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The Effects of Elevated CO_2 on Bog Rush (*Juncus effusus* L.) Growing Near a Natural CO_2 Springs I. Effects on Shoot Anatomy

By

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Summary

TURK B., PFANZ H., VODNIK D., BERNIK R., WITTMANN C., SINKOVIČ T. & BATIČ F. 2002. The effects of elevated CO_2 on bog rush (*Juncus effusus* L.) growing near a natural CO_2 springs I. Effects on shoot anatomy. – Phyton (Horn, Austria) 42 (1): 13–23, with 3 figures. – English with German summary.

Bog rush (*Juncus effusus* L.) plants growing along a CO_2 concentration gradient in a natural CO_2 spring within a roadside ditch were examined. The concentration gradient of CO_2 within the ditch, measured at soil surface, varied from few percents to few tenths of percent, at the vent and at 23 m distance, respectively. Biometrical examination of *Juncus* plants revealed that besides total shoot height also stem width changed with the distance from the main vent, with the shoots being taller and broader with increasing distance. The degree of stem slenderness (Schlankheitsgrad; ratio between stem width and stem length) was much higher at the vent (11.5) than at a distance of 23 m (3.5). The stem cross section area, the epidermal thickness, and the number and area of sclerenchymal bundles increased with increasing CO_2. The sclerenchymal index, defined as the ratio between the area of the sclerenchymal and

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chlorenchymal tissue, ranged from round 0.04 near the vent to 0.15 at maximum distance. Mechanical properties of the shoots, i.e tensile strength (resistance to tearing forces) also varied with the distance from the vent, being lower at the sites closer to the CO_2 spring. No significant difference was found in the lignin content of differently exposed plants.

Zusammenfassung

TURK B., PFANZ H., VODNIK D., BERNIK R., WITTMANN C., SINKOVIČ T. & BATIČ F. 2002. Wirkungen von erhöhtem CO_2 auf Flatterbinsen (*Juncus effusus*) in einer Mofette I. Die Effekte auf die Sprossanatomie. – Phyton (Horn, Austria) 42 (1): 13–23, mit 3 Abbildungen. – Englisch mit deutscher Zusammenfassung.

Flatterbinsen (*Juncus effusus* L.) die in einem CO_2-Gradienten wuchsen, wurden an Hand anatomisch-morphologischer Parameter untersucht. Der CO_2-Gradient wurde von einer Mofette innerhalb eines Straßengrabens gebildet. Die gemessenen CO_2-Konzentrationen reichten von einigen Prozenten direkt an der Öffnung der CO_2-Quelle bis zu einige Zehntels in einer Entfernung von 23 m, wenn direkt an der Bodenoberfläche gemessen wurde. Die biometrischen Untersuchungen ergaben einen klaren Zusammenhang zwischen der Höhe der Pflanzen und dem CO_2-Regime unter dem die Binsen gewachsen waren. Ähnliches zeigte sich auch für den Sproßdurchmesser. Der Schlankheitsgrad (das Verhältnis zwischen Sproßdurchmesser und Pflanzenhöhe) lag bei Werten um 11,5 direkt an der Mofettenöffnung und bei ca. 3,5 in einer Entfernung von 23 m. Die Querschnittsfläche der Sprosse, die Dicke der Epidermis sowie die Anzahl und die Fläche der Gefäßbündel waren bei erhöhtem CO_2-Angebot größer. Der Sklerenchym-Index, als Verhältnis zwischen der Sklerenchymfläche und der Gesamtfläche des Sproßchlorenchymes variierte zwischen Werten um 0,04 an der Hauptöffnung der Mofette bis hin zu Werten von 0,15 am weitest entfernten Wuchsort. Auch die Zugfestigkeit der Sprosse wies eine Abhängigkeit vom Wuchsort auf und war umso geringer, je näher die Pflanzen an der CO_2-Quelle wuchsen. Der Ligningehalt der Pflanzen war in allen Proben annähernd gleich.

Introduction

Carbon dioxide is the substrate of photosynthesis. It normally occurs at concentration round 360 µmol mol^{-1} what is too low for optimal photosynthesis (MURRAY 1997). In greenhouses an artificial increase of CO_2 is therefore often used to increase plant growth and yield (AMTHOR 1995, DRAKE & al. 1997). Since centuries the concentration of CO_2 in the atmosphere is increasing, influencing Earth temperature and plant life in a number of ways (LUO & MOONEY 1999). Beside the production of carbon dioxide by oxidative processes (burning of fossil fuels like oil, brown coal and peat or renewable sources like wood) and its emission into the atmosphere, carbon dioxide is additionally emitted from CO_2 springs (mofettes) by post-volcanic geo-thermal mechanisms (ETIOPE 1997).

