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Acclimation of two distinct plant species, spring barley and Norway spruce, to combined effect of various irradiance and CO_2 concentration during cultivation in controlled environment

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Abstract

The short-term acclimation (10-d) of Norway spruce [*Picea abies* (L.) Karst] to elevated CO_2 concentration (EC) in combination with low irradiance (100 μmol m^{-2} s^{-1}) resulted in stimulation of CO_2 assimilation (by 61 %), increased total chlorophyll (Chl) content (by 17 %), significantly higher photosystem 2 (PS2) photochemical efficiency (F_v/F_m; by 4 %), and reduced demand on non-radiative dissipation of absorbed excitation energy corresponding with enhanced capacity of photon utilisation within PS2. On the other hand, at high cultivation irradiance (1 200 μmol m^{-2} s^{-1}) both Norway spruce and spring barley (*Hordeum vulgare* L. cv. Akcent) responded to EC by reduced photosynthetic capacity and prolonged inhibition of F_v/F_m accompanied with enhanced non-radiative dissipation of absorbed photon energy. Norway spruce needles revealed the expressive retention of zeaxanthin and antheraxanthin (Z+A) in darkness and higher violaxanthin (V) convertibility (yielding even 95 %) under all cultivation regimes in comparison with barley plants. In addition, the non-photochemical quenching of minimum Chl *a* fluorescence (SV_0), expressing the extent of non-radiative dissipation of absorbed photon energy within light-harvesting complexes (LHCs), linearly correlated with V conversion to Z+A very well in spruce, but not in barley plants. Finally, a key role of the Z+A-mediated non-radiative dissipation within LHCs in acclimation of spruce photosynthetic apparatus to high irradiance alone and in combination with EC was documented by extremely high SV_0 values, fast induction of non-radiative dissipation of absorbed photon energy, and its stability in darkness.

Additional key words: β-carotene; carotenoids; chlorophylls; *Hordeum vulgare*; non-radiative dissipation; photosynthesis; *Picea abies*; xanthophyll cycle.

Introduction

In nature some changes in environmental conditions such as quality and intensity of photosynthetically active radiation (PhAR), [CO_2], water availability, and nutrient status can be unfavourable and thus potentially damaging to the photosynthetic apparatus of plants. On that account the plants have evolved numerous strategies of acclimation including protective mechanisms aimed at prevention or minimisation of damage of the photosynthetic apparatus (Niyogi 1999). The extensive literature on the response of plants either to increased [CO_2] or different irradiances alone has been published, but to date little is known about the concurrent effect of CO_2 enrichment and various

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Abbreviations: A – antheraxanthin; $^{Barley}AC_{100}$, $^{Barley}AC_{1200}$, and $^{Spruce}AC_{100}$, $^{Spruce}AC_{1200}$ – spring barley and Norway spruce plants cultivated at irradiance of 100 or 1 200 μmol m^{-2} s^{-1} and ambient CO_2 concentration [350 μmol(CO_2) mol^{-1}]; β-car – β-carotene; CCs – core complexes; CPs – minor light-harvesting chlorophyll proteins; [CO_2] – CO_2 concentration; D – relative efficiency of non-radiative dissipation of absorbed photon energy; DEPS – de-epoxidation state of the xanthophyll cycle pigments; $^{Barley}EC_{100}$, $^{Barley}EC_{1200}$ and $^{Spruce}EC_{100}$, $^{Spruce}EC_{1200}$ – spring barley and Norway spruce plants cultivated at irradiance of 100 or 1 200 μmol m^{-2} s^{-1} and elevated CO_2 concentration [700 μmol(CO_2) mol^{-1}]; F_v/F_m – photochemical efficiency of photosystem 2 in dark-adapted state; HI – high irradiance; LHC(s) – light-harvesting complex(es); P_N – steady-state rate of CO_2 assimilation; PhAR – photosynthetically active radiation; RCs – reaction centres; SV_0 – non-photochemical fluorescence quenching originating within light-harvesting complexes; V – violaxanthin; VAZ – xanthophyll cycle pool, *i.e.* violaxanthin+antheraxanthin+zeaxanthin; Z – zeaxanthin.
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irradiances on plants and, in addition, results of these studies appear contradictory. In principle the long-term effect of elevated [CO_2] (EC) can lead to up-regulation or downward regulation of photosynthetic capacity (Jach and Ceulemans 2000, Woodward 2002), so called "positive acclimation" or "acclimation depression of photosynthesis", accompanied with increased or decreased photochemical energy utilisation within photosystem 2 (PS2). In a study on winter wheat grown for six weeks in EC in controlled environment, Habash *et al*. (1995) showed the up-regulation of photosynthetic activity accompanied with increased PS2 photochemical efficiency mitigating photoinhibition. The prolonged stimulation of photosynthetic activity of spring wheat grown under EC during two months was also confirmed in the field study using free-air carbon enrichment (FACE; Garcia *et al.* 1998). On the contrary, in other studies on cereals a marked acclimation depression of photosynthesis was reported (Tuba *et al.* 1994, Sicher and Bunce 1997, Kurasová *et al.* 2003). Sicher and Bunce (1997) observed a loss of photosynthetic activity in barley and winter wheat grown in open-top chambers at EC within a two-year study.

