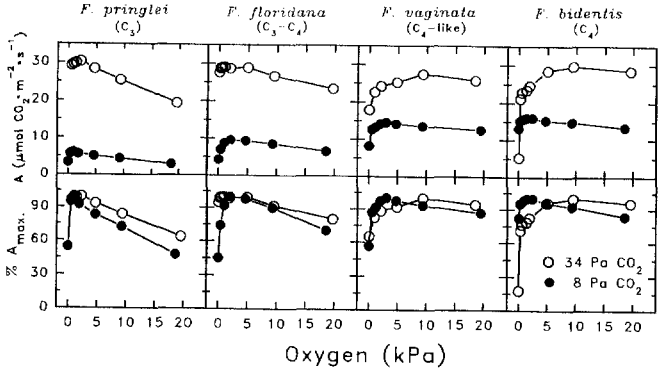

Fig. 1. The responses of photosynthetic CO_2 assimilation (A) in several *Flaveria* species to partial pressure of O_2 at 34 Pa or 8 Pa external CO_2. The conditions were 30 °C, 1200 μmol·m^{-2}·s^{-1}, and 5 ± 1 mbar VPD. At external CO_2 of 34 Pa the corresponding intercellular CO_2 levels (C_i) were 26.0 (± 1.0) Pa for *F. pringlei*, 27.0 (± 0.6) Pa for *F. floridana*, 24.0 (± 0.4) Pa for *F. vaginata*, and 24.5 (± 0.5) Pa for *F. bidentis*. At external CO_2 of 8 Pa the corresponding C_i levels were 7.3 (± 0.2) Pa for *F. pringlei*, 6.4 (± 0.1) Pa for *F. floridana*, 4.0 (± 0.1) Pa for *F. vaginata*, and 4.2 (± 0.2) Pa for *F. bidentis*

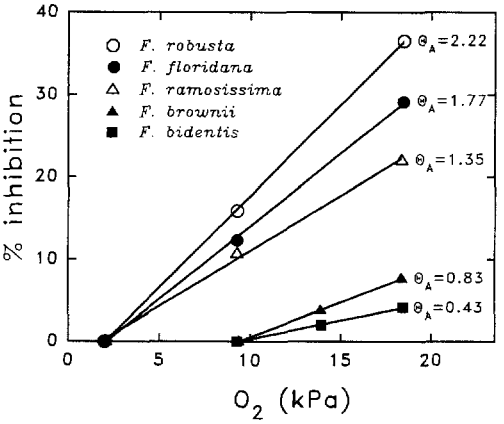

Fig. 2. The percentage inhibition of photosynthesis by O_2 in several *Flaveria* species at atmospheric CO_2 level. For C_4 and C_4-like species, the percentage inhibition was calculated between 14 and 18.6 kPa relative to the rate at 9.3 kPa O_2. For C_3 and C_3–C_4 intermediates, the percentage inhibition was calculated between 9.3 and 18.6 kPa O_2 relative to the rate at 2 kPa O_2. The measurement conditions were 30 °C, 1200 μmol·m^{-2}·s^{-1}, and 5 ± 1 mbar VPD

Increasing O_2 above the optimum partial pressure resulted in inhibition of photosynthesis, especially under low CO_2. For C_3 and intermediates, O_2 inhibition of photosynthesis was calculated from the results with increasing O_2 from 2 to 18.6 kPa. For C_4-like and C_4 species, O_2 inhibition was calculated from measurements of photosynthesis between 9.3 and 18.6 kPa O_2 (Fig. 2). Comparisons of the O_2 inhibition index values (Θ_A, see *Materials and methods*) determined under atmospheric levels of CO_2 show that the magnitude of inhibition of photosynthesis by O_2 decreases progressively from C_3 (2.1–2.2) to C_3–C_4 (1.2–1.6), C_4-like (0.6–0.8) and C_4 species (0.3–0.4) (Fig. 3A).