In the neighbourhood of the mofettes the actual CO_2 concentration may be rather high ranging from several thousand µmol mol^{-1} to 80 and more percent of CO_2 (MEISTER & al. 1999, RASCHI & al. 1997, TOGNETTI & al. 2000). Plants growing at those sites are thus temporarily confronted with exceptionally high CO_2 concentrations which are additionally fluctuating due to local winds and temperature gradients and inversions. With CO_2 reaching certain thresholds (percentage range) it can be rather deleterious to plant. Beside potential anoxia and concomitant problems in water and nutrient supply, harmful effects of CO_2 at the cell level can also be expected. Due to its potentially acidifying properties it will liberate protons in alkaline cellular compartments; the concomitant acidification is likely to inhibit pH-sensitive reactions (WAGNER 1990, PFANZ 1994, PFANZ & HEBER 1986). At lower CO_2 concentrations (about 550–650 μmol mol^{-1}) photosynthesis may be enhanced and so plants are thought to reveal a better growth and higher biomass (see LUO & MOONEY 1999). An increased plant growth should also be paralleled by certain tissue structures that control plant stability and strength. We are therefore interested whether bog rush plants growing in the direct neighbourhood of CO_2 emitting gas vents would differently react within their tissue structures.

Material and Methods

Plant material

Bog rush (*Juncus effusus* L.) plants were naturally growing in an artificial roadside ditch near Stavešinci in NE-Slovenia. The ditch itself has a slight NW inclination so that the CO_2 flow is directed downhill from the main vents, gradually diluting in ambient air and thus forming a CO_2 gradient. Seven plants growing at certain distances from the vent were chosen for analysis. The exact distances from the vent are given in Table 1. Samples were taken in the end of August in 1998 and 1999. From each plant, 15 shoots (stems, adherent leaves and roots) were taken, packed into wetted plastic bags and transported to the lab.

Microscopic analyses

Sections of the stems were cut right above the uppermost ground leaf and prepared for further microscopic examination. Morphological and anatomical parameters of the stem were measured using the Olympus Provis AX 70 microscope and the "Optimas 5.0" image analysing system. Measurements of sclerenchymatous tissue were performed using polarised light microscopy. As the cell walls of the sclerenchymal cells glow whitish under polarised light, the "Optimas" image analysing software was able to identify white cells on the dark background. Using this technique, the number and area of sclerenchyma bundles was determined. Number of vascular bundles was counted in bright field, stem diameter, epidermal and chlorenchymal thickness were measured using eyepiece micrometer. The total stem area, chlorenchymal and pith area, as well as sclerenchymal index (SCLI) and vascular bundles index (VBI) were calculated. The SCLI within the mesophyll is expressed as the ratio between the total sclerenchymal area and the area of the chlorenchymal tissue (total stem area of the cross section minus pith area of the same section) as shown in equation 1,

$$SCLI = \frac{TSB}{\pi\left(\frac{SD}{2}\right)^2 - \pi\left(\frac{SD}{2} - TE - TDC\right)^2} * 100 \qquad \textit{Equation 1}$$

where SCLI = Sclerenchyma Index; TSB = Total Area of Sclerenchyma Bundles [μm^2]; SD = Stem Diameter [μm]; TE = Thickness of Epidermis [μm] and TDC = Total Diameter of Chlorenchyma [μm].

The vascular bundle index is expressed as the area of the chlorenchymal tissue (not including the central pith) per vascular bundle as shown in equation 2,

$$VBI = \frac{\pi\left(\frac{SD}{2}\right)^2 - \pi\left(\frac{SD}{2} - TE - TDC\right)^2}{NVB} * 100 \qquad \textit{Equation 2}$$

where VBI = Vascular Bundle Index and NVB = Number of Vascular Bundles.

Resistance to mechanical tearing forces (tensile strength)

Four samples from each location were taken for measurements of mechanical properties (tensile strength), performed by "*ZWICK* Z50" apparatus from Zwick, Germany, following the EN 10002 standard for non-metallic materials. 20 cm long parts of stems were cut just above the uppermost ground leaf and mounted in the apparatus. The stretching force in N, the deformation in mm and breaking force in N were monitored.