Similarly as in cereals, the different responsiveness of photosynthetic activity to EC varied in deciduous or evergreen trees from positive acclimation to acclimation depression. The FACE study of Gunderson *et al*. (2002) on sweet gum (*Liquidambar styraciflua* L.) trees during three years of CO_2 enrichment by 200 μmol(CO_2) mol^{-1} revealed stimulation of irradiance-saturated CO_2 assimilation both in mid- and upper-canopy foliage. Also in other FACE study, the net primary production of 13-year-old loblolly pine (*Pinus taeda* L.) increased by 25 % after two years of EC treatment (DeLucia *et al.* 1999). The CO_2 enrichment also resulted in increased irradiance-saturated photosynthetic rate of oak (*Quercus rubra* L.) and maple (*Acer rubrum* L.) seedlings grown in sun or shade for 80 d in open-top chambers (Kubiske and Pregitzer 1996). The stimulation of photosynthesis was also found in shade acclimated Norway spruce needles at the end of third vegetation period of cultivation under EC (Kalina *et al.* 2001) in glass domes with adjustable lamella windows (Urban *et al.* 2001). Contrariwise, the complete disappearance of stimulation of CO_2 assimilation was observed in sun exposed needles in upper canopy layer (Kalina *et al.* 2001).

As we have shown recently, the plants of spring barley grown at high irradiance (HI) and CO_2 enrichment by 350 μmol(CO_2) mol^{-1} in controlled environment revealed a pronounced acclimation depression of photosynthesis already after a ten-day cultivation (Kurasová *et al.* 2003). From the long-term field experiments we established that the sun acclimated needles are more susceptible to acclimation depression of photosynthesis under EC than shaded assimilation apparatus of Norway spruce plants (Kalina *et al.* 2001, Marek *et al.* 2002). This agrees with findings that the acclimation depression of photosynthesis is accompanied with a feedback down-regulation of PhAR utilisation in photochemical reactions (Peterson 1991, Marek and Kalina 1996, Kalina *et al.* 2000) and can enhance PS2 sensitivity of sun acclimated plants to photoinhibition (Špunda *et al.* 1998, Hymus *et al.* 2001). The capability of plants to prevent from unfavourable over-reduction of PS2 reaction centres (RCs) in such conditions lies in increased non-radiative dissipation of absorbed PhAR (Melis 1998, Niyogi 1999, Ort 2001). Obviously, the level of non-radiative dissipation of absorbed PhAR closely correlates with the size of the xanthophyll pool and/or de-epoxidation state expressing the extent of violaxanthin conversion to antheraxanthin and zeaxanthin (Osmond *et al.* 1993, Adams and Demmig-Adams 1994, Demmig-Adams *et al.* 1996). According to Huner *et al*. (1993, 1998), cereals acclimate to high excitation pressure (estimated as a high reduction state of quinone molecules within PS2 RCs) by enhancement of photosynthetic activity, whereas especially evergreens respond by increased non-radiative dissipation of absorbed PhAR.

