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Photosynthesis in *Flaveria brownii* A.M. Powell[1]

A C_4-LIKE C_3-C_4 INTERMEDIATE

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ABSTRACT

Leaves of *Flaveria brownii* exhibited slightly higher amounts of oxygen inhibition of photosynthesis than the C_4 species, *Flaveria trinervia*, but considerably less than the C_3 species, *Flaveria cronquistii*. The photosynthetic responses to intercellular CO_2, light and leaf temperature were much more C_4-like than C_3-like, although 21% oxygen inhibited the photosynthetic rate, depending on conditions, up to 17% of the photosynthesis rate observed in 2% O_2. The quantum yield for CO_2 uptake in *F. brownii* was slightly higher than that for the C_4 species *F. trinervia* in 2% O_2, but not significantly different in 21% O_2. The quantum yield was inhibited 10% in the presence of 21% O_2 in *F. brownii*, yet no significant inhibition was observed in *F. trinervia*. An inhibition of 27% was observed for the quantum yield of *F. cronquistii* in the presence of 21% O_2. The photosynthetic response to very low intercellular CO_2 partial pressures exhibited a unique pattern in *F. brownii*, with a break in the linear slope observed at intercellular CO_2 partial pressure values between 15 and 20 μbar when analyzed in 21% O_2. No significant break was observed when analyzed in 2% O_2. When taken collectively, the gas-exchange results reported here are consistent with previous biochemical studies that report incomplete intercellular compartmentation of the C_3 and C_4 enzymes in this species, and suggest that *F. brownii* is an advanced, C_4-like C_3-C_4 intermediate.

To date, nine species in the genus *Flaveria* (Asteraceae) have been characterized as exhibiting photosynthetic and photorespiratory traits intermediate to the C_3- and C_4-syndromes (1, 8, 11, 13). Several of these C_3-C_4 intermediates are capable of assimilating atmospheric CO_2 through the C_4-cycle (3, 15, 20). However, none of them exhibits sufficient integration and compartmentalization of the C_3- and C_4-cycles between the mesophyll and bundle-sheath cells to result in C_4-like carbon isotope fractionation ratios (1, 21, 22). Additionally, none of the C_3-C_4 species exhibit negligible levels of O_2 inhibition of photosynthesis, as is typical of fully expressed C_4 plants (13, 15).

Recently some evidence has been presented to suggest that *Flaveria brownii* is a C_4-like C_3-C_4 intermediate. In past studies, *F. brownii* has been treated as a typical C_4 species (18). However, recently it has been established that the principal photosynthetic carboxylation enzymes, PEP[2] carboxylase and Rubisco, and the decarboxylation enzyme NADP-malic enzyme, are not fully compartmentalized between mesophyll and bundle-sheath cells, as is typically found in C_4 leaves (4, 6, 19). The localization of Rubisco in mesophyll cells, as well as bundle-sheath cells, suggests that atmospheric CO_2 may be assimilated through parallel C_3 and C_4 pathways. $^{14}CO_2$-pulse studies showed only 65 to 75% of the assimilated $^{14}CO_2$ was recovered as malate plus aspartate following pulses of 4 to 8 s (3, 6, 15, although also see 10). Additionally, carbon isotope ratios (measured as $\delta^{13}C$) have been measured between −14.5‰ and −21‰ (DM Keefe, LJ Mets, University of Chicago, personal communication), with most values falling between −16 to −18‰ when the plants were grown in warm, long-photoperiod regimes. A $\delta^{13}C$ value of −17.3‰ was reported recently for *F. brownii*, although the growth conditions for the plant were not specified (22). These values are at the negative end of the range typically attributed to C_4 plants (21). Finally, it was recently reported that PEP carboxylase of *F. brownii* exhibits intermediate kinetic and regulatory properties as compared to the enzyme from C_3, C_3-C_4, and C_4 *Flaveria* species (5).

