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Photosynthetic traits in wheat grown under decreased and increased CO_2 concentration, and after transfer to natural CO_2 concentration

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Abstract

Wheat plants were grown from sowing to day 18 in 26-dm^3 chambers at three different CO_2 concentrations: 150 ($-CO_2$), 350 (C, control), 800 ($+CO_2$) $\mu mol\ mol^{-1}$. Afterwards, plants of the three variants were grown at the same natural CO_2 concentration. Plant characteristics were measured just before the transfer (0 days after CO_2 treatment, DAT), and at 5 - 8 DAT on the 1st leaf, and at 12 - 22 DAT on the 4th leaf. Decreased or increased CO_2 concentrations caused acclimations which persisted after transplantation to natural CO_2 concentration. At 5 - 8 DAT, stomatal density, stomatal conductance (g_s), CO_2 saturated net photosynthetic rate (P_{NsatC}), radiation saturated net photosynthetic rate (P_{NsatI}), and carboxylation efficiency (τ) were higher in $-CO_2$ plants and lower in $+CO_2$ plants than in C plants. As compared with C plants, the photochemical efficiency (α) was lower in $-CO_2$ and higher in $+CO_2$ plants, however, chlorophyll (Chl) *a*, Chl *b*, Chl *a*+*b* and carotenoid contents were lower in both $-CO_2$ and $+CO_2$ plants. On the 4th leaf, which emerged on plant after finishing CO_2 treatments, at 12 - 22 DAT, no differences in stomatal density and g_s between treatments were observed. In $-CO_2$ plants, pigment content and P_{NsatC} were higher, α was lower, and P_{NsatI} and τ were not different from C plants. In contrast, in $+CO_2$ plants, pigment content, P_{NsatI} and τ were lower, and P_{NsatC} and α were unchanged. Leaf area, dry mass, and tiller development increased in $+CO_2$ plants and decreased in $-CO_2$ plants. In the interval between 8 and 22 DAT, lower net assimilation rate in $+CO_2$ than in $-CO_2$ plants was observed.

Additional key words: carboxylation efficiency, carotenoids, chlorophyll, growth analysis, net photosynthetic rate, photochemical efficiency, ribulose 1,5-bisphosphate carboxylase/oxygenase, stomatal conductance, *Triticum aestivum*.

Introduction

The global climate change, especially upward trend in atmospheric CO_2 concentration, has important implication for plant photosynthesis. For modelling plant behaviour in the future atmosphere, the research has been mostly concentrated on the effect of increased CO_2 concentration on gas exchange, pigment content, stomata behaviour,

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Abbreviations: $+CO_2$ plants, C plants and $-CO_2$ plants - plants treated by 800, 350 and 150 $\mu mol(CO_2)\ mol^{-1}$, respectively, for 18 d; c_a - ambient CO_2 concentration; Car - carotenoids; c_i - intercellular CO_2 concentration; DAT - days after CO_2 treatment; DM - dry mass; g_s - stomatal conductance for water vapour transfer; Chl - chlorophyll; I - irradiance; LA - leaf area; LA/LM ratio - leaf area/leaf DM ratio; LAR - leaf area ratio; NAR - net assimilation rate; P_N - net photosynthetic rate; P_{NsatC} - CO_2 saturated P_N; P_{NsatI} - radiation saturated P_N; R_D - dark respiration rate; R_L - rate of CO_2 evolution in the light at zero c_i; RuBP - ribulose-1,5-bisphosphate; RuBPCO - RuBP carboxylase/oxygenase; S/R ratio - shoot/root ratio; Γ_C - compensation CO_2 concentration; Γ_I - compensation irradiance, α - photochemical efficiency; τ - carboxylation efficiency.

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photosynthate formation and alocation, *etc.* (for recent reviews see, *e.g.* Murray 1997, Saralabai *et al.* 1997, Pospíšilová and Čatský 1999a,b). Only several papers have dealt with the effect of decreased CO_2 concentration which was characteristic for the atmosphere in the past (*e.g.* in wheat or in other cereals - Polley *et al.* 1992, 1993a,b, Mayeux *et al.* 1997, in rice - Baker *et al.* 1990, 1992, in bean and soybean - Allen *et al.* 1991, Sage and Reid 1992, Cowling and Sage 1998). Also in present times, plants are sometimes exposed to rather low CO_2 concentration. For example, decreased CO_2 concentration in dense canopies might contribute to a midday depression of photosynthesis (*e.g.* Xu and Shen 1997). Very low CO_2 concentration (near compensation concentration) develops during light period in the rather tightly closed vessels of autotrophic or mixotrophic *in vitro* cultures (*e.g.* Fujiwara *et al.* 1987, Solárová *et al.* 1989, 1996, Šantrůček *et al.* 1991, Niu and Kozai 1997, Kozai *et al.* 1997, Nguyen and Kozai 1998). Similarly, plants are growing under low CO_2 concentration in unventilated hotbeds or greenhouses (*e.g.* Mortensen 1987, Kitaya *et al.* 1998). Therefore, the photosynthetic production of plants grown *in vitro* (Tichá 1996, Solárová and Pospíšilová 1997) and in greenhouses (Critten 1991, Nederhoff 1994) can be improved by rising CO_2 concentration. Plants from high altitudes are also exposed to lack of CO_2 caused by its decreased partial pressure (*e.g.* Billings *et al.* 1961, Körner 1992).