Values of Θ_A, *and CE.* The Θ_A values obtained for the different *Flaveria* species were compared with measurements of CE and Γ. The value of Γ, determined by extrapolation of A/C_i response under low CO_2, and by equilibrium in a closed system, varied considerably among different species of the genus *Flaveria*. As expected, C_3 species had much higher Γ values (ca. 6 Pa) than C_3–C_4 (1.0–3.3 Pa), C_4-like (0.4–0.8 Pa), and C_4 species (0.3–0.5 Pa) (Fig. 3B). On the other hand, there was not much difference in CE between C_3 and C_3–C_4 (ca. 1.3–1.7), whereas the values were much higher in C_4-like and C_4 species (3.8–5.2), except the C_4-like *F. brownii* which exhibited a CE similar to those of C_3–C_4 (Fig. 3C). It is clear that values of Θ_A do not decline as much as values of Γ when comparing the progression from C_3 to C_3–C_4 to C_4-like to C_4 species.

A plot of the values obtained for CE versus the initial products of CO_2 fixation shows that the CE is low in C_3–C_4 species despite significant fixation of CO_2 into C_4 acids in some intermediates while there is a sharp increase in CE values of most C_4-like and C_4 species in parallel with an increase in C_4 photosynthesis (Fig. 4).

Effects of O_2 *partial pressure on* Γ, *and* Φ_{PSII} *and* J_{O_2} *measured at* Γ. With increasing O_2 partial pressures at

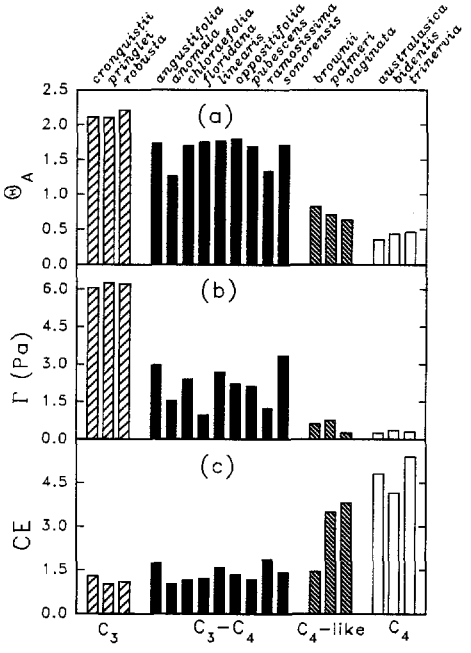

Fig. 3a–c. The O_2 inhibition index for photosynthesis (Θ_A); see Fig. 2 and *Materials and methods* for calculation) (**a**), photosynthetic CO_2 compensation point (Γ) (**b**), and carboxylation efficiency (CE) (**c**) for 18 *Flaveria* species. The measurement conditions were 30 °C, 1200 μmol·m^{-2}·s^{-1}, and 5 ± 1 mbar VPD

300 μmol·m^{-2}·s^{-1} PPFD there is a linear increase in Γ in the C_3 species *F. pringlei* and the intermediates *F. sonorensis* and *F. floridana*; whereas in the C_4 species *F. bidentis* and the C_4-like species *F. vaginata*, Γ remained low and was little effected by increasing partial pressure of O_2 up to 51 kPa (Fig. 5a, also see Ku et al. 1991). However, the increase in Γ with increasing O_2 partial pressure was much less in the intermediate species *F. floridana* than in the C_3 species.

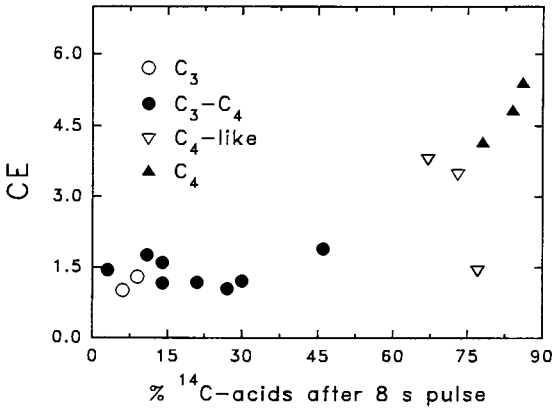

Fig. 4. The relationship between CE and the percentage of ^{14}C label incorporated into malate and aspartate following an 8-s pulse with $^{14}CO_2$ for various *Flaveria* species. The data of CE were from Fig. 3c. The data of percentage of ^{14}C label initially incorporated into malate and aspartate were from Moore et al. (1987)

