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Different responses of Norway spruce needles from shaded and exposed crown layers to the prolonged exposure to elevated CO_2 studied by various chlorophyll *a* fluorescence techniques

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Abstract

The acclimation depression of capacity of photon utilisation in photochemical reactions of photosystem 2 (PS2) can develop already after three months of cultivation of the Norway spruces (*Picea abies* [L.] Karst.) under elevated concentrations of CO_2 (*i.e.*, ambient, AC, + 350 μmol(CO_2) mol^{-1} = EC) in glass domes with adjustable windows. To examine the role that duration of EC plays in acclimation response, we determined pigment contents, rate of photosynthesis, and parameters of chlorophyll *a* fluorescence for sun and shade needles after three seasons of EC exposure. We found different responses of shaded and exposed needles to EC. Whereas the shaded needles still profited from the EC and revealed stimulated electron transport, for the exposed needles the stimulation of both electron transport activity and irradiance saturated rate of CO_2 assimilation (P_{Nmax}) under EC already disappeared. No signs of the PS2 impairment were observed as judged from high values of potential quantum yield of PS2 photochemistry (F_V/F_M) and uniform kinetics of Q_A re-oxidation for all variants. Therefore, the long-term acclimation of the sun-exposed needles to EC is not necessarily accompanied with the damage to the PS2 reaction centres. The eco-physiological significance of the reported differentiation between the responses of shaded and sun exposed needles to prolonged EC may be in changed contribution of the upper and lower crown layers to the production activity of the tree. Whereas for the AC spruces, P_{Nmax} of shaded needles was only less than 25 % compared to exposed ones, for the EC spruces the P_{Nmax} of shaded needles reached nearly 40 % of that estimated for the exposed ones. Thus, the lower shaded part of the crown may become an effective consumer of CO_2.

Additional key words: carotenoids; irradiance; net photosynthetic rate; photosystem 2; *Picea abies*.

Introduction

The rising atmospheric CO_2 concentration is an important ecophysiological topic (Eamus and Jarvis 1989, Ceulemans and Mousseau 1994, Saxe *et al.* 1998). The characterisation of elevated CO_2 effects on production activity of forest trees such as Norway spruce is very important for the estimation of atmospheric CO_2 consumption. The advantage of forests is that they may store the atmospheric carbon dioxide in wood for a long time (Dixon *et al.* 1994). However, the response of trees to elevated CO_2 is very variable. Some tree species profited from higher

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Abbreviations: AC and EC – ambient and elevated CO_2 concentration; ACS and ECS – ambient and elevated shaded needles; ACE and ECE – ambient and elevated exposed needles; Car (*x+c*) – total carotenoids; Chl – chlorophyll; ETR – electron transport rate over photosystem 2; F_0 and F_M – minimum and maximum levels of Chl *a* fluorescence of dark adapted leaves; F_P and F_{2s}, – important points determined from the kinetics of Chl *a* fluorescence induction (maximum and fluorescence intensity after 2 s of red actinic radiation); F_0', F_M', F_S – minimum, maximum, and actual steady state levels of Chl *a* fluorescence of light-adapted leaves; LHC2 – light-harvesting Chl *a/b* complexes of photosystem 2; P_{Nmax} – irradiance saturated rate of CO_2 assimilation; PPFD – photosynthetic photon flux density; PQ – plastoquinone; PS – photosystem; RC – reaction centre; $t_{1/2}$ – half time of $F_0 - F_P$ fluorescence increase; α_1 – the slope of fluorescence 1 – q_P increase; α_2 – the slope of fluorescence 1 – q_P decrease.
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availability of carbon dioxide (Scarascia-Mugnozza *et al.* 1996, Kalina and Ceulemans 1997), whereas others revealed an acclimation depression of photosynthesis (Marek and Kalina 1996, Marek *et al.* 1997, Urban and Marek 1999). For the estimation of whole tree response to elevated CO_2 it is necessary to evaluate a possible differentiation of the response of shaded and exposed parts of tree. Assimilation activity of exposed Norway spruce foliage is higher compared to the shaded one (Marek *et al.* 1999). However, during vegetation season, the exposed needles are frequently exposed to high photosynthetic photon flux density (PPFD), which leads to increased capacity of non-radiative dissipation of absorbed radiation (Špunda *et al.* 1998a, Marek *et al.* 1999). Moreover, the adaptation to high irradiance induces a decrease in the amount of chlorophyll (Chl) and an increase of the Chl *a/b* ratio suggesting a diminution of light-harvesting complexes of PS2 (Chow and Anderson 1987, Lichtenthaler and Buschmann 1987, Špunda *et al.* 1993).