Lignin determination

Total lignin was determined according to the method of Bruce & West 1989.

Gas measurements

Simultaneous measurements of the concentrations of CO_2, CO, O_2, CH_4, and H_2S in the soil and in the air surrounding the vegetation was performed with a portable gas analyser (landfill gas analyser GA 2000, Ansyco, FRG). The landfill gas analyser measures methane and carbon dioxide in % by infrared absorption and CO and H_2S in ppm and O_2 in % by internal electrochemical cell measurements. Holes of 10, 25 and 40 cm depth were drilled into the soil with a borer and the gas-piping tubing was immediately inserted and values recorded. Air measurements were done only during calm periods.

Results and Discussion

Spatial and temporal variability in CO_2 concentrations at natural springs prevents a correct determination of average concentrations of CO_2, which plants are exposed to (Badiani & al. 1999). Therefore only rough estimation of prevailing CO_2 regime can be presented for chosen distances from the vent. On a relatively calm day measured CO_2 concentrations within the ditch ranged from 0.1% to 6.4% when measured directly at the soil surface. Oxygen anti-paralleled the carbon dioxide concentration being lower when CO_2 increased and vice versa (Table 1). The CO_2 con-

Table 1.
The distance of seven locations, at witch the plant samples were collected, from the main vent and estimated concentration of CO_2, O_2 and CO at vent and same seven locations along the ditch at the ground level. The measurements were taken once, in March 2001, on a sunny day with low wind with a portable gas analyser (landfill gas analyser GA 2000, Ansyco, FRG).

			Concentration	
Growing site	Distance	CO_2 [%]	O_2 [%]	CO [µmol mol^{-1}]
Vent	0.0 m	6,4	19,5	3,0
1	0.5 m	5,4	19,6	4,0
2	3.2 m	1,3	20,5	1,5
3	6.4 m	0,3	20,7	2,0
4	8.6 m	0,2	20,7	1,5
5	12.9 m	0,1	20,8	1,5
6	17.6 m	0,1	20,8	1,0
7	23.1 m	0,1	20,8	1,5

atm. pressure 987 hPa
H_2S – not detectable

centration dramatically increased from soil level to various soil depths (10 to 40 cm). Concentrations up to 84% were measured within the rooting depth of *Juncus*. With some exception the CO_2 gradient within the soil followed the expected gradient formed by the uphill located vent. The CO_2 measurements in air at certain heights above soil level were rather difficult to perform due to changing wind speeds and wind directions but were nearly all in a range between 1000 µmol mol^{-1} and 10.000 µmol mol^{-1}. Carbon monoxide ranged between 1 µmol mol^{-1} and 4 µmol mol^{-1}, methane was around 0,1% and H_2S was not to be detected.

Total plant height directly paralleled the CO_2 gradient found within the ditch. The plants growing at half a meter distance from the CO_2-emitting vent were the smallest revealing a mean height of 15 cm; plants at the maximum distance of 23 m grew up to 110 cm. A nearly linear dependency between plant height and its growing site was found (Table 2). The total height of the shoots is the sum of the proper stem and the tip leaf. One plant was formed by roughly 10–20 individual shoots near the vent and over 500 shoots in plants growing at maximum distance. Beside the total height of the plants, increased CO_2 affected plant structure. These effects have not received sufficient attention in the literature and data on morphological and anatomical changes under elevated CO_2 are scarce (see review of PRITCHARD & al. 1999, MASLE 2000, PRITCHARD & al. 1998). To our knowledge (see BADIANI & al. 1999) no detailed anatomical study has been performed in CO_2 spring's plants, which have to cope with huge and frequent diurnal fluctuations of local carbon dioxide conditions. Our re-

Table 2.

Morphological features of plants growing on 7 locations along the ditch (N = 15 per growing site). Shoot height and stem diameter are increasing with the distance from the vent. Plants near the vent are more stout and far from it more slender. As shown in columns 5 and 6, the pit represents the largest part of the stem cross-section area. Following columns represent some features of the bog rush stems based on microscopical examination of stem cross sections made 2 cm above the highest base leaf. Diameter of epidermis was determined using eyepiece micrometer; sclerenchyma bundles were counted by Optimas 5.0 Image analysing system under polarised light microscopy; the same method was used to determine the total area of sclerenchyma bundles; number of vascular bundles was counted under bright field microscopy. (Data represent the mean of 15 measurements ± SD.)