In studies presented here, we examined the photosynthetic responses to oxygen, CO_2, light, and temperature in *F. brownii* leaves to determine whether such responses also reflect a C_3-C_4 intermediate nature. There is some evidence from carbon isotope ratios that the relative expression of C_3 and C_4 pathways in *F. brownii* is under partial environmental control, with C_4 photosynthesis reaching its greatest expression when grown in warm, long-photoperiod regimes (DM Keefe, LJ Mets, University of Chicago, personal communication). In order to establish photosynthetic traits of *F. brownii* in the most C_4-like condition, and thus bias our results as far as possible away from the C_3-C_4 intermediate condition, we conducted experiments during the months of May–July on plants grown in a greenhouse with natural lighting and warm midday temperatures. It was our contention that in order to establish *F. brownii* as a true C_3-C_4 intermediate species, we would have to observe such intermediate photosynthetic traits in plants that had been provided with every opportunity for C_4-cycle expression.

MATERIALS AND METHODS

Plant Material. Plants of *F. brownii* A.M. Powell were established during February 1986 from cuttings of clone B6 (obtained from the University of Chicago). Plants of *F. trinervia* C. Mohr (C_4) were grown from seed collected near Lubbock, TX. Plants of *F. cronquistii* A.M. Powell (C_3) were established from cuttings of clone K1 (obtained from Washington State University). All plants were grown in a greenhouse in Boulder, CO. By May 1986 the cuttings were fully rooted and exhibited vigorous growth. All gas-exchange measurements were conducted between June 15 and July 20, 1986, and again between May 15 and May 30,

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[2] Abbreviations: PEP, phosphoenolpyruvate; Rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase; CE, carboxylation efficiency; C_i, intercellular CO_2 partial pressure; RPP, reductive pentose phosphate.

1987, when photoperiods were approximately 14.5 h. Midday photon flux densities were 750 to 800 μmol m^{-2} s^{-1} on clear days, and midday temperatures were between 27 and 35°C. Plants that were used to examine the light-response of photosynthesis were grown in an unshaded part of the greenhouse where midday photon flux densities were 1500 to 1800 μmol m^{-2} s^{-1}. All plants were watered twice weekly with half-strength Hoagland solution.

Gas-Exchange Measurements. All gas-exchange measurements were conducted with an open, infrared gas analysis system as described in detail previously (15). The protocol for conducting the quantum yield studies has also been described previously (15). The photosynthetic response to light was determined by progressing from the highest to the lowest photon flux density, using cheesecloth screens to modify the incident light intensities. The response of photosynthesis rate to intercellular CO_2 concentration was determined beginning with the highest CO_2 concentration and progressing to the lowest value. In one experiment, the CO_2-response curve was restricted to intercellular CO_2 partial pressures between 70 μbar and the CO_2 compensation point. The temperature dependence of photosynthesis was determined 20 to 37.5°C, beginning with the lowest temperature and progressing to the highest temperature. The leaf-to-air water vapor concentration gradient was maintained 9.5 to 15 mmol H_2O/mol air over the entire temperature range. The temperature response pattern was determined in the presence of 21% O_2 on 1 d and 2% O_2 on the next day. The same leaf was used on both days. Preliminary experiments showed no significant change in photosynthetic rates of several leaves during two successive days. All gas-exchange values were calculated according to von Caemmerer and Farquhar (23).

RESULTS

The photosynthetic response of *F. brownii* to C_i exhibited a pattern that was intermediate to those observed for the C_3 and C_4 species (Fig. 1), although it appeared more C_4-like than C_3-like. Photosynthesis rates at C_i values below 200 μbar were less than the C_4 species, but considerably greater than the C_3 species. The photosynthetic rate appeared to reach CO_2 saturation at a C_i of approximately 300 μbar in the C_4 species, but did not appear to saturate at C_i values as high as 335 μbar in *F. brownii.*

The photosynthetic response to photon flux density in leaves of *F. brownii* appeared to reach saturation at approximately 1600 μmol m^{-2} s^{-1} (Fig. 2). This differed from the pattern exhibited by the C_4 species, *F. trinervia*, which did not completely saturate up to 2000 μmol m^{-2} s^{-1}. The C_3 species, *F. cronquistii* also exhibited saturation at approximately 1600 μmol m^{-2} s^{-1}.

