

LEAF AND PLANT WATER USE EFFICIENCY OF C₄ SPECIES GROWN AT GLACIAL TO ELEVATED CO₂ CONCENTRATIONS

H. WAYNE POLLEY,^{1,*} HYRUM B. JOHNSON,^{*} HERMAN S. MAYEUX,^{*} DANIEL A. BROWN,^{*} AND JAMES W. C. WHITE[†]

^{*}USDA, Agricultural Research Service, Grassland, Soil and Water Research Laboratory, Temple, Texas 76502; and
[†]Institute of Arctic and Alpine Research and Department of Geological Sciences,
 University of Colorado, Boulder, Colorado 80309

Leaf gas exchange was measured on C₄ plants grown from near glacial to current CO₂ concentrations (200–350 μmol mol⁻¹) and from the current concentration to possible future levels (near 700 and 1000 μmol mol⁻¹) to test the prediction that intrinsic water use efficiency (CO₂ assimilation [A]/stomatal conductance to water [g]) would rise by a similar relative amount as CO₂ concentration. Studied were species differing in growth form or life history, the perennial grass *Schizachyrium scoparium* (little bluestem), perennial shrub *Atriplex canescens* (four-wing saltbush), and annual grass *Zea mays* (maize). Contrary to our prediction, leaf A/g of the C₄ species examined was stimulated proportionally more by a given relative increase in CO₂ over subambient than by elevated concentrations. The ratio of the relative increase in A/g to that in CO₂ exceeded unity in *S. scoparium* and, in 1 of 2 yr, in *Z. mays* as CO₂ rose from 200 to 350 μmol mol⁻¹, but declined to near zero in *S. scoparium* and *A. canescens* as CO₂ rose from 700 to 1000 μmol mol⁻¹. At higher CO₂ concentrations, A/g of the C₄ perennials was similar to that expected for C₃ plants. Since much of the potential response of C₄ plants to CO₂ often derives from higher water use efficiency (WUE), these results indicated that potential productivity of some C₄ plants increased relatively more since glaciation than it will in the future. There also were large (>100%) differences in A/g and plant WUE (production/transpiration) at a given CO₂ level among the plants examined that could influence the relative productivities of C₄ species or growth forms and their interactions with C₃ plants.

Introduction

An increase in plant water use efficiency (WUE) is among the most consistent and, perhaps, most important effects of rising atmospheric CO₂ concentration for vegetation in arid and seasonally dry habitats. For C₄ plants in particular, maximum growth response to CO₂ frequently is realized when water limits production and the benefits of higher WUE are expressed (Gifford and Morison 1985; Owensby et al. 1993). The amount by which WUE rises may therefore largely determine the potential response of many C₄ species to atmospheric change.

At the leaf level, WUE or transpiration efficiency may be expressed as the ratio of the rate of net photosynthesis or assimilation (A) to transpiration (E), and approximated by

$$\begin{aligned} \text{WUE} &= \frac{A}{E} = \frac{1}{\Delta w} \times \frac{A}{g} = \frac{1}{\Delta w} \times \frac{(c_a - c_i)g_c}{1.6g_c} \\ &= \frac{1}{\Delta w} \times \frac{c_a \left(1 - \frac{c_i}{c_a}\right)}{1.6}, \end{aligned} \quad (1)$$

where c_a and c_i are external or ambient and leaf intercellular CO₂ concentrations, respectively, 1.6 is the ratio of diffusivities of water vapor and CO₂ in air, Δw is the mole fraction water vapor gradient from leaves to bulk air, and g and g_c are stomatal conductances to water vapor and CO₂, respectively. Sometimes it is

convenient when different species or growth conditions are being compared to remove the influence of Δw and evaluate potential or intrinsic WUE, A/g, where

$$\frac{A}{g} = \frac{c_a \left(1 - \frac{c_i}{c_a}\right)}{1.6}. \quad (2)$$

At least two conclusions follow from eqq. (1) and (2). Both A/E and A/g are (1) positively related to c_a and (2) negatively related to c_i/c_a , a variable that reflects the balance between the supply of CO₂ to the leaf and consumption of CO₂ in photosynthesis.