Growing site	Shoot height [cm]	Stem diameter [mm]	Slenderness (W/H* 100)	Stem section area [mm²]	Pith area [mm²]	Diameter of epidermis [μm]	No. of sclerenchyma bundles	Total area of sclerenchyma bundles [μm²]	No. of vascular bundles per stem
1	15	1.80±0.20	11.97	2.53	2.52	2.19±0.39	25.1±6.2	577.6±389.6	39.3±7.8
2	40	2.75±0.08	6.86	5.92	5.90	1.64±0.08	51.1±6.4	1572.8±426.8	70.3±2.4
3	25	2.03±0.33	8.11	3.23	3.22	2.13±0.16	25.9±7.2	326.0±105.8	42.6±5.8
4	60	2.85±0.08	4.76	6.40	6.38	1.64±0.08	59.4±7.2	2378.8±492.8	73.6±1.5
5	90	2.76±0.26	3.07	5.99	5.98	1.49±0.16	45.7±8.3	1452.3±715.7	66.4±6.8
6	80	2.74±0.09	3.43	5.91	5.89	1.64±0.08	47.1±7.3	1507.4±540.8	65.9±5.6
7	100	3.52±0.20	3.52	9.75	9.73	1.59±0.08	75.9±16.7	3735.1±1559.7	84.1±4.5

search showed that stem width of bog rosh was influenced by carbon dioxide. The closer the plants grew to the source the smaller the actual diameter of the stems (Table 2). The degree of stem slenderness (Schlankheitsgrad) was calculated as the ratio between shoot width and total height of the shoot (W/H*100). Slenderness clearly followed the gradient, with plants growing apart from the ditch revealing a higher slenderness (W/H = 3.52) with those plants growing close to the vent being stouter (W/H = 11.97).

Microscopic analysis also revealed some clear correlations between anatomical features and the CO_2 regime the plants had been grown (distance from the vent). The thickness of epidermal cells ranged from 2.2 μm in plants close to vent to 1.6 μm far from it (Table 2), although the diameter of stem increased in the same direction (Table 2). The plausible rationale for the increased thickness of epidermis is the increased diffusion resistance for CO_2. Smaller epidermal cross-sectional areas (PRITCHARD & al. 1998) or unaltered epidermal anatomy (MASLE 2000) is reported from the short-term experiments with doubled air CO_2 concentration. The number of sclerenchymal bundles in *Juncus* is negatively correlated to the concentration of CO_2 (Table 2). A certain amount of sclerenchymal tissue is always present forming a perivascular envelope, so the number of sclerenchymal bundles is obviously connected to the number of vessels. Nevertheless, a larger number of extravascular sclerenchymal bundles (just below the epidermis) were found in plants growing far from the vent. A similar connection can be seen using the correlation between the total area of the sclerenchymal bundles and the ambient CO_2 concentration (Fig. 2c). Starting with values from about 1000 μm^2 directly at the vent it increased to about 3500 μm^2 at the greatest distance. On the other hand a 30% increase of the proportion of sclerenchyma within the transsectional area of fully expanded *Panicum* leaves is reported after 9 wk exposure to 900 ppm CO_2 (TIPPING & MURRAY 1999). In the same research species-depended effects on vascular tissue were observed. The proportion of vascular tissue increased in C4 *P. antidotale*, decreased in C3/4 intermediate species *P. decipiens* or remain unchanged in C3 *P. tricanthum*. In *Juncus*, the number of vascular bundles increased with the distance (Table 2) from the vent, a fact that is presumably connected with an increasing stem circumference (vessels are arranged in a thin layer of chlorenchymatous tissue). Since the maximum size of a single vessel is limited, a stem increased in size should be supplied by a higher number of supporting vessels.

To elucidate the direct dependence of the sclerenchymal area and the number of vascular bundles to the stem diameter, we used the sclerenchymal index and the vascular bundle index. The sclerenchymal index is negatively correlated to the air mean CO_2 concentration (Fig. 1a). In plants growing near the vent only about 4% of the tissue around the central pith was a proper sclerenchyme; yet, at 23 m distance about 14% of sclerenchymatous tissue were found in the outer rim of stem. Apparently, around the CO_2 vent plants were not forced to invest into stabilising tissue. Quite in contrast, no clear correlation was found between the vascular bundle index and the growing site. Although the number of vessels increased with an increasing stem diameter i.e. with the distance from the vent (Table 2), the vascular bundle index does not follow this pattern (Fig. 1b). It seems that the amount of tissue, which can be supported by one vessel, is not affected by differences in CO_2 concentration.

