PHOTOSYNTHETICA 36 (4): 533-545, 1999

Seasonal changes of selected parameters of CO_2 fixation biochemistry of Norway spruce under the long-term impact of elevated CO_2

O. URBAN and M.V. MAREK

Laboratory of Ecological Physiology of Forest Trees, Institute of Landscape Ecology, Academy of Sciences of the Czech Republic, Poříčí 3b, CZ-603 00 Brno, Czech Republic

Abstract

Twelve-year-old Norway spruce (*Picea abies* [L.] Karst.) trees were exposed to ambient (AC) or elevated (EC) [ambient + 350 μmol(CO_2) mol^{-1}] CO_2 concentrations in open-top-chamber (OTC) experiment under the field conditions of a mountain stand. Short-term (4 weeks, beginning of the vegetation season) and long-term (4 growing seasons, end of the vegetation season) effects of this treatment on biochemical parameters of CO_2 assimilation were evaluated. A combination of gas exchange, fluorescence of chlorophyll *a*, and application of a mathematical model of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) activity was used. The analysis showed that the depression of photosynthetic activity by long-term impact of elevated CO_2 was mainly caused by decreased RuBPCO carboxylation rate. The electron transport rate as well as the rate of ribulose-1,5-bisphosphate (RuBP) formation were also modified. These modifications to photosynthetic assimilation depended on time during the growing season. Changes in the spring were caused mainly by local deficiency of nitrogen in the assimilating tissue. However, the strong

Received 15 February 1999, *accepted* 15 May 1999.
Fax: +420 5 43211560, e-mail: otmar@brno.cas.cz
Abbreviations: Γ* - compensation CO_2 concentration in absence of photorespiration [μmol(CO_2) mol^{-1}]; AC (EC) - treatment exposed to ambient (elevated) CO_2 concentration; ATP – adenosine triphosphate; C_a (C_i, C_c) - ambient (internal, chloroplastic) CO_2 concentration [μmol(CO_2) mol^{-1}]; C_{ip} – transition CO_2 concentration from RuBPCO to RuBP regeneration limitation [μmol(CO_2) mol^{-1}]; Chl - chlorophyll; J_{max} – maximal electron transport rate [μmol m^{-2} s^{-1}]; OTC – open-top-chamber; P_N - net rate of CO_2 assimilation [μmol(CO_2) m^{-2} s^{-1}]; P_{NR} (P_{NJ}) – CO_2 assimilation rate limited by RuBPCO activity (RuBP regeneration) [μmol(CO_2) m^{-2} s^{-1}]; R_d^* - rate of non-photorespiratory CO_2 efflux in the light [μmol(CO_2) m^{-2} s^{-1}]; RuBPCO – ribulose-1,5-bisphosphate carboxylase/oxygenase; S* - the apparent specificity factor of RuBPCO *in vivo* [mol mol^{-1}]; V_{Cmax} (V_{Omax}) – maximal rate of RuBPCO carboxylation (oxygenation) [μmol(CO_2)(O_2) m^{-2} s^{-1}]; V_{RuBP} - rate of RuBP consumption and formation [μmol m^{-2} s^{-1}].
Acknowledgement: The work forms a part of the research supported by grant no. 522/96/1106 of the Grant Agency of the Czech Republic, EN4-CT95-PL951544 of the EU (ECOCRAFT), and contract no. OK 127 of the Ministry of Education of the Czech Republic.

depression of assimilation observed in the autumn months was the result of insufficient carbon sink capacity.

Additional key words: acclimation depression; carbon sink; CO_2 assimilation; elevated CO_2 concentration; electron transport; *Picea abies;* ribulose-1,5-bisphosphate carboxylase/oxygenase.

Introduction

Many previous papers and reviews discussed how the photosynthetic apparatus acclimates to rising atmospheric CO_2 concentrations in time and spatial scale. Plants change their net photosynthetic rate (P_N) in response to an increase of CO_2 concentration (Eamus and Jarvis 1989, Ceulemans and Mousseau 1994, Webber *et al.* 1994, Ceulemans 1997); the degree of this reaction is very variable, and depends on species, growing conditions, mineral nutrition status, and duration of CO_2 enrichment.

Short-term exposure (period of days or weeks) of higher plants to elevated CO_2 often increases photosynthetic CO_2 uptake for two reasons: (*1*) suppressing of the photorespiration, and (*2*) insufficiency to saturate RuBPCO activity by the current atmospheric CO_2 concentration (Stitt 1991, Long and Drake 1992). However, after a long-term period (months or years) of exposure to elevated CO_2 some species reduce the P_N as a result of acclimation depression, referred also as a downward regulation (Kramer 1981, Marek *et al.* 1995).