The temperature dependence of photosynthesis for leaves of *F. brownii* was similar to that reported for C_4 plants, exhibiting an apparent optimum at 35°C in either 2% or 21% O_2 (Fig. 3a). The oxygen inhibition of photosynthesis, expressed as a percentage of the photosynthesis rate observed in 2% O_2, decreased progressively as leaf temperature increased above 25°C (Fig. 3b). Intercellular CO_2 concentrations decreased progressively from the lowest temperature to the temperature optimum (data not shown), which would eliminate the possibility of increased CO_2 concentrations underlying the reduced O_2 inhibition. The photosynthetic response at 21% O_2 to temperature in the C_3 species, *F. cronquistii*, exhibited an optimum at 30°C, whereas the C_4 species, *F. trinervia*, exhibited a temperature optimum of 35°C (Fig. 4).

Gas-exchange rates were measured under a single set of environmental conditions for several leaves of *F. brownii* and compared to F. trinervia (C_4) and *F. cronquistii* (C_3) (Table I). Leaves of *F. brownii* exhibited higher levels of inhibition of photosynthesis by 21% O_2, relative to the C_4 species *F. trinervia*, but considerably lower levels relative to the C_3 species *F. cronquistii*. The 23% inhibition of photosynthesis observed for *F. cronquistii* is lower than the value of 33% reported in a previous study (20). However, the ambient CO_2 concentration (Ca) used in the latter study was 310 μmol/mol, which corresponds to a CO_2 partial pressure of 288 μbar (using a prevailing atmospheric pressure of 93 kPa for Pullman, WA; RK Monson, unpublished data). This is considerably less than the ambient CO_2 partial pressure of 340 μbar used in this study. The higher CO_2 partial pressure used in this study might account for the lower measured O_2 inhibition of photosynthesis. Intercellular CO_2 partial pressures (C_i) and water-use efficiencies were intermediate in *F. brownii* relative to the C_3 and C_4 species, although they were closer in value to the

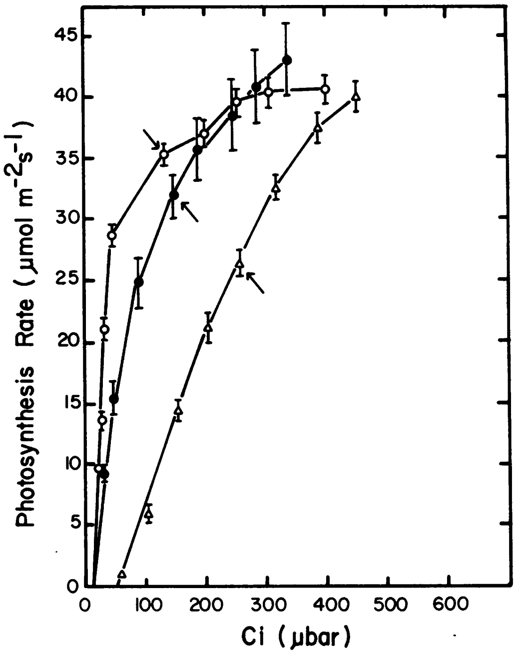

FIG. 1. The response of photosynthesis to intercellular CO_2 partial pressure (C_i) in *F. trinervia* (○), *F. cronquistii* (△), and *F. brownii* (●). Arrows indicate the points obtained when the atmospheric CO_2 partial pressure was 340 μbar. Other environmental conditions during the measurements were the same as listed in Table I. Vertical bars represent ±1 SE, $n = 3$. When error bars are not presented they were smaller than the size of the symbol.