Up to our knowledge the only information on the effects of elevated CO_2 on shaded and exposed leaves is available for crop species (Osborne *et al.* 1998) and *Quercus ilex* (Marek *et al.* 2000). The response of light reactions of photosynthesis to the elevated CO_2 may be easily and rapidly estimated using parameters of Chl *a* fluorescence which reflect the function of PS2 (Peterson 1991, Epron *et al.* 1994, Kalina *et al.* 1997, Špunda *et al.* 1998b). Kalina *et al.* (2000) showed that comprehensive signs of slight impairment of PS2 function of Norway spruce needles developed already within three months of exposure of the trees to EC. Up to now all reports of the EC-induced acclimation depression of Norway spruce photosynthesis were obtained for the sun-exposed needles (Marek *et al.* 1997, Špunda *et al.* 1998b). Information on the effects of different radiation exposure on the acclimation of photosynthetic apparatus to EC within a vertical profile of Norway spruce is missing.

On the basis of several methods (pigment analysis, kinetics of fluorescence induction, the room temperature modulated Chl *a* fluorescence, measurements of Q_A^- reoxidation and S-states kinetics, P_{Nmax}) we attempted to describe a PS2 functional state of shaded and exposed Norway spruce grown in ambient and elevated CO_2 concentration, respectively.

Material and methods

Plants: The experiment was conducted on the Experimental Research site Bílý Kříž in the Moravian-Silesian Beskydy mountains (NE Moravia, Czech Republic, 49°30'N, 18°32'E, 943 m a.s.l.). Young Norway spruces [*Picea abies* (L.) Karst., age 13 years, average height 2.1 m] were grown in two domes with lamella windows (Urban *et al.* 2001). In one of them the trees were grown in ambient atmospheric CO_2 concentration (355±6 μmol mol^{-1}) = ambient (AC) variant. In the second dome the Norway spruces trees were exposed to elevated CO_2 concentration (720±72 μmol mol^{-1}) for three subsequent seasons = elevated (EC) variant. Norway spruce trees in both domes are planted in the same space densities, *i.e.*, *ca.* 10 000 trees per ha, and are characterised by projected leaf area index 2.3 m^2 m^{-2}.

The measurements of irradiance responses of steady state Chl *a* fluorescence parameters were performed on the one-year-old shoots from the upper and lower crown parts (exposed and shaded; E and S variants) of individual trees grown in the AC and EC domes. The shaded and exposed samples were taken from the same part of trees grown under AC and EC to assure the same irradiance exposure of the compared samples. Under direct sunshine the shaded needles were exposed approximately to 10 % PPFD as compared to exposed needles. The one-year-old needles for the measurements of room temperature fluorescence induction and pigment analysis were taken from the same parts of the trees as the shoots used for the measurement of the irradiance-response of steady state Chl *a* fluorescence. All measurements were carried out in September and October 1999.

Pigment analysis: Estimation of the pigment contents [Chl *a*, Chl *b*, and carotenoids, Car (x+c)] was performed spectrophotometrically (*UV550, Unicam*, UK) from pigment extracts in 80 % acetone with a small amount of $MgCO_3$ according to Lichtenthaler (1987). The mixed samples of needles taken from the same parts of the trees were used.

Electron transport rate estimations using room temperature Chl *a* fluorescence and P_{Nmax}: Parameters of the room temperature modulated Chl *a* fluorescence were measured using a *PAM 101, 103* fluorometer (*H. Walz*, Effeltrich, Germany). The dark-adapted shoots with the adaxial surface up were placed inside the leaf assimilation chamber (*PP System*, UK) to assure the AC and/or EC. The shoots were darkened for 60 min. Afterwards, F_0 and F_M were measured and potential quantum yield of PS2 photochemistry [$F_V/F_M = (F_M - F_0)/F_M$] was determined. The PS2 electron transport rate [ETR =Y × PPFD × 0.5 × 0.8, where $Y = (F_M' - F_S)/F_M'$; Genty *et al.* 1989] were estimated in the steady state after 10 min exposure of the shoots to individual incident irradiances (30, 65, 90, 170, 330, 610, 810, and 1 100 μmol m^{-2} s^{-1}). P_{Nmax} was measured using *CIRAS 1* (*PP System*, UK) at the

highest PPFD (1 100 μmol m^{-2} s^{-1}) and under 350 and 700 μmol(CO_2) mol^{-1}, respectively.

