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What is the usual internal carbon dioxide concentration in C_4 species under midday field conditions?

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Abstract

The carbon dioxide concentrating system in C_4 photosynthesis allows high net photosynthetic rates (P_N) at low internal carbon dioxide concentrations (C_i), permitting higher P_N relative to stomatal conductance (g_s) than in C_3 plants. This relation would be reflected in the ratio of C_i to external ambient (C_a) carbon dioxide concentration, which is often given as 0.3 or 0.4 for C_4 plants. For a C_a of 360 µmol mol^{-1} that would mean a C_i about 110–140 µmol mol^{-1}. Our field observations made near midday on three weedy C_4 species, *Amaranthus retroflexus*, *Echinochloa crus-galli*, and *Setaria faberi*, and the C_4 crop *Sorghum bicolor* indicated mean values of C_i of 183–212 µmol mol^{-1} at C_a = 360 µmol mol^{-1}. Measurements in two other C_4 crop species grown with three levels of N fertilizer indicated that while midday values of C_i at high photon flux were higher at limiting N, even at high nitrogen C_i averaged 212 and 196 µmol mol^{-1} for *Amaranthus hypochondriacus* and *Zea mays*, respectively. In these two crops midday C_i decreased with increasing leaf to air water vapor pressure difference. Averaged over all measurement days, the mean C_i across all C_4 species was 198 µmol mol^{-1}, for a C_i/C_a ratio of 0.55. Prior measurements on four herbaceous C_3 species using the same instrument indicated an average C_i/C_a ratio of 0.69. Hence midday C_i values in C_4 species under field conditions may often be considerably higher and more similar to those of C_3 species than expected from measurements made on plants in controlled environments. Reducing g_s in C_4 crops at low water vapor pressure differences could potentially improve their water use efficiency without decreasing P_N.

Additional key words: net photosynthetic rate; nitrogen; stomatal conductance; water use efficiency.

Introduction

The high affinity of phospho*enol*pyruvate carboxylase for carbon dioxide allows CO_2 assimilation rates in C_4 species to be saturated with respect to CO_2 at lower concentrations than is the case in C_3 species, in which the initial fixation of CO_2 is by ribulose-1,5-bisphosphate carboxylase (Laisk and Edwards 1998). Although there are exceptions, photosynthesis in C_4 plants is often saturated at internal CO_2 concentrations (C_i) of 100–150 µmol mol^{-1} (Laisk and Edwards 1998). For C_4 species, stomatal conductances (g_s) which result in C_i values above the saturation level would be wasteful of water without increasing net photosynthetic rates (P_N). Many measurements of C_i on C_4 plants made at ambient CO_2 concentrations (C_a) of 330–360 µmol mol^{-1} have indicated values in the 100–150 µmol mol^{-1} range, leading to the generalization that the C_i/C_a ratio is 0.3–0.4 in C_4 plants (Jones 1983). These results further suggest that high water use efficiency (WUE) may have been an evolutionary priority for C_4 plants. The C_i/C_a ratio of 0.3–0.4 in C_4 species contrasts with the typical ratio of 0.6–0.7 in C_3 species, and reflects the higher ratio of P_N/g_s and higher WUE in C_4 species (Jones 1983, Tanner and Sinclair 1983).

However, most of the measurements indicating C_i/C_a ratios of 0.3–0.4 in C_4 species have been made under laboratory or glasshouse conditions. The measurements presented here suggest that C_i/C_a ratios for C_4 plants under field conditions are often considerably higher. I also investigated the possibility that under N deficient conditions, C_i might increase, and that this might partly explain higher than expected C_i values under field conditions. Leaf to air water vapor pressure difference can affect g_s independently of P_N in C_4 plants (Bunce 1982), and relationships between leaf to air water vapor pressure difference and C_i were also investigated.