The upward trend in atmospheric CO_2 concentration probably has already enhanced the photosynthesis and growth of many plants, however, one of the central issues is to elucidate a mechanism why long-term exposure of plants to elevated CO_2 concentration often results in a down-regulation of photosynthesis, *i.e.*, that photosynthetic rate at elevated CO_2 is lower than it would be expected based on short-term assessment of photosynthetic rates as a function of CO_2 concentration (for reviews see, *e.g.*, Mott 1990, Bowes 1993, Allen 1994, Reining 1994, Sage and Reid 1994, Sharkey 1994). The down-regulation of photosynthesis may be in consequence of *1)* decreased stomatal density and partial stomata closure reducing the CO_2 transport to the sites of carboxylation, *2)* rapid production of photosynthates leading to excess amount of starch in the chloroplasts and feedback inhibition of P_N; this inhibition is dependent on the availability of sinks in the plant and also on the availability of nitrogen to balance enhanced availability of carbon, *3)* reduction in the amount or activity of RuBPCO, *etc.*

On the contrary, in alpine plants adapted to decreased CO_2 partial pressure higher carboxylation efficiency in comparison with lowland plants was found (*e.g.* Körner and Diemer 1994). In primary bean leaves growing under environment simulating *in vitro* culture (CO_2 concentration decreasing with the development of photosynthetic activities from 600 to 150 μmol mol^{-1}), net photosynthetic rates and other photosynthetic activities in mature leaves were higher than in plants grown under natural CO_2 concentration (Čatský *et al.* 1995). These results suggest that photosynthetic traits in mature leaves might be affected by CO_2 concentration during very early phases of ontogeny.

The aim of this research was to confirm or reject the hypothesis that long-term exposure of plants to decreased CO_2 concentration might induce up-regulation of photosynthesis and if this hypothesis is confirmed to compare the mechanisms of acclimation of photosynthesis to elevated and decreased CO_2 concentration. Similarly important seems to determine if the changes induced by elevated or decreased CO_2 concentrations during early phases of plant development persist in the further growth under natural CO_2 concentration.

Materials and methods

Plants: Seeds of winter wheat (*Triticum aestivum* L. cv. Zdar line A, breeding station Uhřetice) were sown on a filter paper moistened with distilled water. After 5 d, seedlings were fixed into glass holders with foam-rubber and placed into Hoagland nutrient solution with microelements (Hewitt 1966). Each 3-dm^3 cultivation vessel contained 80 seedlings. The cultivation vessels were placed in three plexiglass chambers (0.22 × 0.3 × 0.4 m) with low (150 μmol mol^{-1}, -CO_2 plants), natural atmospheric (350 μmol mol^{-1}, C plants) and high (800 μmol mol^{-1}, +CO_2 plants) CO_2 concentrations (Hák and Nátr 1990). CO_2 in the incoming air was firstly depleted by soda lime (*Natrocalcid, Spolana*, Neratovice, Czech Republic), and then CO_2 was added from cylinders through silicon-rubber tubes; CO_2 concentration was regulated by the length of silicon-rubber tubes and by air-flow (Apel 1966). Air exchange in growth chambers was 50 cm^3 s^{-1}, day/night temperature 20/14 °C, and irradiance of 250 μmol m^{-2} s^{-1} during 16-h photoperiod. Relative air humidity was dependent on the leaf area and transpiration of cultivated plants and rose from 70 to 98 %. Plants were grown for 18 d (before developing of fourth leaf) and afterwards CO_2 treatment finished, and plants of all variants grew for 22 d under CO_2 concentration of 350 μmol mol^{-1} and air humidity of 30 - 60 %, other conditions remain unchanged. The experiments finished on the 40th day after sowing.

The parameters studied were measured just before finishing CO_2 treatment (0 DAT, 15 - 18 d after sowing) in the first leaves, 5 - 8 DAT (23 - 26 d after sowing) also in the first leaves, and 12 - 22 DAT (30 - 40 d after sowing) in the fourth leaves. We aimed to determine characteristics of the 1st leaf which developed in the different CO_2 concentrations, and also of the 4th leaf which emerged on plant when CO_2 treatment was already finished.

Leaf area, dry mass and growth analysis: Leaf area (LA) was computed as a product of leaf length and width, and coefficient 0.67. Dry masses (DM) of leaves, stems, and roots were determined after drying at 95 °C to constant mass (about 8 h). Parameters of growth analysis, e.i. shoot DM/root DM ratio (S/R), leaf area/leaf mass ratio (LA/LM), leaf area ratio (LAR), and net assimilation rate (NAR) were calculated according to Květ *et al.* (1971).

Chlorophyll and carotenoid contents were determined in 80 % acetone extracts using spectrophotometer *U 3300* (*Hitachi*, Tokyo, Japan); the content of individual pigments per leaf area was calculated from measured absorbances according to Arnon (1949), Lichtenthaler (1987) and Wellburn (1994).

Stomatal density was determined on imprints from middle parts of adaxial and abaxial leaf sides according to Kubínová (1994).

Gas exchange measurements: The open gas exchange system (Janáček 1996) was used. Leaves of 5 - 7 plants were closed into the assimilation chamber with the copper-constantan thermocouple to measure leaf temperature (air flow rate 6.7 $cm^3 s^{-1}$, temperature 22 °C). Leaves were irradiated by the halogen lamp (*Osram 64571*, 800 W) through the water filter with 0.15 % $CuSO_4$, to improve light spectral composition. Differences in CO_2 concentration between output and input air was measured by the infra-red gas analyzer *Uras 3G* (*Hartmann und Braun*, Frankfurt am Main, Germany), partial pressures of water vapour was calculated from dew point measured by the mirror dew point meter (*Waltz*, Effeltrich, Germany). Gas mixtures with different CO_2 concentration rate were prepared in two gas mixing pumps (*Wösthoff*, Bochum, Germany), different irradiance was obtained by changing distances between light source and the assimilation chamber. The dependence of net photosynthetic rate (P_N) and stomatal conductance (g_s) on intercellular CO_2 concentration (c_i) was measured at irradiance of 500 $\mu mol\ m^{-2} s^{-1}$ in the range of ambient CO_2 concentration (c_a) from 0 to 1000 $\mu mol\ mol^{-1}$. The dependence of P_N and g_s on irradiance (I) was measured at c_a of 350 $\mu mol\ mol^{-1}$ in the range of I from 0 to 800 $\mu mol\ m^{-2} s^{-1}$.

