Photosynthesis of cockspur [Echinochloa crus-galli (L.) Beauv.] at sites of naturally elevated CO₂ 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 CO₂ concentrations. Living cockspur plants can even be found very close to gas-releasing vents where growth is significantly reduced. Plant height correlated well with CO₂ exposure. The δ¹³C value of the CO₂ spring air was −3.9 ‰ and δ¹³C values of high-, medium-, and low-CO₂ plants were −10.14, −10.44, and −11.95 ‰, respectively. Stomatal response directly followed the prevailing CO₂ concentrations, with the highest reduction of stomatal conductance in high CO₂ concentration grown plants. Analysis of the curves relating net photosynthetic rate to intercellular CO₂ concentration (Pₙ₋ₐ-C, curves) revealed higher CO₂ compensation concentration in plants growing at higher CO₂ concentration. This indicates adjustment of respiration and photosynthetic carbon assimilation according to the prevailing CO₂ concentrations during germination and growth. There was no difference in other photosynthetic parameters measured.

Additional key words: carbon dioxide springs; chlorophyll; CO₂ compensation concentration; intercellular CO₂ concentration; net photosynthetic rate; plant height; stomatal conductance; δ¹³C.

Introduction

C₄ plants significantly contribute to the global primary productivity, mainly because of their high productivity in grasslands. In a world with gradually increasing CO₂ concentration ([CO₂]), recognition and understanding of the direct impact of elevated atmospheric [CO₂] (EC) on growth and function of C₄ plants is therefore of great importance and remains a crucial area of interest (Ghannoum et al. 2000).

Studies of the photosynthetic response of C₃ 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 C₄ plants, and on the basis of early experiments, it was assumed that responsiveness to CO₂ should be much lower in C₄ than in C₃ plants. A different reaction of C₃ and C₄ 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 C₄ plants responds positively to EC (e.g. Ziska and Bunce 1997, Wand et al. 1999, Ghannoum et al. 2000). Stimulation of growth of C₄ 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 C₃ plants, C₄ plants exhibit only a limited number of consistent changes in response to EC (Wand et al. 1999), with a reduction of stomatal conductance (gₛ) and an increase in leaf area being the most evident responses. In contrast to C₃ plants a CO₂-induced reduction in photosynthetic capacity has usually not been observed in C₄ plants.

Early research on the response of C₄ 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.,...
Amaranthus sp.). Ziska and Bunce (1997) examined the response of six weedy C₄ species and four C₄ crop species to a doubled [CO₂] (760 μmol mol⁻¹). A significant increase in net photosynthetic rate (Pₙₐ) 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 Pₙ was twice as high in C₄ weeds than in comparable C₃ 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.

CO₂ can significantly influence growth and development of cockspur (Echinochloa crus-galli). Yoshioka et al. (1998) showed that high soil [CO₂] (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 ac-climation (Ziska and Bunce 1997).

At the CO₂ spring area in Stavešinci the high abundance of cockspur (Batil et al. 1999) indicates that this species is able to grow under widely varying [CO₂]. A few years ago, part of Stavešinci CO₂ 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 CO₂ concentrations we have investigated possible physiological adaptations.

Materials and methods

The study was conducted in autumn 2001 at the geothermal CO₂ spring Strmec near Stavešinci, Slovenia (see Kaligarič 2001, Turk et al. 2002). At this site very pure CO₂ is released into the atmosphere by several vents in a flat area of ca. 3 000 m². Air CO₂ 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 CO₂ ranging from 0.4 to 26.0 % (v/v) (Pflanz, 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 C₃ and C₄ grasses and several (pioneer) herbs. The CO₂ ex-posure 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 [CO₂] (low, medium, and high CO₂ 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. CO₂ 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 PN to changing intercellular CO₂ concentrations (Ci) were conducted at 24 °C, 55 % RH, and PFD-saturating conditions (800 μmol m⁻² s⁻¹). PN was measured 10-15 min after CO₂-supply when all parameters and the total coefficient of variation (CV %) indicated stable leaf chamber conditions.

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

\[ P_N = β_0 [1 - \exp(-β_1 (C_i - Γ))] \]

β₀ describes the asymptotic value of the PN-Ci curve and it can be interpreted as CO₂ saturated photosynthesis [μmol m⁻² s⁻¹], β₁ describes the initial slope of the PN-Ci curve and can be interpreted as carboxylation efficiency [mol m⁻² s⁻¹]. Γ is equivalent to the CO₂ compensation concentration [μmol mol⁻¹] in our exponential model.