In the present study we attempted to characterise the main common and/or different features of the acclimation of photosynthetic apparatus of barley and Norway spruce, representing cereals and conifers, to different irradiance and EC during the short-term treatment under controlled environment (growth chamber). The aims of our contribution were: (*1*) to estimate if under the laboratory conditions a rapid acclimation depression of photosynthesis can develop also in Norway spruce exposed to EC; (*2*) to show if the manifestation of acclimation depression of photosynthesis will be more pronounced in combination with excess irradiance even after the short term exposure to EC; (*3*) to evaluate the importance of non-radiative dissipation of absorbed PhAR energy in response of barley and spruce plants to combined effect of HI and EC.

Materials and methods

Plants: The spring barley (*Hordeum vulgare* L.) cv. Akcent was grown in 4 500 cm^3 pots (100 seeds per pot) in soil substrate (*AGRO CS*, Czech Republic) with defined mineral nutrition (each pot contained 1.2 g of ammonia and nitrate nitrogen, 1.1 g P_2O_5, and 1.9 g K_2O). Plants were grown under controlled environment inside growth chamber *HB 1014* (*Bioline-Heraeus,* Germany) at constant temperature of 20 °C, constant 65 % relative humidity, and 16/8 h day/night regime. The measurements were carried out on plants grown under a low and high (100 and 1 200 μmol m^{-2} s^{-1}) irradiance in *HB 1014* (for details see Kurasová *et al.* 2003) for nine days from seed sowing at the ambient [350 μmol(CO_2) mol^{-1}] CO_2 concentration. The same irradiances and the same duration of treatment were applied on barley plants in elevated [700 μmol(CO_2) mol^{-1}] CO_2 concentration. The [CO_2] inside

the growth chamber during the plant cultivation was controlled by infrared CO_2/H_2O analyser (*LI–6262*, *LI-COR*, USA). The primary leaves of 8–9-d-old plants were used for the measurements.

5-year-old spruce seedlings (about 50 cm of average height) were grown in 3 000 cm^3 pots in the same soil substrate as used for barley cultivation and also the other conditions (irradiances, $[CO_2]$, temperature, relative humidity, and day/night regime) inside growth cabinet (*HB 1014*) were identical. The seedlings were acclimated to given irradiance at ambient $[CO_2]$ for 25 d and then $[CO_2]$ was adjusted at 700 μmol(CO_2) mol^{-1} for the next 10 d. All measurements were performed on 1-year-old needles from the two uppermost whorls of the crown where the incident irradiance was as mentioned above. The measurements were carried out at the end of vegetation period (November–February) and in addition the germinating new buds on shoots were regularly nipped.

The low and high irradiances with the same shape of spectrum were achieved by combination of xenon (*Powerstar HQI-T 400 W/D daylight*; *Osram*, Germany) and krypton (*Krypton 100 W LO*; Hungary) lamps. The identity and stability of spectral composition of the radiation incident on the plant samples was checked using spectroradiometer *LI-1800* (*LI-COR*, USA).

Pigment analysis: Two barley leaf segments (3 cm^2 of projection leaf area) from the middle part of leaf blade or spruce needles (100 mg of fresh matter) were used for pigment analysis. The plant material was inserted into polyethylene bags and immediately frozen in liquid nitrogen (77 K) to retain unchanged pigment content induced by dark adaptation or defined irradiance treatment (see below). Then, the pigments were extracted in 80 % acetone with small amount of $MgCO_3$. The supernatant obtained after centrifugation at 480×*g* for 3 min was used for spectrophotometric (*UV/VIS 550*, *Unicam*, England) estimation of contents of Chl *a*, Chl *b*, and total carotenoids according to the equations of Lichtenthaler (1987).