Mathematical model of gas exchange: Net photosynthetic rate (P_N), stomatal conductance (g_s), and intracellular CO_2 concentration (c_i) were computed from gas exchange rates. The P_N-c_i response curves were processed with the FOTOS computer program (Pirochtová and Marek 1991) using the convexity equation (Thornley 1976, Kotvalt and Hák 1987):

$$\Theta P_N^2 - P_N [\tau (c_i - \Gamma_c) + P_{NsatC}] + \tau P_{NsatC} (c_i - \Gamma_c) = 0$$

where Θ is the convexity of the curve (saturation rate), τ is the initial slope of the curve (carboxylation efficiency), Γ_c is CO_2 compensation concentration and P_{NsatC} is net photosynthetic rate saturated by CO_2. The equation for P_N-I response curve is analogous, where c_i is replaced by I and τ is replaced by α (photochemical efficiency of CO_2 uptake: $\alpha = \tau^{-1}$). The values obtained correspond to the limitation theory. In the processing of P_N/c_i response curve (under constant irradiance), the τ expresses limitation of photosynthesis by RuBPC carboxylation capacity, and P_{NsatC} limitation of photosynthesis by RuBP regeneration capacity. In the processing of P_N-I response curve (under constant CO_2 concentration), the initial slope of response curve (α) expresses limitation of photosynthesis by RuBP regeneration capacity and P_{NsatI} limitation of photosynthesis by RuBPC capacity (Marek *et al.* 1995, 1997, Urban *et al.* 1999).

Statistical processing of all results was made by analysis of variances (*ANOVA*) according to Anděl (1985).

Results

Growth: At 0 DAT, LA and DM of roots, stems and leaves were lower in -CO_2 plants and higher in +CO_2 plants than in controls. Similar differences were found also at 8 and 22 DAT, but differences among variants in LA rose with time, while in DM declined (Table 1). LA/LM ratio and LAR were highest in -CO_2 plants and lowest in +CO_2 plants at 0 DAT, but they were lowest in -CO_2 plants and highest in +CO_2 plants at 22 DAT. In the interval 8 to 22 DAT NAR was significantly higher in -CO_2 plants than in +CO_2 plants. Differences in relative growth rate was not found (data not shown). S/R ratio in +CO_2 plants was significantly higher than in -CO_2 plants only at 0 DAT, other differences in S/R were not significant (Table 1). The differences were found also in the development of tillers: +CO_2 plants had higher number of tillers and their LA and DM than C and -CO_2 plants at both 8 and 22 DAT (Table 1).

Table 1. Effects of CO_2 concentration during early phase of ontogeny on plant growth traits measured at 0, 8, and 22 DAT. LA - leaf area, DM - dry mass, LAR - leaf area ratio, LA/LM ratio - leaf area/mass ratio, NAR - net assimilation rate, S/R - shoot/root ratio, DAT - days after CO_2 treatment. Means ± SE, $n = 6 - 8$, different letters denote significant difference between CO_2 treatments at $P = 0.05$, asterisks at $P = 0.01$.