For many species, both C₃ and C₄, c_i/c_a varies little at different CO₂ levels (Morison 1993), and A/g increases by about the same relative amount as does c_a or CO₂ concentration. There are studies, however, in which A/E of C₄ plants changed proportionally much more than or less than did CO₂ concentration (Morison 1993), indicating that c_i/c_a may not have remained stable. Some of the variation reported may reflect experimental differences in temperature, light, or humidity, all of which influence stomatal sensitivity to CO₂ (Morison and Gifford 1983; Morison 1985; Bunce 1993). There is evidence that the effect of elevated CO₂ on C₄ WUE may vary with soil water availability (Gifford and Morison 1985) and that c_i/c_a of C₄ species (Bowman 1991) may vary with nitrogen availability. Substantial variation among C₄ species also occurs in the response of whole-plant WUE (production/transpiration) to elevated CO₂ that is not readily explained by differences in Δw or in other factors, including respiration, that also affect WUE at this level (Morison and Gifford 1984).

Until recently, most of the C₄ species grown at dif-

¹Author for correspondence and reprints. Fax 817-770-6561; E-mail a03lctemple@attmail.com.

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Table 1
EXPERIMENTS WITH C₄ SPECIES GROWN AT DIFFERENT CO₂ CONCENTRATIONS

Experiment and species	CO ₂ treatment (μmol mol ⁻¹)	Other treatments	Duration
1: <i>Schizachyrium scoparium</i>	200–350	Presence/absence of <i>Prosopis</i>	May 1989–May 1990
2: <i>Zea mays</i>	200–350	None	March–May 1991
3: <i>Z. mays</i>	200–350	None	April–May 1992
4: <i>Atriplex canescens</i>	360, 680, 1005	Nitrogen	June 1993–October 1994
<i>S. scoparium</i>	360, 680, 1005	Nitrogen	June 1993–October 1994
<i>Z. mays</i>	360, 680, 1005	None	June–August 1994

Note. Treatments other than CO₂ concentration did not significantly affect leaf gas exchange or plant water use efficiency.

ferent CO₂ concentrations were weedy annuals or agronomic species. Relatively few of the perennial C₄ species that dominate vast tropical and warm temperate grazing lands have been studied. Particularly lacking is information on the response of C₄ WUE to the low atmospheric CO₂ levels that prevailed during much of C₄ evolution and expansion (Ehleringer et al. 1991).

We investigated effects of atmospheric CO₂ from near glacial (200 μmol mol⁻¹) to current concentrations (350 μmol mol⁻¹) and from the current concentration to levels possible in the future, near 700 and 1000 μmol mol⁻¹, on leaf gas exchange variables contributing to intrinsic water use efficiency (*A/g*) of three C₄ species. Studied were *Schizachyrium scoparium* (Michx.) Nash. (little bluestem), *Atriplex canescens* (Pursh) Nutt. (four-wing saltbush), and, for comparison to several previous studies, *Zea mays* (maize). We predicted that *c_i/c_a* would remain constant in plants grown at different CO₂ concentrations, such that *A/g* would increase by the same relative amount as CO₂ concentration. At the plant level and over longer time periods, WUE is influenced by respiration, changes in Δ*w*, and water loss not coupled to CO₂ uptake. Plant WUE of *A. canescens* and *S. scoparium* was measured at the current and elevated CO₂ concentrations to test the prediction that WUE, like *A/g*, would increase by the same proportion as CO₂ concentration.

Material and methods

Results were taken from four experiments (table 1). During the first three experiments (Experiments 1–3), either *Zea mays* or *Schizachyrium scoparium* was grown over a gradient of daytime CO₂ concentration from near 350 to 200 μmol mol⁻¹ in a 38-m-long controlled-environment chamber located in a ventilated greenhouse (Mayeux et al. 1993). During the fourth experiment (Experiment 4), *Atriplex canescens*, *S. scoparium*, and *Z. mays* were grown at nominal CO₂ levels of 360, 700, and 1000 μmol mol⁻¹ in air-conditioned greenhouse bays.

The perennial grass *S. scoparium* is a codominate of the mixed-grass and tallgrass prairies in central North America

(Risser et al. 1981). The evergreen shrub *A. canescens* is widely distributed through the western United States, with a range that extends north to Canada and south to Mexico (Vines 1976).

EXPERIMENTS 1–3: SUBAMBIENT CO₂

Aerial parts of plants grown at low CO₂ concentrations (Experiments 1–3) were enclosed in a transparent and tunnel-shaped polyethylene cover that was attached to the top of a 0.76 m deep and 0.45 m wide soil container. The plastic-lined soil container was divided into 0.6-m-long water-tight compartments. Plants received no fertilizer, and the ca. 12 m³ of fine sandy loam soil (Udic Paleustalfs; Huckabee et al. 1977) in the system was removed and mixed between the first two of the three experiments. Soil water content in each of the closed 0.6-m-long soil compartments was measured weekly through the sides of the wooden soil containers with a Troxler 3218 surface moisture gauge (Troxler Electronics, Research Triangle Park, N.C.).

