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Regular paper

Influence of increasing carbon dioxide concentration on the photosynthetic and growth stimulation of selected C_4 crops and weeds

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Abstract

Plants of six weedy species (*Amaranthus retroflexus, Echinochloa crus-galli, Panicum dichotomiflorum, Setaria faberi, Setaria viridis, Sorghum halapense*) and 4 crop species (*Amaranthus hypochondriacus, Saccharum officinarum, Sorghum bicolor and Zea mays*) possessing the C_4 type of photosynthesis were grown at ambient (38 Pa) and elevated (69 Pa) carbon dioxide during early development (i.e. up to 60 days after sowing) to determine: (a) if plants possessing the C_4 photosynthetic pathway could respond photosynthetically or in biomass production to future increases in global carbon dioxide and (b) whether differences exist between weeds and crops in the degree of response. Based on observations in the response of photosynthesis (measured as A, CO_2 assimilation rate) to the growth CO_2 condition as well as to a range of internal CO_2 (C_i) concentrations, eight of ten C_4 species showed a significant increase in photosynthesis. The largest and smallest increases observed were for *A. retroflexus* (+30%) and *Z. mays* (+5%), respectively. Weed species (+19%) showed approximately twice the degree of photosynthetic stimulation as that of crop species (+10%) at the higher CO_2 concentration. Elevated carbon dioxide also resulted in significant increases in whole plant biomass for four C_4 weeds (*A. retroflexus, E. crus-galli, P. dichotomiflorum, S. viridis*) relative to the ambient CO_2 condition. Leaf water potentials for three selected species (*A. retroflexus, A. hypochondriacus, Z. mays*) indicated that differences in photosynthetic stimulation were not due solely to improved leaf water status. Data from this study indicate that C_4 plants may respond directly to increasing CO_2 concentration, and in the case of some C_4 weeds (e.g. *A. retroflexus*) may show photosynthetic increases similar to those published for C_3 species.

Abbreviations: A – leaf CO_2 assimilation rate; C_a – ambient CO_2; C_i – internal CO_2; CER – carbon exchange rate; DAS – days after sowing; PAR – photosynthetically active radiation

Introduction

Ongoing increases in atmospheric carbon dioxide should stimulate net photosynthesis in C_3 plants by increasing the CO_2 concentration gradient from air to the leaf interior and by decreasing the loss of CO_2 via photorespiration. Alternatively, plants with the C_4 photosynthetic pathway already have an internal biochemical pump for concentrating CO_2 at the site of carboxylation which functions to eliminate the oxygenase component of Rubisco, thereby eliminating photorespiratory carbon loss. Because of these different photosynthetic pathways, it is anticipated that C_4 plants should be saturated at the current atmospheric CO_2 concentration while C_3 plants should continue to respond photosynthetically to ongoing increases in atmospheric carbon dioxide (see Figure 10.10 in Taiz and Zeiger 1991).

Although C_4 species may not be directly stimulated by higher CO_2 levels, they can still show a significant stimulation of photosynthesis as CO_2 rises. This is because increasing CO_2 may also result in stomatal closure. This is a common response in both C_3 and C_4 species and results in an increase in water use efficiency and improved water relations. As a consequence, plants with the C_4 photosynthetic pathway, may show improved photosynthesis and growth under a drought stress condition at elevated carbon dioxide (cf. *Andropogon gerardii*, Knapp et al. 1993). In addition, increased water potential at elevated CO_2 could stimulate growth even in wet soil (e.g. field corn, Rogers et al. 1983), by increasing leaf area without any increase in carbon exchange rate (CER).

However, in examining reviews which document the average response of C_4 plants to a doubling of CO_2 concentration, it is clear that for a number of C_4 species, significant increases in both growth and photosynthesis have been observed (cf. Poorter 1993). Although little effort has been done to separate improved water relations from a direct stimulation of photosynthesis, it seems unlikely that improved water relations can, in all cases, account for the response of C_4 species to elevated carbon dioxide.

Within agricultural systems C_4 species are important as both basic food crops (e.g. maize, sorghum) and as noxious weeds (e.g. pigweed, foxtail). If potential differences in the growth or photosynthetic response of C_4 weeds and crops to elevated CO_2 exist, such differences may have consequences for weed-crop competitive interactions.

In the current study, our principle objective was to quantify the photosynthetic and growth response of C_4 plants to a doubling of the current atmospheric CO_2 concentration in order to determine if increasing CO_2 could stimulate photosynthesis and/or growth directly. However, because of the importance of both C_4 weeds and crops to agricultural systems, a secondary objective was to determine if differential responses to increasing CO_2 occurred between the groups.

Materials and methods

All experiments were conducted from late April through early September 1996 in two glasshouses located at the USDA-ARS Climate Stress Laboratory in Beltsville, Maryland. Each glasshouse was 13.5 m^2 in surface area and transmitted 65% of incoming PAR while temperature and CO_2 concentration were maintained within preset limits. Glasshouses were designed to maintain maximum and minimum temperatures between 31 and 17 °C, respectively. Air temperature was obtained by shielded, aspirated thermocouples near the top of plants in each glasshouse. Blowers constantly circulated air through heat exchangers and produced an air speed of about 0.5 $m\ s^{-1}$ across leaves. Relative humidity inside the glasshouse was not controlled, but was at or near that of ambient, outside air. Carbon dioxide was controlled 24 h day^{-1} by a WMA2 infra-red gas analyzer (PP systems, Haverhill, MA) which injected CO_2 if levels fell below 35 and 70 Pa, respectively, for each glasshouse. CO_2 treatments were switched between glasshouses twice during the study. No significant differences with respect to light or temperature were observed between glasshouses. A 21x datalogger (Campbell Scientific, Logan, UT) recorded PAR, temperature and CO_2 concentration in both glasshouses at 30 second intervals. Average 24-h values of CO_2 concentration were 38±1.7 and 69±2.5 Pa for the ambient and elevated treatments, respectively.

