Photosynthesis in Flaveria brownii A.M. Powell

A C₄-LIKE C₂-C₄ INTERMEDIATE

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ABSTRACT

Leaves of Flaveria brownii exhibited slightly higher amounts of oxygen inhibition of photosynthesis than the C₃ species, Flaveria trinervia, but considerably less than the C₄ species, Flaveria cronquistii. The photosynthetic response to intercellular CO₂, light and leaf temperature were much more C₄-like than C₃-like, although 21% oxygen inhibited the photosynthetic rate, depending on conditions, up to 17% of the photosynthesis rate observed in 2% O₂. The quantum yield for CO₂ uptake in F. brownii was slightly higher than that for the C₃ species F. trinervia in 2% O₂, but not significantly different in 21% O₂. The quantum yield was inhibited 10% in the presence of 21% O₂ 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% O₂. The photosynthetic response to very low intercellular CO₂ partial pressures exhibited a unique pattern in F. brownii, with a break in the linear slope observed at intercellular CO₂ partial pressure values between 15 and 20 μbar when analyzed in 21% O₂. No significant break was observed when analyzed in 2% O₂. When taken collectively, the gas-exchange results reported here are consistent with previous biochemical studies that report incomplete intercellular compartmentation of the C₃ and C₄ enzymes in this species, and suggest that F. brownii is an advanced, C₃-like C₂-C₄ intermediate.

To date, nine species in the genus Flaveria (Asteraceae) have been characterized as exhibiting photosynthetic and photosynthetic traits intermediate to the C₃ and C₄-syndromes (1, 8, 11, 13). Several of these C₂-C₄ intermediates are capable of assimilating atmospheric CO₂ through the C₄-cycle (3, 15, 20). However, none of them exhibits sufficient integration and compartmentalization of the C₃ and C₄-cycles between the mesophyll and bundle-sheath cells to result in C₃-like carbon isotope fractionation ratios (1, 21, 22). Additionally, none of the C₂-C₄ species exhibit negligible levels of O₂ inhibition of photosynthesis, as is typical of fully expressed C₃ plants (13, 15).

Recently some evidence has been presented to suggest that Flaveria brownii is a C₃-like C₂-C₄ intermediate. In past studies, F. brownii has been treated as a typical C₃ species (18). However, recently it has been established that the principal photosynthetic carboxylation enzymes, PEP 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 C₄ leaves (4, 6, 19). The localization of Rubisco in mesophyll cells, as well as bundle-sheath cells, suggests that atmospheric CO₂ may be assimilated through parallel C₃ and C₄ pathways. ¹⁴CO₂-pulse studies showed only 65 to 75% of the assimilated ¹⁴CO₂ 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 δ¹³C) have been measured between -14.5‰ and -21‰ (DM Keefe, LJ Mots, University of Chicago, personal communication), with most values falling between -16 to -18‰ when the plants were grown in warm, long-photoperiod regimes. A δ¹³C 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 C₃ 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 C₃, C₃-C₄, and C₄ Flaveria species (5).