Four individuals of the C₄ perennial grass *S. scoparium*, two grown from tillers and two from seed, were established May 17, 1989, in each of 29 compartments along the 38-m chamber (Experiment 1; Polley et al. 1994). The grass was grown in monoculture or in mixture with one individual of the woody legume *Prosopis glandulosa* Torr. var. *glandulosa* (honey mesquite). Soil was restored weekly to "field capacity" (ca. 20% volumetric water content) during the measurements reported here.

Nine of the 0.6-m-long soil compartments along the 38-m chamber were sown as monocultures with two to three plants of 'Gaspe' yellow flint *Z. mays* on both March 20, 1991 (Experiment 2; Polley et al. 1993b), and April 1, 1992 (Experiment 3; Polley et al. 1995). Soil in each compartment was brought to field capacity weekly.

Photosynthesis by plants enclosed in the 38-m chamber depleted CO₂ in air as it was moved by a blower from the air intake to the outlet of the chamber. A continuous gradient of CO₂ from near 350 to 200 μmol mol⁻¹ was maintained during daylight of each experiment by automatically varying the rate of air flow through the chamber. The CO₂ concentration was measured each minute with an infrared gas analyzer (Binos 67, Leybold-Haeraeus, Hanau, Germany) in air drawn from the chamber outlet and sequentially from five points spaced at 7.6-m intervals along the chamber. Blower

speed was increased each night to exhaust respired CO₂. Nighttime CO₂ concentration averaged 360–400 μmol mol⁻¹.

Drybulb and dewpoint air temperatures were regulated each 7.6 m along the chamber with chilled-water cooling coils and resistance heating elements to track these temperatures within the ventilated greenhouse. Drybulb and dewpoint temperatures immediately before and after points of regulation were measured with fine-wire (25 μm diameter) thermocouples and chilled-mirror hygrometers (DEW-10, General Eastern Instruments, Wobarn, Mass.). Vapor pressure deficit of air was calculated from these records. Photosynthetically active photon flux density was measured during all experiments (Experiments 1–4) on the greenhouse roof with a quantum sensor (LI-190SB) and within greenhouses with 1-m-long line quantum sensors (Li-Cor, Inc., model LI-191SA, Lincoln, Nebr.) mounted above plants. The daily integral of photosynthetic photon flux density (PPFD) incident on plants in the 38-m chamber averaged 55% of that outside the greenhouse, but exceeded 1400 μmol(quantum) m⁻² s⁻¹ near midday. The vapor pressure deficit of air introduced to successive lengths of chamber did not change systematically through 1989 (mean = 1.64 kPa) or 1990 (mean = 1.18 kPa) during Experiment 1 with *S. scoparium* or during Experiments 2 and 3 in 1991 and 1992 with *Z. mays* (mean = 1.46 and 1.27 kPa during Experiments 2 and 3, respectively).

EXPERIMENT 4: ELEVATED CO₂

Atriplex canescens, *S. scoparium*, and *Z. mays* were grown at 360, 680, and 1005 μmol mol⁻¹ CO₂ in air-conditioned greenhouse bays (Experiment 4). The CO₂ concentration and dewpoint temperature of air in each bay were measured each fourth minute with a Li-Cor, Inc., model 6262 infrared gas analyzer (Lincoln, Nebr.). The CO₂ readings were corrected for atmospheric pressure measured with a Druck model DPI 260 pressure indicator (Druck, Inc., New Fairfield, Conn.). Air temperature was measured in the center of each bay with fine-wire thermocouples and was changed seasonally to approximate that outdoors by manually adjusting thermostatic controls.

Atriplex canescens and *S. scoparium* each were grown in a 2:1 (v/v) mixture of washed sand: fine sandy loam soil in 0.25-m-diameter and 0.60-m-deep pots. Thirty pots, each with two individuals, were planted to each species in June 1993. *Atriplex* was grown from seed. A single ramet, each with two to three tillers, from each of two *S. scoparium* individuals was planted per pot.

Ten pots with each species were maintained from planting in each CO₂ treatment. The soil in each pot was covered with 1–2 cm of vermiculite to reduce evaporation. Pots were weighed weekly from June 1993 through January 1994 (first season) and from April 1994 through October 1994 (second season) with a load beam (KIS-3, BLH Electronics, Canton, Mass.) to determine water loss to evapotranspiration. Water was added weekly to restore soil to 20% volumetric moisture content. Transpiration from planted pots was calculated by subtracting water loss from two unplanted pots at each CO₂ level.