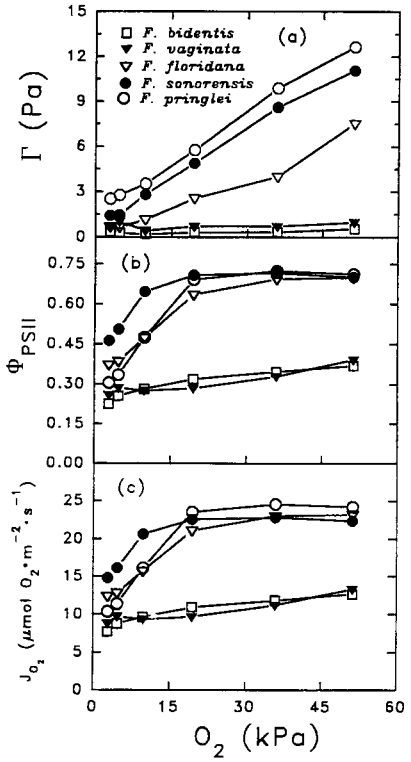

Fig. 5a–c. The effect of O_2 partial pressure on Γ (**a**), on Φ_{PSII} (**b**) and on J_{O_2} (**c**) values measured at Γ in *Flaveria* species representing different photosynthetic groups. The measurement conditions were 30 °C, 300 μmol·m^{-2}·s^{-1}, and 5 ± 1 mbar VPD

The Φ_{PSII} and J_{O_2} values, which are a measure of the quantum yield of PSII and the true rate of O_2 evolution, respectively, increased rapidly with increasing O_2 above 2 kPa in the C_3 and C_3–C_4 species (Fig. 5b, c). In the C_4 and C_4-like species, which have low photorespiration, there was a more gradual increase in Φ_{PSII} and J_{O_2} with increasing O_2, indicating O_2 is much more limited as a sink for electrons derived from oxidation of water via PSII in these species.

Effect of PPFD on Γ, *and on* Φ_{PSII} *and* J_{O_2} *measured at* Γ

In the C_3 species *F. pringlei*, Γ initially decreased with increasing PPFD and then remained reasonably constant (Fig. 6a, also see Ku et al. 1991). The two C_3–C_4 species had Γ values similar to C_3 species at PPFDs below 150 μmol·m^{-2}·s^{-1}, but Γ decreased rapidly in the intermediates with increasing PPFD. At a given PPFD the intermediate *F. sonorensis* had much higher Γ values than the intermediate *F. floridana*. In the C_4 and C_4-like species Γ was very low and remained low over the entire range of PPFDs tested.

At Γ, Φ_{PSII} decreased with increasing PPFD in the same manner for C_3 and intermediates but decreased more rapidly with C_4 and C_4-like species (Fig. 6b). Also, in the C_4 species *F. bidentis* there was a rapid decline in Φ_{PSII} values with increasing PPFD in the absence of CO_2 under low O_2 (0.5 kPa). Using Φ_{PSII} and I_a values as experimental inputs for calculation of J_{O_2} (see *Materials and methods*) the response of J_{O_2} versus PPFD is shown at Γ (Fig. 6c). Whereas J_{O_2} increased with increasing PPFD in the same manner for C_3 and C_3–C_4 species, J_{O_2} was substantially lower in the C_4 species. Moreover, J_{O_2} was very low in *F. bidentis* under 0.5% O_2 and in the absence of CO_2, with maximum rates at about 100 μmol·m^{-2}·s^{-1} PPFD.

