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Photosynthesis of cockspur [*Echinochloa crus-galli* (L.) Beauv.] at sites of naturally elevated CO_2 concentration

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Abstract

High abundance of cockspur (*Echinochloa crus-galli*) at the geothermal carbon dioxide spring area in Stavešinci indicates that this species is able to grow under widely varying CO_2 concentrations. Living cockspur plants can even be found very close to gas-releasing vents where growth is significantly reduced. Plant height correlated well with CO_2 exposure. The $\delta^{13}C$ value of the CO_2 spring air was −3.9 ‰ and $\delta^{13}C$ values of high-, medium-, and low-CO_2 plants were −10.14, −10.44, and −11.95 ‰, respectively. Stomatal response directly followed the prevailing CO_2 concentrations, with the highest reduction of stomatal conductance in high CO_2 concentration grown plants. Analysis of the curves relating net photosynthetic rate to intercellular CO_2 concentration (P_N-C_i curves) revealed higher CO_2 compensation concentration in plants growing at higher CO_2 concentration. This indicates adjustment of respiration and photosynthetic carbon assimilation according to the prevailing CO_2 concentrations during germination and growth. There was no difference in other photosynthetic parameters measured.

Additional key words: carbon dioxide springs; chlorophyll; CO_2 compensation concentration; intercellular CO_2 concentration; net photosynthetic rate; plant height; stomatal conductance; $\delta^{13}C$.

Introduction

C_4 plants significantly contribute to the global primary productivity, mainly because of their high productivity in grasslands. In a world with gradually increasing CO_2 concentration ([CO_2]), recognition and understanding of the direct impact of elevated atmospheric [CO_2] (EC) on growth and function of C_4 plants is therefore of great importance and remains a crucial area of interest (Ghannoum *et al.* 2000).

Studies of the photosynthetic response of C_3 plants showed that, at least in the short term, carbon assimilation could be stimulated by EC (Drake *et al.* 1997). Because of the well-known carbon dioxide concentrating mechanisms of C_4 plants, and on the basis of early experiments, it was assumed that responsiveness to CO_2 should be much lower in C_4 than in C_3 plants. A different reaction of C_3 and C_4 grasses to EC could have an impact on the consequent competitiveness of species in mixed grass communities. However, recent studies have found that growth of many C_4 plants responds positively to EC (*e.g.* Ziska and Bunce 1997, Wand *et al.* 1999, Ghannoum *et al.* 2000). Stimulation of growth of C_4 plants by EC mainly occurs at decreasing soil water availability and increasing leaf-to-air water vapour pressure deficit, air temperature, photon flux density, and nitrogen supply (Ghannoum *et al.* 2000). Nevertheless, when compared to C_3 plants, C_4 plants exhibit only a limited number of consistent changes in response to EC (Wand *et al.* 1999), with a reduction of stomatal conductance (g_s) and an increase in leaf area being the most evident responses. In contrast to C_3 plants a CO_2-induced reduction in photosynthetic capacity has usually not been observed in C_4 plants.

Early research on the response of C_4 plants to EC was focused on several crop species but later it was also extended to forage and range grasses (*e.g. Panicum maximum*) and distinct weeds (*e.g. Echinochloa* sp.,

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Abbreviations: C_i = intercellular concentraction of CO_2; Chl = chlorophyll; EC = elevated CO_2 concentration; g_s = stomatal conductance; GC = gas chromatography; P_N = net photosynthetic rate; PFD = photon flux density; Γ = carboxylation efficiency.

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Amaranthus sp.). Ziska and Bunce (1997) examined the response of six weedy C_4 species and four C_4 crop species to a doubled [CO_2] (760 μmol mol^{-1}). A significant increase in net photosynthetic rate (P_N) in eight of ten species was found. The observed enhancement in carbon assimilation was not paralleled by differences in C-partitioning, senescence, or improved water relations. Interestingly, the stimulation of P_N was twice as high in C_4 weeds than in comparable C_4 crops. Although the rea-son for this difference is still unclear, differential sensi-tivity would have consequences with respect to competi-tion and crop production in agricultural systems.