Five pots per species at each CO₂ level were randomly assigned to each of two nitrogen (N) addition rates (0 or 19 mg wk⁻¹, low and high N, respectively). Nitrogen was supplied weekly as NH₄NO₃ in 100 mL of Hoagland's solution that otherwise lacked N (Hoagland and Arnon 1950; modified by replacing CaSO₄ with NaSO₄ and by supplying phosphorus as a 13:1 w/w mixture of KH₂PO₄:K₂HPO₄). Plants

in the low N treatment received an equivalent amount of Hoagland's solution lacking N. Soils were leached once during the first growing season and twice during the second season to reduce the potential for salt accumulation. One to two well-watered plants of *Z. mays* also were grown from seed planted June 21, 1994, in each of three additional pots per CO₂ level. Each pot containing *Z. mays* received 250 mL of complete Hoagland's solution biweekly.

Schizachyrium scoparium in 10 of 30 pots died before the second growing season. Mortality ranged from 60% of plants grown at 360 μmol mol⁻¹ to 0% of plants grown at 680 μmol mol⁻¹.

Senescent aboveground tissues of *S. scoparium* and *A. canescens* were collected at the end of the first season, during January 1994. Aboveground structures were completely harvested and roots (including crowns) were separated on 12.7-mm mesh screen in October 1994. Tissues were dried to constant mass at 60°C and weighed. Plant WUE was calculated as the ratio of total biomass, including that of senescent aboveground tissues, to transpiration measured gravimetrically.

Mean daytime temperature in greenhouse bays declined from 26°C in June 1993 to 17°C in December 1993, then increased to a maximum of 30°C in August 1994 before declining to 20°C at harvest of perennial species in October 1994. Mean vapor pressure deficit of air during daylight ranged between a low of 0.5 kPa in January 1994 and a high of 2.1 kPa in August 1994. The daily integral of PPFD inside bays averaged 58% of that measured above the greenhouse, although instantaneous PPFD inside the greenhouse approached 90% of that measured outdoors at midday. Standard deviation of CO₂ concentration was calculated daily. The average of these values ranged from 12.6 μmol mol⁻¹ at the lowest CO₂ concentration to 6.9 μmol mol⁻¹ in the midlevel CO₂ treatment.

LEAF GAS EXCHANGE

Net photosynthesis (*A*) and stomatal conductance to water (*g*) were measured on recent expanded leaves of plants grown at subambient CO₂ levels (Experiments 1–3; *Z. mays*, *S. scoparium* established from ramets) during April and May of 1990, 1991, and 1992 with an LCA-2 infrared gas analyzer (Analytical Development Co., Hertfordshire, England). Gas exchange of recent expanded leaves of *A. canescens*, *S. scoparium*, and *Z. mays* grown at the current and elevated CO₂ concentrations (Experiment 4) was measured during August and September 1994 with a portable gas exchange system (Campbell Scientific, Inc., MPH-1000, Logan, Utah) coupled to an infrared analyzer (model 6262, Li-Cor, Inc., Lincoln, Nebr.). Gas exchange was measured on the relatively broad leaves that expanded initially on new branches of *A. canescens* rather than on the needle-like leaves that develop later. All steady-state measurements were taken on well-watered plants and on clear days with near maximum incident light near the CO₂ concentration at which plants were grown. Air temperature and humidity during measurements approximated conditions under which plants grew. Boundary layer conductances of leaves were estimated from filter paper replicas. Gas exchange parameters were calculated using one-sided leaf area (von Caemmerer and Farquhar 1981).

Gas exchange of *S. scoparium* grown over subambient CO₂ concentrations (Experiment 1, *n* = 20) was measured at 31.6° ± 0.5°C, 1391 ± 38 μmol(quantum) m⁻² s⁻¹, and leaf-to-air vapor pressure differences (VPD) of 0.62 ± 0.02