	DAT	$-CO_2$	C	$+CO_2$
LA of whole plant [cm^2]	0	13.5 ± 0.54 a*	17.0 ± 0.47 b*	20.6 ± 1.02 c*
	8	35.4 ± 2.80 a	46.7 ± 2.67 b	60.3 ± 2.13 c*
	22	199.7 ± 6.23 a*	330.4 ± 19.66 b*	459.8 ± 37.96 c*
DM of whole plant [mg]	0	63.2 ± 1.20 a*	84.4 ± 2.99 b*	114.7 ± 5.61 c*
	8	174.2 ± 12.46 a	229.7 ± 14.98 b	278.4 ± 8.48 c
	22	756.8 ± 7.89 a*	994.0 ± 43.76 b	1132.5 ± 33.42 c
DM of roots [mg]	0	13.4 ± 0.49 a	17.8 ± 0.60 b	23.0 ± 1.72 c*
	8	28.5 ± 2.18 a	38.8 ± 2.79 b	45.1 ± 1.70 b
	22	150.6 ± 3.25 a	176.5 ± 16.29 ab	211.7 ± 6.28 b
DM of stems [mg]	0	13.2 ± 0.58 a*	19.2 ± 1.01 b*	28.4 ± 1.64 c*
	8	44.6 ± 4.00 a	60.8 ± 5.07 b	80.6 ± 2.56 c*
	22	224.8 ± 4.18 a*	281.6 ± 10.47 b*	346.5 ± 13.71 c*
DM of leaves [mg]	0	32.8 ± 0.64 a*	43.1 ± 1.37 b*	58.6 ± 2.36 c*
	8	96.6 ± 6.21 a	126.4 ± 7.62 b	148.5 ± 5.94 b
	22	377.7 ± 5.97 a*	530.4 ± 22.33 b	570.1 ± 14.93 b
LAR [cm^2 mg^{-1}]	0	0.214 ± 0.0063 a	0.202 ± 0.0050 b	0.179 ± 0.0013 c*
	8	0.203 ± 0.0066 ns	0.204 ± 0.0046 ns	0.217 ± 0.0089 ns
	22	0.264 ± 0.0057 a	0.331 ± 0.0060 b	0.403 ± 0.0212 c
LA/LM ratio [cm^2 mg^{-1}]	0	0.413 ± 0.0171 a	0.395 ± 0.0072 b	0.351 ± 0.0069 c
	8	0.367 ± 0.0155 ns	0.370 ± 0.0049 ns	0.408 ± 0.0181 ns
	22	0.528 ± 0.0093 a	0.621 ± 0.0117 b	0.801 ± 0.0464 c*
NAR [mg cm^{-2} d^{-1}]	0 - 8	0.609 ± 0.0577 ns	0.608 ± 0.0498 ns	0.549 ± 0.0388 ns
	8 - 22	0.443 ± 0.0217 a	0.378 ± 0.0139 b	0.313 ± 0.0061 c
S/R ratio	0	3.45 ± 0.10 a	3.50 ± 0.07 ab	3.86 ± 0.16 b
	8	4.98 ± 0.15 ns	4.86 ± 0.21 ns	5.11 ± 0.24 ns
	22	4.01 ± 0.13 ns	4.73 ± 0.31 ns	4.33 ± 0.04 ns
Number of tillers	8	0.67 ± 0.333 a	1.50 ± 0.224 ab	1.83 ± 0.167 b
	22	4.33 ± 0.211 a	5.00 ± 0.365 a	6.67 ± 0.211 b*
tillers/main shoot DM ratio	8	0.01 ± 0.007 a	0.08 ± 0.016 b	0.13 ± 0.012 c
	22	0.69 ± 0.024 a	0.84 ± 0.063 a	1.04 ± 0.051 b
tillers/main shoot LA ratio	8	0.05 ± 0.033 a	0.18 ± 0.047 b	0.39 ± 0.063 c
	22	2.10 ± 0.201 a	2.75 ± 0.188 a	4.53 ± 0.383 b*

Chlorophyll and carotenoid contents: In the 1st leaf, low CO_2 treatment decreased Chl *a*, Chl *b*, Chl *a*+*b* and Car contents at 0 and 5 - 8 DAT in comparison with control. Also high CO_2 treatment decreased all pigment contents (except Chl *b*) in the 1st leaf, but less than in the low CO_2. In the 4th leaf at 12 - 22 DAT, pigment contents were lower $+CO_2$ plants than in $-CO_2$ plants but the differences from C plants were not significant (Table 2). Chl *a*/*b* ratio was decreased in $+CO_2$ plants in the 1st leaf, but only a tendency to increase it in $-CO_2$ plants was found (Table 2). Differences in Chl/Car ratio were insignificant (data not shown).

Stomatal density: Low CO_2 treatment increased and high CO_2 treatment decreased stomatal densities of adaxial and abaxial epidermes of the 1st leaf. This was especially marked at 0 DAT. In the 4th leaf, stomatal density did not change (Table 3).

Dependence of stomatal conductance and net photosynthetic rate on internal CO_2 concentration: At 0 DAT, g_s in $-CO_2$ plants was higher than g_s in $+CO_2$ plants; differences from C plants were not significant (Fig. 1*A*). P_N in $+CO_2$ plants was lower than P_N in C, especially at high c_i, other differences in P_N were insignificant (Fig. 1*D*). P_{NsatC} was lower in both $+CO_2$ and $-CO_2$ plants in comparison with C plants, τ was significantly decreased and Γ_C increased only in $+CO_2$ plants (Table 4).

At 5 - 8 DAT, g_s and P_N in $-CO_2$ plants were higher, and g_s and P_N in $+CO_2$ plants were lower than g_s and P_N in C plants (Figs. 1*B*,*E*). P_{NsatC} and τ increased in $-CO_2$ plants, and decreased in $+CO_2$ plants. Γ_C increased, and R_L decreased in $+CO_2$ plants; differences in Γ_C and R_L between $-CO_2$ plants and C plants were insignificant (Table 4).

At 12 - 22 DAT, differences among g_s were insignificant in all the variants (Fig. 1*C*). At high c_i, P_N in -CO_2 plants was higher than P_N in C plants and in +CO_2 plants; at low c_i, P_N in +CO_2 plants was lower than P_N in C plants and in -CO_2 plants (Fig. 1*F*). P_{NsatC} was increased in -CO_2 plants, and τ was decreased in +CO_2 plants. R_L was decreased in +CO_2 plants, and Γ_C was not changed in all the variants (Table 4). No differences in Θ among the variants were found in all DAT (Table 4).

Table 2. Effects of CO_2 concentration during early phase of ontogeny on contents of photosynthetic pigments [μg cm^{-2}]. The 1st leaf was measured at 0 and 5 - 8 DAT, the 4th leaf at 12 - 22 DAT. Means ± SE, *n* = 6 - 13, different letters denote significant difference between CO_2 treatments at *P* = 0.05, asterisks at *P* = 0.01.