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Materials and methods

All plants studied were grown at the South Farm of the Beltsville Agricultural Research Center, in Beltsville, Maryland, USA in 2001–2004. Three weedy C_4 species, *Amaranthus retroflexus* L., *Echinochloa crus-galli* (L.) P. Beauv., and *Setaria faberi* Herrm., and the crop *Sorghum bicolor* (L.) Moench. cv. AT×399×RT×430 were seeded in early June in a plot fertilized with 25 g(N) m^{-2}, 1.8 g(P) m^{-2}, and 4.8 g(K) m^{-2}. Sorghum was thinned to 5 plants m^{-2}, while the weed species underwent self-thinning. Leaf gas exchange measurements were made on these plants in 2001 and 2002.

Two other C_4 crop species, *Amaranthus hypochondriacus* L. and *Zea mays* L. cv. Silver Queen, were grown with three N fertilizer treatments applied to an adjacent field which had received no fertilizer for at least 10 years. Plots received 40, 20, or 3 g(N) m^{-2}, and all plots received 1.8 g(P) m^{-2} and 4.8 g(K) m^{-2}. Plants were in 76 cm rows, at a density of 4.4 plants m^{-2}. There were two replicate plots per species and fertilizer level, and gas exchange measurements were made only on interior plants. Both species were grown for two summers. Destructive harvests were made in each plot in late September to determine total above ground biomass before significant leaf fall occurred and after reproductive growth was nearly complete.

Leaf gas exchange measurements were made near midday on clear days between late June and early September on mature leaves that were fully irradiated. Ambient air temperatures ranged from 25 to 33 °C, the water vapor pressure ranged from 1.7 to 2.8 kPa, and wind speed ranged from 0.5 to 2.5 m s^{-1}. A recently factory calibrated *CIRAS-1* (*PP Systems*, Amesbury, MA, USA) portable photosynthesis system was utilized, with a broad-leaf chamber. The carbon dioxide concentration external to the leaf was maintained at 360±10 µmol mol^{-1} by adjusting the inlet concentration as necessary. Ambient air averaged 360 µmol(CO_2) mol^{-1} at this time of day in this location. The inlet humidity was reduced to 50–80 % of that of the ambient air, depending on g_s, so that the water vapor pressure of the air around the leaf in the chamber was nearly the same as that of the ambient outside air. The leaf chamber was oriented to maximize the photosynthetic photon flux density (PPFD) on the enclosed part of the leaf, and the minimum PPFD was 1 400 µmol m^{-2} s^{-1}. Leaves were left in the chamber until constant g_s and P_N were obtained. This usually required less than 5 min. The gas exchange system does not have chamber heating or cooling, but uses an infrared radiation filter and ventilated heat exchangers. Steady-state leaf temperatures were within ±2 °C of that of the ambient air, depending on g_s. Plots were sampled such that the ambient environmental conditions did not differ systematically with N fertilizer treatment. C_i values were calculated by the system software, assuming a 1 : 1 ratio of g_s on the upper and lower surfaces. The leaf to air water vapor pressure difference was calculated from leaf temperature and the water vapor pressure external to the boundary layer. Leaves were sampled for determination of total N per unit leaf area, with N measured using a *CHN* analyzer by the State of Maryland Soil Testing Laboratory.

For the species other than the *A. hypochondriacus* and *Z. mays*, leaf gas exchange was determined on a minimum of 5 leaves per species on each occasion. For those two species, leaf gas exchange was measured on either 2 or 3 leaves per species from each fertilizer plot on each occasion.

In the N fertilizer experiment with the *A. hypochondriacus* and *Z. mays*, an overall mean value for each plot for a given summer was obtained for C_i, P_N, g_s, P_N/g_s, and leaf N per unit of area. Fertilizer treatment effects were tested using analysis of variance, with 2 replications over 2 years for each species, and means were separated using Tukey's HSD test. For the high N treatment, linear regressions were developed relating C_i, g_s, and P_N to water vapor pressure difference and leaf temperature, using mean values for each measurement date.

Results

The overall mean C_i values for the three C_4 weedy species ranged from 183 to 212 µmol mol^{-1}, with values ranging from 108 to 267 µmol mol^{-1} among days (Table 1). Similar overall mean, minimum, and maximum values also occurred in the three C_4 crop species (Table 1). For C_a = 360 µmol mol^{-1}, the overall mean C_i of 198 µmol mol^{-1} resulted in a C_i/C_a ratio of 0.55.