A total of six weed species, (*Amaranthus retroflexus* L., *Echinochloa crus-galli* (L.) P. Beauv., *Panicum dichotomiflorum* Michaux, *Setaria faberi* Herrm., *Setaria viridis* (L) P. Beauv., *Sorghum halapense* (L) Pers.) and four crop species (*Amaranthus hypochondriacus* L., *Saccharum officinarum* L., *Sorghum bicolor* (L) Moench, and *Zea mays* L.) possessing the C_4 type of photosynthesis were grown at ambient (38) and elevated (69 Pa) carbon dioxide during early development (i.e. all plants were harvested from 30–60 days after sowing, DAS) (Table 1). All species were grown from seed obtained locally (except *S. officinarum* which was obtained from cuttings) in 20 cm diameter (3.5 l) pots filled with vermiculite, with pots rotated weekly to minimize micro-climate effects within the glasshouse. All pots were watered daily to the drip point with a complete nutrient solution (Robinson 1984). After emergence, seedlings were thinned to one plant per pot with six pots per species randomly assigned to one of the two CO_2 treatments. Because of limited space within the glasshouses and the number of species to be examined, different species were grown at different times over the period from April 25 through August 27. Species were grown at random during this time period (i.e. weeds were not grown all at once followed by crops), and values of average daily (24 h) temperature and PAR were consistent between species and groups (e.g. 23.8 vs. 26.5 °C; 23.6 vs. 23.4 mol m^{-2} day^{-1}, for weeds and crops, respectively) (Table 1).

Single leaf photosynthesis (measured as A, the rate of CO_2 assimilation) was determined twice during the vegetative stage of growth for each species. On each

Table 1. List of C_4 crops and weeds used in the experiment. Subtype refers to the different decarboxylation kinetics of CO_2 release within the bundle sheath. Subtypes are named after the enzymes that catalyze the specific decarboxylation reactions: NADP-dependent malic enzyme (NADP-ME); NAD-dependent malic enzyme (NAD-ME) and phosphoenolpyruvate (PEP) carboxykinase (PEP-CK). Temp. and PAR refer to the average 24 h temperature and photosynthetically active radiation during the period of growth for that particular species. Crops and weeds were grown at random over a 4 month period in climate controlled glasshouses. See methods for additional details

Species	Common name	C_4 subtype	Temp. (°C)	PAR (mol $m^{-2}day^{-1}$)
Weeds				
Amaranthus retroflexus	Red-root pigweed	NAD-ME	23.5	22.2
Echinochloa crus-galli	Barnyard grass	NADP-ME	23.5	23.1
Panicum dichotomiflorum	Fall panicum	NAD-ME	23.6	23.9
Setaria faberi	Giant foxtail	NADP-ME	23.5	22.7
Setaria viridis	Green foxtail	NADP-ME	24.5	25.1
Sorghum halapense	Johnson grass	[a]	24.3	24.7
Crops				
A. hypochondriacus	Grain Amaranth	NAD-ME	25.7	23.5
Saccharum officinarum	Sugarcane	NADP-ME	26.3	18.9
Sorghum bicolor	Grain sorghum	NADP-ME	26.9	24.7
Zea mays	Corn	NADP-ME	27.0	26.3

[a] We could find no literature which documented the C_4 subtype of this species

sampling date, assimilation was determined on the uppermost, fully expanded leaf for 6 plants of each species (i.e. 3 per CO_2 treatment). Measurements were made using a portable open gas exchange system incorporating infra-red CO_2 and water vapor analyzers for determining net photosynthetic CO_2 uptake rate and stomatal conductance (CIRAS-1, PP Systems, Haverhill, MA). PAR was supplied by a separate light unit which produced a constant PAR of 2000 μmol m^{-2} s^{-1} for all measurements of gas exchange. The water vapor pressure surrounding the leaf during the measurement was approximately 2.0 kPa, and did not vary between CO_2 treatments or species. Leaf temperature was maintained between 26–28 °C. All data were collected on a PC at 10 s intervals using the remote control operating system software supplied by CIRAS. To determine assimilation for a wide range of CO_2 values, different CO_2 concentrations were obtained using the internal CO_2 mixing system (with CO_2 cartridge) provided with the CIRAS-1 system. The response of leaf assimilation rate (A) to intercellular carbon dioxide concentration (C_i) was determined at six external CO_2 concentrations (C_a) of 12, 24, 38, 70, 108 and 144 Pa. Initially, leaves were measured at their respective growth CO_2 concentration (i.e. 38 or 70 Pa); then C_a was lowered to 12 Pa and increased in steps to 144 Pa. In changing to a new CO_2 concentration, sufficient time was given (10–15 min) to allow for a steady-state condition prior to the measurement of assimilation rate. All measurements were made at approximately the same temperature and humidity as within the glasshouses. Equal numbers of A/C_i responses were determined in the morning and afternoon, but combined for analysis.