Acclimation depression of photosynthesis may be a response to three main factors: (*1*) Reduction of carboxylation efficiency because of decrease of RuBPCO amount and/or activity (Sage *et al.* 1989) mainly associated with changes in nitrogen reallocation (Lloyd *et al.* 1995), RuBPCO gene expression (Winder *et al.* 1992), and carbonic anhydrase activity (Porter and Grodzinski 1984). (*2*) Increasing starch accumulation in chloroplasts which can lead to chloroplast disruption (Sasek *et al.* 1985, Marek *et al.* 1995). Also, an increase in content of phosphorylated intermediates that decreases cytosolic and subsequently chloroplast P_i pools may lead to inhibition of ATP synthesis, RuBP regeneration, and CO_2 assimilation (Besford 1990, Stitt 1991). The decrease in capacity to use absorbed radiant energy as a result of P_i limitation may lead to photoinhibion (Špunda *et al.* 1998) and, in the long-term, to damage and loss of specific membrane proteins (Besford *et al.* 1998). (*3*) Insufficient sinks for the increased amount of produced assimilates. When source synthesis of saccharides exceeds sink requirements, decreased investment of N into photosynthetic proteins may be expected (Webber *et al.* 1994). Moreover, increased investment into the non-photosynthetic organs, *e.g.*, root system, that increases the sink capacity has been observed (Eamus and Jarvis 1989, Stitt 1991, Opluštilová and Dvořák 1997).

Some differences in acclimation amongst different species may be explained by different degrees of intercellular limitation to CO_2 diffusion (Ceulemans 1997). For species such as Norway spruce, where photosynthesis is strongly limited by chloroplastic CO_2 concentration (Priwitzer *et al.* 1998), sensitive responses to elevated CO_2 concentration have been observed in absorption of radiant energy (Špunda *et al.* 1998), P_N (Marek *et al.* 1995, 1997), and in biomass allocation (Opluštilová and Dvořák 1997).

The aim of this paper is to demonstrate new characteristics of the photosynthetic apparatus in trees exposed to elevated double CO_2 concentration (EC) based on a combination of gas exchange and fluorescence measurement techniques and on a mathematical model, to enhance the interpretation of some results presented earlier by Marek *et al.* (1995), and to further explain changes of the reasons of acclimation depression for Norway spruce trees acclimated to EC during the vegetation season.

Materials and methods

Plants and experiment design: Impact of EC on physiological parameters of Norway spruce (*Picea abies* [L.] Karst.) trees (age 12 years, average height 2.5 m) treated in open-top-chambers (OTCs) (Janouš *et al.* 1996) was investigated at the Experimental Research Site Bílý Kříž in the Beskydy Mts. (Czech Republic, 49° 30′N, 18° 32′E, 908 m a.s.l.). Four OTCs contained air with AC [*ca.* 350 μmol(CO_2) mol^{-1}], four OTCs were supplied with EC [*ca.* 700 μmol(CO_2) mol^{-1}] from the spring 1992 to autumn 1995, except during the winter dormancy period (see Marek *et al.* 1995 for details).

Gas exchange measurements: A closed portable photosynthetic system with infra-red gas analyser *Li-6200* (*LI-COR*, USA) was used for measurement of the relationship between the rate of CO_2 assimilation and intercellular CO_2 concentrations (P_N-C_i) under saturating irradiance (1300 μmol m^{-2} s^{-1}). Estimation of the input biochemical model parameters was based on the analysis of the initial linear part of P_N-C_i relationship measured at two low irradiances (*ca.* 100 and 250 μmol m^{-2} s^{-1}) (Farquhar *et al.* 1980). Microclimatic conditions inside the assimilation chamber were kept constant during all the measurements (temperature of needles 20±2 °C, relative air humidity 55±3 %). Marek *et al.* (1995, 1997) gave detailed information about the measuring protocols.

Modulated chlorophyll (Chl) *a* fluorescence: The electron transport rate, required for P_N-C_c estimation, was calculated from the Chl *a* fluorescence (Genty *et al.* 1989). Chl *a* fluorescence was measured using portable chlorophyll fluorometers *PAM 101*, *102*, and *103* (*H. Walz*, Germany). Identical shoots were used for the measurements during the whole growing season. After the dark adaptation of needles (30 min) the original fluorescence value (F_0) was estimated. Following the period of 10 min continuous actinic irradiation (1300 μmol m^{-2} s^{-1}), a saturating pulse of "white light" (0.6 s, 3500 μmol m^{-2} s^{-1}) was applied for the estimation of the maximal level of fluorescence (F_M). Electron transport rate was determined according to Genty *et al.* (1989) using an absorption coefficient of 0.87 for Norway spruce needles (Špunda *et al.* 1993).

Mathematical model of photosynthesis: Photosynthetic gas exchange parameters can be identified with those obtained from biochemical assay by a biochemically based photosynthetic model (Farquhar *et al.* 1980, Caemmerer and Farquhar 1981). This model is based on the stochiometry of the carboxylation and electron transport processes and their relationship with CO_2 assimilation. The model predicts a change

in the P_N-C_i curve as the limitation to photosynthesis changes from RuBPCO (1) to RuBP regeneration (2):

$$P_{NR} = \frac{(C_i - \Gamma^*)\, V_{Cmax}}{C_i + K_C\,(1 + O/K_O)} - R_d^* \qquad (1)$$

$$P_{NJ} = \frac{(C_i - \Gamma^*)\, J_{max}}{4.5\,(C_i + 7/3\,\Gamma^*)} - R_d^* \qquad (2)$$

where P_{NR} (P_{NJ}) is the rate of CO_2 assimilation limited by RuBPCO activity (RuBP regeneration), C_i (O) intercellular CO_2 (O_2) concentration, K_C (K_O) Michaelis-Menten constants for CO_2 and O_2 at 20 °C, respectively, V_{Cmax} (J_{max}) maximal rates of carboxylation (electron transport) estimated by fitting of initial linear (saturated) part of P_N-C_i relationship, Γ^* (R_d^*) compensation CO_2 concentration (rate of CO_2 efflux in the light) in the absence of photorespiration (measured input parameters). The CO_2 concentration (C_{ip}) at the transition from RuBPCO activity to RuBP regeneration limitation occurs at $P_{NR} = P_{NJ}$.