Kinetics of Chl *a* fluorescence at room temperature: Samples containing five one-year old needles were predarkened for 60 min. The needles were oriented on the sample holder so that the fluorescence was excited from the upper side of needles and placed inside the leaf assimilation chamber (*PP System*, UK) to assure the AC and/or EC. During 3 s of red actinic irradiation (incident PPFD was 70 μmol m^{-2} s^{-1}) the rapid Chl *a* fluorescence induction curve was recorded, afterwards the samples were darkened for another 60 min, and finally F_0 and F_M were measured (*PAM 101, 102, 103, H. Walz*, Germany). These measurements were performed at 20 °C. Twelve samples of each variant were measured. Fluorescence induction curves were normalised at the F_0 value and divided by F_V to express the time dependence of $1 - q_P$ [$(1 - q_P)(t) = (F(t) - F_0)/F_V$]. As a generally known parameter characterising the functional size of LHC2 the $t_{1/2}$ was estimated from the measured curves. Moreover, the induction curves were characterised by the slopes of fluorescence $1 - q_P$ increase (α_1; estimated from the linear regression of the curve around the $t_{1/2}$) and fluorescence $1 - q_P$ decrease (α_2; estimated from the linear part of fluorescence decrease from F_P to F_{2s}).

Measurements of Q_A^- reoxidation and S-states kinetics: Leaf version of double-modulation Chl fluorometer (*FL-100, P.S. Instruments*, Czech Republic) (Urban *et al.* 1999) based on the method of Nedbal and Trtílek (1995) was used for measurements of Q_A^- re-oxidation kinetics. Single turnover actinic flashes saturating the reduction of primary quinone acceptor Q_A were generated by the red light-emitting diodes (LEDs; 30 μs duration, 100 % power). The fluorescence values were obtained using 4 measuring flashes per time decade (orange LEDs, 2.5 μs duration, 80 % power) with the first point taken 70 μs after the actinic flash. Negligible actinic effects of the selected measuring radiation and the capacity of the fluorometer to produce single-turnover flashes that saturate Q_A reduction in Norway spruce needles were tested in previous experiments (Urban *et al.* 1999).

Seven to nine Norway spruce needles were fixed on the transparent slide with adaxial surface up and dark adapted (60 min) in the AC atmosphere. Because of fluorometer construction, measuring flashes preferentially excited the upper layers of the needles.

Statistical analysis: All experimental values were tested for significance by a F-test (a two sample test for variances), followed by a *t*-test. The levels of significance $p = 0.05$, $p = 0.01$, and $p = 0.001$ were indicated as *, **, and ***. All calculations and statistical tests were performed using analysis tools from *Microsoft Excel* (version *7.0*).

Results and discussion

Pigment contents and composition: The exposed needles had significantly lower content of Chls and carotenoids as compared to shaded ones in both AC and EC variants (Table 1). Moreover, the higher Chl *a/b* ratio found in exposed needles of both variants confirmed a typical degree of differentiation between sunny and shaded needles (Špunda *et al.* 1998a). Only slight differences in Chl (*a+b*)/Car (*x+c*) ratio between exposed and shaded needles for both AC and EC spruces indicated that the exposed needles of both variants did not suffer from high irradiance stress. The typical response of adaptation of Norway spruce needles to high-irradiance stress is accompanied with low Chl (*a+b*)/Car (x+c) ratio, typically bellow 4 (Špunda *et al.* 1998b). The differences in pigment contents and Chl *a/b* ratio between shaded and exposed needles were generally more pronounced that those between EC and AC needles from either shaded or exposed crown layers. For shaded needles the contents of Chl *a*, Chl *b*, and Car (*x+c*) per dry matter were by 23, 25, and 25 % lower in EC spruces (ECS variant) as com-

Table 1. Chlorophyll (Chl) *a*, Chl *b*, and carotenoid [Car (*x+c*)] contents [g kg^{-1}] and Chl *a/b* and Chl (*a+b*)/Car (*x+c*) ratios for shaded and exposed needles of spruces grown in ambient (AC) or elevated (EC) CO_2 concentration. Mean values from seven measurements. Numbers in brackets represent standard deviation. Values with different superscripts are significantly different at $p = 0.05$ or lower.