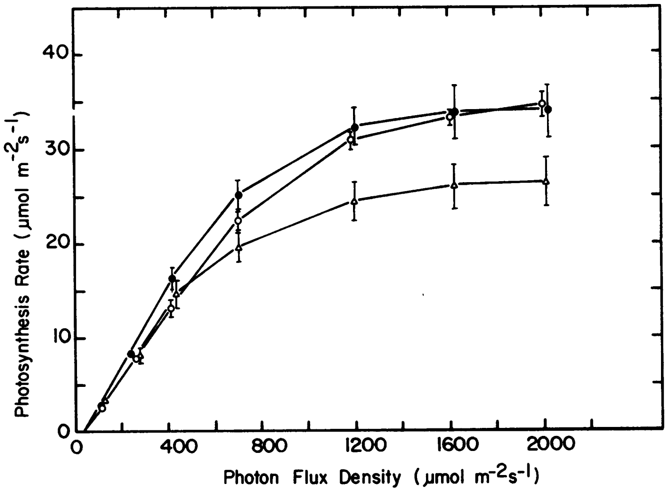

FIG. 2. The photosynthetic response to incident light intensity in *F. brownii* (●), *F. trinervia* (○), and *F. cronquistii* (△). Other environmental conditions during the measurements were the same as listed in Table I. The vertical bars are described in Figure 1; $n = 3$.

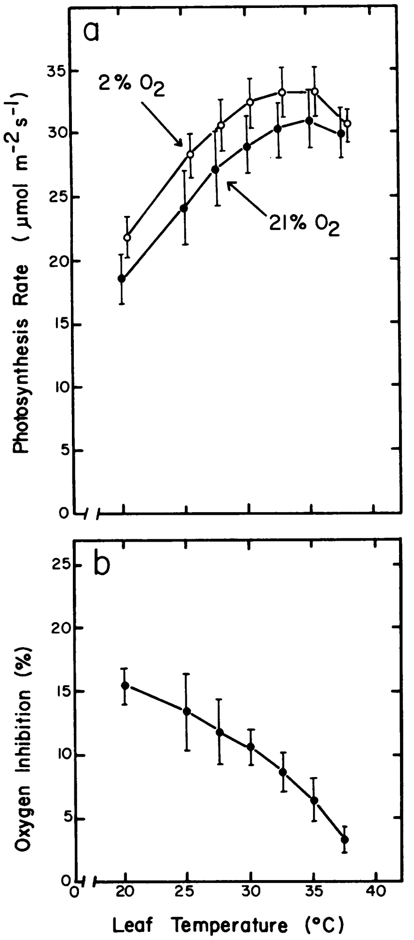

FIG. 3. a, The response of photosynthesis rate to leaf temperature in leaves of *F. brownii* in 2% and 21% O_2; b, the response of oxygen inhibition of photosynthesis, expressed as a percentage of the photosynthesis rate observed in 2% O_2, to leaf temperature in *F. brownii*. The vertical bars are described in Figure 1; $n = 3$.