The estimation of individual carotenoids from the same pigment extract as used for spectrophotometric analysis was performed by a semi-isocratic (according to Kurasová *et al.* 2002; barley) or by gradient reversed-phase HPLC analysis (according to Färber and Jahns 1998; spruce). The gradient HPLC analysis was provided with gradient pump (*Spectra Series P4000*) and the autosampler *AS3000* with 50 mm^3 sample loop (both *TSP Analytical*, USA). The isocratic elution for 10 min with the solvent system of acetonitrile : methanol : 0.1 M Tris (87 : 10 : 3, v/v) was followed by a 2-min linear gradient to second solvent (methanol : *n*-hexan 4 : 1, v/v) at a flow rate of 33.3 mm^3 s^{-1}. The solvents were degassed using vacuum degasser *SCM1000* (*TSP Analytical*, USA). The peak areas at 440 nm were integrated using *ChromQuest* software for *Windows NT* (*ThermoQuest*, Canada) in all chromatograms.

The conversion state of the xanthophyll cycle pigments (DEPS) was calculated as [Z+A]/[V+A+Z] (Adams and Demmig-Adams 1994). The three types of DEPS estimation were provided on plants after: (*a*) 1 h dark-adaptation ($DEPS_{dark}$), (*b*) 10 min exposure to the cultivation irradiance (100 or 1 200 μmol m^{-2} s^{-1}; $DEPS_{act}$), and (*c*) 10 min exposure to HI of 1 870 (barley) or 2 100 (spruce) μmol m^{-2} s^{-1} saturating the V de-epoxidation ($DEPS_{sat}$). The actinic irradiance was provided by a fiber illuminator (*KL 1500 LCD*, *Schott*, Germany).

Gas exchange: An open portable photosynthetic system *CIRAS-1* (*PP Systems*, UK) with an infrared gas analyser was used for measurements of CO_2 assimilation on plants taken during 8 h dark regime inside *HB 1014*. The steady-state CO_2 assimilation rate expressed per projected leaf area was measured on intact barley leaves (3 cm^2) and attached spruce shoots (8 cm^2). The actual CO_2 assimilation rate, P_N [μmol(CO_2) m^{-2} s^{-1}] was estimated on plants exposed to actual cultivation conditions, *i.e.* irradiance (100 or 1 200 μmol m^{-2} s^{-1}) and $[CO_2]$ (ambient or elevated). The maximal CO_2 assimilation capacity (P_{Nsat}) was measured at irradiance and $[CO_2]$ both saturating photosynthesis, that means irradiance of 1 500 (barley) or 1 400 (spruce) μmol m^{-2} s^{-1} and $[CO_2]$ of 1 300 (barley) and 1 900 (spruce) μmol(CO_2) m^{-2} s^{-1}. The irradiances and $[CO_2]$ concentrations suitable for saturation of CO_2 assimilation were preliminary tested (values not shown). P_N and P_{Nsat} were attained at maximal stomatal conductivity approximately 8–10 min after exposure of plants to the corresponding cultivation irradiance and $[CO_2]$. The microclimatic conditions inside the assimilation chamber were kept constant during all measurements as follows: temperature of leaves 24±2.5 °C and relative air humidity 55±2 %.

Chlorophyll fluorescence: Room temperature fluorescence of Chl *a* was measured using a *PAM 101*, *103* fluorometer (*H. Walz*, Effeltrich, Germany) equipped with light-emitting diode (type *USBR*, Stanley; peak wavelength 650 nm) and PIN detector with long-pass filter (*RG 9*, *Schott*) passing fluorescence at wavelengths above 700 nm. The initial fluorescence level (F_0) of the dark-adapted plants was obtained upon excitation with weak modulated measuring beam adjusted to the level not evoking any fluorescence induction related to the reduction of PS2 acceptors. A saturation pulse of 1 s or 800 ms duration at irradiance of 4 000–5 000 μmol m^{-2} s^{-1} at the plant surface was optimal to close all the PS2 RCs and was used for estimation of maximal fluorescence level (F_m). The correct intensity and length of the saturation pulse was checked *via* kinetics of the Chl *a* fluorescence response to the pulse. F_0 and F_m were measured on sufficiently dark-adapted plants (sampled during 8 h dark regime, but after 1 h of darkness minimally) and the maximal photochemical efficiency of PS2 was determined as $F_v/F_m = (F_m - F_0)/F_m$. The three sets of fluorescence experiments were performed:

(*a*) Estimation of steady-state Chl *a* fluorescence parameters was provided under corresponding cultivation irradiance (100 or 1 200 μmol m^{-2} s^{-1}) and under ambient or photosynthesis-saturating [CO_2] on the barley leaf segment or spruce needles (1.5 cm^2). The saturation pulses of the same duration and irradiance as for F_m estimation were used for determination of the maximal fluorescence level during the exposure of plant segment to continuous irradiation (F_m'). To estimate minimal fluorescence of plant segment adapted to actinic irradiance (F_0'), the irradiation was turned off for 3 s and the lowest fluorescence level during this period was regarded as F_0'. The following Chl *a* fluorescence parameters were calculated from F_0' and F_m' measured in steady-state at given irradiance (8–10 min): non-photochemical quenching of F_0 based on Stern-Volmer formalism (SV_0) as $F_0/F_0' - 1$ (Bilger and Björkman 1990), and the relative efficiency of non-radiative dissipation (D) was estimated according to Demmig-Adams *et al.* (1996) as $1 - F_v'/F_m'$.

(*b*) The slow phase of Chl *a* fluorescence induction curve at room temperature (dependence of relative Chl *a* fluorescence on time) was recorded during 10 min at corresponding cultivation irradiance and under ambient or photosynthesis-saturating [CO_2].

(*c*) Relaxation of Chl *a* fluorescence parameters during darkness. The continuous irradiation that corresponded to cultivation irradiance (100 and 1 200 μmol m^{-2} s^{-1}, respectively) was applied for 10 min and SV_0 and D were estimated as in section (*a*). Consequently, the continuous irradiation was switched off and saturation pulses were applied after 10, 20, 60, 90, 180, 240, 300, 420, 540, 660, 780, 900, 1 260, and 1 380 s of darkness for F_m'' estimation. The fluorescence level recorded during darkness period was considered to be F_0''. Chl *a* fluorescence parameters during relaxation in darkness were calculated as $SV_0^{relax} = F_0/F_0'' - 1$ and $D^{relax} = 1 - (F_m'' - F_0''/F_m'')$.

Statistical analysis: The experimental data were tested for significance by a two-sample *F* test for variances followed by a *t*-test. Based on the result of the *F* test a *t*-test for two samples was used assuming either equal variances or unequal variances. All statistical tests were performed using analysis tools from *Microsoft® Excel 97 SR*.

Results and discussion

CO_2 assimilation and extent of the acclimation depression of photosynthesis in barley and spruce plants cultivated under EC: Fig. 1*A,B* demonstrates that the positive acclimation of photosynthetic activity under EC occurred neither for low-irradiance (100 μmol m^{-2} s^{-1}) nor for HI (1 200 μmol m^{-2} s^{-1}) barley plants. The low-irradiance barley cultivated under EC ($^{Barley}EC_{100}$) showed significantly lower P_N (Fig. 1*A*) and CO_2 assimilation capacity lowered by 31 % (P_{Nsat}; Fig. 1*B*) in comparison with low-irradiance barley cultivated under ambient [CO_2] ($^{Barley}AC_{100}$). The EC combined with HI ($^{Barley}EC_{1200}$) induced almost 50 % reduction of P_{Nsat} in comparison with HI barley cultivated under ambient [CO_2] ($^{Barley}AC_{1200}$; Fig. 1*B*). On the contrary to barley, significant stimulation of P_N at EC was observed for low-irradiance spruce plants ($^{Spruce}EC_{100}$), but pursuant to barley no stimulation of P_N under EC was obtained for HI spruce plants ($^{Spruce}EC_{1200}$) (Fig. 1*C*). The positive effect of cultivation under EC disappeared when low-irradiance spruce plants were shortly exposed both to irradiance and [CO_2] saturating photosynthesis (P_{Nsat}; Fig. 1*D*). Then the acclimation depression of photosynthesis, defined as a reduction of P_{Nsat}, occurred. Similarly, the HI-acclimated spruce plants also revealed acclimation depression of CO_2 assimilation, but the decline of P_{Nsat} by 38 % was more pronounced than in $^{Spruce}EC_{100}$ (Fig. 1*D*). The observed different response of low- and high-irradiance spruce plants to EC is in good agreement with field study of Kalina *et al.* (2001) and Marek *et al.* (2002). The sun exposed needles of Norway spruce cultivated under EC in glass domes with lamella windows revealed a slight acclimation depression of photosynthesis, whereas shade needles maintained pronounced stimulation of CO_2 assimilation and capacity of photosynthesis at saturating [CO_2] (Kalina *et al.* 2001, Marek *et al.* 2002).

