

What is the usual internal carbon dioxide concentration in C₄ species under midday field conditions?

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Abstract

The carbon dioxide concentrating system in C₄ 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 C₃ 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 C₄ plants. For a C_a of 360 $\mu\text{mol mol}^{-1}$ that would mean a C_i about 110–140 $\mu\text{mol mol}^{-1}$. Our field observations made near midday on three weedy C₄ species, *Amaranthus retroflexus*, *Echinochloa crus-galli*, and *Setaria faberi*, and the C₄ crop *Sorghum bicolor* indicated mean values of C_i of 183–212 $\mu\text{mol mol}^{-1}$ at $C_a = 360 \mu\text{mol mol}^{-1}$. Measurements in two other C₄ 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 $\mu\text{mol mol}^{-1}$ 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 C₄ species was 198 $\mu\text{mol mol}^{-1}$, for a C_i/C_a ratio of 0.55. Prior measurements on four herbaceous C₃ species using the same instrument indicated an average C_i/C_a ratio of 0.69. Hence midday C_i values in C₄ species under field conditions may often be considerably higher and more similar to those of C₃ species than expected from measurements made on plants in controlled environments. Reducing g_s in C₄ 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 phosphoenolpyruvate carboxylase for carbon dioxide allows CO₂ assimilation rates in C₄ species to be saturated with respect to CO₂ at lower concentrations than is the case in C₃ species, in which the initial fixation of CO₂ is by ribulose-1,5-bisphosphate carboxylase (Laisk and Edwards 1998). Although there are exceptions, photosynthesis in C₄ plants is often saturated at internal CO₂ concentrations (C_i) of 100–150 $\mu\text{mol mol}^{-1}$ (Laisk and Edwards 1998). For C₄ 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 C₄ plants made at ambient CO₂ concentrations (C_a) of 330–360 $\mu\text{mol mol}^{-1}$ have indicated values in the 100–150 $\mu\text{mol mol}^{-1}$ range, leading to the generalization that the C_i/C_a ratio is 0.3–0.4 in C₄ plants (Jones 1983). These results further suggest that high water use efficiency (WUE) may have been an evolution-

nary priority for C₄ plants. The C_i/C_a ratio of 0.3–0.4 in C₄ species contrasts with the typical ratio of 0.6–0.7 in C₃ species, and reflects the higher ratio of P_N/g_s and higher WUE in C₄ species (Jones 1983, Tanner and Sinclair 1983).

However, most of the measurements indicating C_i/C_a ratios of 0.3–0.4 in C₄ species have been made under laboratory or glasshouse conditions. The measurements presented here suggest that C_i/C_a ratios for C₄ 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 C₄ 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(CO₂) 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

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. 1A). There was no significant correlation between P_N and C_i or between P_N and water vapor pressure difference in either species (Fig. 1B). In

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.

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_s , 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 C₄ 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 [$\mu\text{mol mol}^{-1}$]		
		Minimum	Maximum	Mean
<i>Amaranthus hypochondriacus</i>	10	174	245	212
<i>Amaranthus retroflexus</i>	5	146	267	212
<i>Echinochloa crus-galli</i>	3	142	218	183
<i>Setaria faberi</i>	5	108	231	196
<i>Sorghum bicolor</i>	3	136	225	188
<i>Zea mays</i>	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 [$\mu\text{mol m}^{-2} \text{s}^{-1}$]	P _N [$\mu\text{mol m}^{-2} \text{s}^{-1}$]	P _N /g _s [mol mol ⁻¹]	Leaf N [g m ⁻²]	LAI	Shoot mass [g m ⁻²]
<i>Z. mays</i>	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
<i>A. hypochondriacus</i>	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 C₄ 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 C₃ species, discrimination among isotopes of carbon can be used to estimate C_i. However, in C₄ 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 CO₂ (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 C₃ 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 $\mu\text{mol mol}^{-1}$, we obtained C_i/C_a ratios of 0.28–0.37 (*i.e.* C_i = 106–141 $\mu\text{mol mol}^{-1}$) 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 C₄ plants.