A combination of fluorescence and leaf gas exchange techniques allows the calculation of CO_2 concentration at the catalytic site of RuBPCO (C_c) and subsequently the estimation of the dependence of the CO_2 assimilation rate on the CO_2 concentration at the catalytic site of RuBPCO (P_N-C_c) (Epron *et al.* 1995, Priwitzer *et al.* 1998). The model is based on the estimation of the apparent specificity factor of RuBPCO *in vivo* (S^*) from the stoichiometry of electron consumption for carboxylation and oxygenation cycles. The apparent specificity factor of RuBPCO *in vivo* at 20 °C was estimated following Lloyd *et al.* (1995).

Statistics: The P_N-C_i and P_N-C_c curves were fitted using the FOTOS programme (*e.g.*, Marek *et al.* 1995). For each treatment a set of 12 curves and related parameters were obtained and statistically processed. The statistical significance of differences of these parameters between the AC and EC variants were based on the F- and *t*-tests of the mean values. The analysis was carried out using the analytical tools in the EXCEL programme package.

Results

All the presented results were obtained from one-year-old shoots located in the S/SW parts of the crowns, because of their importance to the photosynthetic production and to minimise the effects of needle development during the vegetation season. An effect of EC on photosynthetic characteristics of Norway spruce shoots was investigated during the beginning of the second growing season (May 1993) and during the end of the fourth growing season (September 1995) of the CO_2 enrichment experiment.

May 1993–beginning of the growing season: The differences in P_N-C_c relationships between AC and EC variants (Fig. 1) correspond in detail to the earlier presented P_N-C_i response curves (Marek *et al.* 1995). Lower carboxylation efficiency (initial slope of the P_N-C_c curve) and higher rate of electron transport (RuBP regeneration-limited asymptote of the P_N-C_c curve) were estimated for the EC treatment shoots. Stomatal and intercellular limitations were derived from C_a, C_i, and C_c values (Epron *et al.* 1995) (Table 1). Slightly higher stomatal limitation of assimilation (20 %) and significantly lower intercellular limitation (56.7 %) to CO_2 diffusion were observed for the EC variant compared to the AC treatment (18.8 and 78.0 %, respectively). The supply function for the EC variant was lower by 42 % in comparison with the AC treatment (Table 1). Γ^* and R_d^* in the absence of photorespiration were significantly different for the AC and EC variants (Table 2). These changes depended on the duration of CO_2 enrichment. Short-term EC exposure significantly decreased Γ^* (16.6 %), while the R_d^* value was not changed. Maximal rates of carboxylation (V_{Cmax}) and electron transport (J_{max}) were lower for the AC variant by 14 and 9 %, respectively. Further, short-term exposure to EC caused a decrease of the maximal oxygenation rate, V_{Omax}, by 46 %, an increase of the RuBP regeneration rate, V_{RuBP}, by 29 %, and an increase of the value of the RuBPCO specificity factor *in vivo*, S^*, by 64 % (Table 3).