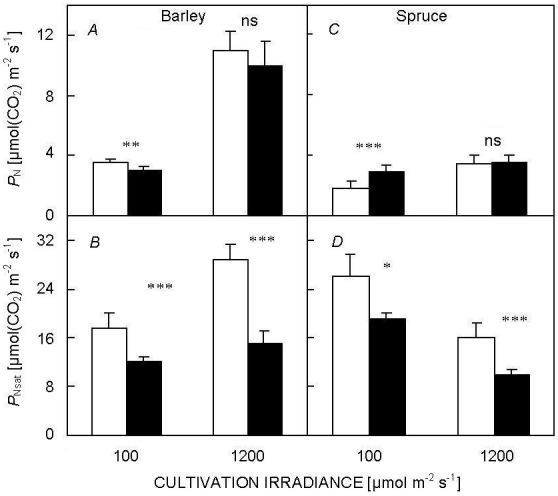

Fig. 1. The steady-state rates of CO_2 assimilation expressed per leaf area on barley (*A*, *B*) and spruce (*C*, *D*) plants cultivated at low and high irradiance (100 and 1 200 μmol m^{-2} s^{-1}) together with ambient (*empty bars*) or elevated (*full bars*) CO_2 concentration. The actual CO_2 assimilation rate (P_N) was estimated at corresponding conditions of cultivation (*A*, *C*). The capacity of CO_2 assimilation (P_{Nsat}) was estimated at irradiance and [CO_2] saturated photosynthesis (see Materials and methods). The differences among individual variants are marked as *, **, ***, and ns for the levels of significance 0.5, 0.01, and 0.001, and non-significant difference, respectively. $n = 8 \pm SD$.

Accordingly, one of differences in the response of photosynthesis for barley and spruce plants to EC lies in stimulation of CO_2 assimilation observed for low-irradiance spruce plants under CO_2 enrichment. We suppose that the accumulation of saccharides related to stimulated CO_2 assimilation under EC occurs and consequently it results in feedback depression of photosynthetic capacity (Kurasová *et al.* 2003). In any case, the extent of acclimation depression is not so pronounced for low-irradiance acclimated spruce plants to affect the photosynthesis at actual growth conditions and thus to reverse the stimulating effect of EC on P_N in contrast to low-irradiance barley plants (Fig. 1*B,D*). In addition, the HI spruce plants cultivated under controlled environment revealed more pronounced manifestation of acclimation depression of photosynthesis as compared to that found previously for the Norway spruce in field conditions (Marek and Kalina 1996, Špunda *et al.* 1998). This fact can be explained by higher daily dose of PhAR for spruce plants acclimated inside growth cabinet, where HI was applied for the duration of 16 h, whereas in the field conditions the exposure of plants to HI occurs maximally during a sunny day. Notably reduced P_{Nsat} confirmed the marked photoinhibition of spruce photosynthesis at high growth irradiance (by 38 and 47 %) in $^{Spruce}AC_{1200}$ and $^{Spruce}EC_{1200}$ in comparison with P_{Nsat} in $^{Spruce}AC_{100}$ and $^{Spruce}EC_{100}$ (Fig. 1*D*). The second reason of pronounced manifestation of acclimation depression in spruce plants cultivated under controlled environment lies probably in insufficient sink-strength to consume the higher amounts of saccharides produced in response to CO_2 enrichment. The role of low sink-strength in occurrence of acclimation depression of photosynthesis is supported by the fact that the measurements on spruce plants were carried out at the end of their vegetation period (November–February) and in addition the germinating new buds were regularly nipped. It is in agreement with findings that the down-regulation of photosynthesis under EC in Scots pine is associated with a seasonal decline in sink-strength as shoot growth is completed (Jach and Ceulemans 2000).