We have used the same instrument to measure midday leaf gas exchange under field conditions in C₃ species, although at C_a = 350 $\mu\text{mol mol}^{-1}$, rather than the 360 $\mu\text{mol mol}^{-1}$ 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 C₃ species was 0.69. These values are in the range expected for C₃ species (Jones 1983).

Higher than expected C_i values for C₄ 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 $\mu\text{mol mol}^{-1}$ (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 $\mu\text{mol mol}^{-1}$. Leakey *et al.* (2004) reported midday C_i as high as 214 $\mu\text{mol mol}^{-1}$ in *Z. mays*. In sorghum, Wall *et al.* (2001) obtained midday P_N averaging about 40 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and g_s of about 500 $\text{mmol m}^{-2} \text{s}^{-1}$ for a C_i of about 220 $\mu\text{mol mol}^{-1}$. Similarly Peng and Krieg (1992) reported P_N for sorghum of about 45 $\mu\text{mol m}^{-2} \text{s}^{-1}$

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

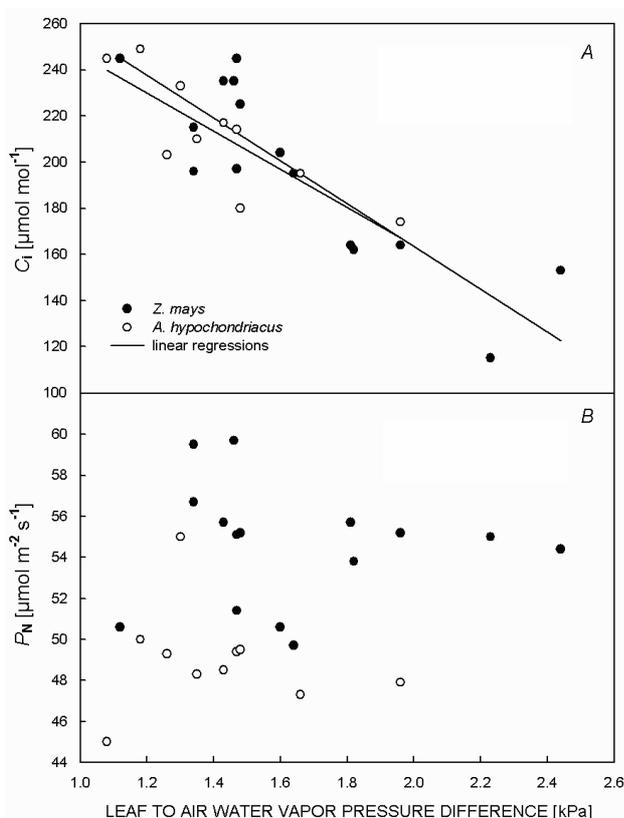


Fig. 1. Midday C_i (A) and net photosynthetic rate (P_N) (B) values for *Amaranthus hypochondriacus* and *Zea mays* and the corresponding leaf to air water vapor pressure difference on different measurement dates. Each point represents a mean value for 4–6 leaves. (A) Linear regressions shown for each 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 $200 \text{ } \mu\text{mol mol}^{-1}$ (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 $250 \text{ } \mu\text{mol mol}^{-1}$. In open top chambers at C_a of $360 \text{ } \mu\text{mol mol}^{-1}$, mean P_N and g_s for *Bouteloua gracilis* over 36 measurement dates averaged 15.9 and $250.0 \text{ mmol m}^{-2} \text{ s}^{-1}$ (LeCain *et al.* 2003), which would give an average C_i of about $258 \text{ } \mu\text{mol mol}^{-1}$. 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 $140 \text{ } \mu\text{mol mol}^{-1}$ 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, Shangguan *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 C₄ 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 C₄ plants may not occur under conditions of low

evaporative demand. There may be considerable scope for reducing g_s and transpiration rate in C₄ crops without reducing P_N , thus increasing their WUE without sacrificing yield potential.