In studies presented here, we examined the photosynthetic responses to oxygen, CO₂, light, and temperature in F. brownii leaves to determine whether such responses also reflect a C₂-C₄ intermediate nature. There is some evidence from carbon isotope ratios that the relative expression of C₃ and C₄ pathways in F. brownii is under partial environmental control, with C₄ photosynthesis reaching its greatest expression when grown in warm, long-photoperiod regimes (DM Keefe, LJ Mots, University of Chicago, personal communication). In order to establish photosynthetic traits of F. brownii in the most C₃-like condition, and thus bias our results as far as possible away from the C₂-C₄ 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 C₂-C₄ intermediate species, we would have to observe such intermediate photosynthetic traits in plants that had been provided with every opportunity for C₄-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 (C₃) were grown from seed collected near Lubbock, TX. Plants of F. cronquistii A.M. Powell (C₄) 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.
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 to intercellular CO₂ concentration was determined beginning with the highest CO₂ concentration and progressing to the lowest value. In one experiment, the CO₂-response curve was restricted to intercellular CO₂ partial pressures between 70 μbar and the CO₂ 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 H₂O/mol air over the entire temperature range. The temperature response pattern was determined in the presence of 21% O₂ on 1 d and 2% O₂ 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 C₃ exhibited a pattern that was intermediate to those observed for the C₃ and C₄ species (Fig. 1), although it appeared more C₄-like than C₃-like. Photosynthesis rates at C₃ values below 200 μbar were less than the C₄ species, but considerably greater than the C₃ species. The photosynthetic rate appeared to reach CO₂ saturation at a C₃ of approximately 300 μbar in the C₃ species, but did not appear to saturate at C₃ 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 C₄ species, F. trinervia, which did not completely saturate up to 2000 μmol m^{-2} s^{-1}. The C₃ 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 C₃ plants, exhibiting an apparent optimum at 35°C in either 2% or 21% O₂ (Fig. 3a). The oxygen inhibition of photosynthesis, expressed as a percentage of the photosynthesis rate observed in 2% O₂, decreased progressively as leaf temperature increased above 25°C (Fig. 3b). Intercellular CO₂ concentrations decreased progressively from the lowest temperature to the temperature optimum (data not shown), which would eliminate the possibility of increased CO₂ concentrations underlying the reduced O₂ inhibition. The photosynthetic response at 21% O₂ to temperature in the C₃ species, F. cronquistii, exhibited an optimum at 30°C, whereas the C₄ 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 (C₃) and F. cronquistii (C₄) (Table I). Leaves of F. brownii exhibited higher levels of inhibition of photosynthesis by 21% O₂, relative to the C₃ species F. trinervia, but considerably lower levels relative to the C₃ 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 CO₂ concentration (Ca) used in the latter study was 310 μmol/mol, which corresponds to a CO₂ 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 CO₂ partial pressure of 340 μbar used in this study. The higher CO₂ partial pressure used in this study might account for the lower measured O₂ inhibition of photosynthesis. Intercellular CO₂ partial pressures (Ca) and water-use efficiencies were intermediate in F. brownii relative to the C₃ and C₄ species, although they were closer in value to the
The response of photosynthesis rate to C4 values below 12 μbar in leaves of *F. trinervia* exhibited a slight inhibition by 21% O2 (Fig. 5a). Carboxylation efficiencies were measured as 1.08 ± 0.02 μmol m⁻² s⁻¹ μbar⁻¹ (n = 3) in 2% O₂, and 0.91 ± 0.10 μmol m⁻² s⁻¹ μbar⁻¹ (n = 3) in 21% O₂. Inhibition of the carboxylation efficiency by 21% O₂ at these extremely low CO₂ partial pressures may be due to a CO₂/O₂ conductance of the bundle-sheath cells that is sufficiently high to permit the diffusion of atmospheric O₂ into, or C₄ acid-derived CO₂ out of, the cells, resulting in some photosynthesis (12, 17). The CO₂ compensation point was measured as 3.1 ± 0.04 μbar (n = 3) in 2% O₂, and 3.4 ± 0.2 μbar (n = 3) in 21% O₂. The response of photosynthesis rate to C4 values near the CO₂ compensation point in leaves of the C₃ species, *F. cronquistii*, exhibited slight curvilinearity in both 2 and 21% O₂ (Fig. 5c). Such a pattern is presumably related to deactivation of Rubisco at very low C₄ values (2, 9). The CO₂ compensation point was 52.5 ± 1.4 μbar (n = 3) in 21% O₂, and 20.5 ± 0.5 μbar (n = 6) in 2% O₂. The high CO₂ compensation point in 2% O₂ 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 CO₂ compensation point in wheat leaves was 50.5 μbar in 21% O₂ and 11.4 μbar in 2% O₂. The value in 2% O₂ 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 CO₂ compensation point in 2% O₂ in *F. cronquistii*, relative to other C₃ species (2), is not currently known. CE in leaves of *F. brownii* were lower than those for the C₄ species, *F. trinervia* but higher than in the C₃ species *F. cronquistii* (Fig. 5b). In *F. brownii* the CO₂ compensation point was measured as 3.7 ± 0.8 μbar (n = 3) in 21% O₂ and 2.0 ± 0.4 μbar (n = 3) in 2% O₂. In leaves of *F. brownii* a change of slope occurred in the linear response of photosynthesis rate to C₄ in the presence of 21% O₂. The result of the change of slope was that photosynthesis rates in 21% O₂ were slightly higher than those in 2% O₂ at C₄'s 15 to 20 μbar. The CE at 21% O₂ 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 CO₂ uptake was not significantly affected by O₂ concentration in the C₄ plant *F. trinervia* (Fig. 6a). In leaves of the C₃ species, *F. cronquistii*, the quantum yield was reduced by 27% in 21% O₂ (Fig. 6c). In leaves of *F. brownii* the quantum yield for CO₂ uptake averaged 0.052 ± 0.001 mol CO₂/mol quanta absorbed (n = 4) in 21% O₂ and 0.058 ± 0.003 mol CO₂/mol quanta absorbed (n = 4) in 2% O₂, indicating a measurable inhibition of the quantum yield by 21% O₂, averaging 10.4 ± 2.9% (n = 4).