Day to day variation in C_i in both *A. hypochondriacus* and *Z. mays* was correlated with water vapor pressure, as C_i decreased linearly with increasing leaf to air water vapor pressure difference (Fig. 1*A*). There was no significant correlation between P_N and C_i or between P_N and vapor pressure difference in either species (Fig. 1*B*). In these data, leaf temperatures only ranged from 27 to 33 °C in *A. hypochondriacus* and between 29 and 34 °C in *Z. mays*, and there was no significant correlation between leaf temperature and water vapor pressure difference in either species, nor between P_N or C_i and temperature (not shown).

In both *A. hypochondriacus* and *Z. mays*, low N fertilization significantly increased C_i, and reduced P_N/g, with no significant effect on P_N (Table 2). Leaf N per area was somewhat reduced by the low N treatment in *A. hypochondriacus*.

Shoot masses increased with N level in both *A. hypochondriacus* and *Z. mays*, with the plants in the

Table 1. Midday values of C_i for six C_4 species. Minimum, maximum, and mean values refer to day to day variation in mean C_i values. 4–6 leaves were measured on each date.

Species	Number of days	C_i [µmol mol^{-1}] Minimum	 Maximum	 Mean
Amaranthus hypochondriacus	10	174	245	212
Amaranthus retroflexus	5	146	267	212
Echinochloa crus-galli	3	142	218	183
Setaria faberi	5	108	231	196
Sorghum bicolor	3	136	225	188
Zea mays	15	117	245	196

Table 2. Effect of nitrogen fertilizer application rate on internal carbon dioxide concentration (C_i), net photosynthetic rate (P_N), the ratio of P_N to stomatal conductance to water vapor (P_N/g_s), leaf N content per unit area, leaf area index (LAI), and shoot mass in *A. hypochondriacus* and *Z. mays*. Means over two years. Within species, numbers within columns followed by different letters are significantly different at p=0.05, using analysis of variance.

Species	N application [g m^{-2}]	C_i [µmol m^{-2} s^{-1}]	P_N [µmol m^{-2} s^{-1}]	P_N/g_s [mol mol^{-1}]	Leaf N [g m^{-2}]	LAI	Shoot mass [g m^{-2}]
Z. mays	40	201 c	52.0 a	0.083 a	2.2 a	3.0 a	1050 a
	20	211 b	54.8 a	0.075 b	2.0 a	2.7 a	911 a
	3	225 a	56.3 a	0.066 c	2.0 a	2.1 b	682 b
A. hypochondriacus	40	215 b	48.8 a	0.063 a	3.0 a	4.0 a	1122 a
	20	233 a	47.8 a	0.052 b	2.8 ab	3.2 b	881 b
	3	239 a	47.0 a	0.048 b	2.6 b	2.5 c	730 c

highest N treatment having about 1.5 times the biomass of those in the low N treatment. The N treatment effects on leaf area index were proportional to the effects on biomass (Table 2).

Discussion

The higher than expected mean values of C_i for the C_4 plants found in this study and relationships between C_i and N deficiency have potentially important implications for WUE (Tanner and Sinclair 1983), but first the validity of the measurements must be established. In C_3 species, discrimination among isotopes of carbon can be used to estimate C_i. However, in C_4 species relationships between C_i and isotopic discrimination can be positive or negative (Henderson *et al*. 1998), or discrimination can be primarily related to variation in bundle sheath leakiness to CO_2 (Meinzer *et al*. 1994, Saliendra *et al*. 1996), so no simple surrogate for gas exchange measurements is available. The reliability of the measurements can instead be examined by comparing results obtained with the same instrument on the same species under glasshouse conditions, on C_3 species under similar field conditions, and by comparisons with data obtained under similar conditions but with different instruments.

We previously measured leaf gas exchange for all of these species under glasshouse conditions using the same instrument (Ziska and Bunce 1997). Using a C_a of 380 µmol mol^{-1}, we obtained C_i/C_a ratios of 0.28–0.37 (*i.e*. C_i = 106–141 µmol mol^{-1}) for the species in the present study, when plants were grown at the ambient carbon dioxide concentration. These C_i/C_a ratios are those expected for C_4 plants.