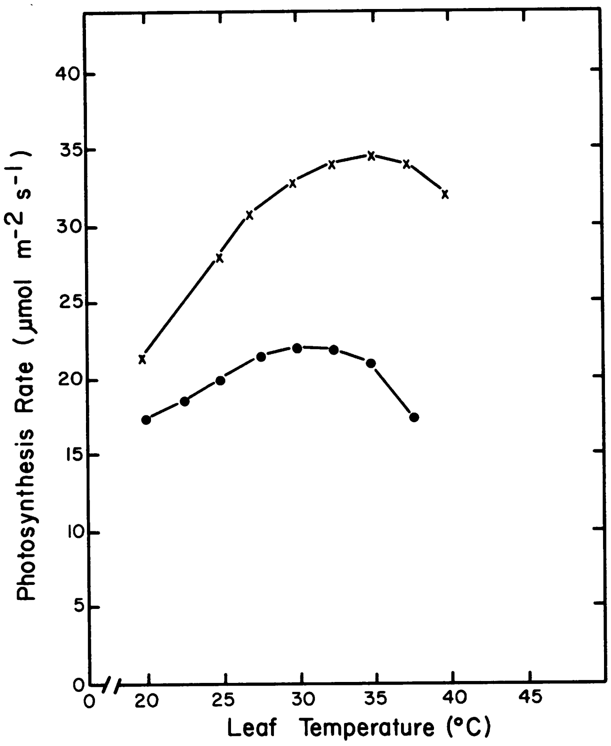

FIG. 4. The response of photosynthesis rate to leaf temperature in leaves of *F. trinervia* (×) and *F. cronquistii* (●) in 21% O_2 and 340 μbar atmospheric CO_2. The presented responses are for a single leaf and are representative of two replicates.

C_4 species.

The response of photosynthesis rate to C_i values below 12 μbar in leaves of *F. trinervia* exhibited a slight inhibition by 21% O_2 (Fig. 5a). Carboxylation efficiencies were measured as 1.08 ± 0.02 μmol m^{-2} s^{-1} μbar^{-1} ($n = 3$) in 2% O_2, and 0.91 ± 0.10 μmol m^{-2} s^{-1} μbar^{-1} ($n = 3$) in 21% O_2. Inhibition of the carboxylation efficiency by 21% O_2 at these extremely low CO_2 partial pressures may be due to a CO_2/O_2 conductance of the bundle-sheath cells that is sufficiently high to permit the diffusion of atmospheric O_2 into, or C_4 acid-derived CO_2 out of, the cells, resulting in some photorespiration (12, 17). The CO_2 compensation point was measured as 3.1 ± 0.04 μbar ($n = 3$) in 2% O_2, and 3.4 ± 0.2 μbar ($n = 3$) in 21% O_2. The response of photosynthesis rate to C_i values near the CO_2 compensation point in leaves of the C_3 species, *F. cronquistii*, exhibited slight curvilinearity in both 2 and 21% O_2 (Fig. 5c). Such a pattern is presumably related to deactivation of Rubisco at very low C_i values (2, 9). The CO_2 compensation point was 52.5 ± 1.4 μbar ($n = 3$) in 21% O_2 and 20.5 ± 0.5 μbar ($n = 6$) in 2% O_2. The high CO_2 compensation point in 2% O_2 was consistently observed in six different replicate experiments with *F. cronquistii*. In order to ensure that the high values were not due to artifacts of the analysis system, we conducted comparative measurements with fully expanded wheat leaves (*Triticum aestivum*). The CO_2 compensation point in wheat leaves was 50.5 μbar in 21% O_2 and 11.4 μbar in 2% O_2. The value in 2% O_2 is only slightly higher than the previously published value of 6 μbar (measured at 20°C; 2). Given that the measurements in the current study were conducted at 30°C, the compensation points for wheat are reasonably comparable between the two studies. The basis for the high CO_2 compensation point in 2% O_2 in *F. cronquistii*, relative to other C_3 species (2), is not currently known. CE in leaves of *F. brownii* were lower than those for the C_4 species, *F. trinervia* but higher than in the C_3 species *F. cronquistii* (Fig. 5b). In *F. brownii* the CO_2 compensation point was measured as 3.7 ± 0.8 μbar ($n = 3$) in 21% O_2 and 2.0 ± 0.4 μbar ($n = 3$) in 2% O_2. In leaves of *F. brownii* a change of slope occurred in the linear response of photosynthesis rate to C_i in the presence of 21% O_2. The result of the change in slope was that photosynthesis rates in 21% O_2 were slightly higher than those in 2% O_2 at C_i's 15 to 20 μbar. The CE at 21% O_2 was measured as 0.21 ± 0.04 μmol m^{-2} s^{-1} μbar^{-1} ($n = 3$) above the break in slope and 0.35 ± 0.01 μmol m^{-2} s^{-1} μbar^{-1} ($n = 3$) below the break. The slopes above and below the break were significantly different (analysis of covariance, $P < 0.05$).