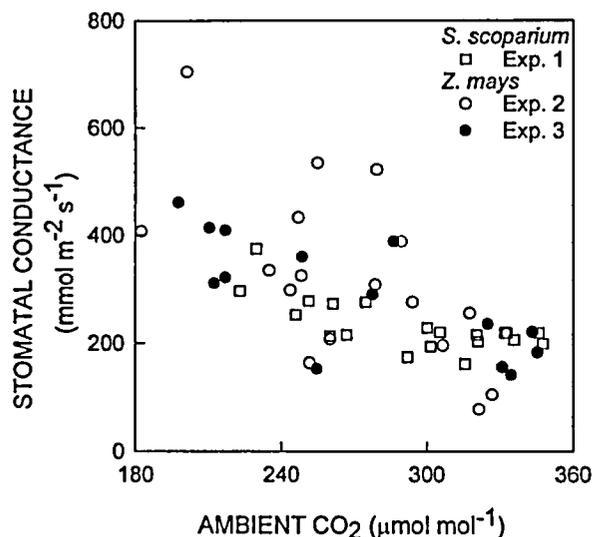


Fig. 1 Stomatal conductance as a function of the daytime CO₂ concentration at which leaf blades of two C₄ grasses were grown and measured during three separate experiments (Experiments 1–3).

kPa (Polley et al. 1994). Single leaves per plant of *Z. mays* were measured at mean leaf temperatures (\pm SE) of $33.7^\circ \pm 0.5^\circ\text{C}$ and $26.3^\circ \pm 0.1^\circ\text{C}$, PPFD of 1406 ± 34 and $1364 \pm 34 \mu\text{mol m}^{-2} \text{s}^{-1}$, and VPD of 1.92 ± 0.19 and 1.38 ± 0.05 kPa during Experiment 2 ($n = 17$) and Experiment 3 ($n = 14$), respectively. Single measurements per plant of *A. canescens* ($n = 60$), *S. scoparium* ($n = 40$), and *Z. mays* ($n = 13$) were taken at mean leaf temperatures (\pm SE) of $31.0^\circ \pm 0.2^\circ$, $32.6^\circ \pm 0.3^\circ$, $32.5^\circ \pm 0.6^\circ\text{C}$, incident PPFD of 1190 ± 19 , 1123 ± 19 , and $1095 \pm 52 \mu\text{mol m}^{-2} \text{s}^{-1}$, and VPD of 2.44 ± 0.04 , 2.91 ± 0.08 , and 2.84 ± 0.16 kPa, respectively, during Experiment 4.

STATISTICAL ANALYSES

Measurements on plants exposed to subambient CO₂ levels (Experiments 1–3) were analyzed by linear regression (Weisberg 1980). Data taken at the current and elevated CO₂ concentrations (Experiment 4) were analyzed with a three-way analysis of variance (ANOVA) that included CO₂ treatment, species, N treatment, and appropriate interactions (Sokal and Rohlf 1981). Student-Newman-Keuls's multiple range test was used to determine significant differences among three or more means. Data were transformed logarithmically before analysis when required to satisfy assumptions of ANOVA. Standard errors for transformed data were omitted in presentation of results using the original scale of measurement.

Results

EXPERIMENTS 1–3: SUBAMBIENT CO₂

Leaf net photosynthesis (A) increased 27%, from 220 to $340 \mu\text{mol mol}^{-1}$ in *Schizachyrium scoparium* (from 21.1 to $26.8 \mu\text{mol m}^{-2} \text{s}^{-1}$, $P = 0.005$, $r^2 = 0.36$, $n = 20$; Experiment 1). Leaf A of *Zea mays* was not significantly affected by CO₂ during Experiment 2 ($n = 17$), but surprisingly was negatively correlated with CO₂, from 200 to $350 \mu\text{mol mol}^{-1}$ during Experiment 3 ($P = 0.03$, $r^2 = 0.33$, $n = 14$; not shown).

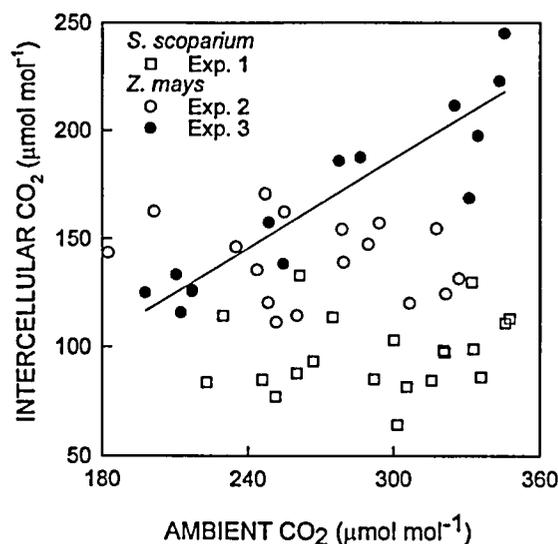


Fig. 2 Relationship of leaf intercellular CO₂ concentration to the ambient CO₂ concentration at which *Schizachyrium scoparium* and *Zea mays* were grown and measured during three experiments (Experiments 1–3). The line is a linear regression of gas exchange measurements for *Z. mays* on CO₂ during Experiment 3 ($r^2 = 0.85$, $P < 0.0001$, $n = 14$).

