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The impact of elevated carbon dioxide on the growth and gas exchange of three C_4 species differing in CO_2 leak rates

Lewis H. Ziska*, Richard C. Sicher and James A. Bunce

Climate Stress Laboratory, USDA-ARS, Bldg 046A, 10300 Baltimore Avenue, Beltsville, MD 20705, USA
**Corresponding author, e-mail: lziska@asrr.arsusda.gov*

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Recent work has suggested that the photosynthetic rate of certain C_4 species can be stimulated by increasing CO_2 concentration, $[CO_2]$, even under optimal water and nutrients. To determine the basis for the observed photosynthetic stimulation, we tested the hypothesis that the CO_2 leak rate from the bundle sheath would be directly related to any observed stimulation in single leaf photosynthesis at double the current $[CO_2]$. Three C_4 species that differed in the reported degree of bundle sheath leakiness to CO_2, *Flaveria trinervia*, *Panicum miliaceum*, and *Panicum maximum*, were grown for 31–48 days after sowing at a $[CO_2]$ of 350 μl l^{-1} (ambient) or 700 μl l^{-1} (elevated). Assimilation as a function of increasing $[CO_2]$ at high photosynthetic photon flux density (PPFD, 1600 μmol m^{-2} s^{-1}) indicated that leaf photosynthesis was not saturated under current ambient $[CO_2]$ for any of the three C_4 species. Assimilation as a function of increasing PPFD also indicated that the response of leaf photosynthesis to elevated $[CO_2]$ was light dependent for all three C_4 species. The stimulation of leaf photosynthesis at elevated $[CO_2]$ was not associated with previously published values of CO_2 leak rates from the bundle sheath, changes in the ratio of activities of PEP-carboxylase to RuBP carboxylase/oxgenase, or any improvement in daytime leaf water potential for the species tested in this experiment. In spite of the simulation of leaf photosynthesis, a significant increase in growth at elevated $[CO_2]$ was only observed for one species, *F. trinervia*. Results from this study indicate that leaf photosynthetic rates of certain C_4 species can respond directly to increased $[CO_2]$ under optimal growth conditions, but that the stimulation of whole plant growth at elevated carbon dioxide cannot be predicted solely on the response of individual leaves.

Introduction

At present, the current atmospheric carbon dioxide concentration, $[CO_2]$, of 360 μl l^{-1} is expected to exceed 600 μl l^{-1} by the end of the 21st century (Houghton et al. 1996). Since carbon dioxide is the sole source of carbon for growth in plants, the response of photosynthesis and growth to increasing $[CO_2]$ has been the subject of a number of studies (cf. Kimball et al. 1993). These studies have focused principally on species with the C_3 photosynthetic pathway since their photosynthetic capacity and potential growth are limited at the current concentration of CO_2 due, in part, to photorespiratory carbon loss (Allen 1990).

Increased growth in response to elevated $[CO_2]$ has been reported for C_4 species (see Poorter 1993 for a review), but this increase is usually attributed to the indirect effects of CO_2 (e.g. stomatal closure with a subsequent improvement in water use efficiency and water potential). However, recent studies of C_4 species have indicated that the growth and photosynthetic rate of some C_4 plants could respond directly to increasing $[CO_2]$ (Ghannoum et al. 1997, Read et al. 1997, Ziska and Bunce 1997, LeCain and Morgan 1998). Although water potential was not measured for all species, most of these recent C_4 studies were conducted under optimal conditions of water and nutrient availability.

If the growth of certain C_4 species is, in fact, stimulated as atmospheric $[CO_2]$ increases, this suggests that some aspect of C_4 photosynthesis may be affected differentially. Present evidence indicates that differences in the conductance of the bundle sheath cells to CO_2 can result in different rates of CO_2 leakage (Hattersley 1982, Ehleringer and Pearcy 1983, Brown and Byrd 1993). Although specific values within a

Abbreviations – A: leaf assimilation rate; $[CO_2]$: carbon dioxide concentration; DAS: days after sowing; PEP-carboxylase: phospholenolpyruvate carboxylase; rubisco: ribulose bisphosphate carboxylase/oxygenase.

subtype vary, in general, the rate of leakage from bundle sheath cells in C_4 plants is thought to be lower in monocots than in dicots (due to the presence of suberized lamella in bundle sheath cells in grasses) and may vary according to decarboxylating subtype (lower in NADP-ME and PCK subtypes than in NAD-ME enzyme type C_4 plants) (Ehleringer and Pearcy 1983). In addition, CO_2 bundle sheath leakiness can also be associated with changes in the ratio of rubisco:PEP-carboxylase activity, with leak rates increasing as the ratio decreases (Saliendra et al. 1996).