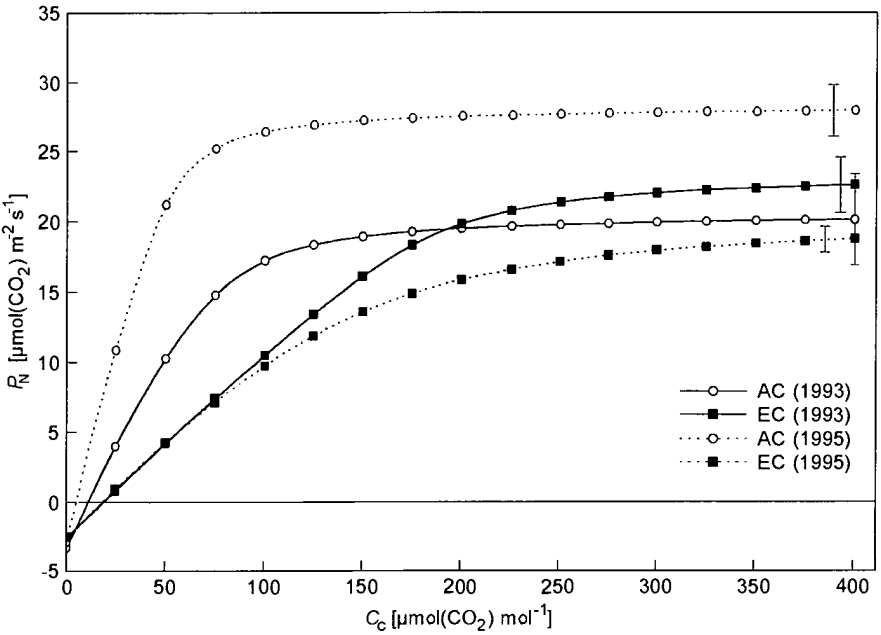

Fig. 1. The relationship between CO_2 concentration at the catalytic site of RuBPCO (C_c) and the rate of CO_2 assimilation uptake (P_N) under saturating irradiance (1300 μmol m^{-2} s^{-1}). AC – control open-top-chambers supplied with ambient CO_2; EC – open-top-chambers with ambient + 350 μmol(CO_2) mol^{-1}. The whisker bars represent ± SD; n = 12.

Table 1. Calculated mean values of CO_2 assimilation rate, P_N [μmol(CO_2) m^{-2} s^{-1}] at internal (C_i) and chloroplastic (C_c) CO_2 concentration [μmol(CO_2) mol^{-1}]. SF - supply function [dimensionless]. AC – control open-top-chambers supplied with ambient CO_2; EC – open-top-chambers with ambient + 350 μmol(CO_2) mol^{-1}.

		C_a	C_i at C_a	$P_N(C_i)$	C_c at C_i	$P_N(C_c)$	SF
1993	AC	350	284	13.7	62	20.0	0.0706
	EC	700	562	18.5	175	22.9	0.0408
1995	AC	350	263	9.3	110	16.7	0.0636
	EC	700	492	9.9	243	12.7	0.0258

September 1995–end of the growing season: P_N-C_c curves (Fig. 1) showed a strong acclimation depression of photosynthesis in the EC treatment (24 %), although the C_c concentration was higher (119 %) than in the AC variant (Table 1). This depression was caused by both decreased carboxylation and decreased electron transport rate (Table 3). Stomatal limitation of assimilation was again higher for the EC treatment (29.7 %) compared to the AC variant (24.9 %). The estimated value of intercellular limitation (64.4 %) was higher by 13.5 % than the intercellular limitation value after short-term EC exposure in May 1993. The long-term EC treatment caused significant increase of Γ^* and decrease of R_d^* (45 and 17 %, respectively) in comparison with the AC variant. Significant depression of V_{Cmax} (60 %), J_{max} (49 %), V_{Omax} (71 %), and V_{RuBP} (12 %), as well as increase of the J_{max}/V_{Cmax} ratio (25 %) were observed for the EC variant compared to the AC one (Table 3). The value of RuBPCO specificity factor decreased by 6.2 % for the EC treatment compared to AC (Table 3).

Calculated values of P_N limited by RuBPCO and RuBP regeneration (Fig. 2) showed distinct limitation to electron transport. Transition CO_2 concentrations (C_{ip}) were 197 and 548 μmol(CO_2) mol^{-1}, respectively, for the AC and EC variants at the beginning of the experiment in 1993. Significant shifts of these values were observed after the long-term exposure at the end of the 1995 growing season: 513 (AC) and 622 (EC) μmol(CO_2) mol^{-1}, respectively.

Table 2. Input parameters of the biochemical model of RuBPCO activity. Γ^* - compensation CO_2 concentration in absence of photorespiration [μmol(CO_2) mol^{-1}]; R_d^* - rate of non-photorespiratory CO_2 efflux in the light [μmol(CO_2) m^{-2} s^{-1}]; AC – control open-top-chambers supplied with ambient CO_2; EC – open-top-chambers with ambient + 350 μmol(CO_2) mol^{-1}. Means ± SD; the same letters indicate significant differences (at the level of 95 %); * highly significant difference (at the level of 99 %); n = 12.

		Γ^*	R_d^*
1993	AC	45.0 ± 1.8 a*	0.65 ± 0.09 e
	EC	38.0 ± 2.3 a*, c*	0.67 ± 0.01 f*
1995	AC	47.0 ± 3.1 b*	0.52 ± 0.06 d*, e
	EC	68.0 ± 3.5 b*, c*	0.43 ± 0.04 d*, f*

Table 3. Calculated parameters of the biochemical model of RuBPCO activity. V_{Cmax} - maximal rate of RuBPCO carboxylation [μmol(CO_2) m^{-2} s^{-1}]; J_{max} - maximal rate of electron transport [μmol(CO_2) m^{-2} s^{-1}]; V_{Omax} - maximal rate of RuBPCO oxygenation [μmol(O_2) m^{-2} s^{-1}]; V_{RuBP} - rate of ribulose-1,5-bisphosphate consumption and formation [μmol m^{-2} s^{-1}]; S^* - apparent specifity factor of RuBPCO *in vivo* [mol mol^{-1}]; AC – control open-top-chambers supplied with ambient CO_2; EC – open-top-chambers with ambient + 350 μmol(CO_2) mol^{-1}.

