

The life of plants under extreme CO₂

Življenje rastlin pri ekstremni koncentraciji CO₂

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Abstract. Highly elevated and fluctuating CO₂ concentrations at the sites with geogenic CO₂ enrichment create unique and often extreme gaseous environment for plant life. In our paper we review the knowledge on plant performance at mofette Stavešinci, which is known for very pure exhalations of CO₂. The responses of root processes and those occurring in aboveground parts of the plants are presented and discussed.

The primary target of elevated CO₂ at NCDS are root- and other belowground processes, while the direct effects on shoots are expected to be minor and only periodical. The successfulness of plants to cope with adverse conditions can be largely dependent on inherent adaptive mechanisms, which can, however, not be regarded specific for the response to elevated CO₂. Some species, for example cockspar grass (*Echinochloa crus-galli*), possess various mechanisms that make them fairly tolerant to extreme mofette environment.

Keywords: natural CO₂ springs, mofettes, soil CO₂ concentration, photosynthesis, respiration.

Introduction

In relation to the problem of global climatic change the effects of elevated CO₂ concentrations ([CO₂]) on plants have been intensively studied in the last years. The responses of plants have been mainly predicted on the basis of experiments where the effects of a doubled present ambient CO₂ concentration, a concentration that can be expected to be reached in the atmosphere in the mid of the 21st century, were compared to a reference state under actual atmospheric concentration. The latter is most commonly considered to be at 350-370 μmol CO₂ mol⁻¹. Besides its long-term increase, however, a certain variability of present [CO₂] can be measured in space and time (short-term variability). Even recent plants can be confronted with carbon dioxide levels well above the average ambient [CO₂]. Such exposure has been reported for forest floor plants on humus rich soils and plants in dense stands during the night period (LARCHER 2003, BLANKE 1997, RASCHI unpublished). In both cases high [CO₂] can be related to a high rate of autotrophic and heterotrophic respiration.

A special environment where [CO₂] can reach very high values are natural CO₂ springs (NCDSs; mofettes). At these sites geogenic CO₂ enriches both soil air and the atmosphere. Especially in the soils, extremely high concentrations can be found (ten-percentage values). Under calm conditions, high [CO₂] can be built up in the atmosphere too. Atmospheric increase strongly depends on topography and meteorological conditions that influence the mixing of air masses. Frequently enrichment can be limited only to a layer close to the soil surface. Overall, unique and sometimes extreme conditions

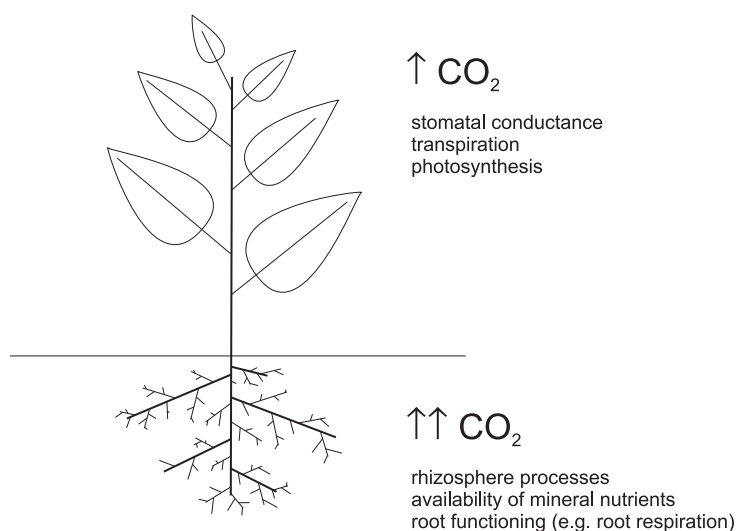


Figure 1: Unique and frequently extreme gaseous conditions are created at mofettes by geogenic CO_2 enrichment. They influence different processes in plants. \uparrow – elevated CO_2 concentration, $\uparrow\uparrow$ – strongly elevated CO_2 concentration.

Slika 1: Geogeni CO_2 ustvarja na mofetah edinstvene in pogostokrat ekstremne plinske razmere. Te vplivajo na različne procese v rastlinah. \uparrow – povečana koncentracija CO_2 , $\uparrow\uparrow$ – močno povečana koncentracija CO_2 .

are created at mofettes influencing different processes in these ecosystems (Fig. 1). In this paper we review the knowledge on plant performance under natural CO_2 enrichment that has been obtained within the research at mofette Stavešinci.