The quantum yield for CO_2 uptake was not significantly affected by O_2 concentration in the C_4 plant *F. trinervia* (Fig. 6a). In leaves of the C_3 species, *F. cronquistii*, the quantum yield was reduced by 27% in 21% O_2 (Fig. 6c). In leaves of *F. brownii* the quantum yield for CO_2 uptake averaged 0.052 ± 0.001 mol CO_2/mol quanta absorbed ($n = 4$) in 21% O_2 and 0.058 ± 0.003 mol CO_2/mol quanta absorbed ($n = 4$) in 2% O_2, indicating a measurable inhibition of the quantum yield by 21% O_2, averaging 10.4 ± 2.9% ($n = 4$).

DISCUSSION

The increased inhibition of photosynthesis by 21% O_2 in *F. brownii*, relative to *F. trinervia*, is consistent with the results of previous comparative studies on biochemical aspects of C_4 photosynthesis in these two species. Studies of $^{14}CO_2$-pulse/$^{12}CO_2$-

Table I. *Gas-Exchange Characteristics of F. brownii, F. trinervia (C_4), and F. cronquistii (C_3)*

Leaf temperature was 30°C for *F. cronquistii*, 32.5°C for *F. brownii*, and 35°C for *F. trinervia*. These temperatures are within 2°C of the respective photosynthetic temperature optima (see Figs. 3a, 4). The atmospheric CO_2 partial pressure was 340 ± 5 μbar, the leaf-to-air water vapor concentration gradient (ΔW) was 14 to 20 mmol H_2O/mol air, and the photon flux density (400–700 nm) was 1500 μmol m^{-2} s^{-1}.

Characteristic	*F. brownii*	*F. trinervia*	*F. cronquistii*
Photosynthesis rate (μmol m^{-2} s^{-1})			
21% O_2	27.6 ± 1.0[a]	37.3 ± 1.2	21.5 ± 0.8
2% O_2	29.6 ± 1.2	37.2 ± 1.1	27.9 ± 1.2
% inhibition by 21% O_2	7.0 ± 1.0	−1.3 ± 0.9	23.0 ± 0.7
Stomatal conductance (mmol m^{-2} s^{-1})	295 ± 31	384 ± 111	470 ± 41
Intercellular CO_2 partial pressure (μbar)	164 ± 7	141 ± 5	245 ± 5
Water-use efficiency (μmol CO_2/mmol H_2O)	5.5 ± 0.1	6.4 ± 0.1	4.0 ± 0.2

[a] Values are mean ± SE for *F. brownii* and *F. trinervia*, $n = 5$. For *F. cronquistii*, $n = 8$.