		V_{Cmax}	J_{max}	V_{Omax}	V_{RuBP}	J_{max}/V_{Cmax}	S^*
1993	AC	83.8	101.6	21.6	18.2	1.04	552.9
	EC	97.9	111.8	11.6	23.5	1.33	907.1
1995	AC	57.9	114.9	15.6	18.7	1.98	613.3
	EC	23.4	58.6	4.6	16.4	2.47	575.3

Discussion

Trees are characterised by their enormous potential for acclimation and adaptation, so it must be emphasised that "short-" and "long-term" are relative terms in relation to the life duration of a tree (Ceulemans and Mousseau 1994).

P_N-C_c relationship: The most convincing evidence for acclimation of photosynthesis in elevated CO_2 concentration comes from study of the *in situ* P_N-C_i response curve (Long 1991, Marek *et al.* 1995). Our P_N-C_c response curves also document and support ideas about acclimation depression of photosynthesis (Fig. 1). Slower increase of the initial part of the P_N-C_c curve for the EC variant in 1993 (Fig. 1), the document of RuBPCO kinetic changes, is mainly caused by inhibited photorespiration (Long and Drake 1992). Because short-term exposure to EC only slightly reduced stomatal conductance of Norway spruce trees (Marek 1998), C_i (Table 1) increased above the transition point (C_{ip}) of RuBPCO activity and capacity for RuBP regeneration co-limitation. Thus, on transfer of leaves from ambient to double CO_2 concentration, there will be an excess of RuBPCO activity (Long 1991). If control mechanisms allow optimisation of all steps of photosynthesis, a decline of RuBPCO activity would be expected. Decreased RuBPCO activity in relation to CO_2 influence was estimated by gas-exchange measurements from P_N-C_i (Marek *et al.* 1995) and P_N-C_c response curves (Fig. 1), as well as by direct isotope measurement of RuBPCO activity (Besford *et al.* 1990). RuBPCO activity decreased (Table 4) with the duration of CO_2 treatment (Marek 1998). Results of the measurements at the end of growing seasons showed that RuBPCO activity decreased by 12 % in 1993 for the EC variant, while it was 40 % lower in 1995 (Table 4). The loss of RuBPCO activity is often associated with nitrogen and phosphorus depletion in the photosynthetic tissue, which was also observed for Norway spruce needles (Table 4). The decreased requirement for RuBPCO in an elevated CO_2 atmosphere allows transfer of N investment from RuBPCO into RuBP regeneration, *i.e.*, into enzymes of the Calvin cycle and chloroplast membrane proteins of electron transport (Webber *et al.* 1994). The result of this was higher saturated CO_2 assimilation rate in comparison

Table 4. The concentration of selected elements and saccharides in needles in September 1993 and in September 1995 [g kg^{-1}(DM)]. Values of elements are estimated on structural mass basis (structural mass = *minus* saccharides); RuBPCO activity [μmol kg^{-1}(FM) s^{-1}]. AC – control open-top-chambers supplied with ambient CO_2; EC – open-top-chambers with ambient + 350 μmol(CO_2) mol^{-1}. Means ± SD; the same letters indicate significant differences (at the level of 95 %); * highly significant difference (at the level of 99 %); n = 12. Values from Marek (1998).

		N	P	K	Glucose	Saccharose	Starch	Total sacch.	RuBPCO
1993	AC	15.80±0.08 a	2.08±0.04 c	6.1±1.2	1.08±0.06 e	1.20±0.30	0.13±0.01 h	4.08±0.60 j*	32.5±0.3 l
	EC	14.30±0.06 a	1.82±0.05 c	5.2±0.9	2.23±0.07 e	1.80±0.40	0.15±0.01 h	5.42±0.70 j*	29.0±0.7 l
1995	AC	16.00±0.10 b*	2.11±0.06 d*	6.0±1.6	1.19±0.30 f*	1.35±0.30 g*	0.18±0.04 i*	4.25±1.01 k*	34.0±0.5 m*
	EC	13.80±0.08 b*	1.44±0.04 d*	5.5±1.7	3.00±0.50 f*	2.35±0.50 g*	0.48±0.02 i*	7.32±0.90 k*	20.2±0.8 m*

to trees planted at AC (Fig. 1, *solid lines*) in the spring 1993. Increased redistribution of nitrogen to the RuBP regeneration processes was supported by recalculation of the CO_2 assimilation rate per needle dry mass [$\mu mol(CO_2)\ kg^{-1}\ s^{-1}$] using specific leaf area (SLA) values (not shown). Smaller differences between the AC and EC variants were estimated for the RuBPCO limited phase and larger differences in the RuBP regeneration limited phase of CO_2 assimilation curves. Thus, the type of acclimation response observed in spring 1993 can be defined as strictly N-supply limited acclimation.