	Shaded		Exposed	
	AC	EC	AC	EC
Chl *a*	4.11 (0.24)^{d}	3.17 (0.19)^{c}	2.35 (0.11)^{b}	1.55 (0.16)^{a}
Chl *b*	1.40 (0.07)^{d}	1.05 (0.06)^{c}	0.69 (0.03)^{b}	0.48 (0.05)^{a}
Car (*x+c*)	1.08 (0.07)^{d}	0.81 (0.05)^{c}	0.61 (0.03)^{b}	0.40 (0.04)^{a}
Chl *a/b*	2.94 (0.06)^{a}	3.02 (0.05)^{b}	3.42 (0.05)^{c}	3.24 (0.08)^{d}
Chl (*a+b*)/Car (*x+c*)	5.08 (0.09)^{c,b,a}	5.20 (0.07)^{d}	5.01 (0.10)^{a}	5.05 (0.17)^{b,a}

pared with AC ones (ACS variant). In spite of lower pigment content for ECS needles the ratios of Chl *a/b* and Chl (*a+b*)/Car (*x+c*) were nearly the same for shaded needles of EC and AC spruces. For exposed needles the larger differences in pigment content were found between the trees grown in EC and AC. In this case, the contents of Chl *a*, Chl *b*, and Car (*x+c*) per dry matter were by 34, 30, and 34 % lower for ECE than ACE variant. The Chl *a/b* ratio lower by 5.25 % was determined for ECE needles as compared to ACE needles, however, Chl (*a+b*)/Car (*x+c*) was again nearly the same for these needles (Table 1). The above-mentioned differences in pigment content between individual needle variants can be attributed mainly to the different accumulation of assimilates in ACS, ECS, ACE, and ECE needles. The exposed needles revealed a doubled dry mass per unit needle area for both AC and EC variants as compared to the shaded ones. Moreover, the exposed and shaded needles from EC spruces had significantly higher dry mass per unit needle area than the needles from the corresponding crown layer of the AC spruces. Hence, both the high irradiance and EC contributed to the accumulation of assimilates.

Different degree of stimulation of photosynthetic activities under EC for shoots from exposed and shaded crown layers: The capacity of photon utilisation in PS2 photochemical reactions was monitored using the dependency of steady state electron transport rate (ETR) through PS2 on PPFD at the end of third vegetation period of cultivation under EC. For exposed shoots no significant differences were observed between AC and EC variants from the irradiance response curves measured under the corresponding cultivation CO_2 concentration within the whole range of applied irradiances (Fig. 1). Hence, the pronounced stimulation of PS2 electron transport capacity typical for the primary stimulation of photosynthesis under EC disappeared. The irradiance-saturated rates of ETR were estimated from the irradiance responses (Table 2). In agreement with above mentioned statement, nearly the same ETR_{max} values were estimated for ECE variant under 700 μmol(CO_2) mol^{-1} and ACE variant under 350 μmol(CO_2) mol^{-1}. The measurements under the opposite CO_2 concentrations to the cultivating ones revealed a 40 % lower ETR_{max} for ECE variant under 350 μmol(CO_2) mol^{-1} compared to AE variant. Similar results were obtained from the measurements of irradiance responses of P_N. Almost the same irradiance-saturated assimilation rates (P_{Nmax}) were estimated under 700 μmol(CO_2) mol^{-1} for exposed shoots of both AC and EC spruces (Table 2). Moreover, under 350 μmol(CO_2) mol^{-1} the P_{Nmax} for ECE variant was by 50 % depressed as compared to the ACE one.