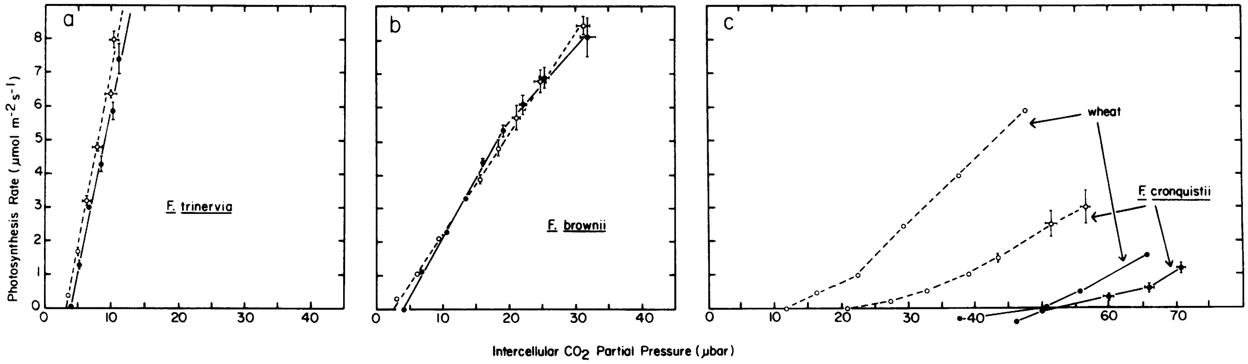

FIG. 5. The photosynthetic response to low intercellular CO_2 partial pressures in 2% O_2 (○) and 21% O_2 (●) for *F. trinervia* (a), *F. brownii* (b), and *F. cronquistii* and wheat (c). Other environmental conditions during the measurements were the same as listed in Table I. Vertical bars and horizontal bars are ± 1 SE as described in Figure 1; $n = 3$.

chase patterns (3, 6, 15, 20) and enzyme compartmentation ((4, 6, 16, 19) have revealed incomplete expression of C_4 photosynthesis in *F. brownii*, and fully expressed C_4 photosynthesis in *F. trinervia*. Also, one of the key enzymes of C_4 photosynthesis, PEP carboxylase, in *F. brownii* is intermediate to the enzyme in C_3 and other C_3-C_4 intermediate compared with C_4 *Flaverias* in kinetic and regulatory properties (5). When taken together, these previous results, along with those presented here, confirm the C_3-C_4 intermediate nature of *F. brownii*. Unlike most other C_3-C_4 intermediates in the genus *Flaveria*, however, *F. brownii* possesses photosynthetic traits much more closely allied with C_4 plants than with C_3 plants.

Since Rubisco also occurs in the mesophyll cells of *F. brownii*, the increased O_2 inhibition of photosynthesis in *F. brownii*, relative to fully expressed C_4 plants, may be due exclusively to mesophyll oxygenase activity. Alternatively, the increased O_2 inhibition could be due, in part, to a relatively high bundle-sheath conductance to atmospheric O_2 or C_4 acid-derived CO_2, thus permitting competition between O_2 and CO_2 for active sites of Rubisco in the bundle-sheath cells (17).

The response of photosynthesis to intercellular CO_2 partial pressures exhibited a pattern that was C_4-like, yet still intermediate between C_4 and C_3 *Flaveria* species. The CO_2 response suggests a CO_2-concentrating mechanism in leaves of *F. brownii*, though once again, it is apparently not as effective as in fully expressed C_4 species, such as *F. trinervia*.

The relatively high photosynthetic temperature optimum in *F. brownii* is also suggestive of a CO_2-concentrating mechanism and concomitant reduced O_2 inhibition of photosynthesis as leaf temperature increases, relative to C_3 plants. Decreases in the percentage O_2 inhibition of photosynthesis as temperature increases clearly demonstrates a reduction in the competitive interaction between CO_2 and O_2. In C_3 plants the percentage of O_2 inhibition of photosynthesis typically increases with increasing leaf temperature to a maximum near 40°C or higher (14). Whether the reduced O_2 sensitivity of photosynthesis in *F. brownii* at higher temperatures is due to increased initial fixation of atmospheric CO_2 through the C_4-cycle relative to the C_3-pathway awaits further investigation.

The quantum yield for CO_2 uptake was slightly higher in 2% O_2 in *F. brownii*, compared to the C_4 plant *F. trinervia*. This is presumably due to stimulation of the RPP pathway by low O_2 in the mesophyll cells of *F. brownii*. Inhibition of the quantum yield by 21% O_2 in *F. brownii* is, once again, likely due to incomplete enzyme compartmentation and exposure of the RPP pathway in the mesophyll cells to atmospheric O_2.