Root growth and functioning

It is clear that natural geogenic CO_2 enrichment primarily influences gaseous regime of the soil. Surprisingly, however, a vast majority of studies of NCDs plants have neglected the possible below-ground effects of elevated $[\text{CO}_2]$. Our measurements at the mofette Stavešinci (NE, Slovenia), which is known for very pure CO_2 exhalations, showed that mofette soils can be exposed to very high or even extreme CO_2 concentrations, that have fairly stable temporal and spatial patterns (VODNIK & al. 2006). High CO_2 concentrations influence the properties of the soil; they decrease soil pH and redox potential, displace oxygen from the soil profile, affect the availability of mineral nutrients and soil microbial life (MAČEK 2004, VIDEMŠEK & al. submitted). In the work by JAMNIK (2005) a high correlation between spatial pattern soil CO_2 concentration and spatial pattern of soil pH was found when both parameters were measured on a small scale (sampling grid with 0.5 m resolution). Similarly, concomitant measurements of soil $[\text{CO}_2]$ and $[\text{O}_2]$ revealed a high negative correlation between both gases (VODNIK & PFANZ unpublished). At least for strongly enriched soils it can therefore be expected that direct effects of CO_2 on plants are combined with hypoxia or anoxia.

Although severely inhibited in growth, the plants of different species are able to sustain these severe conditions. A high $[\text{CO}_2]$ could directly inhibit root growth and functions like aerobic respiration. MAČEK & al. (2005) studied the sensitivity of **root respiration** in seven NCDs grass species. By using liquid phase measurements (Clark-type oxygen electrodes) and potassium hydrogencarbonate addition

a high CO₂ environment was simulated. Under elevated CO_{2(aq)} a clear species specific decrease in root respiration was found (Fig. 2).

At 8.3 mM CO_{2(aq)}, which would correspond to roughly 21% (v/v) of CO₂ in the air phase, the respiration was inhibited for 26%, 31% and 41% in *E. crus-galli*, *S. pumila* and *D. glomerata*, respectively. In the latter two species, a significant inhibition was found even at 4.2 mM CO_{2(aq)}. It can be generally concluded that significant negative effects of CO₂ on root respiration can be expected only at high concentrations that rarely occur in the 'normal' soils but can be easily detected at the sites with the geogenic CO₂ enrichment. At these sites, species having higher respiratory tolerance to elevated [CO₂] could be in advantage (e.g. *E. crus-galli*). The mechanisms of higher CO₂ tolerance have not been elucidated, but might be related to ones underlying plant tolerance to the insufficient supply of oxygen (hypoxia).

Since elevated [CO₂] soil concentrations can be combined with hypoxia it is namely clear, that adaptive and acclimative responses known for plants living at oxygen deficiency (e.g. in flooded soil) would be of benefit in mofettes. Indeed, many plant species that grow at NCDSS are the ones well known for high tolerance to hypoxia (e. g. *Phragmites australis* most known in Italian mofette