As expected, for shaded needles the ETR measured under cultivation CO_2 concentration reached hardly half of that observed for exposed needles of both AC and EC spruces and the saturating irradiance was shifted to the lower levels as compared to the exposed needles (Fig. 1). On the contrary to the exposed needles, for the shaded shoots the significantly higher ETR values were observed under the cultivation CO_2 concentration for ECS variant. For the highest irradiances of 810 and 1 100 μmol m^{-2} s^{-1} the ETR values were by 43 % higher for the ECS shoots in comparison with the ACS ones (Fig. 1). Hence, the shoots from the shaded crown layer of spruces cultivated under EC still revealed the effects of primary stimulation of photon utilisation in photosynthetic reactions. No significant differences were observed between ETR_{max} values of ECS and ACS shoots neither under 700 μmol(CO_2) mol^{-1} nor under 350 μmol(CO_2) mol^{-1} (Table 2). Moreover, the P_{Nmax} was by 20 % higher for ECS variant than for the ACS one, not only under 700 μmol(CO_2) mol^{-1} but even under 350 μmol(CO_2) mol^{-1}. This seems to indicate that the stimulation of photosynthetic activity of shaded needles under prolonged exposure to EC is even more pronounced than that induced by sudden increase of CO_2 concentration. Hence, the contribution of the shaded crown layers to the whole tree photosynthesis may increase under the prolonged EC exposure. Whereas for the AC spruces P_{Nmax} was 4 times lower in shaded needles as compared to exposed ones, for the shaded needles of EC variant we observed only 2.5 fold lower values. Higher availability of CO_2 probably mitigates the suppression of photosynthetic capacity caused by sub-optimal sun irradiance. This is in agreement with Marek *et al.* (2000),

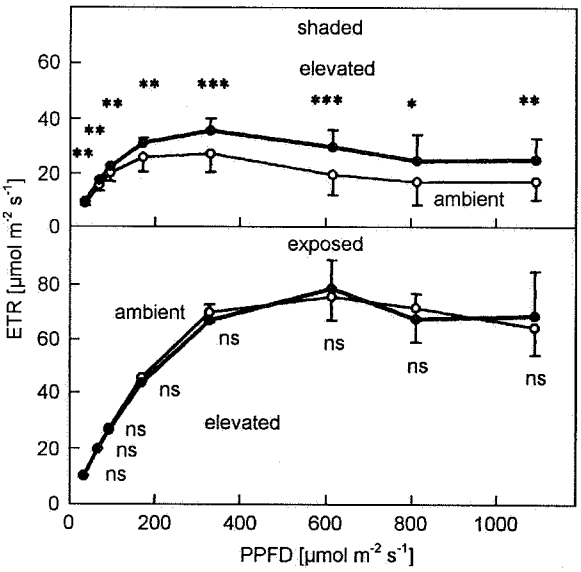

Fig. 1. The photosynthetic photon fluence density (PPFD) responses of steady state electron transport rate through PS2 (ETR) in shaded (*upper panel*) or exposed (*lower panel*) shoots grown in AC (*thin line; open circles*) or EC (*thick line; full circles*). Measurements after three years of exposure of trees to elevated CO_2. Error bars are ± SD (n = 12). $^{*}p$ = 0.05, $^{**}p$ = 0.01, $^{***}p$ = 0.001, ns – not significant.

Table 2. Irradiance-saturated rates of electron transport (ETR_{max}) and photosynthetic CO_2 assimilation (P_{Nmax}) [μmol m^{-2} s^{-1}] for the shoots from exposed and shaded crown layers of the Norway spruces cultivated at ambient (AC) or elevated (EC) CO_2 concentrations. The irradiance-saturated rates were estimated from the irradiance response curves measured at 350 μmol(CO_2) mol^{-1} (ETR_{max350} or $P_{Nmax350}$) and 700 μmol(CO_2) mol^{-1} (ETR_{max700} or $P_{Nmax700}$). Mean values from twelve measurements. Numbers in brackets represent standard deviation. Values with different superscripts are significantly different at $p = 0.05$ or lower.

	Shaded		Exposed	
	AC	EC	AC	EC
ETR_{max350}	27.0 (6.7)^{b,a}	24.5 (4.8)^{a}	75.8 (8.7)^{c}	45.3 (11.7)^{d}
ETR_{max700}	37.6 (8.5)^{b,a}	35.4 (4.3)^{a}	81.7 (9.6)^{d,c}	78.8 (10.4)^{c}
$P_{Nmax350}$	2.8 (0.5)^{a}	3.4 (0.7)^{b,a}	12.0 (2.5)^{d}	6.0 (1.2)^{c}
$P_{Nmax700}$	5.9 (1.0)^{a}	7.0 (1.2)^{b,a}	16.0 (2.5)^{c}	17.6 (1.6)^{d,c}

who reported enhanced sink effects on the photosynthetic activity of shaded leaves of *Quercus illex* trees grown under EC.