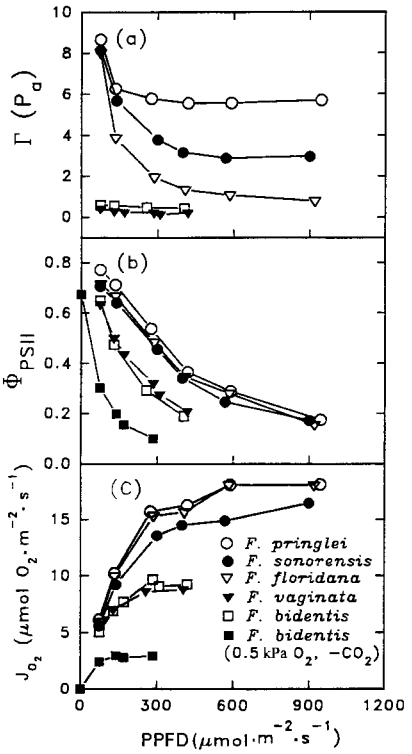

Fig. 6a–c. The effect of PPFD on Γ (**a**), Φ_{PSII} measured at Γ (**b**), and J_{O_2} measured at Γ (**c**) in *Flaveria* species representing different photosynthetic groups. The measurement conditions were 30 °C, 19.5 kPa and 5 ± 1 mbar VPD. Results are also shown with *F. bidentis* in the absence of CO_2 with 0.5% kPa O_2 (**b**, **c**)

Discussion

Optimum O_2 level for photosynthesis

There is a dual effect of O_2 on photosynthesis in the various photosynthetic types of *Flaveria* species, and the O_2 requirement for maximum photosynthesis is dependent on the species and CO_2 level. Under normal atmospheric levels of CO_2, the level of O_2 required for maximum rates of photosynthesis is similar in the C_4 plant *F. bidentis* and C_4-like *F. vaginata* (ca. 9 kPa) whereas the C_3–C_4 and C_3 *Flaveria* species have a much lower requirement (ca. 2 kPa). Under similar conditions the C_4 plant maize requires ca. 9 kPa O_2 for maximal photosynthesis (Dai et al. 1993), similar to that of *F. bidentis*, both of which are NADP-malic enzyme-type C_4 species. The C_4-like species *F. vaginata* has a high capacity for C_4 photosynthesis (ca. 92% of atmospheric CO_2 fixed through the C_4 pathway; Moore et al. 1989). Thus, it is clear that species having high levels of C_4 photosynthesis have a higher O_2 requirement than intermediates or C_3 species. This correlation between a high O_2 requirement and C_4 photosynthesis may be associated with the generation of ATP which is required by the C_4 cycle. This ATP may be produced through O_2-dependent pseudocyclic photophosphorylation (Huber and Edwards 1975) or O_2-dependent poising of the electron-transport chain such that a proper balance of linear and cyclic electron transport is established to supply ATP for CO_2 fixation (Ziem-Hanck and Heber 1980). Under a low atmospheric level of CO_2 (8 Pa) the optimum O_2 partial pressure for photosynthesis decreased from 9 kPa to 3 kPa in the C_4 and C_4-like species. This may be due to a drop in the requirement for ATP as the rate of CO_2 fixation drops with a CO_2 limitation.

Oxygen inhibition of photosynthesis relative to Γ values and carboxylation efficiency

The degree of inhibition of photosynthesis by supraoptimum levels of O_2, as reflected by the O_2 inhibition index (Θ_A), varies considerably in the genus *Flaveria* (Figs. 2, 3A). The progressive decrease of Θ_A from C_3 to C_3–C_4 to C_4-like and C_4 species suggests a gradation of photorespiratory activity among these *Flaveria* species. In our earlier study (Ku et al. 1991), C_4 *Flaveria* were reported to have no apparent photorespiration based on the lack of O_2 inhibition or slight stimulation of photosynthesis by O_2 when A was measured at 2% versus 21% O_2. However, it is clear from this and our recent study with maize (Dai et al. 1993, 1995) that C_4 plants exhibit a degree of photorespiration based on inhibition of photosynthesis above the optimum O_2 partial pressure. For the same reason, the degree of inhibition of photosynthesis by O_2 in the C_4-like species *F. brownii*, *F. palmeri* and *F. vaginata* was underestimated based on previous measurements under 2% versus 21% O_2 (Ku et al. 1991) (calculated average Θ_A values for the three species based on previous results from Ku et al. 1991, under 2% versus 21% O_2 is 0.4 compared to 0.7 in the present analysis).