To determine if growth at elevated CO_2 resulted in improved water relations, leaf water potential was measured in the dewpoint mode using a HR-33 microvoltmeter and six insulated C-51 sample chambers (Wescor, Inc. Logan, UT). Water potential measurements were determined during the morning (0900–1000) and afternoon (1300–1600) for six sunlit leaves for each of three species, *A. retroflexus, A. hypochondriacus,* and *Z. mays* with data combined from both time periods for analysis.

Within 24 h following the second A/C_i measurement, all plants of a given species were cut at ground level and separated into leaf laminae, stems (sheaths for monocotyledons) and roots. Leaf area was determined photometrically with a leaf area meter (Li-Cor Corp., Li-3000, Lincoln, NE). 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) and weighed.

The effect of elevated CO_2 on gas exchange parameters at the growth CO_2 concentration, water poten-

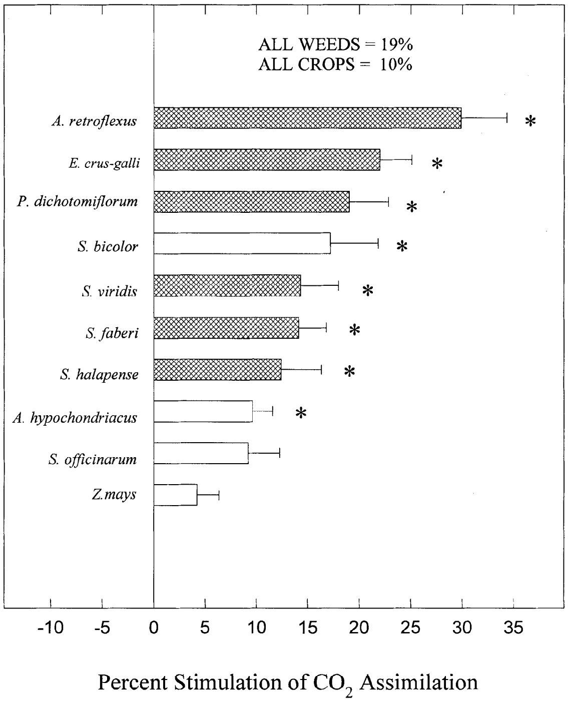

Figure 1. The percent stimulation of single leaf photosynthesis (measured as leaf CO_2 assimilation rate μmol m^{-2} s^{-1}) for selected weed (hatched bars) and crop species (open bars) grown and measured at elevated CO_2 (69 Pa) relative to ambient CO_2 (38 Pa). $*$ indicates a significant difference relative to the ambient CO_2 concentration (Students unpaired t-test), *n* = 10–12.

tial and dry weight were analyzed by species using a Students unpaired t-test. Comparisons between crops and weeds as a whole were made using a one-factor ANOVA. Comparisons between ambient and elevated CO_2 for short-(measurement CO_2) and long-term (growth CO_2) exposure for a given species were made using a 2 way ANOVA with means separated by a least squares means table. Unless otherwise stated, differences were determined as significant at the $P<0.05$ level.

Results

In the present experiment, averaged for both sampling periods, leaf photosynthetic rate measured at the growth CO_2 concentration was significantly increased at elevated relative to ambient CO_2 concentration for eight out of ten C_4 species (Table 2). When ranked, *A. retroflexus* showed the largest response to elevated CO_2 with an average increase in photosynthesis of 30%, while no response could be detected for *Z. mays* and *S. officinarum* (Figure 1). All of the weedy C_4 species responded significantly to the increase in CO_2 concentration compared to half of the C_4 crop species tested. When averaged by group, weeds showed a 19% stimulation of photosynthetic rate, while crop photosynthesis increased by 10% (Figure 1). (The difference between crops and weed in the stimulation of photosynthesis was significant at $P = 0.04$).

By comparing photosynthetic rates under short-term exposure to the different growth CO_2 concentrations, photosynthetic acclimation could be determined. Should the photosynthetic rate at a given measurement CO_2 differ with growth CO_2 treatment, acclimation could be said to have occurred. Short-term measurements at each of the treatment CO_2 concentrations did not indicate photosynthetic acclimation to elevated CO_2 had occurred for any C_4 species which demonstrated a enhanced photosynthetic response at the elevated CO_2 treatment (i.e. all species except *Z. mays* and *S. officinarum*) (Table 1). In addition, (with the exception of *S. faberi*), the A/C_i response was independent of the treatment CO_2 concentration for both crop (Figure 2a, b) and weedy species (Figure 3a, b) at either sampling period. For *S. faberi*, acclimation did occur as evidenced by a significant decline in the maximum rate of photosynthesis for the elevated grown leaves at 38 DAS.

For all species, assimilation continued to increase up to a C_i value of ca. 20 Pa (Figures 2 and 3). With the exception of *S. faberi*, no significant change in either the initial slope of the A/C_i response or the upper portion of the curve occurred with long-term growth at elevated CO_2 (Table 3, Figures 2 and 3) for any species. The ratio of intercellular CO_2 to growth CO_2 (C_i/C_a) also was unaffected by CO_2 concentration. For both crops and weeds, average stomatal conductance (g_s) decreased ca. 50% at the elevated relative to the ambient CO_2 concentration (Table 3). No significant differences in initial slope, C_i/C_a or g_s were observed between crops and weeds at either growth CO_2 concentration (Table 3).