Kinetic characterization of Q_A reduction and reoxidation for the exposed and shaded needles based on Chl *a* fluorescence induction measured under the cultivation CO_2 concentration: The kinetics of fluorescence induction during several seconds of actinic irradiation (Kautsky phenomenon) is a source of valuable information on the functional state of PS2 (Lichtenthaler and Rinderle 1988). The typical Chl *a* fluorescence induction kinetics for shaded and exposed needles from AC and EC spruces were expressed as proportion of reduced Q_A during induction [$(1 - q_P)(t) = (F(t) - F_0)/F_V$] (Fig. 2). The medium irradiance (around 70 μmol m^{-2} s^{-1}) was not sufficient to completely reduce the plastoquinone (PQ) pool, hence F_P was lower than F_M. The increase of fluorescence from F_0 to F_P corresponded to the kinetics of closure of PS2 RCs. This part of fluorescence induction is usually characterised by the half time of the fluorescence rise from F_0 to F_P ($t_{1/2}$) which bears an approximate information on LHC2 size (Öquist and Wass 1988, Špunda *et al.* 1998b). However, this parameter is partially influenced by the proportion of Q_B^- non-reducing PS2 RCs. Similar information may be obtained from the slope of $1 - q_P$ increase (α_1, determined from the linear part of $1 - q_P$ fluorescence increase around the $t_{1/2}$ point of fluorescence induction) which is proportional to the kinetics of closure of Q_B^- reducing PS2 RCs. The following decrease of the fluorescence to F_{2s} reflects particularly the re-oxidation of PQ pool. A rapid formation of ΔpH may contribute to the non-photochemical quenching of fluorescence from F_P to F_{2s} levels, too. However, under a sub-saturating red actinic irradiance (70 μmol m^{-2} s^{-1}) only a slight non-photochemical quenching of F_M was observed after 2 s of actinic irradiation (about 10 %, values not shown) in agreement with Špunda *et al.* (1998b). As the quenching of F(t) during decreasing phase of the Kautsky phenomenon was more pronounced (Fig. 2), we suggest that the photochemical quenching prevails. Hence, this part of fluorescence induction curve bears mainly information about the activity of electron transport through PQ pool. This may be characterised by the slope of $1 - q_P$ decrease [α_2, determined from the linear part of fluorescence decrease at the time when the fluorescence intensity is about $F_{2s} + (F_P - F_{2s})/2$]. Clear differences are seen between the shaded and exposed needles of both variants (Table 3). The significantly longer $t_{1/2}$ slowed kinetics of Q_A reduction (reduced slope α_1) and more rapid Q_A re-oxidation (increased slope α_2) for exposed needles as compared to

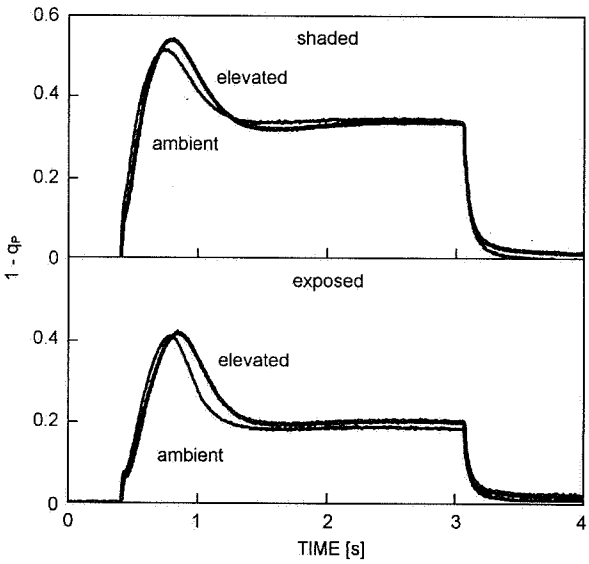

Fig. 2. Chlorophyll *a* fluorescence induction kinetics converted to $1 - q_P$ induced by actinic radiation for shaded (*upper panel*) or exposed (*lower panel*) needles from Norway spruce grown in AC (*thin line*) or EC (*thick line*). Mean of twelve curves is presented. Measurements after three years of exposure of trees to elevated CO_2.