References

- Adam, N.R., Owensby, C.E., Ham, J.M.: The effect of CO₂ enrichment on leaf photosynthetic rates and instantaneous water use efficiency of *Andropogon gerardii* in the tallgrass prairie. – *Photosynth. Res.* **65**: 121-129, 2000.
- Ashraf, M., Shabaz, M., Mahmood, S., Rasul, E.: Relationships between growth and photosynthetic characteristics in pearl millet (*Pennisetum glaucum*) under limited water deficits conditions and with enhanced nitrogen supplies. – *Belg. J. Bot.* **134**: 131-144, 2001.
- Bunce, J.A.: Low humidity effects on photosynthesis in single leaves of C₄ plants. – *Oecologia* **54**: 233-235, 1982.
- Bunce, J.A.: Afternoon inhibition of photosynthesis in maize. 1. Evidence and relationship to stand density. – *Field Crops Res.* **24**: 251-260, 1990.
- Bunce, J.A.: Effects of humidity on short-term responses of stomatal conductance to an increase in carbon dioxide concentration. – *Plant Cell Environ.* **21**: 115-120, 1998.
- Bunce, J.A.: Acclimation to temperature of the response of photosynthesis to increased carbon dioxide concentration in *Taraxacum officinale*. – *Photosynth. Res.* **64**: 89-94, 2000.
- Bunce, J.A.: Effects of water vapor pressure difference on leaf gas exchange in potato and sorghum at ambient and elevated carbon dioxide under field conditions. – *Field Crops Res.* **82**: 37-47, 2003.
- Evans, J.R.: Photosynthesis and nitrogen relationships in leaves of C₃ plants. – *Oecologia* **78**: 9-19, 1989.
- Grace, J., Lloyd, J., Miranda, A.C., Miranda, H., Gash, J.H.C.: Fluxes of carbon dioxide and water vapour over a C₄ pasture in south-western Amazonia (Brazil). – *Aust. J. Plant Physiol.* **25**: 519-530, 1998.
- Grantz, D.A.: Effect of cool temperatures on photosynthesis and stomatal conductance in field-grown sugarcane in Hawaii. – *Field Crops Res.* **22**: 143-155, 1989.
- Henderson, S., Caemmerer, S. von, Farquhar, G.D., Wade, L., Hammer, G.: Correlation between carbon isotope discrimination and transpiration efficiency in lines of the C₄ species *Sorghum bicolor* in the glasshouse and the field. – *Aust. J. Plant Physiol.* **25**: 111-123, 1998.
- Hirasawa, T., Hsiao, T.C.: Some characteristics of reduced leaf photosynthesis at midday in maize growing in the field. – *Field Crops Res.* **62**: 53-62, 1999.
- Jones, H.G.: *Plants and Microclimate*. – Pp. 151-152. Cambridge University Press, Cambridge – London – New York – New Rochelle – Melbourne – Sydney 1983.
- Laisk, A., Edwards, G.E.: Oxygen and electron flow in C₄ photosynthesis: Mehler reaction, photorespiration and CO₂ concentration in the bundle sheath. – *Planta* **205**: 632-645, 1998.
- Leakey, A.D.B., Bernacchi, C.J., Dohleman, F.G., Ort, D.R., Long, S.P.: Will photosynthesis of maize (*Zea mays*) in the US corn belt increase in future [CO₂] rich atmospheres? An analysis of diurnal courses of CO₂ uptake under free-air concentration enrichment (FACE). – *Global Change Biol.* **10**: 951-962, 2004.
- LeCain, D.R., Morgan, J.A., Mosier, A.R., Nelson, J.A.: Soil and plant water relations determine photosynthetic responses of C₃ and C₄ grasses in a semi-arid ecosystem under elevated CO₂. – *Ann. Bot.* **92**: 41-52, 2003.
- Lee, T.D., Tjoelker, M.G., Ellsworth, D.S., Reich, P.B.: Leaf gas exchange responses of 13 prairie grassland species to elevated CO₂ and increased nitrogen supply. – *New Phytol.* **150**: 405-418, 2001.