Enhanced photosynthesis and growth of a given C_4 species with increasing CO_2 concentration could therefore be proportional to the CO_2 leak rate, since any CO_2 that leaks from the bundle sheath cells will reduce the efficiency of C_4 photosynthesis. Although leak rates within a given decarboxylation type may vary, few studies have measured specific rates of CO_2 leakage from the bundle sheath. Previously published work by Hatch et al. (1995) using a pulse-chase procedure to estimate the leakage of CO_2 from bundle sheath cells at steady-state photosynthesis, reported the lowest CO_2 leak rates in the C_4 monocots *Panicum maximum* (L.) (PEP-CK subtype) and *Panicum miliaceum* (L.) (NAD-ME subtype), with the highest leak rates (approximately $2.5\times$ those of the two *Panicum* species) for *Flaveria trinervia* (Spreng.) (NADP-ME subtype) (see Tables IV and V, Hatch et al. 1995).

Using these contrasting C_4 species, our principal hypothesis in the current experiment was to determine if the response of single leaf photosynthesis and subsequent plant growth at elevated CO_2 was proportional to the reported rates of CO_2 leakage from the bundle sheath for these three species.

Materials and methods

All experiments were conducted in controlled environment chambers located at the Climate Stress Laboratory, USDA-ARS, Beltsville, MD, using three C_4 species: *Flaveria trinervia* (Spreng.) (NADP-ME subtype), *Panicum maximum* (L.) (PEP-CK subtype), and *Panicum miliaceum* (L.) (NAD-ME subtype). Seed for both *Panicum* species was obtained from the Valley Seed Company (Fresno, CA) and seed for *F. trinervia* from Professor Scott Holaday at Texas Tech University (Lubbock, TX).

For each controlled environment chamber (EGC, Chagrin Falls, OH), the $[CO_2]$ was controlled by continuous flushing with CO_2-free air, then re-injected with CO_2 to maintain the desired concentration. Carbon dioxide concentration was maintained by an absolute infra-red gas analyzer (WMA-2 PP Systems, Haverhill, MA) which activated a solenoid valve to control the injection of pure CO_2. Carbon dioxide set points were 350 (ambient) and 700 $\mu l\ l^{-1}$ (elevated, 100% above ambient). Actual $[CO_2]$, based on a 24-h average was 354 ± 11 and $708 \pm 18\ \mu l\ l^{-1}$. All plants received 14 h of 800 $\mu mol\ m^{-2}\ s^{-1}$ photosynthetic active radiation (PPFD) at the upper leaf level from a mixture of incandescent, high pressure sodium and metal halide lamps (GE Corp., Glen Ellen, VA). Day/night temperatures were maintained at 25°C, and average daily relative humidity (RH) exceeded 60%. Temperature, $[CO_2]$, and RH inside the chambers were monitored and recorded at 1 min intervals by an EGC network datalogger (EGC Corp.) in conjunction with a PC. It can be argued that the growth temperature is low for a C_4 species or that the plants received insufficient irradiance. However, the mean daily temperature for July in the Beltsville area was 25.7°C, and the June, July, and August mean was 24.6°C. Daily PPFD for the experimental plants was ca 40 moles m^{-2}, which is consistent with the outdoor PPFD received on cloudless summer days in this location.

Three to five seeds were sown in 20-cm diameter (3.5 l) pots filled with vermiculite. Pots were rotated weekly to minimize microclimate effects within the growth chamber. All pots were thinned to one seedling within 5 days after emergence. For each experiment, 12–15 pots of a given C_4 species were assigned to each CO_2 treatment. All pots were watered daily with complete nutrient solution containing 13.5 m*M* nitrogen (Robinson 1984).