Measurement of Θ_A shows that the O_2 inhibition of photosynthesis in the intermediate species is only slightly lower than that in C_3 species; however, Γ values of intermediate species are much lower than that of C_3 plants, suggesting they have low apparent photorespiration. This can be explained by C_3–C_4 species having direct O_2 inhibition of CO_2 fixation in mesophyll cells via Rubisco through O_2 competition with CO_2, and the compartmentation of photorespiratory CO_2 release and its refixation in the bundle-sheath cells of C_3–C_4 species. In C_3–C_4 species most of the Rubisco is in the mesophyll cells, whereas glycine decarboxylase is mainly compartmentalized in the bundle-sheath cells (Hylton et al. 1988; Moore et al. 1988; Rawsthorne et al. 1988). The confinement of photorespiratory release of CO_2 to bundle-sheath cells is proposed to be an important evolutionary development in minimizing loss of CO_2 by photorespiration (Monson et al. 1984). This biochemical modification of glycolate metabolism appears to be common in all intermediates, with or without C_4 photosynthesis, and may be the very first step in improving the efficiency of C_3 photosynthesis. Although the intermediate *F. floridana* has a partially functional C_4 cycle (Moore et al. 1987) its Θ_A value indicates it is not effectively concentrating CO_2 in bundle-sheath cells (see discussion regarding Θ_A versus CO_2 concentration around Rubisco in Dai et al. 1993, 1995).

The similar CE between C_3 species and most intermediate species further supports the notion that intermediate species, with or without a partially functional C_4 pathway, are incapable of effectively concentrating CO_2 in the bundle-sheath cells. The high CE values of C_4-like (except *F. brownii*) and C_4 species are consistent with their having a functional C_4 cycle which increases the CO_2 level at the site of Rubisco in the leaf bundle-sheath cells. When the data of the percentage of ^{14}C initially incorporated into the C_4 acids (a measure of C_4 capacity) from our earlier study (Moore et al. 1987) were compared with CE for the various *Flaveria* species, there was a curvilinear relationship between these two (Fig. 4). Lower CE occurs in many intermediates, even in those with a relatively high capacity for synthesis of malate and aspartate as the initial photosynthetic products via the C_4 pathway. This suggests that although some of the intermediates have a partial C_4 cycle, they still have low carboxylation efficiency due either to the leakage of bundle-sheath cells, concentrating inorganic carbon in bundle-sheath cells predominantly as bicarbonate rather than CO_2, or inefficiency of C_4 photosynthesis due to lack of a strict compartmentation of key photosynthetic enzymes. Also, the lower CE value in the C_4-like species *F. brownii* is not due to lack of capacity to concentrate inorganic carbon in the leaf (Moore et al. 1987), but may be limited by the form of inorganic carbon accumulated or Rubisco content as this species has a low capacity for CO_2 assimilation (Krall et al. 1991).

Effect of O_2 and PPFD on Φ_{PSII} and J_{O_2} at Γ

Several representative intermediate *Flaveria* species were compared to their C_3 and C_4 counterparts for the effects

of O_2 and PPFD on Φ_{PSII} and J_{O_2} at Γ. These included *F. sonorensis*, which has low apparent photorespiration (reduced Γ) without C_4 photosynthesis (Moore et al. 1987; Ku et al. 1991), *F. floridana*, which has low apparent photorespiration with a partially functional C_4 cycle (30–50% initial products of $^{14}CO_2$ into C_4 acids; Monson et al. 1986; Moore et al. 1987), and *F. vaginata*, a C_4-like species with a high capacity for C_4 photosynthesis (ca. 80–90% initial products of CO_2 fixation into C_4 acids, Moore et al. 1989).