Chl determination: Photosynthetic pigments were extracted from frozen leaf material (second leaf) with acetone. Mg(OH)₂·CO₂ was added to avoid acidification and a concomitant phaeophytinisation 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 sepa-rated in a GC column (90 °C, flow 0.83 cm⁻³ s⁻¹). The
results are expressed as relative δ value:

$$\delta = \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \times 1000 \text{ [‰]}$$

where $R$ is $^{13}\text{C}/^{12}\text{C}$ 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 $\delta^{13}\text{C} = -31.8 \text{ ‰}$ (IAEA-CH-7) and graphite with $\delta^{13}\text{C} = -16.1 \text{ ‰}$ (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 In-transformed prior the analyses. For model-parameters $\beta_0$, $\beta_1$, and $\Gamma$, 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 $\delta^{13}\text{C}$ value of the air sampled at one of the main vents was $-3.9 \text{ ‰}$, while $\delta^{13}\text{C}$ of the normal air is reported to range from $-7$ to $-9 \text{ ‰}$ V-PDB (Mook 1986). The $\delta^{13}\text{C}$ value was higher for the smallest (high CO$_2$) and medium plants ($-10.14$ and $-10.44 \text{ ‰}$, respectively) when compared to the tallest individuals ($-11.95 \text{ ‰}$) (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. $\delta^{13}\text{C}$ of air sampled directly at the gas vent was $-3.9 \text{ ‰}$.

<table>
<thead>
<tr>
<th>Site</th>
<th>CO$_2$ exposure $\delta^{13}\text{C}$ [‰]</th>
<th>Mean height [cm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$-10.14 \pm 0.45$</td>
<td>15.4 ± 2.8</td>
</tr>
<tr>
<td>2</td>
<td>$-10.44 \pm 0.14$</td>
<td>34.0 ± 2.4</td>
</tr>
<tr>
<td>3</td>
<td>$-11.95 \pm 0.22$</td>
<td>66.0 ± 2.4</td>
</tr>
</tbody>
</table>

![Figure 1](image1.png)

**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$_2$O) 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$^{-1}$).

![Figure 2](image2.png)

**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 $P_N = \beta_0 \left(1 - \exp[-\beta_1 (C_i - \Gamma)]\right)$. ○ 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. Thus, no diffe-
references in carboxylation efficiency were found (Table 2, Fig. 2A).

Closer analysis of the $P_N$-Ci curves of cockspur revealed differences for the lower part of the curve. When $\Gamma$ values were compared for different groups of plants, a shift of $\Gamma$ was observed (Fig. 2B). Values of 5.2, 7.2, and 9.0 $\mu$mol mol$^{-1}$ CO$_2$ were recorded for LC, MC, and HC plants, respectively.

Table 2. Photosynthetic parameters ($P_N =$ net photosynthetic rate, $\Gamma =$ CO$_2$ compensation concentration, CE = carboxylation efficiency) of *Echinochloa crus-galli* plants from different sites at the moofette 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.

<table>
<thead>
<tr>
<th>Plant height [cm]</th>
<th>Site 1</th>
<th>Site 2</th>
<th>Site 3</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorophyll [g kg$^{-1}$ (f.m.)]</td>
<td>1.45 ± 0.12</td>
<td>1.57 ± 0.14</td>
<td>2.02 ± 0.12</td>
<td>ANOVA $p &lt; 0.0001$</td>
</tr>
<tr>
<td>$P_N$ [$\mu$mol m$^{-2}$ s$^{-1}$]</td>
<td>12.43 ± 1.75 a</td>
<td>11.11 ± 1.03 a</td>
<td>14.34 ± 1.37 a</td>
<td>t-test ns</td>
</tr>
<tr>
<td>$P_N$ 350</td>
<td>15.25 ± 2.06 a</td>
<td>13.79 ± 0.99 a</td>
<td>15.73 ± 1.26 a</td>
<td>t-test ns</td>
</tr>
<tr>
<td>$P_N$ 700</td>
<td>18.02 ± 1.82 a</td>
<td>15.81 ± 0.98 a</td>
<td>17.29 ± 1.20 a</td>
<td>t-test ns</td>
</tr>
<tr>
<td>$P_N$ 2000</td>
<td>9.01 ± 5.84 a</td>
<td>7.17 ± 2.50 b</td>
<td>5.16 ± 3.05 b</td>
<td>t-test, $p &lt; 0.01$</td>
</tr>
<tr>
<td>$\Gamma$ [mol $\mu$mol$^{-1}$]</td>
<td>0.013 ± 0.003 a</td>
<td>0.015 ± 0.002 a</td>
<td>0.019 ± 0.003 a</td>
<td>t-test, $p &lt; 0.01$</td>
</tr>
</tbody>
</table>
| CE [mol m$^{-2}$ s$^{-1}$] | 578

**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 $-5.2\%$ (Pezdíc et al. 1995). $\delta^{13}$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. $\delta^{13}$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 moofette 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 $P_N$-Ci 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 $P_N$-Ci 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 moofette site in Stavešinci showed that reductions of photosynthetic
capacity and carboxylation efficiency could occur in several plant species (Pfaniz, unpublished, Vodnik et al. 2002).

In cockspur a significant shift of $\Gamma$ 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 $\Gamma$ considerably increased (36, 93, and 144 $\mu$mol 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 photosynthesis that both could help explain observed adaptations.

References