Significant increases in total biomass at the end of the experimental period were observed in four C_4 species (Table 4). The increase in total biomass with elevated CO_2 concentration was not associated with a consistent increase in leaf area, or changes in partitioning between leaves, stem or roots (Table 4). As with photosynthesis, *A. retroflexus* showed the largest increase in biomass with elevated CO_2 (ca. 25%). If ranked, four of the six weeds tested showed a sig-

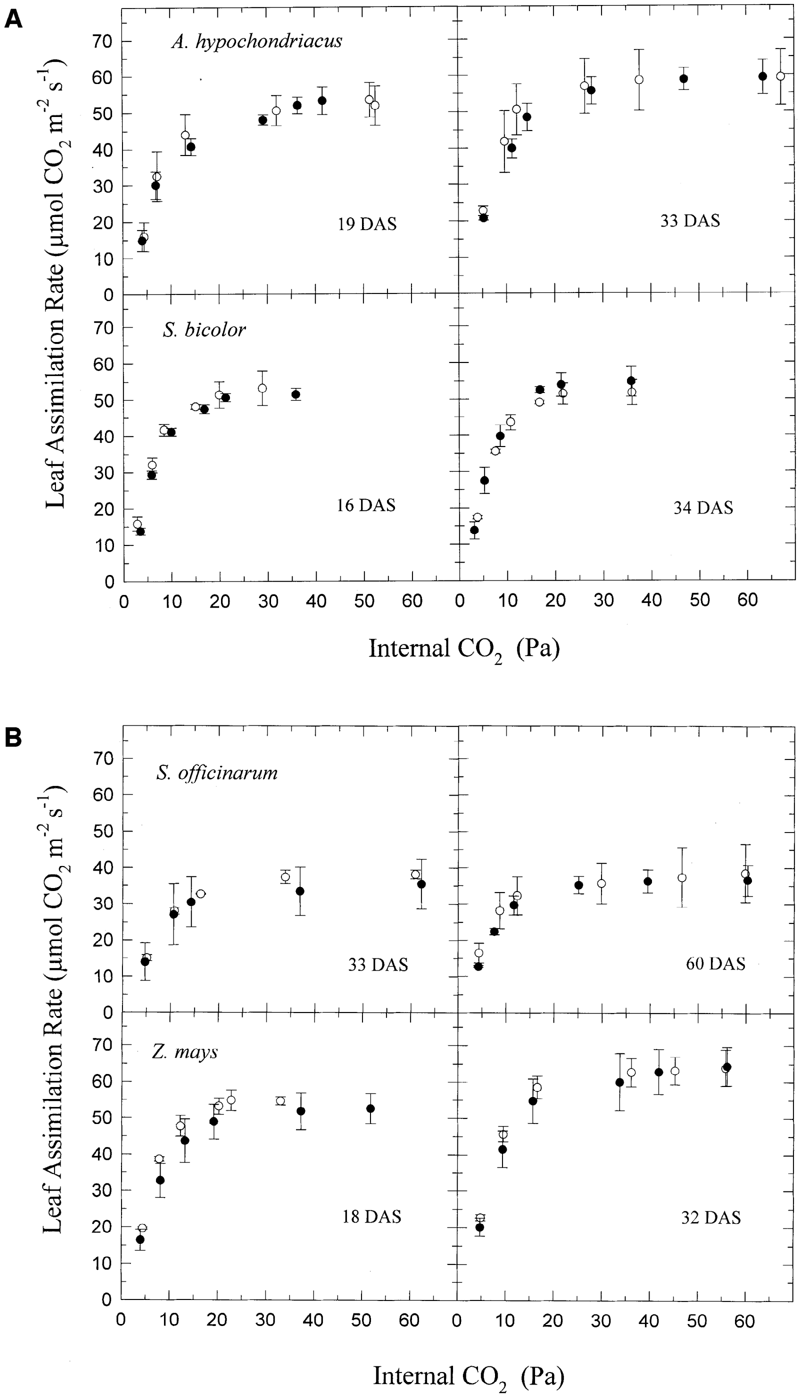

Figure 2. Response of leaf photosynthesis (measured as leaf CO_2 assimilation rate, A) to a range of internal CO_2 concentrations (C_i) for single leaves of four crop species *A. hypochondriacus, S. bicolor* (A) and *S. officinarum, Z mays* (B) grown at either ambient (○, 38 Pa) or elevated (●, 69 Pa) CO_2. DAS indicates the number of days after sowing in which the measurements were taken. *n* = 3, bars are ± SE.