- Livingston, N.J., Guy, R.D., Sun, Z.J., Ethier, G.J.: The effects of nitrogen stress on the stable carbon isotope composition, productivity and water use efficiency of white spruce (*Picea glauca* (Moench) Voss) seedlings. – *Plant Cell Environ.* **22**: 281-289, 1999.
- Meinzer, F.C., Plaut, Z., Saliendra, N.Z.: Carbon isotope discrimination, gas exchange, and growth of sugarcane cultivars under salinity. – *Plant Physiol.* **104**: 521-526, 1994.
- Meinzer, F.C., Zhu, J.: Nitrogen stress reduces the efficiency of the C₄ CO₂ concentrating systems, and therefore quantum yield, in *Saccharum* (sugarcane) species. – *J. exp. Bot.* **49**: 1227-1234, 1998.
- Niu, S.L., Jiang, G.M., Li, Y.G., Gao, L.M., Liu, M.Z.: Diurnal gas exchange and superior resources use efficiency of typical C₄ species in Hunshandak Sandland, China. – *Photosynthetica* **41**: 221-226, 2003.
- Peng, S., Krieg, D.R.: Gas exchange traits and their relationship to water use efficiency of grain sorghum. – *Crop Sci.* **32**: 386-391, 1992.
- Ranjith, S.A., Meinzer, F.C., Perry, M.H., Thom, M.: Partitioning of carboxylase activity in nitrogen-stressed sugarcane and its relationship to bundle sheath leakiness to CO₂, photosynthesis and carbon isotope discrimination. – *Aust. J. Plant Physiol.* **22**: 903-911, 1995.
- Saliendra, N.Z., Meinzer, F.C., Perry, M., Thom, M.: Association between partitioning of carboxylase activity and bundle sheath leakiness to CO₂, carbon isotope discrimination, photosynthesis, and growth in sugarcane. – *J. exp. Bot.* **47**: 907-914, 1996.
- Shangguan, Z.P., Shao, M.A., Dyckmans, J.: Nitrogen nutrition and water stress effects on leaf photosynthetic gas exchange and water use efficiency in winter wheat. – *Environ. exp. Bot.* **44**: 141-149, 2000.
- Siegwolf, R.T.W., Matyssek, R., Saurer, M., Maurer, S., Günthardt-Georg, M.S., Schmutz, P., Bucher, J.B.: Stable isotope analysis reveals differential effects of soil nitrogen and nitrogen dioxide on the water use efficiency in hybrid poplar leaves. – *New Phytol.* **149**: 233-246, 2001.
- Tanner, C.B., Sinclair, T.R.: Efficient water use in crop production: Research or re-search. – In: Taylor, H.M., Jordan, W.R., Sinclair, T.R. (ed.): *Limitation to Efficient Water Use in Crop Production*. Pp. 1-28. Amer. Soc. Agron., Crop Sci. Soc. Amer., Soil Sci. Soc. Amer., Madison 1983.
- Tognetti, R., Johnson, J.D.: The effect of elevated atmospheric CO₂ concentration and nutrient supply on gas exchange, carbohydrates and foliar phenolic concentration in live oak

- (*Quercus virginiana* Mill.) seedlings. – Ann. Forest Sci. **56**: 379-389, 1999.
- Wall, G.W., Brooks, T.J., Adam, N.R., Cousins, A.B., Kimball, B.A., Pinter, P.J., Lamorte, R.L., Triggs, L., Ottman, M.J., Leavitt, S.W., Mathias, A.D., Williams, D.G., Webber, A.N.: Elevated atmospheric CO₂ improved sorghum plant water status by ameliorating the adverse effects of drought. – New Phytol. **152**: 231-248, 2001.
- Watson, D.J.: The physiological basis of variation in yield. – Adv. Agron. **4**: 101-145, 1952.
- Wilson, K.B., Bunce, J.A.: Effects of carbon dioxide concentration on the interactive effects of temperature and water vapour on stomatal conductance in soybean. – Plant Cell Environ. **20**: 230-238, 1997.
- Ziska, L.H., Bunce, J.A.: Influence of increasing carbon dioxide concentration on the photosynthetic and growth stimulation of selected C₄ crops and weeds. – Photosynth. Res. **54**: 199-208, 1997.