A different type of CO_2 uptake limitation was observed in autumn 1995 (Fig. 1, *dashed lines*). The end-product inhibition of photosynthetic metabolism by both P_i (31.8 %) and N (13.6 %) limitation (Table 4) together with decreased RuBPCO activation energy by elevated CO_2 concentration (Sage *et al.* 1989) indicates an excess of some components of the photosynthetic apparatus (Stitt 1991). This allows investment of assimilates to non-photosynthetic plant organs, such as the root system (Opluštilová and Dvořák 1997), and thus to increase the sink capacity. Because the P_N was decreased over the whole C_c interval (Fig. 1) for EC trees, both RuBPCO activity and capacity for RuBP regeneration were decreased. Thus, photosynthesis was strictly limited by insufficient sinks in this case (Webber *et al.* 1994). Long and Drake (1992) gave evidence for higher photosynthetic acclimation to elevated CO_2 concentration for plants that were least able to use additional saccharides in respiration, growth, and storage, *i.e.*, in sink-limited plants. From this point of view, the mentioned physiological reactions class Norway spruce as a strong sink-limited tree species (Marek *et al.* 1995, 1997, Opluštilová and Dvořák 1997, Špunda *et al.* 1998).

Moreover, complete data sets of P_N-C_i and P_N-C_c relationships obtained during three subsequent growing seasons (1993, 1994, 1995) in spring and autumn, presented by Marek (1998), show seasonal periodicity of downward regulation of photosynthesis caused by a deficiency of nitrogen (spring) and by lack of active sinks for increased production of assimilates (autumn). However, a small number of long-term exposure values (over three-years) in the literature show no similar trend for other coniferous or broadleaved tree species (Ceulemans and Mousseau 1994).

Biochemical photosynthesis model: Maximal rates of carboxylation (V_{Cmax}) and electron transport (J_{max}) were calculated from the initial linear slope and saturated part of the P_N-C_i response curves (Eqs. 1 and 2). Results presented earlier by Marek *et al.* (1995) did not discriminate separate parts of the P_N-C_i curves and thus must be interpreted as the actual rate values (Caemmerer and Farquhar 1981, Brooks and Farquhar 1985).

Results obtained in spring 1993 show differences in co-limitation of photosynthesis by RuBPCO activity and RuBP regeneration between the AC and EC variants (Fig. 2). The AC variant was characterised by larger limitation to the electron transport because P_{NJ} was saturated at lower C_{ip} in the AC treatment compared to the EC treatment.

By contrast, short-term EC exposure caused larger limitation to RuBPCO: P_{NJ} at saturating C_i concentration represents 75 % of the P_{NR} value for the EC variant,

while P_{NJ} represents only 46 % of P_{NR} for the AC treatment. This result again supports ideas about nitrogen reallocation from RuBPCO to enzyme systems connected with the utilisation of assimilates. Long-term EC treatment (autumn 1995) decreased differences of co-limitation by RuBP regeneration and RuBPCO activity: the P_{NJ} value at saturated C_i represented 84 % of P_{NR} value for the EC and 70 % for the AC variant.

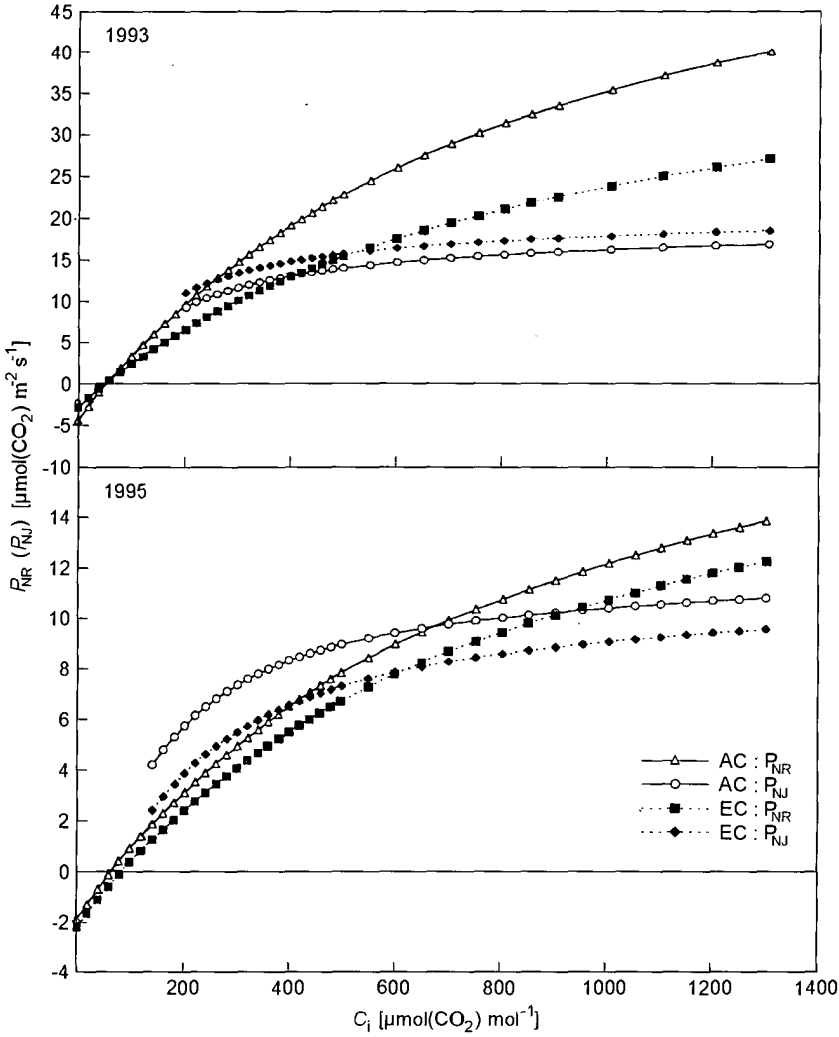

Fig. 2. Relationship between internal CO_2 concentration (C_i) and the rate of CO_2 assimilation limited by RuBPCO activity (P_{NR}) and RuBP regeneration (P_{NJ}) under saturating irradiance (1300 μmol m^{-2} s^{-1}). The curves correspond to the mean P_N-C_c curves. AC – control open-top-chambers supplied with ambient CO_2; EC – open-top-chambers with ambient + 350 μmol(CO_2) mol^{-1}.