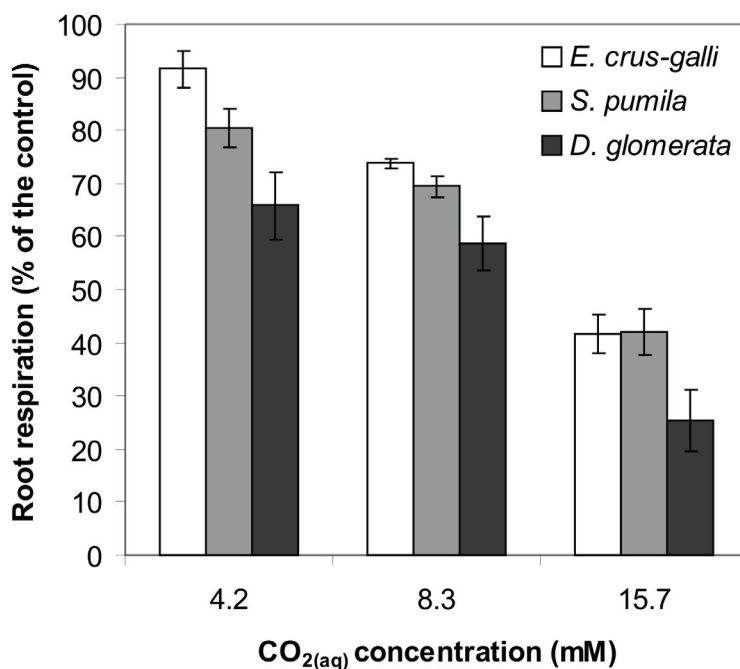


Figure 2: Root respiration of chamber grown *Echinochloa crus-galli* (L.) PB., *Setaria pumila* (Pior.) Roem & Schult. and *Dactylis glomerata* L. seedlings under different CO_{2(aq)} concentrations during the measurements. Values are given as percentages of the control respiration (16.5 ± 3.4, 28.3 ± 2.3 and 29.6 ± 2.2 nmol O₂ g⁻¹DW s⁻¹ for *E. crus-galli*, *S. pumila* and *D. glomerata*, respectively), measured at ambient CO₂ concentration in the measuring cuvette. Means ± SE are presented, n = 5 (see MAČEK & al. 2005 for details).

Slika 2: Dihanje korenin sejank vrst *Echinochloa crus-galli* (L.) PB., *Setaria pumila* (Pior.) Roem & Schult. in *Dactylis glomerata* L. pri povečani koncentraciji CO_{2(aq)} med meritvijo. Vrednosti so podane kot odstotne vrednosti dihanja korenin v primerjavi s kontrolo, merjeno pri ambientalni koncentraciji CO₂ v merilni kivetki (16,5 ± 3,4, 28,3 ± 2,3 in 29,6 ± 2,2 nmol O₂ g⁻¹DW s⁻¹, ločeno za vrste *E. crus-galli*, *S. pumila* in *D. glomerata*). Podana so povprečja ± SN, n = 5 (podrobneje opisano v MAČEK & sod. 2005).

Il' Bossoleto; *Agrostis stolonifera*, *Juncus effusus* and *Echinochloa crus-galli* in Stavešinci). These species possess adaptive metabolic and morpho-anatomical mechanisms that enable them to cope with adverse gaseous conditions. One of the most evident responses to hypoxia is an increased porosity of the roots. Oxygen deficiency induces the formation of gas transducing channels in the root cortex (aerenchyma), which deliver atmospheric oxygen from the surface, i. e. via the shoot, to the root and rhizosphere. By this way the negative effects of hypoxia on root aerobic respiration and soil mineral nutrients availability can be mitigated and the action of toxic ions can be limited. It is indicated that similar responses can be found in plants exposed to natural CO₂ enrichment, as revealed from the anatomical studies of the roots of maize grown under low and high soil CO₂ concentration (Fig.3; VIDEMŠEK 2004, VIDEMŠEK & al. 2006).

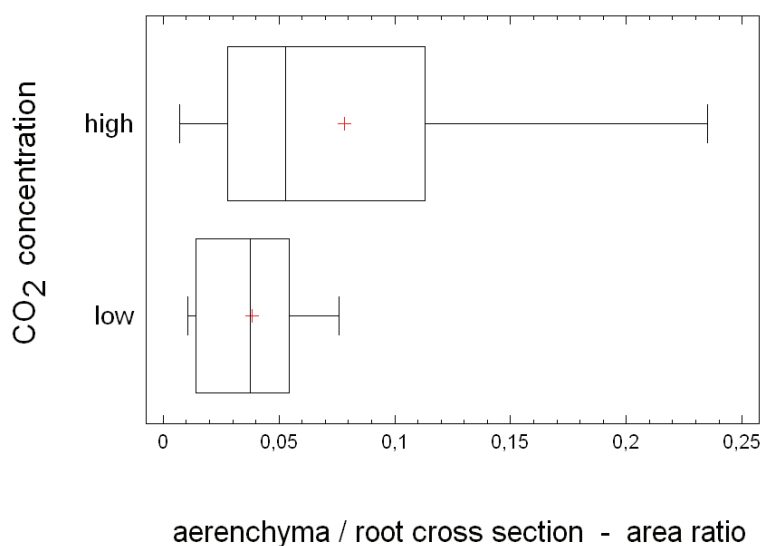


Figure 3: Aerenchyma in the roots of maize (*Zea mays* L.) growing in the soil with different geogenic CO₂ enrichment, natural CO₂ spring Stavešinci (NE Slovenia); low [CO₂] < 1%; high [CO₂] > 15 % (adapted from VIDEMŠEK & al. 2006, with permission). For a general response of maize to naturally elevated CO₂ concentrations see also VODNIK & al. 2005.

