Responses of the Lichen *Cladonia convoluta* to High CO_2 Level and Heavy Metal Treatment

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Despite of the downward acclimation of photosynthesis in *C. convoluta*, increased net photosynthesis and carbon balance can be anticipated in response to elevated atmospheric CO_2 level. CO_2 exchange measurement seems to be more indicative when detecting heavy metal stress than fluorescence parameters. Among these, the relative fluorescence decrease ratio (RFd690) shows damage first, suggesting that the primary attack site for heavy metal ions is CO_2 fixation and reaction centres are harmed last. Long-term elevated CO_2 ameliorates partly this damage by improving C-balance to a greater extent in the heavy-metal stressed lichens.

Introduction

Atmospheric CO_2 concentration is estimated to double from pre-industrial levels by the middle of the next century. Its effects on plants are under intense investigation. Being the key substrate of photosynthesis, most of the research focuses on assimilation and primary production under the predicted scenarios. Photosynthesis can show upward (Arp and Drake, 1991) or downward (Jarvis, 1993) regulation in response to elevated CO_2 level. Decreases in assimilation capacity in downward regulated plants are usually due to decreases in rubisco level and/or activity (Sicher *et al.,* 1994). Studies on lichens are extremely scarce in this context. Despite of marked downward regulation of CO_2 fixation increased lipid storage could be found in *Parmelia sulcata* (Balaguer *et al.,* 1996). Tuba *et al.* (1998) found that short-term elevated CO_2 level increases carbon gain during desiccation in *Cladonia convoluta.*

Interactions of elevated CO_2 concentration with different abiotic stress factors have been widely investigated. It can help plants to cope with drought (Scarasciamugnozza *et al.,* 1996; Arp *et al.,* 1998) mostly by decreasing stomatal conductance, with heat stress (Wayne *et al.,* 1998), with salinity (Reuveni *et al.,* 1997) and even with air pollutants like SO_2 (Lee *et al.,* 1997) or O_3 (McKee *et al.,* 1995). There are some reports, which provide evidence that increased CO_2 concentration may act at the antioxidant system. It decreases intrinsic oxidative stress (Schwanz *et al.,* 1996) and strengthens acclimation at the onset of stress (Polle *et al.,* 1997).

Although lichens are used to biomonitor aerial dispersion of heavy metals (Garty *et al.,* 1997) using their ability to tolerate elevated quantities of heavy metals (Nash, 1975) and the heavy metal content of *Cladonia convoluta* correlated with soil contamination as well (Chettri *et al.,* 1997), heavy metals are by no means harmless to them. These ions denaturate enzymes and block their functional groups as direct toxic effects. Heavy metals also decrease chlorophyll content especially at low pH (Garty *et al.,* 1992). Besides this they also induce free radical toxicity (Gadd, 1993). Although there has been an extensive research done on the effect of heavy metals on lichens, it has not been investigated under the future high CO_2 environment yet. For this purpose we chose two metals with different toxicity (Wells and Brown, 1995) but having a similarly high importance in environment quality.

In this work we aimed to reveal the effects of the long-term elevated CO_2 level on the photosynthetic responses of the highly desiccation tolerant lichen *C. convoluta*, because lichens have rarely been the targets of such investigations. We also intended to determine whether elevated CO_2 level

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ameliorates heavy-metal induced damage of this already highly stress-tolerant lichen species.

Materials and Methods

The lichen *Cladonia convoluta* (Lam.) P. Cout. was collected in *Festuca vaginata* grassland near Fülöpháza, Hungary (19 ° 14′ E; 47 ° 30′ N, some 70 km SSE of Budapest at 130 m a.s.l.).

Lichens with their original soil substrates were transplanted into boxes (three replicates) and put in Plexiglas chambers (one chamber for the fumigation treatment and one for the control) situated in the botanical garden in Gödöllõ, some 30 km NE of Budapest. High-CO_2 (700 $\mu l\ l^{-1}$) treatment was maintained as described in previous studies (Tuba *et al.*, 1995). Since the chambers were closed on the top, plants had to be sprayed every morning with [1] deionized water (control); [2] 350 $\mu mol\ l^{-1}$ Pb^{2+}; [3] 350 $\mu mol\ l^{-1}$ Cd^{2+} or [4] 175 $\mu mol\ l^{-1}$ Pb^{2+} + 175 $\mu mol\ l^{-1}$ Cd^{2+} (combined treatment). Metals were given as nitrate salts. Exposure lasted for one month.