Previous studies indicate that a reasonable measure of the true rate of O_2 evolution can be obtained from application of modulated chlorophyll fluorescence (Cornic and Briantais 1991; Krall et al. 1991; Krall and Edwards 1992; Edwards and Baker 1993; Cornic 1994). Analyses with both C_3 and C_4 plants indicate that the true rate of O_2 evolution predicted from chlorophyll fluorescence is correlated with independent analysis of rates of whole-chain electron flow associated with Rubisco activity in vivo.

C_3 response. The linear increase in Γ with increase in O_2 partial pressure in the C_3 species *F. pringlei* is typical of the predicted response for C_3 species. This is explained on the basis of the kinetic properties of Rubisco where $v_c/v_o = S_{rel}\,[CO_2]/[O_2]$ (Liang et al. 1974).

At Γ^* (the CO_2 compensation point in the absence of dark-type mitochondrial respiration), $v_c/v_o = S_{rel}\,[\Gamma^*]/[O_2]$. Since at Γ^*, $v_c = 0.5\,v_o$, $\Gamma^* = 0.5\,[O_2]/S_{rel}$. Thus, a plot of Γ^* versus O_2 will exhibit a linear response intercepting at zero. The higher values of Γ at low O_2 as observed experimentally for *F. pringlei* (Fig. 5) suggest a contribution of dark-type mitochondrial respiration to the CO_2 compensation point. In contrast to the linear dependence of Γ on O_2 up to 51 kPa in *F. pringlei*, J_{O_2} reached a maximum rate around atmospheric levels of O_2 (at 18.6 kPa). In C_3 and C_4 plants, J_{O_2} is largely dependent on Rubisco activity, specifically the sum of carboxylase and oxygenase activities (i.e. relative to Rubisco $J_{O_2} = v_c + v_o$) where the consequence of both carboxylase and oxygenase activity is utilization of the equivalent of ca. 2 NADPH per RuBP (Krall and Edwards 1992; Edwards and Baker 1993), and $v_c + v_o$ equals the rate of RuBP utilization. The increase in J_{O_2} which occurs as a consequence of increase in O_2 in *F. pringlei* must be due to both an increase in v_c and v_o of Rubisco in mesophyll cells since, as discussed above, at Γ, v_c/v_o remains constant with increasing O_2. Saturating rates of J_{O_2} at 18.6 kPa O_2 (see Fig. 5) likely occur due to a limitation on the rate of RuBP regeneration at 300 μmol quanta $\cdot m^{-2} \cdot s^{-1}$. With respect to light intensity, increasing the PPFD is expected to have little effect on Γ in C_3 plants due to low refixation of photorespired CO_2; the initial drop in Γ in *F. pringlei* with increasing light may be associated with refixation of CO_2 from dark-type mitochondrial respiration.

The true rate of O_2 evolution calculated from fluorescence analysis at Γ can be compared with results from previous studies with C_3 species. In the present study, light saturation of O_2 evolution occurred at a PPFD of 600 μmol $\cdot m^{-2} \cdot s^{-1}$ in *F. pringlei* (maximum rate of ca. 18 μmol $\cdot m^{-2} \cdot s^{-1}$) versus light saturation at a PPFD of 800 μmol $\cdot m^{-2} \cdot s^{-1}$ in *Hirschfeldia incana* (maximum rate of ca. 50 μmol $\cdot m^{-2} \cdot s^{-1}$) (determined from O_2 isotope analysis, Canvin et al. 1980), and at a PPFD of ca. 400 μmol $\cdot m^{-2} \cdot s^{-1}$ in wheat (ca. 10–20 μmol $\cdot m^{-2} \cdot s^{-1}$) (oxygen isotope and fluorescence analysis; Biehler and Fock 1995). Thus, light-saturated rates of O_2 evolution in these studies occurred at 400–800 μmol quanta $\cdot m^{-2} \cdot s^{-1}$; the variation in maximum rates might be due to differences in maximum capacity for carbon assimilation per leaf area (e.g. Rubisco content). With increasing partial pressure of O_2, maximum rates of O_2 evolution (ca. 25 μmol $\cdot m^{-2} \cdot s^{-1}$) occurred around atmospheric levels of O_2 in the present study with *F. pringlei* at a PPFD of 300 μmol $\cdot m^{-2} \cdot s^{-1}$, compared to a maximum rate (ca. 35 μmol $\cdot m^{-2} \cdot s^{-1}$) at 28% O_2 with *H. incana* at a PPFD of 400 μmol $\cdot m^{-2} \cdot s^{-1}$ (determined from oxygen isotope analysis; Canvin et al. 1980).