To determine if photosynthesis of a given C_4 species was saturated at the current level of CO_2, single leaf photosynthesis (measured as A, the rate of CO_2 assimilation) was determined as a function of changing $[CO_2]$ and PPFD twice during the vegetative growth of each species. Assimilation was determined on the uppermost, fully expanded leaf for 2 plants of each species (i.e. 2 per CO_2 treatment) using a differential infra-red carbon dioxide analyzer (model 6252, Li-Cor Corp., Lincoln, NE) in an open system attached to two single leaf cuvettes. Temperature, humidity, and $[CO_2]$ were set to approximate those of the growth chamber. The gas stream was humidified by bubbling it through a water bath at a given dew point, and humidity was monitored by a dew point hygrometer (Hygro M-1, General Eastern Co., Cambridge, MA). Mass flow controllers (Tylan Corp.) were used to mix dry CO_2-free air with tanks of 100% CO_2 to obtain a desired $[CO_2]$ within a cuvette. Supplemental lighting was supplied by a GE 150-W cool-beam floodlight (GE Corp., Cleveland, OH) attached to a variable transformer to obtain a desired PPFD. The response of photosynthesis to light (PPFD) was determined by measuring CO_2 assimilation under ambient growth conditions (i.e. either 350 or 700 $\mu l\ l^{-1}$ CO_2, 25°C, and 800 $\mu mol\ m^{-2}\ s^{-1}$ PPFD), then lowering the PPFD value to 100 $\mu mol\ m^{-2}\ s^{-1}$, then increasing the PPFD value (after a 20–30 min equilibration time at each setting) to 200, 400, 800, 1600, and 2100 $\mu mol\ m^{-2}\ s^{-1}$. The response of photosynthesis to external $[CO_2]$ was determined using the same leaves the following day. Photosynthesis was determined initially as the CO_2 assimilation rate at the growth CO_2 concentration (C_a) at saturating light intensity (i.e. 1600 $\mu mol\ m^{-2}\ s^{-1}$). The C_a was then reduced to 90 $\mu l\ l^{-1}$ and increased in steps to 180, 360, 720, 1080, and 1440 $\mu l\ l^{-1}$. Sufficient time (usually 20–30 min) was given after the C_a was changed to allow equilibration to occur. At the end of this measurement, leaf lamina within a cuvette was cut and leaf area determined with a leaf area meter (Li-Cor, Model 3100, Lincoln, NE).

Since CO_2 leak rates from the bundle sheath could reflect changes in the ratio of ribulose bisphosphate carboxylase/oxygenase (rubisco) to phosphophenol pyruvate carboxylase (PEP-carboxylase), rubisco and PEP-carboxylase activities

were determined for each species and $[CO_2]$ at 25°C for leaves of the same position and age as were used in gas exchange. Leaf segments (2.0–4.0 cm^2) were collected from lamina of the uppermost fully expanded leaves of plants that were fully illuminated for a minimum of 4 h prior to sampling. Samples were quenched directly in liquid N_2 to stop metabolism. The frozen tissue was extracted immediately or was stored at −80°C in small aluminum foil envelopes. Frozen samples were extracted at 0°C in a ground glass tissue homogenizer with 1–2 ml extraction buffer containing 50 m*M* Bicine-NaOH (pH 8.1), 5 m*M* $MgCl_2$, 1 m*M* EDTA, 10% (v/v) glycerol, 1% (w/v) soluble PVP (Plasdone-c30, Calbiochem-Behring, La Jolla, CA), 0.01% Triton X-100, 2% (w/v) bovine serum albumin (BSA), and 5 m*M* dithiothreitol (DTT). Homogenates were transferred to 2.0 ml polypropylene centrifuge tubes and spun for 2 min in a microcentrifuge (Eppendorf model 5415C, Brinkmann Instruments, Westbury, NY). Supernatant fractions were rapidly frozen in liquid N_2 and were used immediately after thawing.

Phosphoenolpyruvate carboxylase (PEP-carboxylase) activity was measured spectrophotometrically at 25°C according to Uedan and Sugiyama (1976). The standard assay contained 50 m*M* Tris-HCl (pH 8.0), 5 m*M* $MgCl_2$, 4 m*M* PEP, 10 m*M* $NaHCO_3$, 0.15 m*M* NADH, 2 units of malate dehydrogenase, and 5–10 µl of extract.

Initial and total rubisco activities were measured radiometrically by a modification of previously described procedures (Sicher et al. 1995). Assays were initiated by injecting 25 µl of thawed extract into 175 µl activation buffer at 0°C containing 50 m*M* Bicine-NaOH (pH 8.1), 5 m*M* $MgCl_2$, 12.5 m*M* $NaHCO_3$, 2% BSA, 1.25 m*M* [^{14}C] $NaHCO_3$ (13 Gbq mol^{-1}). Assays were terminated after 30 s with 0.2 ml 0.5 *M* HCl, and acid stable ^{14}C was measured in a liquid scintillation counter (model 2800, Beckman Instruments, Fullerton, CA). During initial experiments, PEP-carboxylase activity was lost at a rate of 1–2% per min at 0°C. This was stabilized by adding 2% BSA during the extraction step. Moreover, initial rubisco activity was greater than total activity unless a positive effector (1.25 m*M* 6-phosphogluconate) was added during the CO_2 and Mg^{2+} dependent activation step. Unless otherwise indicated, values reported are for fully activated enzymes per unit leaf surface area.