**DISCUSSION**

The increased inhibition of photosynthesis by 21% O₂ in *F. brownii*, relative to *F. trinervia*, is consistent with the results of previous comparative studies on biochemical aspects of C₄ photosynthesis in these two species. Studies of ¹⁴CO₂-pulse/¹²CO₂-
Table 1. Gas-Exchange Characteristics of F. brownii, F. trinervia (C4), and F. cronquistii (C3)

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 CO2 partial pressure was 340 ± 5 μbar, the leaf-to-air water vapor concentration gradient (ΔW) was 14 to 20 mmol H2O/mol air, and the photon flux density (400–700 nm) was 1500 μmol m⁻² s⁻¹.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>F. brownii</th>
<th>F. trinervia</th>
<th>F. cronquistii</th>
</tr>
</thead>
<tbody>
<tr>
<td>Photosynthesis rate (μmol m⁻² s⁻¹)</td>
<td>27.6 ± 1.0*</td>
<td>37.3 ± 1.2</td>
<td>21.5 ± 0.8</td>
</tr>
<tr>
<td>2% O2</td>
<td>29.6 ± 1.2</td>
<td>37.2 ± 1.1</td>
<td>27.9 ± 1.2</td>
</tr>
<tr>
<td>% inhibition by 21% O2</td>
<td>7.0 ± 1.0</td>
<td>-1.3 ± 0.9</td>
<td>23.0 ± 0.7</td>
</tr>
<tr>
<td>Stomatal conductance (mmol m⁻² s⁻¹)</td>
<td>295 ± 31</td>
<td>384 ± 111</td>
<td>470 ± 41</td>
</tr>
<tr>
<td>Intercellular CO2 partial pressure (μbar)</td>
<td>164 ± 7</td>
<td>141 ± 5</td>
<td>245 ± 5</td>
</tr>
<tr>
<td>Water-use efficiency (μmol CO2/mmol H2O)</td>
<td>5.5 ± 0.1</td>
<td>6.4 ± 0.1</td>
<td>4.0 ± 0.2</td>
</tr>
</tbody>
</table>

* 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 CO2 partial pressures in 2% O2 (C) and 21% O2 (•) 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.

Though expressed C4 inhibition of photosynthesis typically increases with increasing leaf temperature to a maximum near 40°C or higher (14). Whether the reduced O2 sensitivity of photosynthesis in F. brownii at higher temperatures is due to increased initial fixation of atmospheric CO2 through the C4-cycle relative to the C3-pathway awaits further investigation.

The quantum yield for CO2 uptake was slightly higher in 2% O2 in F. brownii, compared to the C4 plant F. trinervia. This is presumably due to stimulation of the RPP pathway by low O2 in the mesophyll cells of F. brownii. Inhibition of the quantum yield by 21% O2 in F. brownii is, once again, likely due to incomplete enzyme compartmentation and exposure of the RPP pathway in the mesophyll cells to atmospheric O2.

The unique pattern exhibited in the photosynthetic response to very low C4 values in F. brownii (Fig. 5b) might reflect a complex interaction between the RPP pathway and the C4 cycle, and the activation state of Rubisco. The fact that the break in slope is apparent in 21% O2, but not in 2% O2, 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 C4 in this species.

Two previous studies indicated that F. brownii is unique among other C4 Flaveria species in having a higher CO2 compensation point (1, 11). However, the CO2 compensation point of F. brownii obtained in this study is similar to that of the C4 species F. trinervia. An earlier study using a different method to determine the compensation point also reported values comparable to fully-expressed C4 species (7). Thus, despite a low, but measurable O2 inhibition of photosynthesis, F. brownii exhibits very little or no apparent photorespiration. Presumably, this is due to an efficient recycling of the photorespiratory CO2 through the C3- and C4-cycles. Relative to F. brownii, most other C3-C4 intermediate Flaverias have higher CO2 compensation points,
higher O₂ inhibition of photosynthesis, and lower capacity for C₄ photosynthesis (8). *F. brownii* is, by far, the most advanced C₃-C₄ intermediate yet examined in the genus in terms of development of the C₄ syndrome.

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LITERATURE CITED