Table 2. Photosynthesis (as CO_2 assimilation rate, μmol CO_2 m^{-2} s^{-1}) for ambient and elevated CO_2 grown C_4 crops and weeds each measured at ambient and elevated CO_2. Different letters within a row indicate significant differences. Both sampling periods were combined for analysis. (Least squares means, $n = 6$)

Species	38 Pa		-Grown-	69 Pa	
	38 Pa	69 Pa	-Measured-	38 Pa	69 Pa
Weeds					
A. retroflexus	34.5b	40.6b		38.6b	44.9a
E. crus-galli	34.9b	42.4a		32.7b	42.6a
P. dichotomiflorum	36.6b	42.0a		37.6b	43.3a
S. faberi	36.4b	43.9a		33.7b	41.5a
S. viridis	34.8b	43.2a		34.0b	39.6a
S. halapense	39.8b	46.6a		36.2b	44.6a
Crops					
A. hypochondriacus	47.1b	53.6a		44.8b	51.6a
S. officinarum	32.5ab	36.6a		31.6b	35.5a
S. bicolor	42.5b	48.6a		40.4b	50.0a
Z. mays	52.4a	56.9a		49.2a	54.5a

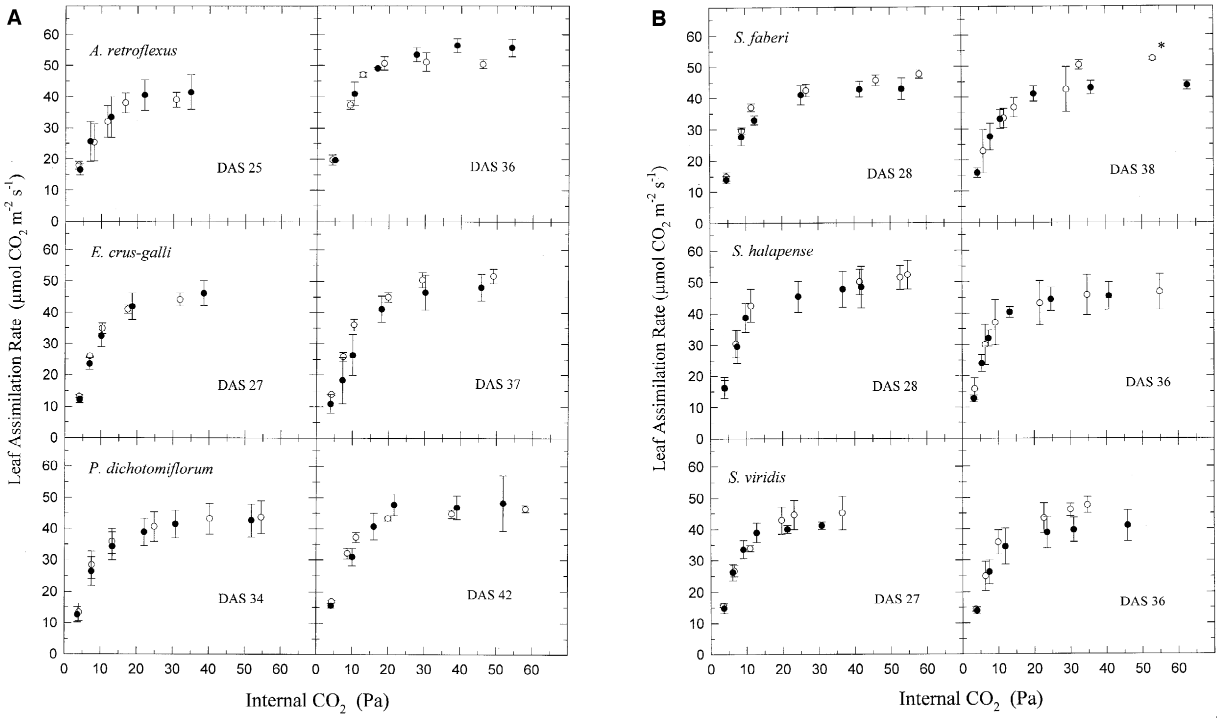

Figure 3. Same as Figure 2, but for six weedy species, *A. retroflexus*, *E. crus-galli*, *P. dichotomiflorum* (A) and *S. faberi*, *S. halepense*, *S. viridis* (B).

nificant stimulation of biomass with increasing CO_2, while none of the C_4 crops tested showed a significant response (Figure 4). As with photosynthesis, the increase in total biomass with elevated CO_2 was significant for weeds as a group, but not significant for crops.

Table 3. Initial slope of the A/C_i response (determined from the first two points of the A/C_i response), ratio of C_i/C_a, and stomatal conductance at C_a for ambient and elevated grown C_4 crops and weeds. Both sampling periods were combined for analysis

Species		Ambient grown			Elevated grown	
	Initial slope μmol m^{-2} s^{-1} /μmol mol^{-1}	C_i/C_a	g_s mmol m^{-2} s^{-1}	Initial slope μmol m^{-2} s^{-1} /μmol mol^{-1}	C_i/C_a	g_s mmol m^{-2} s^{-1}
Weeds						
A. retroflexus	0.27	0.34	353	0.35	0.37	197
E. crus-galli	0.41	0.29	261	0.38	0.28	139
P. dichotomiflorum	0.39	0.30	300	0.32	0.32	156
S. faberi	0.21	0.31	279	0.24	0.36	135
S. viridis	0.22	0.28	316	0.23	0.28	144
S. halapense	0.24	0.26	230	0.24	0.27	131
Crops						
A. hypochondriacus	0.50	0.36	379	0.43	0.43	166
S. officinarum	0.25	0.36	295	0.26	0.43	149
S. bicolor	0.49	0.28	302	0.61	0.26	147
Z. mays	0.52	0.37	459	0.43	0.38	210