To determine if growth at elevated CO_2 resulted in improved water relations, leaf water potential was measured in the dew point mode using a HR-33 microvoltmeter and six insulated C-51 sample chambers (Wescor Inc., Logan, UT). Water potential measurements were determined during the afternoon (13:00–15:00 h) for six leaves from each species and CO_2 treatment.

All species (4–6 pots per species per CO_2 treatment) were harvested at the end of the vegetative stage. This corresponded to panicle initiation (PI) at 32 days after sowing (DAS) for *P. miliaceum*, 48 DAS for *P. maximum* and floral bud appearance at 48 DAS for *F. trinervia*. All plants of a given species and CO_2 treatment were cut at ground level and separated into leaf laminae, stems (sheaths for monocotyledons), and roots. Total leaf area was determined photometrically with a leaf area meter (Li-Cor Corp.). Dry weights were obtained separately for leaves, stems, and roots. Material was dried at 55°C for a minimum of 72 h (or until dry weight was constant), then weighed.

Because only one pair of chambers was available, the entire experiment (including gas exchange and biochemical measurements) was repeated, and pooled data from the two runs are presented. The effect of CO_2 treatment on the light and CO_2 response of photosynthesis, activities of rubisco and PEP-carboxylase, water potential, and dry weight at harvest were analyzed by species using a Student's unpaired t-test. Unless otherwise stated, comparisons between ambient and elevated CO_2 concentration as a function of C_4 species were made using a two-way ANOVA with means separated by a least square means table (SuperANOVA, Berkeley, CA).

Results

At the growth PPFD (i.e. 800 µmol m^{-2} s^{-1}), single leaf photosynthesis showed a significant stimulation at elevated relative to ambient $[CO_2]$ for both *F. trinervia* and *P. miliaceum*, but not *P. maximum* ($P = 0.10$) (Table 1). Averaging all photosynthetic data ($[CO_2]$ and PPFD responses) taken at saturating PPFD (> 1600 µmol m^{-2} s^{-1}) showed significant increases in leaf assimilation rates for *F. trinervia* (+16%), *P. miliaceum* (+18%), and *P. maximum* (+24%) in response to elevated $[CO_2]$ (Table 1). The observed increase in leaf photosynthesis was independent of any improvement in daytime (13:00–15:00 h) water potentials for *F. trinervia* (−1.23, −1.21 MPa), *P. miliaceum* (−0.73, −0.67 MPa), or *P. maximum* (−0.95, −0.93 MPa) at the ambient and elevated $[CO_2]$, respectively. Values of leaf water potential were consistent with the well watered/fertilized condition of these plants.

Long-term growth at a given CO_2 concentration can alter leaf assimilation characteristics from the ambient condition, indicating photosynthetic acclimation. To determine changes in photosynthetic acclimation as a consequence of $[CO_2]$, assimilation rates from leaves of each species and CO_2 treatment were determined over a wide range of mea-

Table 1. Average rates of single leaf photosynthesis (determined as CO_2 assimilation) ± SE for *F. trinervia*, *P. miliaceum*, and *P. maximum* grown at ambient (350 µl l^{-1}) and elevated (700 µl l^{-1}) CO_2 concentrations. Photosynthetic rates were averaged for measurements at 800 µmol m^{-2} s^{-1} (growth PPFD) and at saturating light (i.e. >1 600–2 100 µmol m^{-2} s^{-1}). * indicates a significant increase in single leaf photosynthesis relative to the ambient CO_2 concentration for a given PPFD (Student's t-test assuming unequal variances). n = 8.