The biochemical parameters (Table 3) showed significant time-depending seasonal decrease of V_{Omax}. The *in vivo* apparent specificity factor of RuBPCO (S^*) defines the relative rates of photosynthesis and photorespiration (Epron *et al.* 1995). Significantly higher S^* for the EC variant after short-term exposure supports the idea of strong depression of photorespiration at the RuBPCO level, and thus reduced N flux and amino acid synthesis in needles *via* decreased glycollate formation resulting from low RuBPCO oxygenase activity (Ceulemans and Mousseau 1994). Long-term EC exposure (four growing seasons) lead to decrease of the specificity factor S^* to the primary value in spring 1993 (Table 3). This decrease was mainly caused by the expressively decreased P_N (Table 2). It can be presumed that continuing EC treatment may lead to further decline in the value of S^*. Reduced P_N may reflect a lower N flux and larger reallocation of N (Table 4) from RuBPCO and other Calvin cycle enzymes, such as 3-phosphoglycerate kinase and NADP-3-phosphate-glyceraldehyde-dehydrogenase (Besford 1990). Local phosphorus deficiency in needles is associated with excess of production of assimilates (Table 4) and corresponds to RuBPCO decarboxylation owing to reduced activity of RuBPCO activase (Portis 1990).

Long-term exposure of trees to EC influenced the ratios between C_a, C_i, and C_c (Table 1). The significant increase of intercellular limitation to CO_2 diffusion for the EC variant may be caused by decrease in carbonic anhydrase activity that catalyzes the interconversion of CO_2 to HCO_3^-, and may facilitate diffusion of CO_2 from the intercellular air space to RuBPCO (Porter and Grodzinski 1984, Webber *et al.* 1994).

Trees growing in a forest stand are characterized by differentially formed assimilation apparatus induced by the distribution of photosynthetically active radiation within the canopy layer (Norman and Jarvis 1975). The RuBPCO synthesis (Winder *et al.* 1992, Webber *et al.* 1994), nitrogen distribution (Lloyd *et al.* 1995), and chloroplastic CO_2 concentration (Priwitzer *et al.* 1998) are related to the position within the canopy layer. Thus, different acclimation reactions for the sun and shade foliage under the influence of EC are presumable. It means that seasonal acclimation could be influenced by vertical distribution of some differences of the relations between assimilation function and EC effects. These circumstances should be entered to the models of forest carbon capacity destination.

References

Besford, R.T.: The greenhouse effect: Acclimation of tomato plants growing in high CO_2, relative changes in Calvin cycle enzymes. – J. Plant Physiol. **136**: 458-463, 1990.

Besford, R.T., Mousseau, M., Matteucci, G.: Biochemistry, physiology and biophysics of photosynthesis. - In: Jarvis, P.J. (ed.): European Forests and Global Change. The Likely Impacts of Rising CO_2 and Temperature. Pp. 29-78. Cambridge University Press, Cambridge 1998.

Brooks, A., Farquhar, G.D.: Effect of temperature on the CO_2/O_2 specificity of ribulose-1,5-bisphosphate carboxylase/oxygenase and the rate of respiration in the light. Estimates from gas-exchange measurements on spinach. – Planta **165**: 397-406, 1985.

Caemmerer, S. von, Farquhar, G.D.: Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. - Planta **153**: 376-387, 1981.

Ceulemans, R.: Direct impacts of CO_2 and temperature on physiological processes in trees. - In: Mohren, G.M.J., Kramer, K., Sabaté, S. (ed.): Impacts of Global Change on Tree Physiology and

Forest Ecosystems. Pp. 3-14. Kluwer Academic Publishers, Dordrecht - Boston - London 1997.

Ceulemans, R., Mousseau, M.: Effects of elevated atmospheric CO_2 on woody plants. – New Phytol. **127**: 425-446, 1994.

Eamus, D., Jarvis, P.G.: The different effects of increase in the global atmospheric CO_2 concentration on natural and commercial temperate trees and forest. - Adv. Ecol. Res. **19**: 1-55, 1989.

Epron, D., Godard, D., Cornic, G., Genty, B.: Limitation of net CO_2 assimilation rate by internal resistances to CO_2 transfer in the leaves of two tree species (*Fagus sylvatica* L. and *Castanea sativa* Mill.). - Plant Cell Environ. **18**: 43-51, 1995.

Farquhar, G.D., Caemmerer, S. von, Berry, J.A.: A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. - Planta **149**: 78-90, 1980.