Slika 3: Aerenhimi v koreninah koruze (*Zea mays* L.), ki je rasla v tleh z različno talno koncentracijo CO₂, (low-mala [CO₂] < 1%; high-velika [CO₂] > 15 %), na območju naravnega izvira CO₂ Stavešinci (SV Slovenija) (prirejeno po VIDEMŠEK & al. 2006, z dovoljenjem). Splošni odziv koruze na naravno povečanje CO₂ je opisan v članku VODNIK & al. 2005.

Shoot growth and functioning

A direct effect of elevated CO₂ concentrations on the above ground parts of the plants can be expected in natural CO₂ springs where an accumulation of CO₂ in lower layers of the atmosphere is enabled by topography. A typical example is a karstic dolina Il' Bossoleto which is known for extreme nocturnal [CO₂] enrichment (VAN GARDINGEN & al. 1995). Even at flat area mofettes, however, the CO₂ concentrations at canopy height may reach extreme values under calm, non-windy conditions (up to 3000 -10,000 μmol mol⁻¹ in Stavešinci mofette; PFANZ & al. 2004).

Photosynthesis

The photosynthetic carbon assimilation of plants, growing at NCDSs, has been subjected to quite intensive research. The main interest was to obtain information on the long term effects of elevated [CO₂] that could help when predicting future carbon balance of terrestrial ecosystems. Gas exchange measurements that had been performed in nineties revealed inconsistent photosynthetic response of mofette plants to elevated [CO₂]. There has been also no adaptive photosynthetic strategy found for autotrophic plants growing under extreme CO₂ conditions in natural CO₂ spring areas (BADIANI & al. 1999).

Latter measurements at Stavešinci mofette clearly showed some common features of the photosynthetic performance in several plant species, growing under elevated CO₂. In these experiments, plants with different exposure to geogenic CO₂ enrichment were selected on the basis of the soil CO₂ concentration, measured in their rooting horizon (depth of 20 cm) (PFANZ & al. 2004). Gas exchange measurements were done on individuals that had been exposed to different rates of soil CO₂ enrichment during their growth at the mofette (< 1% of CO₂ in the soil = low, >20 % of CO₂ = high). The results of these measurements are summed in Table 1.

Table 1: Photosynthetic characteristics of the plants, growing at the Stavešinci mofette. Parameters were derived from *A-Ci* curves, measured by Li-6400 (Licor, Lincoln, USA) gas exchange system. The differences between high CO₂ (20-50% of CO₂ in the soil, depth 20 cm) grown plants and control plants of the same species, growing at non-enriched sites within the mofette area, are shown.

Preglednica 1: Značilnosti fotosinteze pri rastlinah z območja mofete Stavešinci. Fotosintezni parametri so bili odčitani iz *A-Ci* krivulij, ki smo jih izmerili z merilnim sistemom Li-6400 (Licor, Lincoln, ZDA). Prikazane so razlike v fotosinteznih lastnostih rastlin, ki na mofeti rastejo pri veliki koncentraciji CO₂, napram rastlinam z lokacij znotraj mofete, kjer obogatitve tal oz. atmosfere z geogenim CO₂ ni.

Photosynthetic response of plants under elevated [CO ₂]	Plant species	Reference
Lower rate of CO ₂ saturated photosynthesis	<i>/Phleum pratense/</i>	PFANZ & al. 2007
	<i>/Setaria pumila/</i>	VODNIK & al. 2002
	<i>/Zea mays/</i>	VODNIK & al. 2005
	<i>/Juncus effusus, Alopecurus pratensis, Plantago major/</i>	PFANZ unpublished
	<i>/Dactylis glomerata, Solidago gigantea/</i>	HLADNIK unpublished
Decrease in carboxylation efficiency	<i>/Phleum pratense/</i>	PFANZ & al. 2007
	<i>/Setaria pumila/</i>	VODNIK & al. 2002
	<i>/Zea mays/</i>	VODNIK & al. 2005
Increase in CO ₂ compensation point	<i>/Phleum pratense/</i>	PFANZ & al. 2007
	<i>/Echinochloa crus-galli/</i>	VODNIK & al. 2002

Evidently the photosynthesis of mofette plants is adversely affected by CO₂ enrichment which could be the result of direct and/or indirect action of high CO₂ concentrations.