Pigment contents or ratio	DAT	-CO_2	C	+CO_2
Chlorophyll *a* content	0	25.10 ± 0.59 a	29.70 ± 0.47 b	27.30 ± 0.63 c
	5 - 8	27.60 ± 0.63 a	29.80 ± 0.49 b	27.50 ± 0.47 a
	12 - 22	31.20 ± 0.87 a	27.50 ± 1.38 ab	25.70 ± 1.64 b
Chlorophyll *b* content	0	6.69 ± 0.15 a*	8.35 ± 0.12 b	8.10 ± 0.15 b
	5 - 8	7.36 ± 0.21 a*	8.48 ± 0.11 b	8.21 ± 0.12 b
	12 - 22	7.86 ± 0.37 a	6.79 ± 0.39 ab	6.40 ± 0.40 b
Chlorophyll *a* + *b* content	0	32.10 ± 0.71 a*	38.10 ± 0.57 b	35.40 ± 0.76 c
	5 - 8	35.00 ± 0.71 a	38.20 ± 0.56 b	35.70 ± 0.55 a
	12 - 22	39.00 ± 0.99 a	34.30 ± 1.73 ab	32.10 ± 2.03 b
Carotenoid content	0	5.96 ± 0.14 a*	7.38 ± 0.14 b	6.72 ± 0.15 c
	5 - 8	6.57 ± 0.26 a	7.46 ± 0.12 b	6.90 ± 0.16 ab
	12 - 22	7.57 ± 0.25 a	7.03 ± 0.32 ab	6.26 ± 0.34 b
Chlorophyll *a*/*b* ratio	0	3.61 ± 0.06 a	3.56 ± 0.04 a	3.37 ± 0.05 b
	5 - 8	3.76 ± 0.12 a	3.51 ± 0.05 ab	3.36 ± 0.04 b
	12 - 22	4.04 ± 0.23 ns	4.07 ± 0.10 ns	4.02 ± 0.08 ns

Table 3. Effects of CO_2 concentration during early phase of ontogeny on stomatal density. The 1st leaf was measured at 0 and 5 - 8 DAT, the 4th leaf at 12 - 22 DAT. Means ± SE, *n* = 50 - 150, different letters denote significant difference between CO_2 treatments at *P* = 0.05, asterisks at *P* = 0.01.

	DAT	-CO_2	C	+CO_2
abaxial side [mm^{-2}]	0	29.4 ± 0.39 a*	25.9 ± 0.34 b*	23.7 ± 0.38 c*
	5 - 8	25.8 ± 0.60 a	24.6 ± 0.55 ab	23.3 ± 0.57 b
	12 - 22	24.8 ± 0.38 ns	25.3 ± 0.43 ns	25.6 ± 0.44 ns
adaxial side [mm^{-2}]	0	45.9 ± 0.67 a*	40.1 ± 0.50 b*	37.1 ± 0.51 c*
	5 - 8	43.4 ± 0.53 a*	39.7 ± 0.42 b	36.6 ± 0.44 c
	12 - 22	38.5 ± 0.43 ns	38.6 ± 0.43 ns	38.1 ± 0.49 ns

Dependence of stomatal conductance and net photosynthetic rate on irradiance: In contrast to previous experimental series, at 0 DAT, g_s in -CO_2 plants was lower than that in C plants and in +CO_2 plants, but g_s in C plants and in +CO_2 plants did not differ (Fig. 2*A*). P_N in +CO_2 plants was lower than in C plants and in -CO_2 plants, especially at high I, while differences between P_N in C plants and in -CO_2 plants were insignificant (Fig. 2*D*). P_{NsatI} was decreased in +CO_2 plants, while P_{NsatI} in C plants and in -CO_2 plants did not differ (Table 5).

At 5 - 8 DAT, g_s and P_N in -CO_2 plants were higher, and g_s and P_N in +CO_2 plants were lower than g_s and P_N in C plants (Figs. 2*B*,*E*). P_{NsatI} was increased in -CO_2 plants, and decreased in +CO_2 plants; α was decreased in -CO_2 plants, and increased in +CO_2 plants. Γ_I was increased in +CO_2 plants, while differences in Γ_I between -CO_2 plants and C plants were insignificant (Table 5).

At 12 - 22 DAT, any difference among g_s in all the variants was not significant (Fig. 2*C*). At low I, P_N in -CO_2 plants was higher than in C plants and in +CO_2 plants; at high I, P_N in +CO_2 plants was lower than in C plants and in -CO_2 plants (Fig. 2*F*). P_{NsatI} was decreased in +CO_2 plants, and α was decreased in -CO_2 plants. R_D was increased in -CO_2 plants, and Γ_I was not changed in all the variants (Table 5).

Discussion

At low CO_2 and saturating irradiance, P_N is limited by the diffusion of CO_2 from ambient air to sites of carboxylation, and by the capacity of RuBPCO to carboxylate RuBP. The slope of initial response of P_N to c_i (often termed carboxylation efficiency - τ) is dependent on RuBPC activity. It progressively declines as c_i increases (usually above 200 μmol mol^{-1}). P_N is enhanced by increasing c_i to a value (about 1 000 μmol mol^{-1}) when the capacity for RuBP regeneration is limiting. P_N may be also limited by consumption of triosophosphates, but we do not suppose this type of limitation in our experimets, because it occurs only at very high CO_2 and irradiance (for reviews see, *e.g.*, Sage 1994, Drake *et al.* 1997). In addition to directly increasing the rate of carboxylation, increased CO_2 concentration increases the rate of CO_2 fixation by depressing RuBP oxygenation and thus photorespiration.