C_4 and C_4-like response. Both the C_4 and C_4-like species maintain a low Γ with increasing O_2 or increasing light, indicating a very low apparent photorespiration. However, there is a small increase in J_{O_2} with increasing O_2 or increasing PPFD, indicating some photorespiration is occurring in these species. This photorespiration is not apparent in measurement of Γ due to refixation of photorespired CO_2. This suggests that in these species the C_4 cycle does not concentrate CO_2 in the bundle-sheath cells at a sufficiently high level to prevent photorespiration. Since Rubisco is confined to bundle-sheath cells in C_4 species, and largely confined to these cells in C_4-like species, the contribution of $v_c + v_o$ to the J_{O_2} activity associated with Rubisco is a function of bundle-sheath cell activities. These results are consistent with other evidence for measurable photorespiration in the C_4 species maize under CO_2-limited conditions (Dai et al. 1993, 1995). The rates of O_2 evolution by *F. bidentis* in the present study at Γ are also similar to those measured in the C_4 dicotyledon, *Amaranthus edulis*, by the oxygen isotope method of analysis where an increase in J_{O_2} with increasing O_2 was observed (Canvin et al. 1980). In the present study, without CO_2 and under 0.5 kPa O_2 the rate of O_2 evolution in *F. bidentis* was low and saturated at low PPFD. Under this condition whole-chain electron flow may be limited mainly to the assimilation of nitrate and refixation of respired CO_2.

C_3–C_4 intermediate response. The decrease of Γ values with increasing light intensity in the intermediates may be due to a higher refixation of photorespired CO_2 during photosynthesis at higher PPFD (Ku et al. 1991). Interestingly, the C_3 and C_3–C_4 species have similar Γ values at very low light intensities. It suggests that under low PPFD the C_3–C_4 species may lose their refixation capacity since the energy supply becomes limiting. Also, dark respiration may contribute to Γ, causing it to be higher under low light. At a given O_2 partial pressure or PPFD, Γ is lower in the intermediate *F. sonorensis* than in the C_3 *F. pringlei*, particularly under high PPFD, but J_{O_2} is similar. The lower Γ value in *F. sonorensis* would limit v_c in mesophyll cells, but the additional photochemistry associated with refixation of CO_2 via Rubisco in bundle-sheath cells of *F. sonorensis* could explain equivalence in J_{O_2} values between

the two species. In intermediates like *F. sonorensis*, Rubisco and the C_3 pathway are considered to function in mesophyll cells in the same way as in C_3 plants. However, the glycine formed as a consequence of RuBP oxygenase activity in mesophyll cells is decarboxylated in bundle-sheath cells where the CO_2 is refixed by RuBP carboxylase. Photorespiration and refixation of photorespired CO_2 utilizes reductive power and allows photochemistry (J_{O_2}) to continue in the intermediate at values equivalent to that of the C_3 plant. In comparison to the C_3 plant at Γ, under a given O_2 and light level in the intermediate species v_c will be lower in the mesophyll cell (MC) due to the lower CO_2 around Rubisco (lower Γ) while v_o will be higher, plus refixation of CO_2 in bundle-sheath cells (BSC) in the intermediate utilizes extra reductive power. As a consequence, in the intermediates at Γ with respect to Rubisco activity $J_{O_2} = v_{c(MC)} + v_{o(MC)} + v_{c(BSC)}$.