In contrast, g of both species was strongly and negatively related to CO₂ (fig. 1). The decline in g compensated for the effect of rising CO₂ (c_a) on leaf c_i in *S. scoparium* (Experiment 1) and *Z. mays* (Experiment 2), and c_i remained relatively constant (fig. 2). As a result, c_i/c_a declined from 0.66 and 0.41 to 0.39 and 0.30 in *Z. mays* and *S. scoparium*, respectively, as CO₂ rose from 220 to $325 \mu\text{mol mol}^{-1}$, and A/g increased relatively more than did the CO₂ concentration (fig. 3). Leaf A/g increased 82% (from 53.4 to $97.2 \mu\text{mol mol}^{-1}$) in *Z. mays* and 81% (from 70.4 to $127.2 \mu\text{mol mol}^{-1}$) in *S. scoparium* with a 48% increase in CO₂ concentration from 220 to $325 \mu\text{mol mol}^{-1}$ CO₂. The more commonly reported linear relationship between c_i and c_a (fig. 2) that resulted in a near constant c_i/c_a across CO₂ levels and similar relative increase in A/g (55%) and CO₂ concentration (70%) was obtained for *Z. mays* during Experiment 3 (fig. 3).

EXPERIMENT 4: ELEVATED CO₂

Nitrogen treatment did not significantly influence leaf gas exchange or plant WUE of *Atriplex canescens* or *S. scoparium* grown at the current and elevated CO₂ levels and thus will not be discussed further. There was no consistent effect of CO₂ on g of *A. canescens* or on photosynthetic rates of either the grass or shrub (table 2). Stomatal conductance of *S. scoparium* was lower at elevated CO₂ levels than at the current concentration. Averaged across species, however, A/g increased 95% (from 74.7 to $145.5 \mu\text{mol mol}^{-1}$) as concentration of CO₂ rose from 350 to $700 \mu\text{mol mol}^{-1}$, but did not change significantly from 700 to $1000 \mu\text{mol mol}^{-1}$ (145.5 to $123.0 \mu\text{mol mol}^{-1}$, respectively).

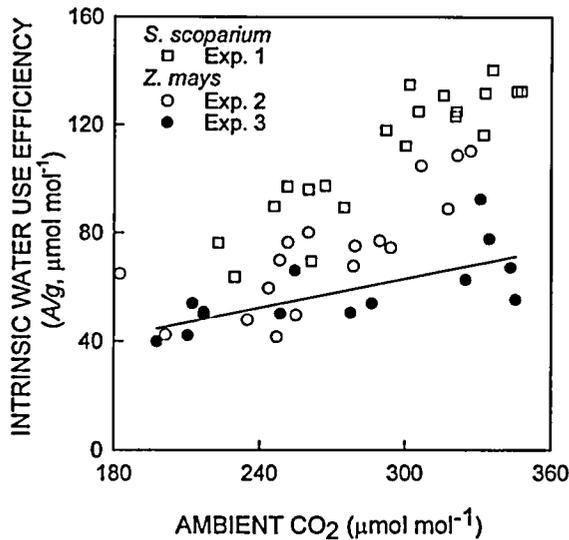


Fig. 3 Relationship of leaf intrinsic water use efficiency (A/g) to the ambient CO_2 concentration at which *Schizachyrium scoparium* and *Zea mays* were grown and measured during three experiments (Experiments 1–3). The line is a linear regression through data for *Z. mays* during Experiment 3 that illustrates a similar relative increase in A/g and CO_2 concentration ($r^2 = 0.51$, $P = 0.004$, $n = 14$).

At each CO_2 level, c_i/c_a was lower and A/g was higher in *S. scoparium* than in *A. canescens*. Across CO_2 treatments, A/g was 78% higher in the grass than shrub (mean = 160.6 and 90.4 $\mu\text{mol mol}^{-1}$ for *S. scoparium* and *A. canescens*, respectively). Limited measurements on *Z. mays* grown during the same experiment indicated no significant effect of CO_2 on c_i/c_a (mean = 0.52, $n = 13$).