For Stavešinci mofette it is known that a very sharp gradient of [CO₂] exists at the soil/atmosphere interface (VODNIK & al. 2006) and that long term enrichment of the atmosphere is much lower than that of the soil air. It is therefore to presume that the direct negative effects of elevated atmospheric [CO₂] concentrations on photosynthesis are minor and most probably occur only periodically. On the other hand the soil [CO₂] are high or even extreme and far more stable (VODNIK & al. 2006, PFANZ & VODNIK unpublished) and could have an important indirect effect on autotrophic carbon assimilation.

The reductions of photosynthetic rates and decreases in carboxylation efficiency can be well related to the lower content of mineral nutrients, in particular nitrogen, in the leaves of high CO₂ grown plants. Such decrease was found for different species, *Phleum pratense*, *Dactylis glomerata*, *Solidago gigantea*, *Zea mays*, *Juncus effusus* (PFANZ & al. 2004). Also COOK & al. (1998) reported on nitrogen deprivation and photosynthetic reduction in *Nardus stricta* plants growing at a CO₂ spring on Iceland.

The same effect of soil CO₂ enrichment can even be observed in fertilized plants. In maize field experiment, that was performed within the Stavešinci mofette area, the leaf N content significantly decreased at the parts of the field that were enriched by geogenic CO₂, despite fertilization (VODNIK & al. 2005). It is to presume that the main factor influencing the mineral nutrition was a reduced availability of minerals in the soil with high CO₂ and low O₂ concentrations. Reductive conditions in the soil strongly affect chemical reactions in the soil, which can decrease the availability of essential elements, for example nitrogen (via denitrification), sulphate etc. and promote toxicity of other elements (e.g. manganese). When the oxygen is depleted from the soil an additional factor would be lower metabolic capacity of the roots for the mineral uptake (see also MAČEK & al. 2005). In non-fertilized mofette soils the nutrient availability would also be strongly affected by the lower input of organic carbon to the soil, due to lower biomass production, and by the lower rate of mineralization.

The observed photosynthetic response can be frequently correlated to a lower content of chlorophyll in the leaves of the plants exposed to geogenic CO₂ enrichment (VODNIK & al. 2002, PFANZ & al. 2004). Highly elevated CO₂ concentrations are reflected also in the increased levels of some stress related compounds in the leaves (VODNIK & al. 2005).

Another aspect on photosynthetic carbon assimilation is the regulation of gas diffusivity between the leaf and the atmosphere. Plants growing at the mofettes are confronted with fluctuating and extreme air CO₂ concentrations and have even more difficult task, compared to plants from 'normal' sites', when regulating CO₂ uptake and water loss.

Stomatal conductance and transpiration

Plants have sophisticated regulation of stomata that allow them to achieve suitable stomatal conductance under given environmental conditions. The [CO₂] in surrounding air is one of the key factors that influence stomatal conductance, it is sensed in substomatal cavity by undisclosed mechanism.

General assumption is that higher [CO₂] leads to reduction of stomatal conductance. When [CO₂] is suddenly increased, which can be expected for the mofette conditions, stomata respond by relatively fast closure (Fig. 4a). In our research dynamics of the stomatal response was compared for different grassland species from the Stavešinci mofette. A species specific response was observed. Plants of cocksbur (*E. crus-galli*) responded to CO₂ increase by faster stomatal closure than other species (Fig. 4b) (HLADNIK & VODNIK unpublished data). When the response of plants from low- and high- CO₂ environments was compared for selected plant species, no differences were found in dynamics of the response (data not shown).

Conclusions

CO₂ emissions at natural CO₂ springs create unique and frequently extreme gaseous conditions for plants. The negative effects of highly elevated [CO₂] can be observed on different levels, high [CO₂] influences growth, photosynthesis, respiration, mineral nutrition etc. Our research at Stavešinci revealed that the responses of plants can be very well correlated to the soil CO₂ concentration. Since, in addition, the CO₂ enrichments of the atmosphere in this particular mofette are minor and only periodical, it is clear that the root- and other belowground processes are the primary target of the geogenic CO₂. This is largely reflected in disturbances of mineral nutrition and leads to secondary effects such as photosynthetic inhibition.