Species	CO_2 treatment	PPFD (µmole m^{-2} s^{-1})	
		800	2 100
		Photosynthetic rate (µmol CO_2 m^{-2} s^{-1})	
F. trinervia	Ambient	37.0 ± 1.45	44.1 ± 2.1
	Elevated	42.4 ± 1.93*	51.2 ± 2.1*
P. miliaceum	Ambient	34.7 ± 0.57	48.1 ± 2.8
	Elevated	38.3 ± 1.82*	53.9 ± 1.4*
P. maximum	Ambient	38.3 ± 3.13	44.7 ± 2.8
	Elevated	43.1 ± 1.62 ($P = 0.10$)	55.5 ± 2.1*

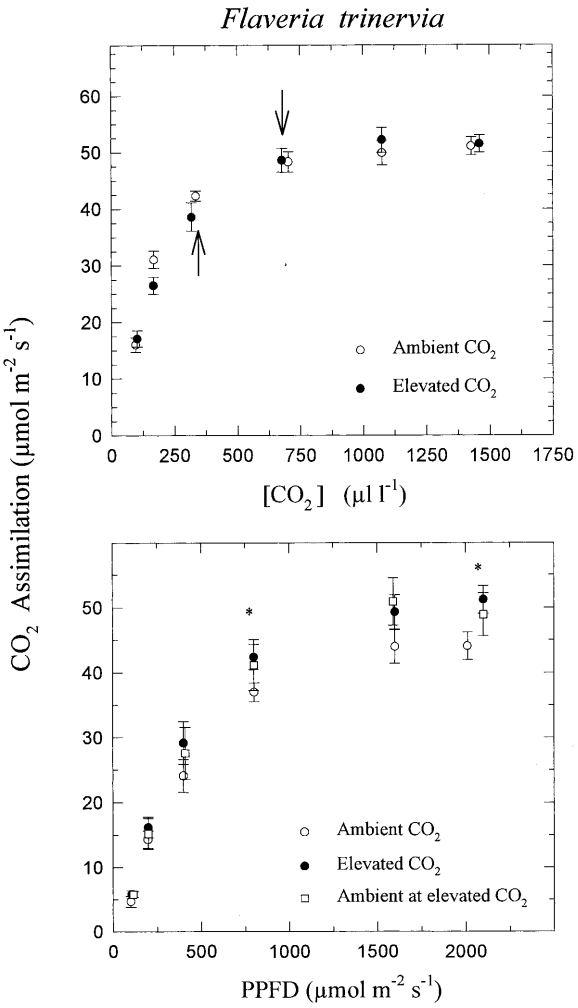

Fig. 1. Response of leaf photosynthesis (measured as leaf CO_2 assimilation rate, A) to a range of external CO_2 concentrations (C_a) (upper graph, measured at a common PPFD of 1600 μmol m^{-2} s^{-1}) and PPFD values (lower graph) for single leaves of *Flaveria trinervia* (NADP-ME decarboxylating C_4 subtype) grown at either ambient (○, 350 μl l^{-1}) or elevated (●, 700 μl l^{-1}) CO_2 concentrations, or ambient grown leaves tested at elevated $[CO_2]$ (□). * indicates a significant difference between growth CO_2 treatments (i.e. grown and tested at ambient or elevated $[CO_2]$) at the $P = 0.05$ level for a given PPFD value (Student's t-test). Arrows in upper graph indicate measurement at the respective growth CO_2 concentration. Bars are ± SE. Each point is the average of eight measurements taken over two runs of the same experiment.

sured $[CO_2]$ and PPFD values. The response of assimilation to external CO_2 concentrations indicated that the overall response was not altered as a function of the treatment $[CO_2]$ for any of the three species (Figs. 1–3). All species, however, indicated that leaf photosynthesis was not saturated at the ambient $[CO_2]$ (i.e. 350 μl l^{-1}) (Figs. 1–3). Growth at elevated $[CO_2]$ did alter the response to PPFD for all three species. Although separation at lower PPFDs (< 500 μmol m^{-2} s^{-1}) between CO_2 treatments was more apparent for *F. trinervia* (Fig. 1), significant differences in leaf assimilation at the elevated relative to the ambient growth $[CO_2]$ were observed only at PPFDs of 800 μmol m^{-2} s^{-1} or higher (Figs. 1–3). Overall, the relative stimulation of leaf photosynthesis at the elevated $[CO_2]$ increased slightly from a PPFD of 800 to a saturating PPFD of 2100 μmol m^{-2} s^{-1} when averaged for all three species (+12–18%, Table 1). No significant change in either the initial slope of the $[CO_2]$ response or the response to PPFD was observed at the elevated relative to the ambient $[CO_2]$ for any species (data not shown).