CO_2 can significantly influence growth and development of cockspur (*Echinochloa crus-galli*). Yoshioka *et al.* (1998) showed that high soil [CO_2] (3 %) can be very effective in enhancing germination of cockspur seeds, while in this study no effective promotion was found for other environmental variables tested (fluctuating temperature, PFD, water). Photosynthetic activity and growth were also stimulated in response to EC (Potvin *et al.* 1984, Ziska and Bunce 1997). The stimulation of *E. crus-galli* continued for some time (at least for weeks) without clear evidence of photosynthetic acclimation (Ziska and Bunce 1997).

At the CO_2 spring area in Stavešinci the high abundance of cockspur (Batič *et al.* 1999) indicates that this species is able to grow under widely varying [CO_2]. A few years ago, part of Stavešinci CO_2 spring area was cultivated as maize field. In growth sites with high concentrations of gas escaping from the soil, the yield was dramatically reduced. Under these conditions *Echinochloa* proved to be very competitive. One year after farming was stopped, the abundance of cockspur dramatically decreased. Yet, it still successfully grows at different sites in the area. Living plants can even be found very close to gas-releasing vents. As *Echinochloa* seems to tolerate very high and fluctuating CO_2 concentrations we have investigated possible physiological adaptations.

Materials and methods

The study was conducted in autumn 2001 at the geothermal CO_2 spring Strmec near Stavešinci, Slovenia (see Kaligarič 2001, Turk *et al.* 2002). At this site very pure CO_2 is released into the atmosphere by several vents in a flat area of *ca.* 3 000 m^2. Air CO_2 concentration depends very much on weather (wind) and it can fluctuate from 0.036 % to at least 1 %. Growing plants of different species were found at locations with soil CO_2 ranging from 0.4 to 26.0 % (v/v) (Pfanz, unpublished, Vodnik *et al.* 2002). The soil is a reduced gley on quaternary alluvium, consisting of silty clay material derived from different parent rocks. The vegetation at the study area, at which agriculture was stopped in 1998, consists of several C_3 and C_4 grasses and several (pioneer) herbs. The CO_2 exposure of plants differs according to the irregular distribution of the gas releasing vents and cracks. Individual plant height is highly variable, the smallest plants can be found in the close neighbourhood to the vents.

We selected for our research plants of *Echinochloa crus-galli* (L.) Beauv. exposed to different [CO_2] (low, medium, and high CO_2 grown plants) on the basis of plant height and distance of grass tufts from gas releasing vents. Gas exchange measurements and sampling for chlorophyll (Chl) and carbon isotope discrimination studies were performed in the first week of August 2001.

Gas exchange measurements of intact second leaves that were still attached to the plants were made with a portable photosynthetic system *LI-6400* (*LI-COR*, Lincoln, USA). Five individuals plants were measured for every site. CO_2 response curves were measured at 24 °C, 55 % relative humidity (RH), and PFD of 800-1 000 μmol m^{-2} s^{-1}. The response of P_N to changing intercellular CO_2 concentrations (C_i) were conducted at 24 °C, 55 % RH), and PFD-saturating conditions (800 μmol m^{-2} s^{-1}). P_N was measured 10-15 min after CO_2-supply when all parameters and the total coefficient of variation (CV %) indicated stable leaf chamber conditions.

The non-linear regression models were fitted to P_N as response variable and C_i as explanatory variable. The best fits for P_N-C_i curves were obtained using exponential function with three parameters:

$$P_N = \beta_0 [1 - \exp(-\beta_1 (C_i - \Gamma)].$$

β_0 describes the asymptotic value of the P_N-C_i curve and it can be interpreted as CO_2 saturated photosynthesis [μmol m^{-2} s^{-1}]. β_1 describes the initial slope of the P_N-C_i curve and can be interpreted as carboxylation efficiency [mol m^{-2} s^{-1}]. Γ is equivalent to the CO_2 compensation concentration [μmol mol^{-1}] in our exponential model.

Chl determination: Photosynthetic pigments were extracted from frozen leaf material (second leaf) with acetone. $Mg_2(OH)_2CO_3$ was added to avoid acidification and a concomitant pheophytinisation of the Chls. Pigments were determined spectrophotometrically and contents calculated using the equations of Lichtenthaler (1987).

Carbon isotope discrimination: Leaves used for gas exchange measurements were sampled for discrimination analysis. Plant samples were oven dried (Peterson and Howarth 1987), treated with HCl vapour to remove carbonate contaminants, washed thoroughly with distilled water, and re-dried again.