The intermediate *F. floridana*, which as noted above has a partially functioning C_4 cycle, has a lower Γ value, at a given PPFD and O_2 level, than *F. sonorensis*, an intermediate without a C_4 cycle. However, the response of J_{O_2} to increasing PPFD, or increasing O_2, is similar to that of the C_3 *F. pringlei*. This suggests that, at Γ, *F. floridana* has a high level of photorespiration and a high capacity for refixation of photorespired CO_2 despite evidence for partial function of a C_4 cycle. As with *F. sonorensis*, J_{O_2} with respect to Rubisco in *F. floridana* equals $v_{c(MC)} + v_{o(MC)} + v_{c(BSC)}$. The low Γ in *F. floridana* results in a low CO_2 concentration around Rubisco in the mesophyll cells and increased v_o (i.e. increased photorespiration). Since up to 70% of the initial products of photosynthesis in *F. floridana* occurs directly via Rubisco in mesophyll cells (Moore et al. 1987), under low Γ values, much of the Rubisco of the leaf would be subjected to conditions allowing high oxygenase activity and high rates of photorespiration. While there is evidence that *F. floridana* actively accumulates inorganic carbon in the leaf, it does not appear to be very effective in C_4 photosynthesis since its carboxylation efficiency is the same as that of *F. pringlei*. If the inorganic carbon pool exists predominantly as bicarbonate rather than CO_2, due to equilibration via carbonic anhydrase, it could be less effective since CO_2 is the form used by Rubisco (see Moore et al. 1987).

Estimates of rates of photochemistry at Γ can be compared to that under normal atmospheric conditions. At Γ with increasing PPFD, maximum rates of J_{O_2} of ca. 18 μmol $O_2 \cdot m^{-2} \cdot s^{-1}$ were obtained at ca. 600 μmol quanta $\cdot m^{-2} \cdot s^{-1}$ with the C_3 species *F. pringlei* and intermediate species *F. sonorensis* and *F. floridana*. By comparison rates of ca. 50 μmol $O_2 \cdot m^{-2} \cdot s^{-1}$ occur under normal atmospheric levels of CO_2 and O_2 in these same species (based on Φ_{PSII} values of 0.62, and I_a of 640 μmol quanta $\cdot m^{-2} \cdot s^{-1}$, with an incident light of 800 μmol quanta $\cdot m^{-1} \cdot s^{-1}$ and leaf absorptance of 0.8, see Figs. 1, 3, and 4, Krall et al. 1991). Thus, at Γ the rates of whole-chain electron flow in C_3 and intermediate species are about 1/3 of that under normal conditions, which indicates substantial photochemistry can occur when CO_2 is limiting (e.g. under water stress or high temperatures), unlike species having C_4 photosynthesis.

Conclusions

The maximum rate of photosynthesis in these *Flaveria* species has a unique dependence on O_2 and CO_2 levels which is related to the photosynthetic type. A decrease in the partial pressure of CO_2 reduced the O_2 requirement for photosynthesis, especially in C_4-like and C_4 species. It suggests that the O_2 requirement for photosynthesis is related to the C_4 pathway which may require additional ATP by pseudocyclic or cyclic photophosphorylation. In contrast to C_3, the Γ values in C_3–C_4 species were much lower; however, the CE in these species is similar to that in C_3 species. Also, measurements of the quantum yields of PSII and calculated true rates of O_2 evolution at Γ under varying partial pressures of O_2 or varying light were similar between C_3 and C_3–C_4 species. The results suggest the intermediates examined (*F. floridana* and *F. sonorensis*) are reducing photorespiration by refixation of photorespired CO_2. Thus, the low apparent photorespiration (i.e. low Γ) of these intermediates compared to the C_3 species in response to increasing PPFD or varying O_2 can be accounted for by refixation of photorespired CO_2 rather than by limited rates of RuBP oxygenase and photorespiration. In C_4 plants, the combination of C_4 cycle function and the localization of Rubisco in bundle-sheath cells results in very low Γ values, and a condition at Γ which limits not only both RuBP carboxylase and oxygenase activities but also J_{O_2} associated with whole-chain electron flow.

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