Although leaf assimilation rates increased at the elevated $[CO_2]$, this increase was not associated with any significant change in rubisco or PEP-carboxylase activity (Table 2). There was a tendency for the ratio of PEP-carboxylase to rubisco activity to decrease at the elevated CO_2 treatment for all species, but this difference was not significant ($P = 0.24$). Overall, the high activity ratio of PEP-carboxylase to rubisco was consistent with the C_4 pathway of photosynthesis.

At the growth PPFD, the relative increase in leaf assimilation at elevated $[CO_2]$ did not differ between the three species (i.e. +10–15%); however, only *F. trinervia* showed a

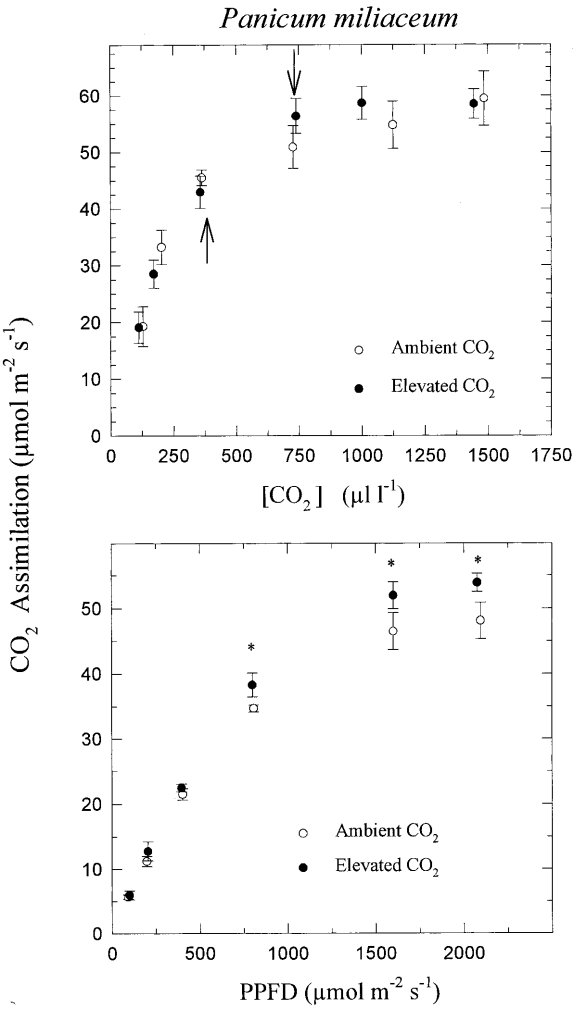

Fig. 2. Same as Fig. 1, but for *Panicum miliaceum* (NAD-ME decarboxylating subtype). Ambient grown leaves were not measured at elevated CO_2 for the PPFD response (lower graph).

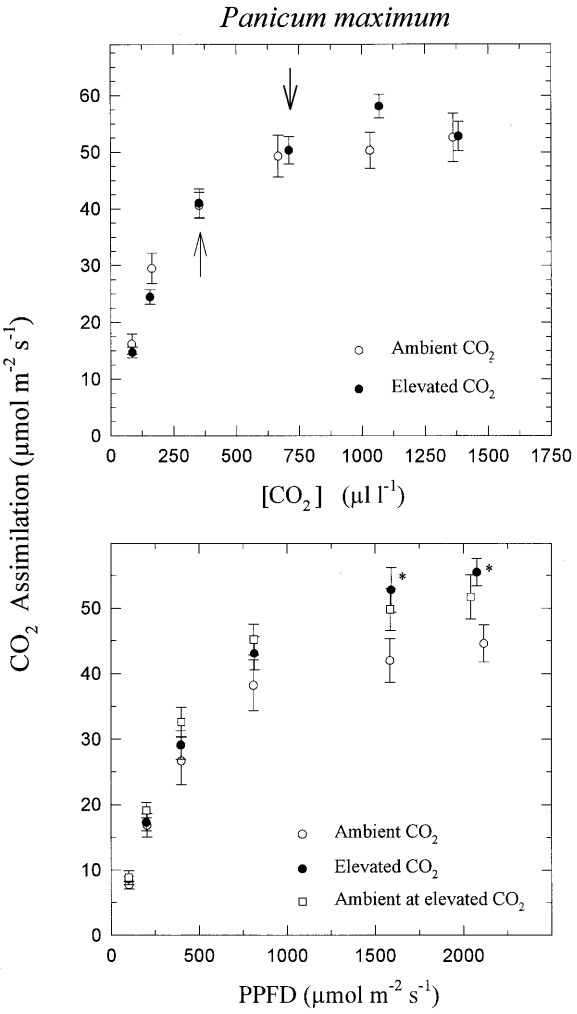

Fig. 3. Same as Fig. 1, but for *Panicum maximum* (PEP-CK decarboxylating subtype).

significant stimulation of total plant biomass (+ 50%) at the elevated [CO_2] by the end of the vegetative period (Table 3). The observed increase in plant biomass at elevated CO_2 was associated with significant increases in leaf area and weight, as well as stem and root weight, with the largest relative increase observed for stem weight (+ 80%) (Table 3).