A continuous flow isotope ratio mass spectrometer *Europa 20-20* (preparation module *ANCA SL*, *PDZ Europa*, U.K.) was used to determine the stable isotopic composition of C. Samples were combusted in elemental analyser in tin capsules and gases produced were separated in a GC column (90 °C, flow 0.83 cm^3 s^{-1}). The results are expressed as relative δ value:

$$\delta = \left(\frac{R_{sample}}{R_{s\tan dard}} - 1 \right) \times 1000 \quad [‰]$$

where R is $^{13}C/^{12}C$ ratio of the sample and the standard, respectively. The results for carbon are reported relative to the *V-PDB* (*Vienna-Pee Dee Belemnite*) standard (Coplen 1996). Laboratory working standards (urea solution) are calibrated *versus* polyethylene with $\delta^{13}C$ = –31.8 ‰ (*IAEA-CH-7*) and graphite with $\delta^{13}C$ = –16.1 ‰ (*USGS24*), reference materials which are regularly used to control the accuracy of the measurement. Reference materials are calibrated *versus NBS19* limestone. For blank subtraction, empty *GF/C* filters (1 mg) treated in the same way as samples are analysed for C and N isotopic compositions. Reproducibility of the analysis was determined on triplicate analyses of the same sample and was better than ±0.2 ‰.

Statistical analysis: Gas exchange results were ln-transformed prior the analyses. For model-parameters β_0, β_1, and Γ, differences between sites were tested by using *t*-test. Growth parameters were analysed by one-way *ANOVA*. All statistical analyses and calculations were performed using *Statgraphics Plus 4.0* (*Manugistics*, USA).

Results

Carbon isotope discrimination revealed that carbon released from the vents in Stavešinci is heavier than atmospheric carbon. The $\delta^{13}C$ value of the air sampled at one of the main vents was –3.9 ‰, while $\delta^{13}C$ of the normal air is reported to range from –7 to –9 ‰ V-PDB (Mook 1986). The $\delta^{13}C$ value was higher for the smallest (high CO_2) and medium plants (–10.14 and –10.44 ‰, respectively) when compared to the tallest individuals (–11.95 ‰) (see Table 1).

Table 1. Plant height and carbon isotope analysis of *E. crus-galli* leaves sampled at different sites at the natural CO_2 spring Stavešinci, NE Slovenia. Means ± one standard error, n = 3 for discrimination analysis, n = 5 for growth measurements. $\delta^{13}C$ of air sampled directly at the gas vent was –3.9 ‰.

Site	CO_2 exposure $\delta^{13}C$ [‰]	Mean height [cm]
1	–10.14 ± 0.45	15.4 ± 2.8
2	–10.44 ± 0.14	34.0 ± 2.4
3	–11.95 ± 0.22	66.0 ± 2.4

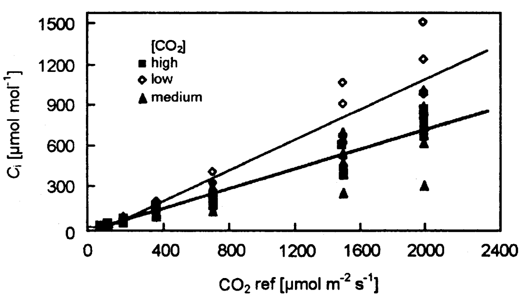

Fig. 1. A comparison of reference CO_2 concentration (CO_2 ref, concentration of CO_2 in the measuring chamber of *LI-6400*) and intercellular CO_2 concentration (C_i) in cockspur differently exposed to naturally elevated CO_2 concentration.

A comparison of measuring (CO_2 ref) and intercellular CO_2 concentrations revealed significantly lower g_s of site 1 and 2 plants when compared to plants from low CO_2 environment (site 3) (Fig. 1). The g_s measured at 350 μmol(CO_2) mol^{-1} was 0.359±0.035, 0.209±0.025, and 0.196±0.036 mol(H_2O) m^{-2} s^{-1} for plants growing at low (LC), medium (MC), and high (HC) [CO_2]. Similar differences were found when g_s measured at 700, 1 500, and 2 000 μmol CO_2 mol^{-1} was compared. At 700 μmol(CO_2) mol^{-1}, for example, g_s in HC plants (0.106 mol m^{-2} s^{-1}) was similar to g_s found in LC plants exposed to much higher CO_2 concentration [2 000 μmol(CO_2) mol^{-1}].