The unique pattern exhibited in the photosynthetic response to very low C_i values in *F. brownii* (Fig. 5b) might reflect a complex interaction between the RPP pathway and the C_4 cycle, and the activation state of Rubisco. The fact that the break in slope is apparent in 21% O_2, but not in 2% O_2, suggests that it is related to the oxygenase activity of Rubisco. Further experimentation at the biochemical level is needed to resolve the patterns of photosynthesis at low C_i in this species.

Two previous studies indicated that *F. brownii* is unique among other C_4 *Flaveria* species in having a higher CO_2 compensation point (1, 11). However, the CO_2 compensation point of *F. brownii* obtained in this study is similar to that of the C_4 species *F. trinervia*. An earlier study using a different method to determine the compensation point also reported values comparable to fully-expressed C_4 species (7). Thus, despite a low, but measurable O_2 inhibition of photosynthesis, *F. brownii* exhibits very little or no apparent photorespiration. Presumably, this is due to an efficient recycling of the photorespiratory CO_2 through the C_4- and C_3-cycles. Relative to *F. brownii*, most other C_3-C_4 intermediate *Flaverias* have higher CO_2 compensation points,

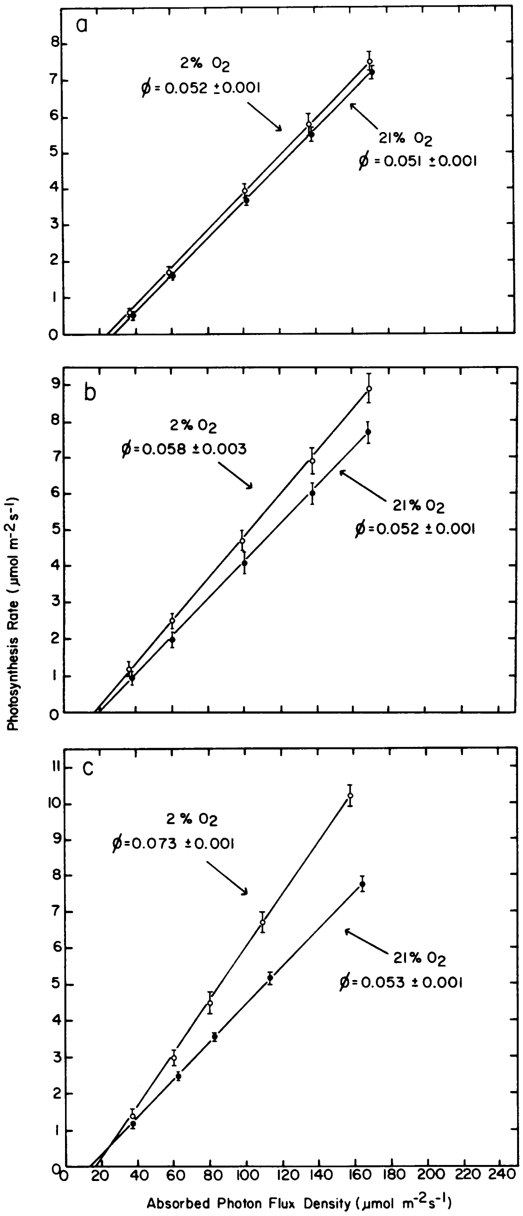

FIG. 6. The response of photosynthesis to low levels of absorbed light in *F. trinervia* (a), *F. brownii* (b), and *F. cronquistii* (c) in 2% and 21% O_2. The quantum yield for CO_2 uptake is indicated as Ø and has units of mol CO_2 mol^{-1} absorbed quanta. Vertical bars represent ± 1 SE; n = 3.

higher O_2 inhibition of photosynthesis, and lower capacity for C_4 photosynthesis (8). *F. brownii* is, by far, the most advanced C_3-C_4 intermediate yet examined in the genus in terms of development of the C_4 syndrome.

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