We have used the same instrument to measure midday leaf gas exchange under field conditions in C_3 species, although at C_a = 350 µmol mol^{-1}, rather than the 360 µmol mol^{-1} used in this study. Wilson and Bunce (1997) obtained a mean C_i/C_a ratio of 0.78 in soybean. Winter wheat and barley had mean C_i/C_a ratios of 0.67 and 0.61, respectively (Bunce 1998), and *Taraxacum officinale* had a mean C_i/C_a ratio of 0.71 (Bunce 2000). Overall mean C_i/C_a ratio for the C_3 species was 0.69. These values are in the range expected for C_3 species (Jones 1983).

Higher than expected C_i values for C_4 species under field conditions have also been reported or can be estimated from reported values of P_N and g_s, for studies which have used different types of instruments. For *Z. mays*, I obtained afternoon C_i values of about 220 µmol mol^{-1} (Bunce 1990) using different instruments, and midday C_i calculated from the data in Figs. 1 and 3 of Hirasawa and Hsiao (1999) was about 190–230 µmol mol^{-1}. Leakey *et al*. (2004) reported midday C_i as high as 214 µmol mol^{-1} in *Z. mays*. In sorghum, Wall *et al*. (2001) obtained midday P_N averaging about 40 µmol m^{-2} s^{-1} and g_s of about 500 mmol m^{-2} s^{-1} for a C_i of about 220 µmol mol^{-1}. Similarly Peng and Krieg (1992) reported P_N for sorghum of about 45 µmol m^{-2} s^{-1}

and g_s of about 450 mmol m^{-2} s^{-1}, for a C_i of about 190 µmol mol^{-1}, assuming C_a = 350 µmol mol^{-1}. For sugarcane, Grantz (1989) reported midday P_N and g_s values that would give a C_i of about 230 µmol mol^{-1}.

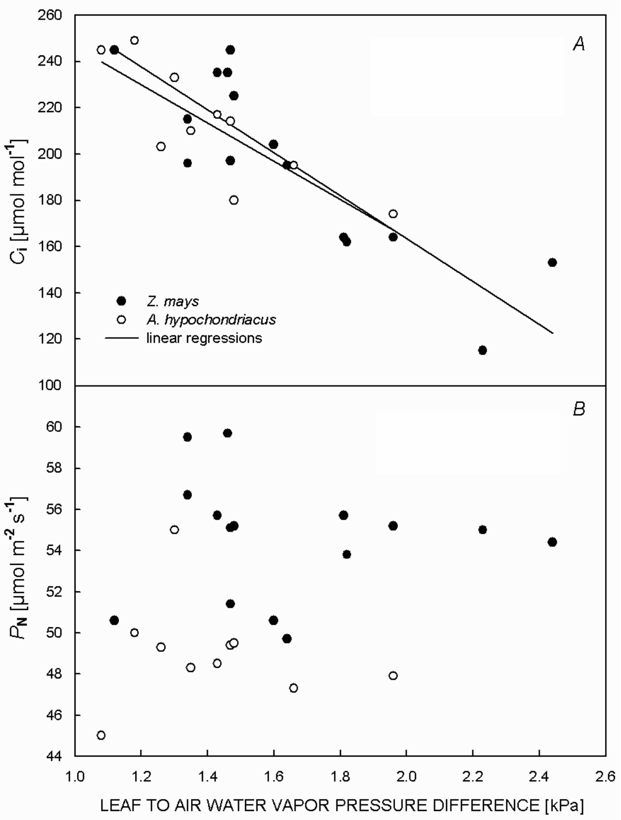

Fig. 1. Midday C_i (*A*) and net photosynthetic rate (P_N) (*B*) values for *Amaranthus hypochondriacus* and *Zea mays* and the corresponding leaf to air water vapor pressure difference on different measurement dates. Each point represents a mean value for 4–6 leaves. (*A*) Linear regressions shown for each species had r^2 = 0.68 for *A. hypochondriacus* and 0.74 for *Z. mays*. (*B*) Correlation between these variables was not significant for either species.