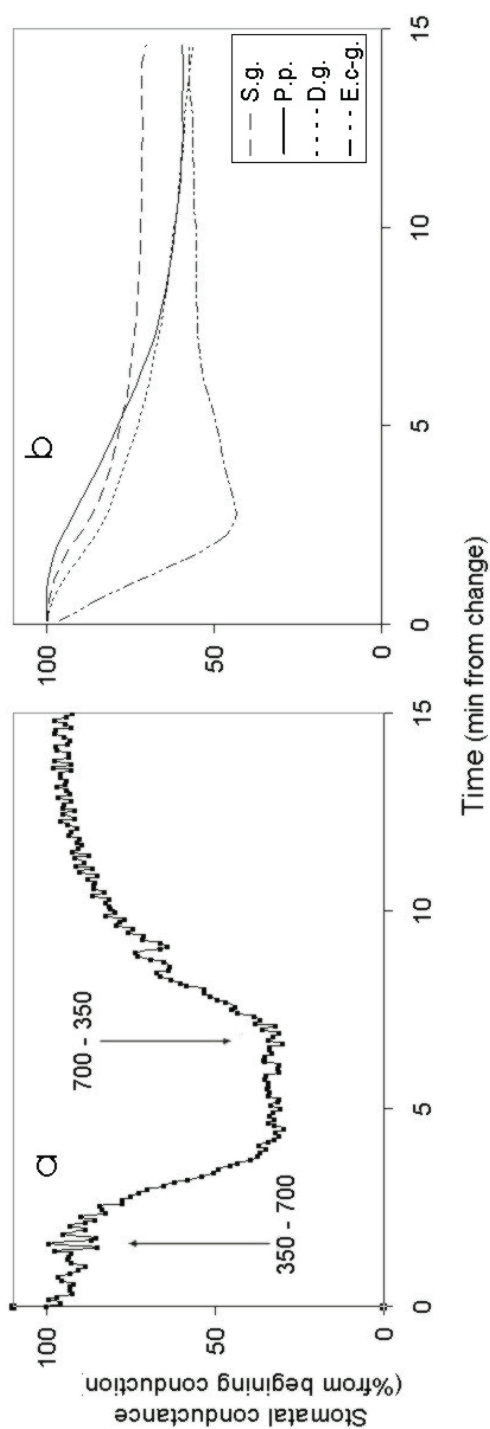


Figure 4: a) Stomatal response of maize (*Zea mays* L.) to the sudden changes of [CO₂] in surrounding air. Arrows and accompanying numbers indicate time and the level [$\mu\text{mol mol}^{-1}$] of CO₂ concentration change (HLADNIK & VODNIK 2007, with permission). b: Stomatal response of *Echinochloa crus-galli* (E.c-g.), *Dactylis glomerata* (D.g.), *Phleum pratense* (P.p) and *Solidago gigantea* (S.g) to sudden change in [CO₂] in surrounding air. Change from ordinary to doubled [CO₂] was achieved at moment 0. Stomatal response in both experiments was measured with Li-6400 system (LICOR, Lincoln, ZDA) at 26°C, 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity and relative humidity of 35%. Data were logged in 5 second intervals.

Slika 4: a) Odziv listnih rež koruze (*Zea mays* L.) na spremembo [CO₂] v okoliškem zraku. Puščice in spremljajoče številke označuje čas in obseg spremembe [CO₂] v $\mu\text{mol mol}^{-1}$ (HLADNIK & VODNIK 2007, z dovoljenjem). b: Odziv listnih rež *Echinochloa crus-galli* (E.c-g.), *Dactylis glomerata* (D.g.), *Phleum pratense* (P.p) in *Solidago gigantea* (S.g) na hitro spremembo [CO₂] iz običajne na podvojeno koncentracijo. Sprememba koncentracije CO₂ je bila dosežena v času 0. Odziv smo merili z merilnim sistemom Li-6400 (LICOR, Lincoln, ZDA) pri 26° C, jakosti svetlobe 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in relativni vlažnosti 35%. Podatki so bili beleženi v intervalu 5 sekund.

To some extent plant can cope with adverse growing conditions at the mofettes. Their successfulness can be largely dependent on inherent adaptive mechanisms, which can, however, not be regarded specific for the response to elevated CO₂. They most commonly represent a response strategy to other stressors, that are more common than the highly elevated CO₂ concentrations. Some species, for example cockspear grass (*Echinochloa crus-galli*) possess various mechanisms that make them fairly tolerant to extreme mofette environment.

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