the shaded ones confirmed both the smaller LHC2 size and higher capacity of electron flow through PS2 as compared to the shaded needles. The differences between AC and EC needles were less pronounced. No significant differences between EC and AC needles were found for α_1 that may indicate that EC did not cause a pronounced change in the LHC2 size for both shade and exposed needles. However, the reduced electron transport capacity

through PS2 may be documented for the exposed needles of the spruces cultivated under EC from the decreased kinetics and extent of Q_A re-oxidation (Fig. 2, Table 3). For the ECE variant the α_2 was by 25 % reduced as compared to the ACE one, whereas for the ECS variant this parameter was slightly higher than for ACS. Hence, similarly as from the steady state measurements of ETR the analysis of fluorescence induction curve supports the idea that the photochemical de-excitation of PS2 RCs is still stimulated for the shaded needles but becomes slightly reduced in the exposed needles under EC. The fact that reduction of PS2 electron transport capacity for ECE needles is observed also from the fluorescence induction measured on the dark-adapted needles implies that the capacity of electron transport through PQ pool itself becomes reduced, not only the consumption of ATP and NADPH in the Calvin cycle reactions.

Functional state of PS2 RCs for the exposed and shaded needles: The F_V/F_M values measured with exposed needles of both AC and EC trees were close to 0.82 which indicated nearly the optimum potential quantum yield of PS2 photochemistry and highly active PS2 RCs (Table 2). The F_V/F_M values for shaded needles of both variants were slightly, but non-significantly, lower (about 0.81). This implied that exposed needles of the EC trees did not show the signs of increased susceptibility to photoinhibition, which contradicts the previous reports on the effects of EC on the photosynthetic apparatus of Norway spruce (Špunda *et al.* 1998b, Kalina *et al.* 2000).

Table 3. Parameters of chlorophyll (Chl) *a* fluorescence induction kinetics in shaded or exposed spruce needles grown in ambient (AC) or elevated (EC) CO_2. F_V/F_M – potential quantum yield of PS2 photochemistry, $t_{1/2}$ – half time of $F_0 - F_P$ fluorescence increase, α_1 – the slope of fluorescence 1 – q_P increase ($r^2 > 0.95$), α_2 – the slope of fluorescence 1 – q_P increase ($r^2 > 0.82$). Mean values from twelve measurements. Numbers in brackets represent standard deviation. Values with different superscripts are significantly different at $p = 0.05$ or lower.

	Shaded		Exposed	
	AC	EC	AC	EC
F_V/F_M	0.807 (0.009)^a	0.815 (0.009)^b	0.821 (0.017)^d	0.819 (0.011)^c
$t_{1/2}$	0.080 (0.013)^a	0.120 (0.019)^b	0.142 (0.022)^c	0.173 (0.015)^d
α_1	1.802 (0.146)^c	1.865 (0.136)^{d,c}	1.429 (0.105)^b	1.286 (0.169)^a
α_2	-0.548 (0.046)^a	-0.600 (0.070)^{c,b}	-0.804 (0.141)^d	-0.597 (0.024)^{b,a,c}

The functional state of PS2 RCs was further monitored using kinetics of Q_A^- re-oxidation following single turnover flash (Fig. 3). In contrast to the Q_A^- re-oxidation observed during continuous sub-saturating actinic excitation observed from fluorescence induction (Fig. 2), the Q_A^- re-oxidation following a single turnover flash is not dependent on the electron transport through PQ pool and bears more direct information on the functional state of PS2 RCs themselves. It is possible to separate the kinetics of Q_A^- re-oxidation into three parts. The most rapid (a few milliseconds or faster; first phase) fluorescence decay is due to the electron transfer from Q_A^- to Q_B (or Q_B^-) in active RCs of PS2, whereas in inactive PS2 centres the oxidation of Q_A^- is much slower (seconds; third phase) (Chylla and Whitmarsh 1989). In the range of hundreds of milliseconds, a mid-phase component characterises the active PS2 RCs with non-occupied Q_B binding site. We did not observe any change in the rapid fluorescence decay phase among shaded and exposed needles of both AC and EC trees (Fig. 3). This may indicate similar proportions of active PS2 RCs. All differences are within the range of experimental error. These results support the above mentioned results of F_V/F_M showing that both the ECE and ECS variants revealed the same high proportion of active PS2 RCs as ACE and ACS ones.