Table 4. Leaf area and dry weight at the final harvest of selected C_4 crops and weeds grown at ambient or elevated CO_2

Species	CO_2	DAS	Leaf area	Leaf Wt.	Stem Wt.	Root Wt.	Total
	Pa		cm^2	-g-			
Weeds							
A. retroflexus	38	37	2055	9.9	4.1	4.4	18.4
	69	37	2110	12.0	4.6[a]	6.3[a]	22.9[a]
E. crus-galli	38	38	1393	4.2	7.3	4.2	15.7
	69	38	1655[a]	4.9[a]	9.0[a]	4.9[a]	18.8[a]
P. dichotomiflorum	38	43	946	3.6	2.4	1.4	7.4
	69	43	1099[a]	4.5[a]	3.0	1.9	9.2[a]
S. faberi	38	39	1561	5.3	6.1	4.6	15.9
	69	39	1925[a]	6.2[a]	6.6	4.6	17.4
S. viridis	38	37	2333	9.1	9.0	6.2	24.3
	69	37	2571[a]	10.8[a]	12.2[a]	5.9	28.9[a]
S. halapense	38	37	2132	7.9	9.1	6.5	23.5
	69	37	2323	8.5	9.7	7.1	25.3
Crops							
A. hypochondriacus	38	34	2493	12.2	5.8	7.0	25.0
	69	34	2611	12.9	6.8	8.2	27.9
S. officinarum	38	61	3616	37.3	49.4	37.1	124.0
	69	61	4110	39.8	48.3	44.3	132.0
S. bicolor	38	35	2144	6.9	5.6	7.1	19.6
	69	35	2118	6.9	6.0	7.5	20.4
Z. mays	38	33	2301	8.9	8.7	6.2	23.8
	69	33	2384	8.9	9.7	5.9	24.5

[a] Indicates a significant difference at the elevated relative to the ambient CO_2 concentration on a per plant basis for a given species ($P<0.05$, Students unpaired t-test).

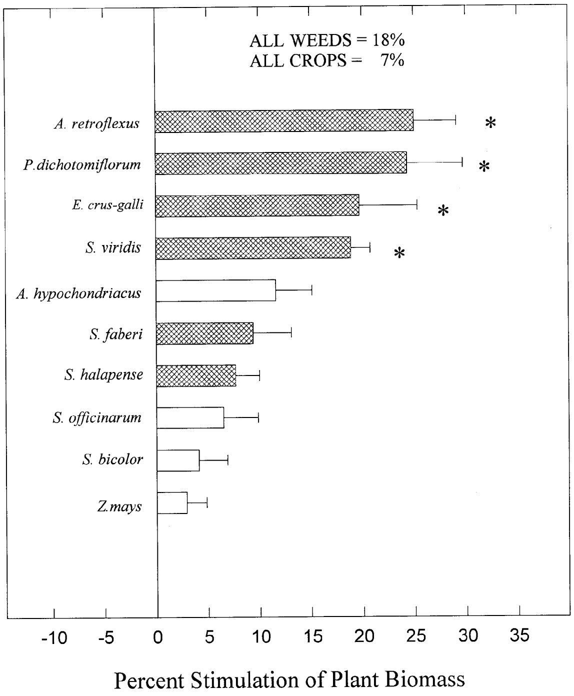

Figure 4. The percent stimulation of total plant biomass for selected weed and crop species grown and measured at elevated CO_2 (69 Pa) relative to ambient CO_2 (38 Pa). Symbols are same as Figure 1. $*$ Indicates a significant difference relative to the ambient CO_2 concentration (Students unpaired t-test), *n* = 6.

Table 5. Mean daytime leaf water potentials for three selected species, *A. hypochondriacus, A. retroflexus* and *Z. mays* at ambient and elevated CO_2

Species	CO_2 concentration		
	38	Pa	69
	---------MPa---------		
A. hypochondriacus	–0.82±0.04		–0.86±0.06
A. retroflexus	–0.89+0.06		–0.96+0.09
Z. mays	–0.91±0.02		–0.88±0.03

Measurements of leaf water potential for the C_4 dicots, *A. retroflexus, A. hypochondriacus* and the C_4 monocot, *Z. mays*, are consistent with the well-watered condition of the plants (Table 5). However, elevated CO_2 did not result in an improved water potential for any species (Table 5).

Discussion

C_4 plants, possessing an internal 'CO_2 pump' have the ability to concentrate CO_2 at the site of carboxylation with a subsequent decrease in the oxygenation of RuBP and loss of photorespiratory CO_2. As a consequence, increasing the external CO_2 concentration above current ambient levels should have little effect on net photosynthesis in C_4 plants. This hypothesis is consistent with the observations of a number of researchers who have reported CO_2 assimilation values for a wide range of C_4 crops and weeds to be saturated at current CO_2 levels (e.g. a C_a of 35 Pa, a C_i of about 15 Pa) (cf. Greer et al. 1995; Tissue et al. 1995; Harley and Ehleringer 1987; Lin and Ehleringer 1983; Mauney 1979; De Wit 1978; Pearcy 1977). Although higher photosynthetic rates or increased biomass for C_4 plants have been reported with increasing CO_2 concentrations, such increases are usually attributed to changes in partitioning, accelerated inflorescence development, delayed leaf senescence or improved water potentials resulting from stomatal closure at high CO_2 (cf. Knapp et al. 1993; Potvin and Strain 1985; Carter and Petersen 1983). However, these hypotheses do not completely explain the increased photosynthetic rate and/or biomass noted at elevated CO_2 in the current experiment for eight of ten species examined. The observed increase in photosynthetic rate and biomass in the present study was not associated with differences in partitioning, senescence or improved water potentials between CO_2 treatments. In addition, little evidence of photosynthetic acclimation to elevated CO_2 was observed, suggesting that the stimulation of photosynthesis could continue for weeks (e.g. *E. crus-galli*) or months (e.g. *S. officinarum*).