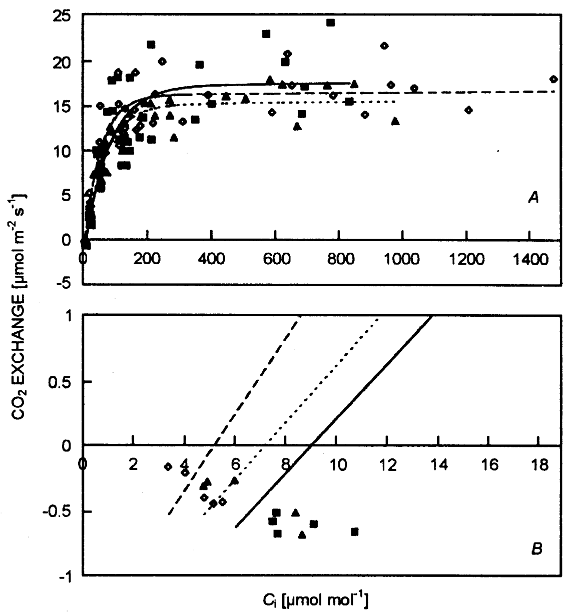

Fig. 2. P_N-C_i curves (*A*) of cockspur differently exposed to naturally elevated CO_2 concentration. Five individuals were measured for every location (exposure). Models were calculated using equation $P_N = \beta_0 \{1 - \exp[-\beta_1 (C_i - \Gamma)]\}$. ◊ low [$CO_2$], ▲ medium [$CO_2$], and ■ high [$CO_2$]. (*B*) Shift of CO_2 compensation concentration.

In plants growing at HC the content of leaf Chl was decreased by *ca.* 25 % (Table 2). The reduction of leaf Chl was not accompanied by lower P_N. No difference in P_N measured at 350, 700, or 2 000 μmol mol^{-1} CO_2 was found for LC, MC, and HC plants. In addition, no differences in carboxylation efficiency were found (Table 2, Fig. 2*A*).

Closer analysis of the P_N-C_i curves of cockspur revealed differences for the lower part of the curve. When Γ values were compared for different groups of plants, a shift of Γ was observed (Fig. 2*B*). Values of 5.2, 7.2, and 9.0 μmol mol^{-1} CO_2 were recorded for LC, MC, and HC plants, respectively.

Table 2. Photosynthetic parameters (P_N = net photosynthetic rate, Γ = CO_2 compensation concentration, CE = carboxylation efficiency) of *Echinochloa crus-galli* plants from different sites at the mofette Stavešinci, NE Slovenia. Means ± standard errors, $n = 5$. For a single parameter values followed with different letters are statistically different ($p<0.05$). ns = non-significant.

		Site 1	Site 2	Site 3	Statistical significance
Plant height [cm]		10-25 cm (15.4 ± 2.8)	30-40 cm (34.0 ± 2.4)	60-75 cm (66.0 ± 2.4)	ANOVA $p < 0.0001$
Chlorophyll [g kg^{-1} (f.m.)]		1.45 ± 0.12	1.57 ± 0.14	2.02 ± 0.12	ANOVA $p < 0.01$
P_N [μmol m^{-2} s^{-1}]	P_N 350	12.43 ± 1.75 a	11.11 ± 1.03 a	14.34 ± 1.37 a	*t*-test ns
	P_N 700	15.25 ± 2.06 a	13.79 ± 0.99 a	15.73 ± 1.26 a	*t*-test ns
	P_N 2 000	18.02 ± 1.82 a	15.81 ± 0.98 a	17.29 ± 1.20 a	*t*-test ns
Γ [μmol mol^{-1}]		9.01 ± 5.84 a	7.17 ± 2.50 b	5.16 ± 3.05 b	*t*-test $p < 0.01$
CE [mol m^{-2} s^{-1}]		0.013 ± 0.003 a	0.015 ± 0.002 a	0.019 ± 0.003 a	*t*-test $p < 0.01$