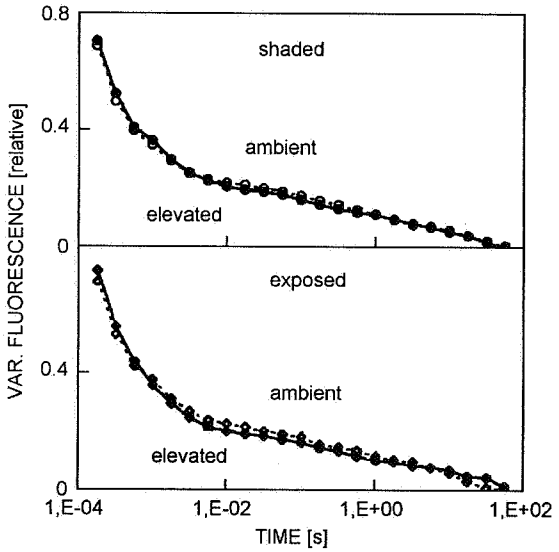

Fig. 3. Relative variable fluorescence $(F - F_0)/(F_M - F_0)$ following an actinic flash that reduced the primary quinone acceptor Q_A in needles of Norway spruce. The *open circles* represent an average of 10 measurements with shaded needles treated in AC (*upper panel*); *full circles* – shaded needles treated in EC (*upper panel*); open diamonds – exposed needles in AC (*lower panel*); *full diamonds* – exposed needles in EC (*lower panel*).

Conclusion: In a previous report (Kalina *et al.* 2000) we showed that the acclimation depression of capacity of photon utilisation in PS2 photochemical reactions can develop already after three months of the cultivation of the Norway spruces under EC (700 μmol mol^{-1}) in domes with lamella windows. In agreement with our findings on the effects of prolonged EC on the photosynthetic apparatus of Norway spruce in the open top chamber experiment (Špunda *et al.* 1998b), the acclimation depression of photosynthetic activity in the needles of spruces grown in EC was accompanied with the impairment of PS2 function. However, these results were obtained for the spruces growing in the nutrient-poor soil, which is typical for mountain areas. The shortage of the nitrogen and phosphorus may cause the acclimation depression of the photosynthetic activities under EC (Kalina and Ceulemans 1997). In order to confirm or refute this affirmation the trees were irrigated by 25 g *Silvamix forte* (9 April, 1998) and by 20 g *Ureaform* (16 April, 1998) per tree supplying thus the tree with optimum mineral nutrition before the start of the second vegetation period of the EC cultivation. The signs of acclimation depression disappeared under these conditions and the stimulation of photosynthetic activities lasted for the following two seasons of the EC treatment. Even at the end of the third season the impairment of the PS2 function was not observed for the sunny needles of EC trees as judged from the high potential of PS2 photochemistry (Table 3) and non-altered kinetics of Q_A^- re-oxidation (Fig. 3).

However, even under the optimum conditions the long-term response of the shaded and sun-exposed needles to EC considerably differed. The stimulation of ETR in the shaded needles of EC spruces remained the same as the short-term stimulation of the ETR during sudden exposure of the shaded needles of AC spruces to the doubled CO_2 concentration (Table 2). Similarly, the irradiance-saturated photosynthetic CO_2 assimilation was even higher in the ECS variant than that obtained after the exposure of the ACS needles to the doubled CO_2 concentration. On the contrary, the stimulation of both ETR_{max} and P_{Nmax} in the exposed needles of EC spruces almost completely disappeared. The eco-physiological importance of this finding lies in the changed contribution of the lower and upper crown layers to the photosynthetic production of the tree under the long-term EC exposure. The hypothesis that the lower shaded part of the crown may become a strong sink for the atmospheric CO_2 should be now studied during longer time period with attention to the profiles of the photosynthetic activities and biomass production.

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