The measurements were made on the upper young parts of the lichen thallus. To ensure full photosynthetic activity the samples were kept close to the optimum water contents (130–150% of DM) for 24 h at a photosynthetic photon flux density (PPFD) of 400 $\mu mol\ m^{-2}\ s^{-1}$ at 20 °C before all gasexchange and chlorophyll-fluorescence measurements on fully hydrated material. Chlorophyll fluorescence was recorded with a modulated one-channel Hansatech fluorometer. We calculated the maximal photosystem (PS)II quantum efficiency [Fv/Fm = (Fm-Fo)/Fm] and vitality index, or relative fluorescence decrease ratio measured at 690 nm [RFd = (Fm-Fs)/Fs] (Lichtenthaler, 1988). Dark respiration and light-saturated net photosynthesis rates were measured using an IRGA system (Type LCA2, ADC Co. Ltd., Hoddesdon, U.K.), operated in differential mode at a PPFD of 400 $\mu mol\ m^{-2}\ s^{-1}$ (see Tuba *et al.*, 1996). The CO_2 concentrations of 350 and 700 $\mu mol\ mol^{-1}$ for the IRGA measurements were produced by a gas diluter (GD 600, ADC Co. Ltd., Hoddesdon, U.K.) (see Tuba *et al.*, 1998). Element analysis (Al, Cd, Cr, Cu, Fe, Ni, Pb, V, Zn) was carried out with ICP-AES technique preceded by dry cleaning and high pressure and temperature digestion of the plant material with 1:1 (v/v) ratio of concentrated HNO_3 and concentrated H_2O_2 (Tuba and Csintalan, 1993). Measurements were performed in three replicates except for the element content data where we had five samples digested and analyzed.

Results and Discussion

CO_2-treatment did not cause significant differences in heavy metal content. Pb content was raised from 13.4±3.6 to 1274±49 $\mu mol\ g^{-1}$ dwt, Cd content from 1.13±1.01 to 581±31 $\mu mol\ g^{-1}$ dwt by the corresponding treatments. Simultaneous treatment with the half dose of the two metals resulted in 692±55 $\mu mol\ g^{-1}$ dwt Pb and 343±19 $\mu mol\ g^{-1}$ dwt Cd concentration. Berg *et al.* (1995) noted that the uptake efficiency for Cd is 40–65% of that for Pb in the case of mosses. *C. convoluta* fell within this range with 45.6% or 49.6% at the combined treatment. Heavy metal treatments reduced Ca-content by 18.6% ($p = 3.78 \cdot 10^{-6}$) and K-content by 10.4% ($p = 7.74 \cdot 10^{-4}$). K-loss is a generally used measure of heavy metal toxicity, however inhibition of photosynthesis in small poikilohydric plants can occur without membrane leakage in Cd-treatment (Brown and Wells, 1990). Decreased Ca-content can be explained by its displacement by Cd from the extracellular binding sites (Brown and Beckett, 1985). When lichens are immersed in a solution of metal ions uptake is nearly complete within an hour (Nieboer *et al.*, 1976). Since metals were given as dissolved ions and drying out lasted at least four hours in the cool autumn weather, we do not assume any errors due to significant amount of externally stored heavy metal containing particles. We can neither tell the ratio of cell wall-bound ions nor the ratio of ions taken up by the two different symbionts. However, Brown and Sidhu (1992) suggest that the cell wall does not prevent but only delays heavy metal induced impairments in mosses.

The chlorophyll fluorescence parameters did not show any acclimation to the elevated CO_2 concentration at the photochemistry level (Table I). PSII activity (presented by Fv/Fm) did not reflect any change due to the heavy metal treatments either. According to Atal *et al.* (1991) water splitting is more harmed by Cd than PSII. Hence one can obtain significant Fv/Fm values without prolonged electron supply from water. RFd690, however, which corresponds to the whole thylakoid

Table I. Fv/Fm and RFd690 values of *Cladonia convoluta* grown at present (350 µmol mol^{-1}) and future elevated (700 µmol mol^{-1}) CO_2 concentration and different heavy metal treatments. control; Pb: 350 µmol l^{-1} $Pb(NO_3)_2$; Cd: 350 µmol l^{-1} $Cd(NO_3)_2$; Pb+Cd: 175 µmol l^{-1} $Pb(NO_3)_2$ + 175 µmol l^{-1} $Cd(NO_3)_2$.

CO_2 level		Fv/Fm	st.dev.	RFd690	st.dev.
Present CO_2	Control	1.184	0.096	0.581	0.058
	Pb	0.995	0.098	0.580	0.026
	Cd	1.024	0.202	0.562	0.035
	Pb+Cd	0.903	0.070	0.569	0.043
Elevated CO_2	Control	1.120	0.057	0.548	0.063
	Pb	0.985	0.115	0.571	0.023
	Cd	0.953	0.111	0.533	0.035
	Pb+Cd	0.837	0.046	0.534	0.023

membrane activity being influenced by CO_2 fixation (Lichtenthaler, 1988) was slightly lowered by the heavy metal treatments. This supports Krupa and Baszynski's view (1995) that heavy metals harm Calvin cycle first. This decrease was significant only in case of the combined (Pb + Cd) treatments.