The second aspect of the different acclimation response of barley and spruce photosynthetic apparatus is the fact that barley plants revealed higher P_N with higher ability to increase CO_2 assimilation to acclimate to HI cultivation than spruce plants (compare Fig. 1*A* and 1*C*). Therefore we suggest in agreement with Huner *et al.* (1993, 1998) that the ability of spruce plants to acclimate to changing environment and to prevent possible excessive over-reduction of PS2 RCs in comparison with barley plants does not lie mainly in pronounced enhancement of their photosynthetic capacity.

Response in pigment composition: $^{Barley}EC_{100}$ and $^{Barley}EC_{1200}$ exhibited a slightly suppressed total Chl content per dry matter, increased relative amount of carotenoids (judged from the decreased Chl $a + b$/Car $x + c$ ratio), and equivocal LHCs (as shown by an almost unchanged Chl a/b) in comparison with $^{Barley}AC_{100}$ and $^{Barley}AC_{1200}$ (Table 1). Similarly, the growth of wheat at EC under field conditions had no apparent effect on the polypeptide composition of different leaves at the completion of blade emergence (Nie *et al.* 1995). Under EC the total Chl content usually decreases (DeLucia *et al.* 1985, Špunda *et al.* 1998, Kalina *et al.* 2001). In agreement, we observed the decrease of total Chls by 25 % and carotenoids by 33 % in $^{Spruce}EC_{1200}$ relative to $^{Spruce}AC_{1200}$ (Table 1). The markedly suppressed pigment contents can not be explained entirely by enhancement of dry matter under EC because the ratio of dry to fresh matter of $^{Spruce}EC_{1200}$ needles was only non-significantly increased by 9 % in comparison with $^{Spruce}AC_{1200}$ (values not shown). Conversely, the stimulation of Chl and carotenoid contents was proved in $^{Spruce}EC_{100}$ in comparison with $^{Spruce}AC_{100}$. In addition, the influence of EC on Chl a/b and Chl $a+b$/Car $x+c$ ratios was different for barley and spruce plants (Table 1).

No differences induced by EC in amount of xanthophyll cycle pigments (VAZ), but the increased amount of lutein (L) were established both in $^{Barley}EC_{100}$ and $^{Barley}EC_{1200}$ in comparison with $^{Barley}AC_{100}$ and $^{Barley}AC_{1200}$ (Table 1). EC also evoked enhancement of β-carotene (β-car) content that was by 68 % higher in $^{Barley}EC_{100}$ than in $^{Barley}AC_{100}$. Thus, HPLC analysis confirmed that increased relative amount of carotenoids for barley cultivated under EC was related particularly to the accumulation of β-car (at low-irradiance) and L (at HI) (Table 1). In comparison with barley the most evident differences of the EC effect on carotenoid composition in spruce needles were the decreased amounts of L and VAZ (Table 1). $^{Spruce}EC_{100}$ revealed a significant 54 % increase in β-car content in comparison with $^{Spruce}AC_{100}$. Taking this in account together with the observed higher total Chl content for $^{Spruce}EC_{100}$, the higher Chl a/b ratio (Table 1) might correspond rather to increased amount of RCs than to diminution of LHCs in these plants. In contrast, in spruce plants the HI combined with EC induced a diminution of LHCs (Table 1) and probably also CPs relative to CCs as can be judged from the increase in β-car/total xanthophylls ratio that roughly estimates the ratio of CCs to CPs and LHCs (Demmig-Adams 1998, Kurasová *et al.* 2002). The loss of total Chls coinciding with an approximately 40 % loss of both LHC2 and CP29 proteins was also established for Scots pine exposed to combined effect of cold and HI during winter (Ottander *et al.* 1995).