Genty, B., Briantais, J.-M., Baker, N.R.: The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. – Biochim. biophys. Acta **990**: 87-92, 1989.

Janouš, D., Dvořák, V., Opluštilová, M., Kalina, J.: Chamber effects and responses of trees in the experiment using open top chambers. – J. Plant Physiol. **148**: 332-338, 1996.

Kramer, P.J.: Carbon dioxide concentration, photosynthesis, and dry matter production. – BioScience **31**: 29-33, 1981.

Lloyd, J., Grace, J., Miranda, A.C., Meir, P., Wong, S.C., Miranda, H.S., Wright, I.R., Gash, J.H.C., McIntyre, J.: A simple calibrated model of Amazon rainforest production based on leaf biochemical properties. - Plant Cell Environ. **8**: 1129-1145, 1995.

Long, S.P.: Modification of the response of photosynthetic productivity to rising temperature by atmospheric CO_2 concentrations: has its importance been underestimated? - Plant Cell Environ. **14**: 729-739, 1991.

Long, S.P., Drake, B.G.: Photosynthetic CO_2 assimilation and rising atmospheric CO_2 concentrations. - In: Baker, N.R., Thomas, H. (ed.): Crop Photosynthesis: Spatial and Temporal Determinants. Pp. 69-103. Elsevier Science Publishers, Amsterdam 1992.

Marek, M.V.: [Effects of Long-Term Impact of Elevated Atmospheric CO_2 Concentration on Photosynthetic and Production Characteristics of Norway Spruce.] – Thesis, Brno 1998. [In Czech.]

Marek, M.V., Kalina, J., Matoušková, M.: Response of photosynthetic carbon assimilation of Norway spruce exposed to long-term elevation of CO_2 concentration. - Photosynthetica **31**: 209-220, 1995.

Marek, M.V., Šprtová, M., Kalina, J.: The photosynthetic irradiance-response of Norway spruce exposed to a long-term elevation of CO_2 concentration. - Photosynthetica **33**: 259-268, 1997.

Norman, J.M., Jarvis, P.G.: Photosynthesis in Sitka spruce (*Picea sitchensis* (Bong.) Carr.). V. Radiation penetration theory and a test case. – J. appl. Ecol. **12**: 839-878, 1975.

Opluštilová, M., Dvořák, V.: Growth processes of Norway spruce in elevated CO_2 concentration. - In: Mohren, G.M.J., Kramer, K., Sabaté, S. (ed.): Impacts of Global Change on Tree Physiology and Forest Ecosystems. Pp. 53-58. Kluwer Academic Publishers, Dordrecht - Boston - London 1997.

Porter, M.A., Grodzinski, B.: Acclimation to high CO_2 in bean. Carbonic anhydrase and ribulose bisphosphate carboxylase. – Plant Physiol. **74**: 413-416, 1984.

Portis, J.R., Jr.: Rubisco activase. – Biochim. biophys. Acta **1015**: 15-28, 1990.

Priwitzer, T., Urban, O., Šprtová, M., Marek, M.V.: Chloroplastic carbon dioxide concentration of Norway spruce (*Picea abies* [L.] Karst.) needles relates to the position within the crown. - Photosynthetica **35**: 561-571, 1998.

Sage, R.F., Sharkey, T.D., Seemann, J.R.: Acclimation of photosynthesis to elevated CO_2 in five C_3 species. - Plant Physiol. **89**: 590-596, 1989.

Sasek, T.W., DeLucia, E.H., Strain, B.R.: Reversibility of photosynthetic inhibition in cotton after long-term exposure to elevated CO_2 concentrations. - Plant Physiol. **78**: 619-622, 1985.

Sharkey, T.D., Socias, X.: CO_2 responses of photosynthesis in elevated CO_2. - In: Alscher, R., Wellburn, A. (ed.): Gaseous Pollutants and Plant Metabolism. Elsevier Applied Sci., London 1994.

Špunda, V., Kalina, J., Čajánek, M., Pavlíčková, H., Marek, M.V.: Long-term exposure of Norway spruce to elevated CO_2 concentration induces changes in photosystem II mimicking an adaptation to increased irradiance. – J. Plant Physiol. **152**: 413-419, 1998.

Špunda, V., Kalina, J., Nauš, J., Kuropatwa, R., Mašláň, M., Marek, M.: Responses of photosystem 2 photochemistry and pigment composition in needles of Norway spruce saplings to increased radiation level. - Photosynthetica **28**: 401-413, 1993.

Stitt, M.: Rising CO_2 levels and their potential significance for carbon flow in photosynthetic cells. - Plant Cell Environ. **14**: 741-762, 1991.

Webber, A.N., Nie, G.-Y., Long, S.P.: Acclimation of photosynthetic proteins to rising atmospheric CO_2. - Photosynth. Res. **39**: 413-425, 1994.

Winder, T.L., Anderson, J.C., Spalding, M.H.: Translational regulation of the large and small subunits of ribulose bisphosphate carboxylase/oxygenase during induction of the CO_2-concentrating mechanism in *Chlamydomonas reinhardtii.* – Plant Physiol. **98**: 1409-1414, 1992.