Biomass of *S. scoparium* was higher at elevated than at the current CO_2 concentration, but CO_2 did not significantly affect production of *A. canescens* (table 3). Averaged across CO_2 treatments, plant WUE of the C_4 grass was 2.5 times that of the shrub. Water use

efficiency was not, however, significantly affected by CO_2 concentration in either species.

Discussion

Over both subambient and elevated CO_2 concentrations, changes in leaf A/g or potential WUE of the C_4 species examined derived largely from changes in g . There was little or no positive effect of CO_2 at any concentration on A . Photosynthesis of some C_4 species (Rogers et al. 1983; Ziska et al. 1990; Knapp et al. 1994b; Tissue et al. 1995), but not all (Sionit and Patterson 1984; Morgan et al. 1994), approached saturation at the current CO_2 level, especially when plants had been well watered.

The decline in g did not stabilize c_i/c_a in these C_4 species as CO_2 rose, contrary to the pattern often observed in C_3 plants (Morison 1993; Polley et al. 1993a) and in some C_4 species (Kirkham et al. 1991; Tissue et al. 1995). Leaf c_i remained relatively stable and leaf c_i/c_a declined as CO_2 rose from near glacial to present levels in the perennial grass *Schizachyrium scoparium* and, in 1 of 2 yr, in the annual *Zea mays*. When c_i/c_a remained constant, as it often did in C_3 plants (Polley et al. 1993a), leaf A/g increased by the same relative amount as did CO_2 concentration. In these C_4 species, the relative increase in A/g was ca. 1.7 times that in external CO_2 over subambient concentrations. Others have reported that *Z. mays* may maintain leaf c_i constant across CO_2 levels (Dubbe et al. 1978; Louwse 1980), resulting in a decline in c_i/c_a as CO_2 rises, although this has not previously been demonstrated in plants grown at subambient concentrations. Conductance and stomatal sensitivity to CO_2 tend to be greater at the relatively low values of VPD that prevailed during these measurements (Morison and Gifford 1983). It is not obvious, however, that VPD should affect the response of c_i/c_a to CO_2 . Indeed, Morison (1985) concluded from a compilation of 80 observations of stomatal response to CO_2 that conductance measured at

Table 2

LEAF GAS EXCHANGE OF ATRIPLEX CANESCENS AND SCHIZACHYRIUM SCOPARIUM MEASURED AT NEAR THE CO_2 CONCENTRATION THAT PREVAILED DURING GROWTH

Parameter	<i>Atriplex</i>			<i>Schizachyrium</i>		
	350	700	1000	350	700	1000
	$(\mu\text{mol mol}^{-1} \text{CO}_2)$			$(\mu\text{mol mol}^{-1} \text{CO}_2)$		
A ($\mu\text{mol m}^{-2} \text{s}^{-1}$) ...	12.0 ^C (1.2)	16.0 ^{ABC} (1.7)	15.7 ^{ABC} (1.2)	20.6 ^A (1.2)	14.4 ^{BC} (1.3)	19.6 ^{AB} (1.5)
g ($\text{mmol m}^{-2} \text{s}^{-1}$) ...	190.0 ^A	153.0 ^{AB}	164.0 ^{AB}	189.0 ^A	79.0 ^C	122.0 ^B
c_i/c_a	0.68 ^B	0.71 ^B	0.80 ^A	0.46 ^P	0.55 ^C	0.71 ^B
A/g ($\mu\text{mol mol}^{-1}$) ...	61.0 ^C	109.2 ^B	100.6 ^B	108.9 ^B	181.3 ^A	160.5 ^A

Note. Values for a given parameter (leaf net photosynthesis [A], stomatal conductance to water [g], ratio of leaf intercellular to ambient CO_2 concentration [c_i/c_a], intrinsic water use efficiency [A/g]) do not differ significantly ($P > 0.05$) if followed by the same uppercase letter (Student-Newman-Keuls's test), ± 1 SE in parentheses, $n = 8$ and 12 measurements for *Schizachyrium* at 350 and 1000 $\mu\text{mol mol}^{-1}$, respectively, and 20 measurements for remaining categories. Arithmetic means of g are shown, but statistical analyses were performed after logarithmic transformation.