To test the mechanical properties of the *Juncus* stems, the tensile strength of the shoots was determined. As expected, the absolute force needed to tear a bog rush stem apart was positively correlated with stem diameter, but also the force expressed in N per mm^2 shows the same tendency. Average tearing forces needed to rupture the stem ranged from 2.0 N mm^{-2} (at 0.5 m) to 7.4 N mm^{-2} at the maximum distance of 23 m. But the correlation between the tearing force and sclerenchymal index is not all that clear (Fig. 3). The plants analysed at location 3 had only half of sclerenchymal area as compared to plants from location 1, but were almost twice as strong. It was therefore assumed that the strength of the cell walls

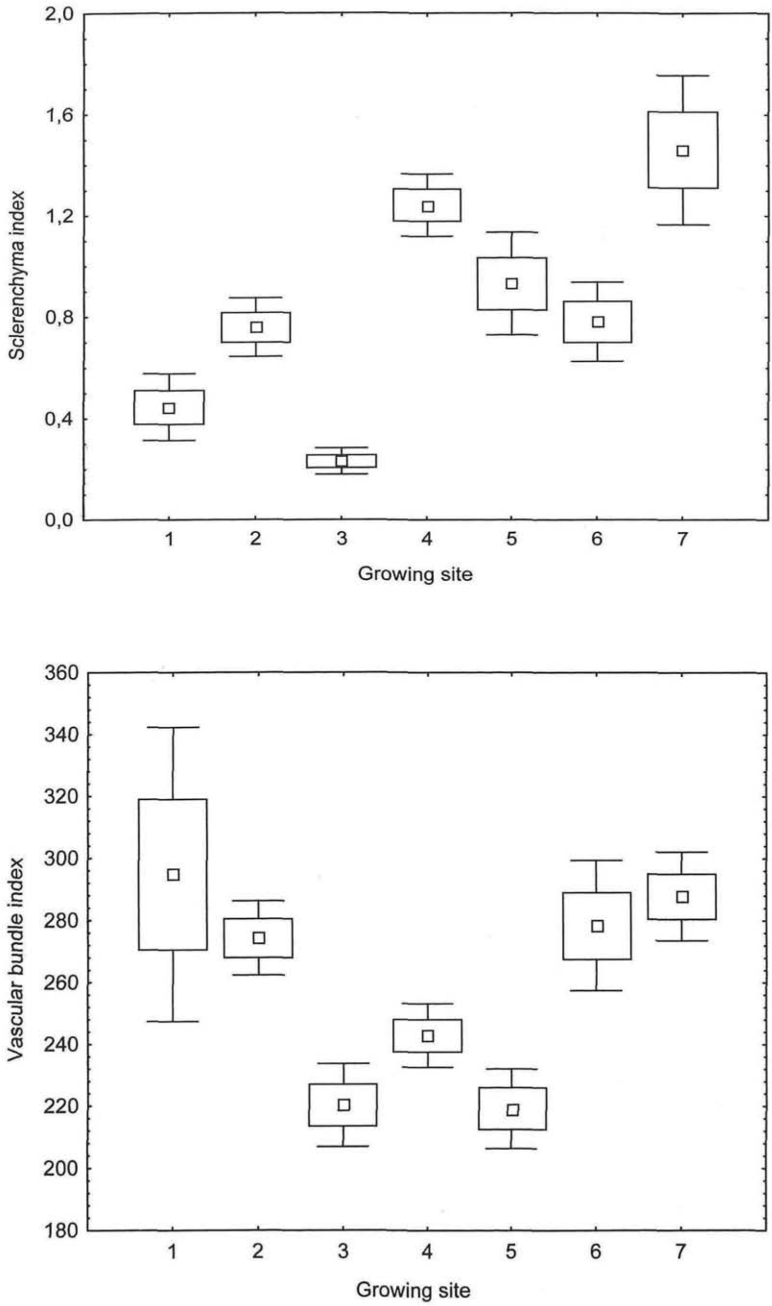

Fig. 1. Sclerenchyma index (a) was calculated according to equation 1 presented in text and vascular bundle index (b) according to formula 2.