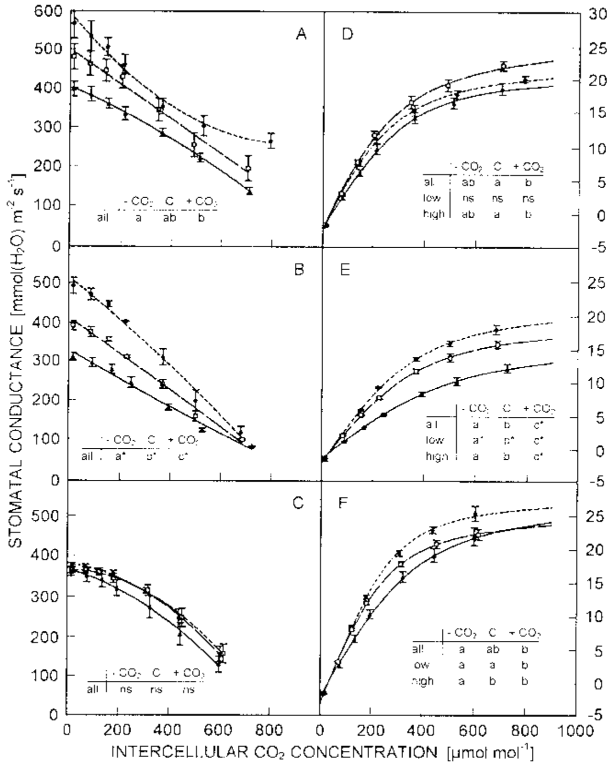

Fig. 1. Effects of different CO_2 concentrations at early phase of ontogeny ($-CO_2$ plants - *filled diamonds, short-dashed line*, C plants - *empty squares, long-dashed line*, and $+CO_2$ plants - *filled triangles, solid line*) on stomatal conductance (*A* - *C*) and net photosynthetic rate (*D* - *F*) in dependence on intercellular CO_2 concentration (c_i) measured at irradiance of 500 μmol m^{-2} s^{-1}. 1st leaf was measured at 0 (*A*,*D*) and 5 - 8 DAT (*B*,*E*). 4th leaf at 12 - 22 DAT (*C*,*F*). Means ± SE, $n = 4 - 5$. different letters in little table in each sub-figure means significant difference between CO_2 treatments at $P = 0.05$. asterisks at $P = 0.01$. Rows represent intervals of c_i during measurement for *ANOVA* analysis: low - lower than 300 μmol mol^{-1}, high - higher than 300 μmol mol^{-1}. all - whole range of c_i.

Since natural CO_2 concentration is not saturating for the photosynthesis of C_3 plants, a short-term enhancement of CO_2 concentration increases P_N. However, the long-term exposure to increased CO_2 concentration did not always increase P_N. On the contrary, increased CO_2 concentration often lead to down-regulation of photosynthesis (for reviews see, *e.g.*, Allen 1994, Reining 1994, Sage and Reid 1994, Sharkey 1994). Also in our experiments P_N in $+CO_2$ plants was lower than P_N of C and $-CO_2$ plants when measured under the same ambient CO_2 concentration.

One of the causes of the down-regulation of photosynthesis may be decreased stomatal density and/or partial stomata closure reducing the CO_2 transport to the sites of carboxylation. Stomatal density at 0 and 5 - 8 DAT was lower in $+CO_2$ plants than in C plants. These results were in agreement with data reported in the literature for many plant species (for review see, *e.g.*, Woodward and Kelly 1995, Drake *et al.* 1997, Pospíšilová and Čatský 1999a,b), however, in wheat Estiarte *et al.* (1994), Tuba *et al.* (1994), and Greiner de Mothes (1996) did not found changes in stomatal density induced by CO_2 enrichment. Nevertheless, decreased g_s in wheat plants grown under elevated CO_2 concentration was observed by Masle *et al.* (1990), Šantrůček *et al.* 1994, Tuba *et al.* 1994, Samarakon *et al.* 1995, and Greiner de Mothes (1996). Similarly in our experiments, g_s was mostly lower in $+CO_2$ plants. The exception was g_s at 0 DAT in experiment following dependence of g_s on I. The reason could be the temporary wilting due to much lower relative humidity and higher irradiance in the chamber of gas-exchange system than in the cultivation chambers.