The CO_2 exchange rates changed in response to CO_2- and heavy metal treatments. Since we aimed to investigate the long-term effects of elevated CO_2 level to control and heavy metal stressed plants, they had to be measured at their own exposure CO_2 concentration. From this aspect, short-term effects presented by the plants grown at 350 and measured under 700 ppm CO_2 are of secondary interest. These can be compared to those grown and measured under high CO_2 in order to detect the presence or the direction of acclimation within the photosynthetic apparatus.

Lead treatment decreased net carbon assimilation by 50 and 40% at present and future CO_2 concentration, respectively (Fig. 1). Cadmium had an even more drastic effect with decreases of about 85 and 70%. Replacing half of the Cd by Pb reduced the damage to 75 and 60%. Although lead was taken up to a greater extent than cadmium, its toxicity was milder. This can be explained by its inactivation as extra- and intracellular lead-phosphate precipitation (Koeppe, 1981). Net CO_2 assimilation was enhanced by elevated CO_2 concentration to a greater extent in the heavy metal stressed plants (except for the Pb-treatment).

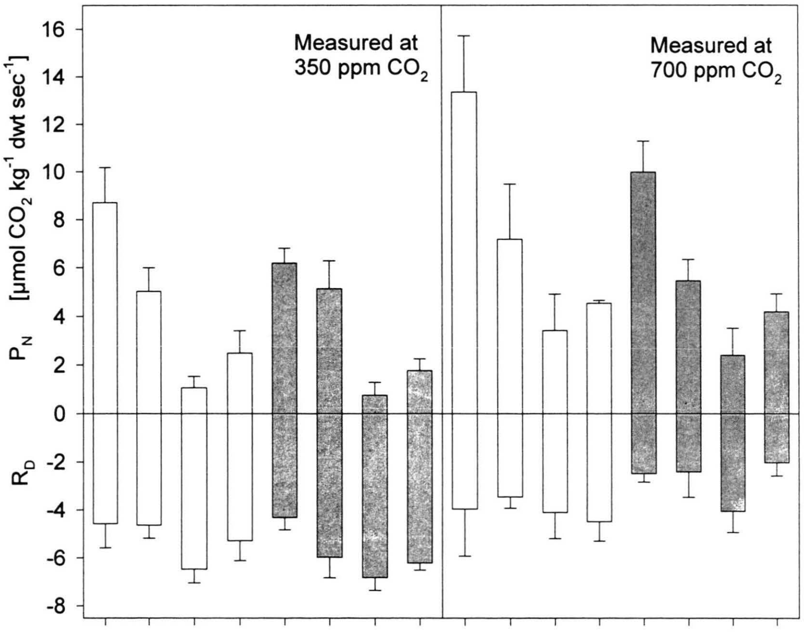

Fig. 1. Net CO_2 assimilation (P_N) and dark respiration (R_D) values of *Cladonia convoluta* grown at 350 (open bars) and 700 (hatched bars) in µmol mol^{-1} CO_2 and different heavy metal treatments. ∅: "control"; Pb: 350 µmol l^{-1} $Pb(NO_3)_2$; Cd: 350 µmol l^{-1} $Cd(NO_3)_2$; Pb+Cd: 175 µmol l^{-1} $Pb(NO_3)_2$ + 175 µmol l^{-1} $Cd(NO_3)_2$. Error bars represent standard deviation of three replicates.

When comparing net assimilation values measured at the same CO_2 concentration, those grown at high CO_2 have a lower rate indicating downward acclimation of photosynthesis. However, this acclimation appears only in the control plants. We suggest that heavy metal treatment caused a greater variance in the net assimilation values than CO_2 exposure and hid its effects.

When comparing the lichens measured at their own CO_2 exposure level, we can find a significant decrease in dark respiration in control and all metal treatments due to the high CO_2 (Fig. 1). Heavy metals can increase respiration rate in plants (Poskuta *et al.,* 1996). In this case plants receiving high concentration of Cd indicate this. This phenomenon was attributed to the uncoupling of the mitochondrial membrane (Brown and Wells, 1990). According to Ryan (1991), elevated CO_2 suppresses dark respiration because high intercellular CO_2 might affect indirectly respiratory enzymes and intercellular pH. This view supports our data, which represent rather a prompt effect of high CO_2 on dark respiration.

P_N/R_D (net assimilation divided by dark respiration) values representing overall carbon balance (Meenks *et al.,* 1991) doubled in the control and Pb-treated samples while they increased 3.6-fold at Cd-treated lichen and 4.3-fold at the combined treatment due to the long-term elevated CO_2 concentration.

These results affirm earlier observations (Tuba *et al.,* 1998) that future high CO_2 level might be beneficial for desiccation tolerant cryptogamic plants by improving their carbon balance. This may be even more the case in the presence of a stress factor, namely heavy metal pollution. We would like to point out that elevated CO_2 can ameliorate partly the deleterious effects of heavy metal stress.

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