Table 2. Average rates of maximum enzymatic activity (μmol m^{-2} s^{-1}) for rubisco and PEP-carboxylase for *F. trinervia*, *P. miliaceum*, and *P. maximum* grown at ambient (350 μl l^{-1}) and elevated (700 μl l^{-1}) CO_2 concentrations. The ratio of initial to fully activated rubisco was greater than 90% for all species with no effect observed for CO_2 concentration. P/R represents the ratio of PEP-carboxylase to rubisco activity for a given species and CO_2 concentration. See Materials and methods for additional details. n = 6.

Species	rubisco		PEP-C		P/R	
	350	700	350	700	350	700
F. trinervia	34.6	32.7	235.1	205.7	6.3	6.0
P. miliaceum	10.0	10.2	131.7	133.8	14.3	13.5
P. maximum	22.5	22.3	174.0	157.3	8.0	7.3

Discussion

A number of studies have shown enhanced photosynthesis and/or growth in response to elevated [CO_2] in C_4 species (reviewed by Poorter 1993); however, few studies have separated a direct effect of CO_2 from reports that have suggested that the beneficial effect of elevated CO_2 on C_4 species is an indirect consequence of improved water relations (Rogers et al. 1983, Knapp et al. 1993). Data from the current study suggest that some C_4 species may be able to respond directly to increasing [CO_2], independently of any improvement in leaf water potential at the higher [CO_2].

Is the ability of a given C_4 leaf to respond photosynthetically to enhanced [CO_2] related to increased CO_2 "leakiness" from the bundle sheath? Different subtypes have been proposed to have different conductances to CO_2, based, in part, on anatomy (e.g. the suberized lamella of NADP-ME grass species should reduce CO_2 leakage) (Hattersley 1982, Ehleringer and Pearcy 1983); but short-term estimates of bundle sheath conductance do not always support this hypothesis (Henderson et al. 1992, Hatch et al. 1995).

Although bundle sheath conductance was not measured directly in the current study, the ratio of PEP-carboxylase to rubisco has been shown to influence the degree of bundle sheath leakiness with leakiness decreasing at lower PEP-carboxylase/rubisco (or higher rubisco/PEP-carboxylase) activities (Saliendra et al. 1996). However for the three species tested, differences in the ratio of PEP-carboxylase to rubisco did not correlate to the relative degree of photosynthetic enhancement at elevated [CO_2]. Similarly, previously published values of CO_2 leak rates from the bundle sheath for these same species (Hatch et al. 1995) were not correlated with the relative stimulation of single leaf photosynthesis at the elevated [CO_2] in the current experiment. While a broader range of species needs to be examined, rates of CO_2 leakage per se do not appear to be correlated with the ability of a given C_4 species to respond photosynthetically to elevated [CO_2]. Results from the current experiment are consistent with a recent study that demonstrated that the relative enhancement of photosynthesis and growth in response to increasing CO_2 for six C_4 species was unrelated to decarboxylation subtype and the presumed degree of bundle sheath leakiness (LeCain and Morgan 1998).

Is the ability of a given C_4 species to respond photosynthetically to enhanced [CO_2] related to PPFD? There are only a few studies that have examined the interaction between increasing [CO_2] and PPFD in C_4 species. Recent work with *Panicum antidotale* (a C_4 tropical grass) indicated that leaf photosynthesis at ambient [CO_2] was saturated at an irradiance of 400 μmol m^{-2} s^{-1} but not at 1200 μmol m^{-2} s^{-1} (Ghannoum et al. 1997). Earlier work demonstrated that the photosynthetic response of the C_4 species, *Echinochloa crusgalli*, *Digitaria sanguinalis*, *Eleusine indica*, and *Setaria faberi* to increasing [CO_2] was also PPFD dependent (see Fig. 3, Sionit and Patterson 1984). These findings are consistent with the current data reported here for *F. trinervia*, *P. miliaceum*, and *P. maximum*.