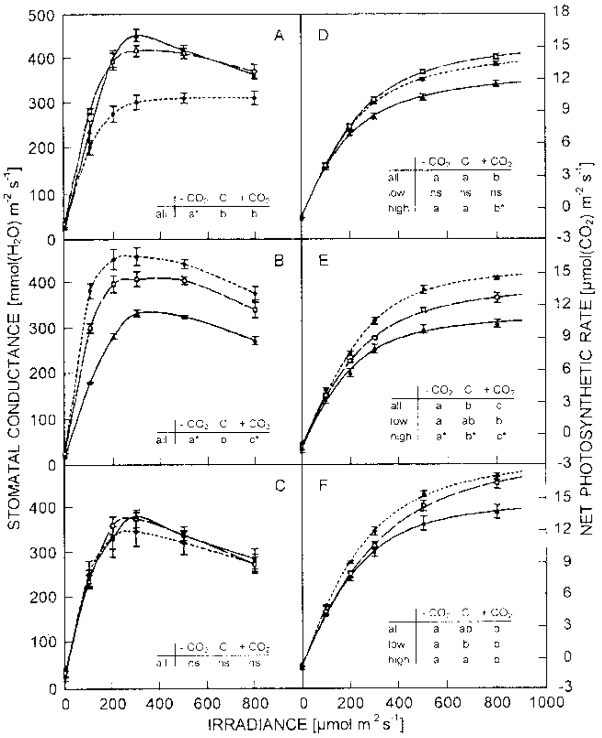

Fig. 2. Effects of CO_2 concentration at early phase of ontogeny ($-CO_2$ plants - *filled diamonds, short-dashed line*, C plants - *empty squares, long-dashed line*, and $+CO_2$ plants - *filled triangles, solid line*) on stomatal conductance (*A* - *C*) and net photosynthetic rate (*D* - *F*) in dependence on irradiance (I) measured at natural atmospheric CO_2 concentration of 350 μmol mol^{-1}. 1st leaf was measured at 0 (*A,D*) and 5 - 8 DAT (*B,E*), 4th leaf at 12 - 22 DAT (*C,F*). Means ± SE, n = 4 - 5, different letters in little table in each sub-figure means significant difference between CO_2 treatments at $P = 0.05$, asterisks at $P = 0.01$. Rows represent intervals of I during measurement for *ANOVA* analysis: low - lower than 300 μmol m^{-2} s^{-1}, high - higher than 300 μmol m^{-2} s^{-1}, all - whole range of I.

The values obtained from FOTOS programme showed that $+CO_2$ treatment decreased both the RuBPCO carboxylation capacity and the RuBP regeneration capacity (Tables 4, 5). The depression of P_{Nsat} as acclimation to high CO_2 concentration was observed by, *e.g.*, Šantrůček *et al.* (1994), Pearson and Brooks (1995), Šantrůčková *et al.* (1999), the depression of τ was observed *e.g.* by Sage *et al.* (1989) and Tuba *et al.* (1994). In addition, Chl *a* and Car contents were decreased in $+CO_2$ plants. The decrease in chlorophyll content under CO_2 enrichment was observed, *e.g.*, by Patterson and Flint (1982) and Tuba *et al.* (1994), the other authors did not report any difference (Campbell *et al.* 1990, Visser *et al.* 1997). Down-regulation of photosynthesis was also found to be associated with the accumulation of large amounts of sugars in wheat leaves (Ghildiyal *et al.* 1998), but this is not probable in our experiments, because plants were cultivated at relatively low irradiance.

In previous experiments focusing on the effects of subambient CO_2 concentration on P_N, only P_N *in situ* was usually measured and increase in P_N with increasing growth concentration from subambient to ambient was observed (*e.g.* Baker *et al.* 1990, Polley *et al.* 1992, 1993a,b, Cowling and Sage 1998). Therefore, a very important finding is the stimulation of P_N in $-CO_2$ plants when measured at natural CO_2 concentration. However, supplemental observations showed that $-CO_2$ plants were the very sensitive to the sudden decrease in air humidity, and they lose high amount of water. Therefore stimulation of P_N in these plants in comparison with controls was not observed at 0 DAT but only at 5 - 8 and 12 - 22 DAT. In $-CO_2$ plants at 5 - 8 DAT, the increased P_N was connected with higher stomatal density, higher g_s, and increased

RuBPCO carboxylation capacity and RuBP regeneration capacity, but the pigment contents were increased only at 12 - 22 DAT and decreased at 0 and 8 - 12 DAT. At 12 - 22 DAT, the increased P_N was connected with increased RuBP regeneration capacity, but not with stomatal density or g_s. Higher stomatal density and/or g_s in plants grown under subambient CO_2 concentration were reported, *e.g.*, by Woodward and Bazzaz (1988), Polley *et al.* (1996), and Cowling and Sage (1998). On the contrary, only slight or no changes were reported by Malone *et al.* (1993).

Table 4. Effects of CO_2 concentration during early phase of ontogeny on leaf photosynthesis (model analysis of P_N - c_i response curves using FOTOS programme). The 1st leaf was measured at 0 and 5 - 8 DAT, the 4th leaf at 12 - 22 DAT. P_{NsatC} - CO_2 saturated net photosynthetic rate, R_S - rate of CO_2 evolution in the light at zero c_i, Γ_C - compensation CO_2 concentration, τ - carboxylation efficiency. Θ - saturation rate.. Means ± SE, $n = 4 - 5$, different letters denote significant difference between CO_2 treatments at $P = 0.05$, asterisks at $P = 0.01$.

	DAT	$-CO_2$	C	$+CO_2$
P_{NsatC} [μmol m^{-2} s^{-1}]	0	23.9 ± 0.85 a	28.1 ± 0.58 b	22.2 ± 1.06 a
	5 - 8	21.9 ± 0.75 a	19.8 ± 0.36 b	16.8 ± 0.33 c*
	12 - 22	29.1 ± 0.48 a	25.5 ± 0.83 b	28.4 ± 0.97 ab
R_S [μmol m^{-2} s^{-1}]	0	2.80 ± 0.110 ns	2.76 ± 0.086 ns	2.59 ± 0.189 ns
	5 - 8	2.46 ± 0.154 a	2.18 ± 0.098 a	1.67 ± 0.104 b
	12 - 22	3.71 ± 0.125 a	3.44 ± 0.120 a	2.67 ± 0.208 b
Γ_C [μmol mol^{-1}]	0	34.3 ± 0.66 a	31.5 ± 0.78 a	39.6 ± 1.21 b*
	5 - 8	40.9 ± 2.43 a	42.9 ± 1.33 a	52.9 ± 3.05 b
	12 - 22	34.8 ± 0.68 ns	34.5 ± 0.98 ns	35.9 ± 2.61 ns
τ [mol m^{-2} s^{-1}]	0	0.082 ± 0.0020 a	0.088 ± 0.0030 a	0.066 ± 0.0035 b*
	5 - 8	0.059 ± 0.0035 a	0.051 ± 0.0007 b	0.031 ± 0.0003 c*
	12 - 22	0.107 ± 0.0038 a	0.100 ± 0.0015 a	0.076 ± 0.0080 b
Θ	0	0.671 ± 0.0633 ns	0.607 ± 0.0761 ns	0.781 ± 0.0280 ns
	5 - 8	0.761 ± 0.0548 ns	0.737 ± 0.0224 ns	0.734 ± 0.0512 ns
	12 - 22	0.761 ± 0.0302 ns	0.780 ± 0.0241 ns	0.752 ± 0.0776 ns