Similarly, in non-crop C_4 species, *Andropogon gerardi* grown at C_a often had C_i values in excess of 200 µmol mol^{-1} (Adam *et al.* 2000). Niu *et al.* (2003) presented two diurnal curves of P_N and g_s of *Agriophyllum squarrosum* which indicate midday C_i values in excess of 250 µmol mol^{-1}. In open top chambers at C_a of 360 µmol mol^{-1}, mean P_N and g_s for *Bouteloua gracilis* over 36 measurement dates averaged 15.9 and 250.0 mmol m^{-2} s^{-1} (LeCain *et al.* 2003), which would give an average C_i of about 258 µmol mol^{-1}. In measuring canopy gas exchange in a C_4 pasture, Grace *et al.* (1998) found apparent C_i/C_a ratios as high as 0.6. While lower C_i values also sometimes occurred in some of these studies and in other studies, as they did in the present study, it is clear that midday C_i in excess of 140 µmol mol^{-1} occurred for measurements made with many different gas exchange systems and for many C_4 species.

Low leaf N content often reduces P_N by reducing the content of photosynthetic enzymes per unit of area (Evans 1989, Ranjith *et al.* 1995). Nitrogen deficiency would therefore tend to increase C_i and reduce WUE at the leaf level unless there was a corresponding reduction in g_s. Reductions in leaf level WUE under N deficiency have frequently been reported (*e.g.* Livingston *et al.* 1999, Tognetti and Johnson 1999, Shanguan *et al.* 2000, Ashraf *et al.* 2001, Lee *et al.* 2001), although in other cases lower g_s has kept WUE unchanged (*e.g.* Siegwolf *et al.* 2001) or even increased it (Ranjith *et al.* 1995, Meinzer and Zhu 1998). Crops are most often grown at N levels high enough that N deficiency primarily affects growth by changing leaf area rather than P_N (Watson 1952). Our data indicate that N deficiency can cause C_i to increase, and the P_N/g_s ratio to decrease, even when P_N is not reduced.

The decrease in C_i with increasing water vapor pressure difference in *A. hypochondriacus* and *Z. mays* occurred without any decrease in photosynthesis. This presumably reflects the fact that even the lowest C_i values were saturating to photosynthesis. Because of the large change in C_i with vapor pressure difference observed, information on field values of vapor pressure difference could be important in estimating normal operating C_i values. However, the different values of water vapor pressure difference presented here resulted from day to day variation in environment and occurred both for the whole shoot and for the individual leaves in which gas exchange was measured. Much less change in leaf gas exchange occurred when changes in water vapor pressure difference were imposed on individual leaves rather than the whole shoot in sorghum and potato (Bunce 2003). I have observed the same phenomenon in *Z. mays.* In such cases, the vapor pressure difference of the whole shoot is probably the crucial determinant of C_i, and not the vapor pressure difference of the measured leaf, as long as the leaf vapor pressure difference is not drastically altered by the measurement process. High vapor pressure difference for the whole shoot presumably reduces P_N by reducing leaf water potential (Bunce 1990, 2003, Hirasawa and Hsiao 1999). Regardless of the mechanisms involved, water vapor pressure difference should be considered an important variable in estimates of the normal operating C_i of C_4 plants. Based on the small plot size (less than 100 m^2 area) used in this study, and the imposition of a high boundary layer conductance in the leaf chamber, it is unlikely that leaves or shoots in the present study were exposed to unnaturally low vapor pressure differences, which could have resulted in unnaturally high C_i values.

The data presented here indicate that midday C_i values in mature leaves of C_4 plants may often be higher than expected from measurements made in controlled environments. But since the data cover a maximum of 15 d per species over two years, they can not be taken to represent average values for a growing season. However, the fact that high values of C_i did commonly occur suggests that C_4 species do not always operate at high WUE. The relationship between C_i and water vapor pressure deficits indicates that the potentially high WUE in C_4 plants may not occur under conditions of low evaporative demand. There may be considerable scope for reducing g_s and transpiration rate in C_4 crops without reducing P_N, thus increasing their WUE without sacrificing yield potential.

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