At the growth PPFD, two of the three species (*F. Trinervia* and *P. miliaceum*) showed a significant stimulation of leaf photosynthesis at elevated [CO_2], with no difference

Table 3. Average leaf area and dry weight per plant at the final harvest of *F. trinervia*, *P.miliaceum*, and *P. maximum* grown at ambient (350 μl l^{-1}) and elevated (700 μl l^{-1}) CO_2 concentrations. DAS = days after sowing. * within a column indicates a significant difference (Student's t-test) for that species. n = 8–12.

Species	CO_2	DAS	Leaf area (cm^2)	Leaf wt. (g)	Stem wt. (g)	Root wt. (g)	Total (g)
F. trinervia	Ambient	48	2 288	9.27	7.87	8.29	25.43
	Elevated	48	3 279*	12.75*	14.17*	12.02*	38.94*
P. miliaceum	Ambient	31	3 263	14.09	17.11	12.98	46.39
	Elevated	31	3 596	13.86	16.01	9.16	39.20
P. maximum	Ambient	48	5 184	23.53	30.91	13.53	67.96
	Elevated	48	4 861	22.13	30.32	14.14	66.58

in the relative stimulation between species. A small enhancement in photosynthesis over the lifetime of the plant could translate into a dry weight increase. However, only *F. trinervia* showed a significant stimulation (+ 50%) of whole plant growth at elevated $[CO_2]$. Lack of a growth response cannot be explained by the appearance of photosynthetic acclimation in the other *Panicum* species. Lack of photosynthetic acclimation was consistent with no observed change in either PEP-carboxylase or rubisco activity between CO_2 concentration treatments for a given species.

Although examining the assimilation response of single leaves at differing CO_2 concentrations is useful for determining potential changes in photosynthetic capacity, the leaf response cannot always be used to predict the carbon dynamics and growth response at the whole plant level. Additional factors at the whole plant level such as respiration (see Amthor 1991), leaf initiation and expansion, branching, or tillering may influence the sensitivity of the growth response to $[CO_2]$ (Luo et al. 1994, Moya et al. 1998). The photosynthetic response of lower, shaded leaves within the plant may also influence the whole plant response. For example, in the current experiment, the trend for increased assimilation rates at the elevated $[CO_2]$ measured at lower PPFDs (200–400 μmol m^{-2} s^{-1}) in *F. trinervia* may have been a factor in the whole plant growth response.

For C_4 species, one additional possibility that deserves consideration is whether leaf age and development alter the sensitivity of photosynthesis and/or growth to higher CO_2 levels. It has been shown that bundle sheath cell walls of young and senescent maize leaves have a relatively high conductance which could result in low $[CO_2]$ around rubisco (Dai et al. 1995). This is evident in results with maize that show that young leaves have substantial photorespiration under normal atmospheric conditions and require a lower O_2 partial pressure to achieve maximum photosynthetic rates (Dai et al. 1995, He and Edwards 1996). Consequently, it is possible that young C_4 leaves could be more responsive to increasing $[CO_2]$ and therefore contribute to the growth stimulation at the whole plant level (Ghannoum et al. 1997). Interestingly, one of the unique features of *F. trinervia* is the large number of meristems and high percentage of young, developing leaves. It is conceivable that this may have contributed to the overall growth stimulation at elevated $[CO_2]$ observed in this species. This possibility deserves additional study.

Data from the current experiment indicated that leaf photosynthesis, at least for some C_4 species, is not saturated at current $[CO_2]$ (e.g. Taiz and Zieger 1991) and can respond as $[CO_2]$ increases, depending on PPFD. The ability to respond directly to $[CO_2]$ was not associated with a measured improvement in leaf water potential or alteration of activities of the primary carboxylating enzymes, PEP-carboxylase, or rubisco at elevated $[CO_2]$. In addition, the sensitivity of the photosynthetic response to increasing $[CO_2]$ appeared to be independent of the rate of CO_2 leakage from the bundle sheath (as determined indirectly from the ratio of PEP-carboxylase to rubisco enzyme activities and from previously published CO_2 leak rates for these species). Although not fully explained by the stimulation of single leaf photosynthesis, the growth response of one C_4 species, *F. trinervia*, to elevated $[CO_2]$ was substantial (+ 50%). In general, differences between the C_3 and C_4 photosynthetic pathways still indicate a larger relative response of C_3 to C_4 plants with increased $[CO_2]$. However, it should not be assumed that C_4 plants are incapable of responding directly to elevated CO_2.

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