Table 5. Effects of CO_2 concentration during early phase of ontogeny on leaf photosynthesis (model analysis of P_N – I response curves using FOTOS programme). The 1st leaf was measured at 0 and 5 - 8 DAT, the 4th leaf at 12 - 22 DAT. P_{NsatI} - irradiance saturated net photosynthetic rate. R_D - rate of dark respiration Γ_I - compensation irradiance. α - photochemical efficiency of CO_2 uptake. Θ - saturation rate. Means ± SE. $n = 4 - 5$. different letters denote significant difference between CO_2 treatments at $P = 0.05$, asterisks at $P = 0.01$.

	DAT	$-CO_2$	C	$+CO_2$
P_{NsatI} [μmol m^{-2} s^{-1}]	0	15.5 ± 0.41 a	16.2 ± 0.28 a	13.6 ± 0.46 b
	5 - 8	16.3 ± 0.24 a	15.0 ± 0.37 b	11.7 ± 0.24 c*
	12 - 22	20.2 ± 0.28 a	20.8 ± 0.48 a	15.5 ± 0.67 b
R_D [μmol m^{-2} s^{-1}]	0	1.00 ± 0.010 ns	1.01 ± 0.012 ns	1.05 ± 0.020 ns
	5 - 8	1.28 ± 0.039 ns	1.32 ± 0.031 ns	1.49 ± 0.086 ns
	12 - 22	1.16 ± 0.090 a	0.95 ± 0.024 ab	0.95 ± 0.010 b
Γ_I [μmol m^{-2} s^{-1}]	0	18.2 ± 0.83 ns	18.6 ± 0.76 ns	18.0 ± 0.35 ns
	5 - 8	23.9 ± 1.00 a	24.9 ± 1.17 a	29.9 ± 0.86 b
	12 - 22	17.6 ± 0.89 ns	17.3 ± 1.29 ns	18.2 ± 0.59 ns
α [mol(phot.) mol^{-1}(CO_2)]	0	18.1 ± 0.68 ns	18.3 ± 0.67 ns	17.1 ± 0.22 ns
	5 - 8	18.8 ± 0.47 a	19.5 ± 0.09 b	20.2 ± 0.04 c
	12 - 22	15.3 ± 0.47 a	18.1 ± 0.83 b	19.1 ± 0.48 b
Θ	0	0.675 ± 0.0264 ns	0.701 ± 0.0929 ns	0.599 ± 0.0227 ns
	5 - 8	0.697 ± 0.0452 ns	0.636 ± 0.0565 ns	0.686 ± 0.0172 ns
	12 - 22	0.643 ± 0.0182 ns	0.591 ± 0.0145 ns	0.751 ± 0.0407 ns

Several results indicate a tendency to an increase or a decrease in respiration rate of -CO_2 and +CO_2 plants, respectively, but the differences were significant only in few measurements. The effect of elevated CO_2 concentration on respiration was reported, *e.g.*, by Gifford *et al.* (1985) and Bunce (1994).

Photosynthetic parameters were measured on the 1st leaf which developed under different CO_2 treatments at 0 DAT and 5 - 8 DAT. As these parameters (absolute values as well as the differences among treatments) showed great similarity in these two sampling dates, we can suppose that the CO_2 concentration at the beginning of leaf ontogeny is very important for its further functioning. This was in agreement with previous experiments with bean plants (Čatský *et al.* 1995). Some differences in gas exchange induced by different CO_2 treatments were even found in the 4th leaf which emerged after finishing CO_2 treatment, *e.g.*, the increased P_N, RuBP regeneration capacity, and Chl content in -CO_2 plants or the decreased P_N and RuBPCO carboxylation capacity in +CO_2 plants. In the 4th leaf, it was not demonstrated any change in stomatal conductance and in stomatal density. Properties of stomata are probably less stable than those of photosynthetic apparatus because also changes in g_s induced by growth under high air humidity disappeared immediately after transfer to low air humidity and *vice versa* (Pospíšilová 1996).

The growth parameters of plants during different CO_2 treatments were affected by different P_N *in situ* and assimilate distribution. The increased growth under CO_2 enrichment and the decrease under CO_2 starvation are well known. Changes in growth parameters found in wheat were in agreement, *e.g.*, with changes in soybean induced by growth under six CO_2 concentrations ranging from 160 to 990 μmol mol^{-1} (Allen *et al.* 1990). The differences in growth parameters in wheat continued also after finishing CO_2 treatment. Only LA/M ratio, LAR and NAR were lower in +CO_2 plants than in -CO_2 plants. The differences in NAR were probably due to corresponding differences in P_N. Imai and Murata (1978a,b) reported similar differences in NAR and also in gas exchange parameters.

In this paper, we proved down-regulation and up-regulation of photosynthesis induced by growth at low and high CO_2 concentrations, respectively. In these acclimations, both the stomatal and biochemical limitations of photosynthesis took part. It was also demonstrated that changes in physiological traits induced in very early phase of ontogeny persisted also after finishing CO_2 treatment when plants grew under the same conditions. Some differences were found not only in leaves directly affected by different CO_2 concentration, but also in leaves emerged on plants after finishing CO_2 treatments.

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