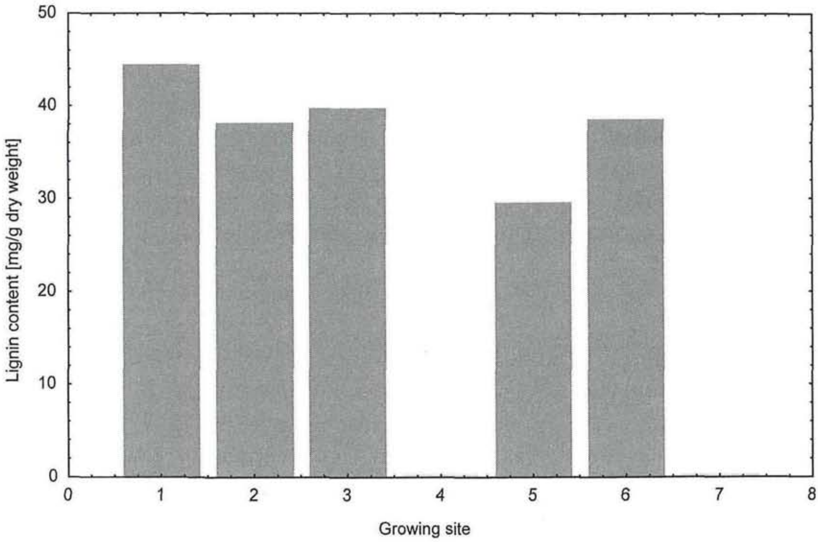

Fig. 2. Lignin content in shoots of bog rush taken on 5 locations (location 4 and 7 are missing).

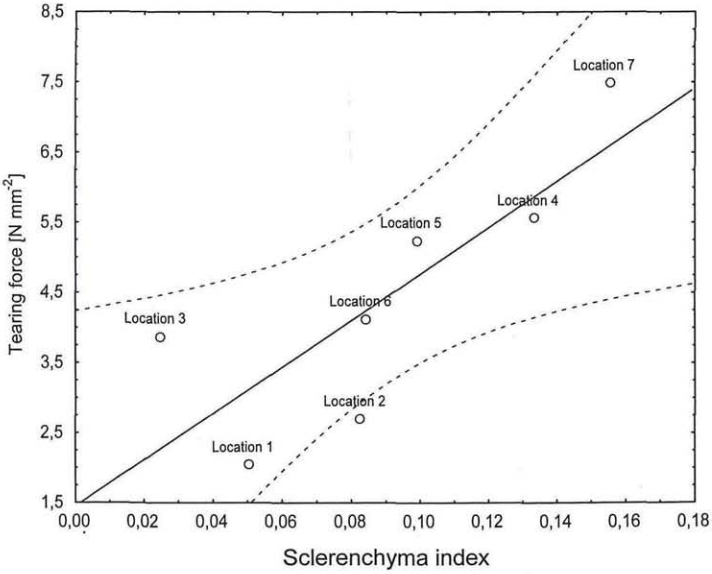

Fig. 3. Correlation between sclerenchyma index and tearing force needed to break the stem. The measurements were done using „ZWICK ZO507TH3A“ apparatus. (N = 4 per growing site; solid line ÷ regression; dotted line ÷ 95% confidence interval)

is related to its content in lignin. Interestingly, there were no significant differences in the lignin content between the bog rush stems growing at the different locations along the CO_2 concentration gradient (Fig. 2). The total lignin therefore does not necessarily play a role in determining plant stability in juncaceous plants. Whether a change in lignin composition had occurred, remains to be elucidated. Clearly, the number of vascular bundles and the total amount of sclerenchymatous tissues was increased (Table 2). As mechanical properties like tensile strength depend on the occurrence and expressiveness of vascular bundles, different tearing strengths are to be understood although lignin was rather constant.

Tensile strength of the *Juncus* stems might be more correlated to cellulose content and overall structural orientation of cellulose fibrils in the cell walls, but this was not jet analysed in our present study.

Natural source of CO_2 in a roadside ditch proved to be very well suited for research of long term effects of high CO_2 concentration on plants, since it releases very pure CO_2 without significant admixture of toxic gases. Clear correlation between some morphological and anatomical parameters of *Juncus effusus* plants and CO_2 gradient (distance from the vent) was found. In further studies different plant species/ecotypes should be involved, to elucidate the universality of CO_2 effect on plant structure.

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