Table 3
BIOMASS PRODUCTION AND WATER USE EFFICIENCY (WUE) OF *ATRIPLEX CANESCENS*
AND *SCHIZACHYRIUM SCOPARIUM* GROWN AT THREE CO₂ CONCENTRATIONS

Parameter	<i>Atriplex</i>			<i>Schizachyrium</i>		
	360 ($\mu\text{mol mol}^{-1} \text{CO}_2$)	680	1005	360 ($\mu\text{mol mol}^{-1} \text{CO}_2$)	680	1005
Biomass (g)	141.4 ^A (11.4)	95.3 ^{AB} (5.5)	137.9 ^A (7.4)	81.9 ^B (9.4)	131.6 ^A (14.8)	143.7 ^A (21.4)
WUE (g L ⁻¹) ..	4.42 ^B (0.33)	5.07 ^B (0.22)	4.93 ^B (0.40)	10.35 ^A (1.96)	12.92 ^A (0.65)	11.83 ^A (1.58)

Note. Values for a given parameter (biomass production or plant WUE [biomass/transpiration]) do not differ significantly ($P > 0.05$) if followed by the same uppercase letter (Student-Newman-Keuls's test), ± 1 SE in parentheses, $n = 4$ and 6 for *Schizachyrium* at 360 and 1005 $\mu\text{mol mol}^{-1}$, respectively, and 10 for remaining categories.

double the present concentration was a constant proportion of g at today's CO₂ level. Data obviously are limited, but results indicate that the glacial to present rise in atmospheric CO₂ concentration elicits a greater relative increase in potential WUE in some C₄ species than in many C₃ plants.

Conversely, the potential benefit of increasing CO₂ above today's level for WUE of *S. scoparium* and *Atriplex canescens* was reduced or even completely negated by an increase in c_i/c_a that reflected the meager response of both A and g in these species to elevated CO₂. Stomatal conductance of *A. canescens* was not affected by CO₂. Conductance declined somewhat at elevated CO₂ in *S. scoparium*. Independent measurements with a diffusion porometer indicated that g of *S. scoparium* may also have declined as CO₂ rose from near 700 to 1000 $\mu\text{mol mol}^{-1}$ (from 62.3 to 35.2 $\text{mmol m}^{-2} \text{s}^{-1}$; H. B. Johnson, unpublished data). In any case, the decrease in g caused by elevating CO₂ by 350 to 650 $\mu\text{mol mol}^{-1}$ was similar in magnitude to that measured when CO₂ was increased less than 150 $\mu\text{mol mol}^{-1}$ above the glacial level. Qualitatively consistent with the limited response of A/g to elevated CO₂ was the absence of a significant CO₂ effect on whole-plant WUE of *S. scoparium* and *A. canescens* (table 3).

At currently "typical" values of c_i/c_a (ca. 0.4 and 0.7 for C₄ and C₃ plants, respectively; Wong et al. 1979), C₄ species have ca. a twofold advantage in potential WUE over C₃ species. That advantage was eroded or eliminated by the increase in c_i/c_a of C₄ species at elevated CO₂. In the longer term, plastic (Knapp et al. 1994a) or perhaps evolutionary declines in stomatal density (Peñuelas and Matamala 1990) may permit C₄ plants to further reduce g and increase A/g and plant WUE as CO₂ increases, though no change in stomatal density was noted in *S. scoparium* grown over glacial to current CO₂ concentrations (Malone et al. 1993) or in Experiment 4 of this study (H. B. Johnson, unpublished data). The benefits of a further decrease in g to plant WUE in nature likely would be marginal, however. At the low rates of conductance typical for

C₄ species, the magnitude of any decline in g would be small.

Contrary to our prediction, leaf A/g of the C₄ species examined was stimulated proportionally more by a given relative increase in CO₂ over subambient than elevated concentrations. The ratio of the relative increase in A/g to that in CO₂, for example, sometimes exceeded unity over subambient concentrations and declined to near zero as CO₂ rose from 700 to 1000 $\mu\text{mol mol}^{-1}$. Because photosynthesis in many C₄ species responds little to CO₂, much of the positive growth response of C₄ plants to CO₂ often occurs when water is limiting and benefits of an increase in WUE are expressed (Gifford and Morison 1985; Owensby et al. 1993). Our results therefore indicate that potential productivity of some C₄ plants increased relatively more since glaciation than it will in the future. The C₄ species studied also differed substantially in potential or whole-plant WUE at a given CO₂ concentration. Perhaps coincidentally, they also differed in growth form or life history. Averaged across CO₂ treatments, for example, A/g was 78% higher and whole-plant WUE was 150% higher in the grass *S. scoparium* than in the shrub *A. canescens*. These differences could further influence the relative productivities of C₄ species or growth forms and their interactions with C₃ species, especially in water-limited environments.

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