Although a direct stimulation of increasing CO_2 on growth or photosynthetic rate may seem contradictory to what is known concerning the C_4 pathway, the current data are consistent with some reports which show a significant stimulation of either photosynthesis or growth in C_4 plants as CO_2 concentration rises (Johnson et al. 1993; Black 1986; Sionit and Paterson 1984; Wong 1979; Ludlow and Wilson 1971; Moss et al. 1961). In these and the current study, photosynthesis in selected C_4 species does not saturate until C_a values approach 60–80 Pa (e.g. see Figure 3, Black 1986). As a consequence, photosynthesis or biomass can increase by 40–60% with a doubling of CO_2 concentration (cf. *A. retroflexus* and *E. indica*, Black 1986; Sionit and Paterson 1984). Such increases are on a par with the observed stimulation of photosynthesis and growth in some C_3 species (see Poorter 1993 and Kimball 1983 for reviews).

Clearly, growth conditions can differ between experiments. However, there does not appear to be one set of environmental or growth conditions which are associated with either those experiments which do (or those which do not) demonstrate a significant stimulation with elevated CO_2. Alternatively, if the C_4 pathway is not always tightly controlled with respect to maintaining a high internal concentration of CO_2, a continued response to increasing CO_2 concentration could be observed, with such a response, as in the current experiment, being species specific.

As determined by Ehleringer and Pearcy (1983), different rates of CO_2 leakage from bundle sheath cells in C_4 plants should be lower in monocots than in dicots (due to the presence of suberized lamella in bundle sheath cells in grasses) and vary according to decarboxylation subtype (lower in NADP-malic enzyme and PCK subtypes than in NAD-malic enzyme type C_4 plants). Can such differences be correlated to the relative response of a given C_4 species to elevated CO_2? In the present experiment, species which should show a strong relative response to elevated CO_2 based on CO_2 leakage rates (i.e. dicots with a NAD-malic enzyme subtype) differ substantially (e.g. *A. retroflexus* and *A. hypochondriacus*) as do species (e.g. *Z. mays* and *E. crus-galli*) which should show a weaker response (i.e. monocots with a NADP-malic enzyme subtype). While a broader range of species and subtypes needs to be examined, it does not seem likely that rates of CO_2 leakage, per se, provide a complete explanation for the sensitivity of C_4 species to increased CO_2 concentration.

Although C_4 plants can have a direct response to increasing CO_2 levels, the exact mechanism is unclear. Ward (1987), in examining the relationship between temperature and photosynthesis in *Z. mays* has suggested that variation in the rate of CO_2 delivery to the bundle sheath relative to the capacity for net assimilation by C_3 photosynthesis could determine the photosynthetic response of C_4 plants to CO_2 concentration. Such variation could be associated with environmental cues such as light, nutrition or temperature which could favor the activity and concentration of PEPCase relative to Rubisco (or vice-versa). For example, Wong (1979), demonstrated that *Z. mays* could respond photosynthetically to elevated CO_2, but only if nitrogen was adequate. Additional work is need to elucidate the role of environmental parameters and nutrition in influencing both carboxylation kinetics and potential photosynthetic acclimation (e.g. *S. faberi*). Such information could serve as the basis for predicting the relative growth stimulation of C_4 species as atmospheric CO_2 continues to increase.

Irrespective of mechanism, it seems clear from the current data that substantial variation in the response to elevated CO_2 exists within C_4 species, especially between weeds and crops. Such variation does not seem to be related to consistent changes in either g_s or in the ratio of C_i/C_a between crops and weeds as CO_2 increases. While the source of such variation is unknown, differential sensitivity to increasing CO_2 could have consequences with respect to competition and crop production in agricultural systems. It is generally acknowledged that while the C_4 pathway occurs in relatively few plants, C_4 species are disproportionally over-represented in lists of major weeds. For example, of the 76 ‘worst’ weeds, 42% are C_4 plants (Paterson 1985). Greater stimulation of C_4 weeds at higher CO_2 concentrations could not only influence C_4 weed/C_4 crop interactions (e.g. *S. halapense* and *S. bicolor*); but, in addition, the response of some C_4 weeds (e.g. *A. retroflexus* in the current study) are greater than those reported for some C_3 crops (e.g. tomato, see Table 4.8, Acock and Allen 1985). It is unfortunate that, to date, no studies of the relative yield response at elevated CO_2 of either C_3 or C_4 crops in competition with C_4 weeds have been reported under field conditions.