Discussion

Values of $\delta^{13}C$ measured for natural carbon dioxide spring Stavešinci are comparable to similar sources of CO_2 measured in some other places in Slovenia, *e.g.* Rogaška Slatina with –5.2 ‰ (Pezdič *et al.* 1995). ^{13}C enrichment was found also at other CO_2 rich mineral springs (Raschi *et al.* 1997, Miglietta *et al.* 1998, Badiani *et al.* 2000). The release of CO_2 from the vents to the atmosphere leads to an increase in atmospheric CO_2 as well as to a change in the stable carbon isotope ratio. Since C_4 plants have a conservative discrimination *vs.* ^{13}C under a range of environmental conditions (Farquhar 1983) the isotopic composition of plant material well reflects the average isotope ratio of the air and indicates prevailing CO_2 regime during growth. Discrimination analysis of *Echinochloa* leaves from the Stavešinci mofette yielded higher $\delta^{13}C$ values than measured normally for C_4 plants (Farquhar 1983). We therefore conclude that plants from all three sampling sites were exposed to the spring air to a certain extent. This indicates differences in CO_2 exposure and confirms that plant height correlates with the CO_2 regime. The latter is known from the work done by Pfanz *et al.* (unpublished) who proved a good correlation between the height of timothy grass (*Phleum pratense*) and soil CO_2 concentration measured directly at the rooting zone around the plants.

On the other hand, carbon isotope discrimination analysis revealed only minor differences between the barnyard plants from sampling sites 1 and 2, which differ in average height of 15.4 and 34.0 cm, respectively. During gas exchange measurements these both groups of plants showed also similar stomatal behaviour (decreased g_s) when exposed to different CO_2 concentrations. According to Wand *et al.* (1999) reduced g_s is one of the most consistent responses of C_4 plants to elevated [CO_2]. A decrease in transpiration rate and in g_s is frequently reported for plants growing at natural carbon dioxide springs (*e.g.* Tognetti *et al.* 1996, 1998, 1999, Bettarini *et al.* 1998). The response of cockspur found in our study indicates that stomatal functioning can directly reflect the degree of CO_2 exposure. This effect was also found in the study on timothy grass in which differently exposed plants selected on the basis of soil CO_2 (high, medium, and low CO_2 exposure) showed a gradual stomatal response. The higher was the CO_2 concentration measured in the rooting horizon, the more limited was stomatal diffusion at high air CO_2 concentration (Pfanz *et al.* unpublished). Measurements in Stavešinci indicate a stomatal response that directly follows the prevailing CO_2 conditions.

Despite a decreased g_s and lower Chl content in HC plants we found no difference in P_N for plants from all three sites, nor there were any differences in carboxylation efficiency. Similar results were obtained by Ziska and Bunce (1997) who compared the P_N-C_i curves of different C_4 plants grown at ambient (39 Pa) or elevated (69 Pa) CO_2. For seven of eight species examined (including *E. crus-galli*) no significant change in either the initial slope of P_N-C_i response or the upper portion of the curve (exception *Setaria faberi*) was observed at elevated [CO_2]. The general lack of photosynthetic acclimation in C_4 plants (see Ghannoum *et al.* 2000) can be explained by the lower content of ribulose-1,5-bisphosphate carboxylase/oxygenase protein (relative to C_3 plants), high growth rates, and limited accumulation of non-structural saccharides (Wand *et al.* 1999, Ghannoum *et al.* 2000). In general, photosynthetic down-regulation is rarely reported for plants growing at carbon dioxide springs (Raschi *et al.* 1997, Badiani *et al.* 1999, but see Cook *et al.* 1998). However, recent research at the mofette site in Stavešinci showed that reductions of photosynthetic capacity and carboxylation efficiency could occur in several plant species (Pfanz, unpublished, Vodnik *et al.* 2002).

In cockspur a significant shift of Γ was observed. This is in good agreement to what was found in timothy grass grown at the same CO_2 spring. In the latter species also Γ considerably increased (36, 93, and 144 μmol mol^{-1}) within the increasing CO_2 gradient. Obviously both species had adapted to these extreme CO_2 conditions and had adjusted respiration and photosynthetic carbon assimilation according to the prevailing CO_2 regime during germination and growth. Unfortunately, up to now there is little information on the respiratory behaviour of plants growing at natural CO_2 springs (see Tognetti and Johnson 1999) and there is no study of photorespiration that both could help explain observed adaptations.

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