In conclusion, while 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 levels, it should not be assumed that C_4 plants are incapable of responding to elevated concentrations of carbon dioxide. Such responses appear to be independent of any improvement in water relations even in the absence of drought (e.g. *Z. may, A. retroflexus* and *A. hypochondriacus*). Direct effects on photosynthesis and/or growth to elevated CO_2 could indicate changes in the ability of a given C_4 species to concentrate CO_2 at the site of carboxylation; however, additional details are needed. In any case, interspecific variation in the response of C_4 plants, particularly between crop and weeds may have eco-physiological consequences which could alter crop production in a future higher CO_2 world.

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References

Acock B and Allen LH Jr. Crop responses to elevated carbon dioxide concentrations. In: Strain BR and Cure JD (eds) Direct Effects of Increasing Carbon Dioxide on Vegetation. United States Department of Energy, DOE/ER-0238, pp 286

Black CC (1986) Effects of CO_2 concentration on photosynthesis and respiration of C_4 and CAM plants. In: Enoch HZ and Kimball BA (eds) Physiology, Yield and Economics, pp 29–40. CRC Press, Boca Raton, FL

Carter DR and Peterson KM (1983) Effects of a CO_2-enriched atmosphere on the growth and competitive interaction of a C_3 and a C_4 grass. Oecologia 58: 188–193

De Wit CT (1978) Stimulation of Assimilation, Respiration and Transpiration of Crops. Halsted Press, John Wiley and Sons, New York, 140 pp

Ehleringer J and Pearcy RW (1983) Variation in quantum yield for CO_2 uptake among C_3 and C_4 plants. Plant Physiol 73: 555–559

Greer DH, Laing WA and Campbell BD (1995) Photosynthetic responses of thirteen pasture species to elevated CO_2 and temperature. Austr J Plant Physiol 22: 713–722

Harley PC and Ehleringer J (1987) Gas exchange characteristics of leaves of four species of grain amaranth. Field Crops Res 17: 141–153

Johnson HB, Polley HW and Mayeux HS (1993) Increasing CO_2 and plant-plant interactions: Effects on natural vegetation. Vegetatio 104/105: 157–170

Kimball BA (1983) Carbon dioxide and agricultural yield: An assemblage and analysis of 430 prior observations. Agronomy J 75: 779–788

Knapp AK, Hamerlyn CK and Owensby CE (1993) Photosynthetic and water relations response to elevated CO_2 in the C_4 grass, *Andropogon gerardii*. Int J Plant Sc 154: 459–466

Lin ZF and Ehleringer J (1983) Photosynthetic characteristics of *Amaranthus tricolor*, a C_4 tropical leafy vegetable. Photosynth Res 4: 171–178

Ludlow MM and Wilson GL (1971) Photosynthesis of tropical pasture plants. I. Illuminance, carbon dioxide concentration, leaf temperature, and leaf air vapor pressure difference. Aust J Biol Sci 24: 449–470

Mauney JR, Guinn G, Fry KE and Hesketh JD (1979) Correlation of photosynthetic carbon dioxide uptake and carbohydrate accumulation in cotton, soybean, sunflower and sorghum. Photosynthetica 13: 260–266

Moss DN, Musgrave RB and Lemon EF (1961) Photosynthesis under field conditions. III. Some effects of light, carbon dioxide, temperature and soil moisture on photosynthesis, respiration and transpiration of corn. Crop Sci 1: 83–87

Paterson DT (1985) Comparative eco-physiology of weeds and crops. In: Duke SO (ed) Reproduction and Ecophysiology, pp 101–129. CRC Press, Boca Raton, FL

Pearcy RW (1977) Acclimation of photosynthetic and respiratory carbon dioxide exchange to growth temperature in *Atriplex lentiformis* (Torr.) Wats. Plant Physiol 59: 795–799

Poorter H (1993) Interspecific variation in the growth response of plants to an elevated ambient CO_2concentration. Vegetatio 104/105: 77–97

Potvin C and Strain BR (1985) Effects of CO_2 enrichment and temperature on growth in two C_4 weeds, *Echinochloa crus-galli* and *Eleusine indica*. Can J Bot 63: 1495–1499

Robinson JM (1984) Photosynthetic carbon metabolism in leaves and isolated chloroplasts from spinach plants grown under short and intermediate photosynthetic periods. Plant Physiol 75: 397–409

Rogers HH, Bingham GE, Cure HD, Smith JM and Suran KA (1983) Responses of selected plant species to elevated carbon dioxide in the field. J Environ Quality 12: 569–74

Sionit N and Paterson DT (1984) Responses of C_4 grasses to atmospheric CO_2 enrichment. I. Effect of irradiance. Oecologia 65: 30–34

Taiz L and Zeiger E (1991) Plant Physiology. Benjamin Cummings, New York, 559 pp

Tissue DT, Griffin KL, Thomas RB and Strain BR (1995) Effects of low and elevated CO_2 on C_3 and C_4 annuals. II. Photosynthesis and leaf biochemistry. Oecologia 101: 21–28

Ward DA (1987) The temperature acclimation of photosynthetic responses to CO_2 in *Zea mays* and its relationship to the activities of photosynthetic enzymes and the CO_2 concentrating mechanism of C_4 photosynthesis. Plant, Cell Environ 10: 407–411

Wong SC (1979) Elevated atmospheric partial pressure of CO_2 and plant growth. I. Interactions of nitrogen nutrition and photosynthetic capacity in C_3 and C_4 plants. Oecologia 44: 68–74