The extent of de-epoxidation of xanthophylls and the photochemical efficiency of PS2: A negligible effect of EC on the de-epoxidation state of xanthophylls, estimated under growth irradiance or irradiance saturating V de-epoxidation ($DEPS_{act}$ and $DEPS_{sat}$), was found out both in low- and high-irradiance barley plants (Fig. 2*A,B*). On the contrary, the combined effect of EC and HI resulted in strikingly slowed-down Z+A epoxidation during the dark

Table 1. The pigment contents in barley and spruce plants cultivated at low and high irradiance (100 and 1 200 μmol m^{-2} s^{-1}) together with ambient [350 μmol(CO_2) mol^{-1}; AC_{100}, AC_{1200}] or elevated [700 μmol(CO_2) mol^{-1}; EC_{100}, EC_{1200}] CO_2 concentration. The total chlorophyll (Chl *a+b*) and carotenoid (Car *x+c*) contents per dry matter were determined spectrophotometrically in 1 h dark-adapted leaves and Chl *a/b* and Chl *a+b*/Car *x+c* ratios were calculated. The contents of lutein (L), β-carotene (β-car), and xanthophyll cycle pigments (VAZ) expressed on Chl *a+b* basis were estimated by semi-isocratic (barley) and gradient (spruce) HPLC analysis in the same pigment extracts as used for spectrophotometric analysis. Means (*n*) from ten (barley) and six (spruce) measurements ± standard deviations (SD) are given. Statistical differences between AC and EC conditions are presented by symbols *, **, ***, and ns which indicate difference at 0.05, 0.01, 0.001 levels of significance and a non-significant difference, respectively.

		Chl *a+b* [g kg^{-1}]	Car *x+c*	Chl *a/b*	Chl *a+b* / Car *x+c*	L [mmol mol^{-1}(Chl)]	β-car	VAZ
Barley	AC_{100}	17.25 ± 0.93**	3.14 ± 0.29*	3.14 ± 0.03^{ns}	5.52 ± 0.27***	176.8 ± 2.8***	78.3 ± 13.7***	59.9 ± 2.5^{ns}
	EC_{100}	13.65 ± 1.55	2.76 ± 0.32	3.17 ± 0.04	4.96 ± 0.11	187.3 ± 2.9	131.3 ± 9.4	60.3 ± 4.6
	AC_{1200}	6.58 ± 0.29*	1.98 ± 0.12 ^{ns}	3.97 ± 0.10*	3.33 ± 0.23*	181.4 ± 9.1***	139.5 ± 33.8^{ns}	183.7 ± 18.0^{ns}
	EC_{1200}	5.81 ± 1.11	1.86 ± 0.32	3.72 ± 0.21	3.12 ± 0.13	240.8 ± 12.9	170.7 ± 6.6	188.5 ± 17.1
Spruce	AC_{100}	2.66 ± 0.22**	0.55 ± 0.02*	2.89 ± 0.13**	4.81 ± 0.27***	248.0 ± 15.6***	64.3 ± 15.7**	72.7 ± 6.6***
	EC_{100}	3.12 ± 0.06	0.58 ± 0.01	3.06 ± 0.03	5.37 ± 0.07	189.0 ± 11.9	99.0 ± 13.5	49.0 ± 2.2
	AC_{1200}	2.84 ± 0.17***	0.79 ± 0.07***	2.93 ± 0.05***	3.61 ± 0.16***	327.8 ± 8.2**	80.5 ± 15.3^{ns}	113.4 ± 7.4**
	EC_{1200}	2.13 ± 0.18	0.53 ± 0.05	3.14 ± 0.04	4.05 ± 0.18	300.7 ± 13.5	94.0 ± 16.1	88.3 ± 12.1

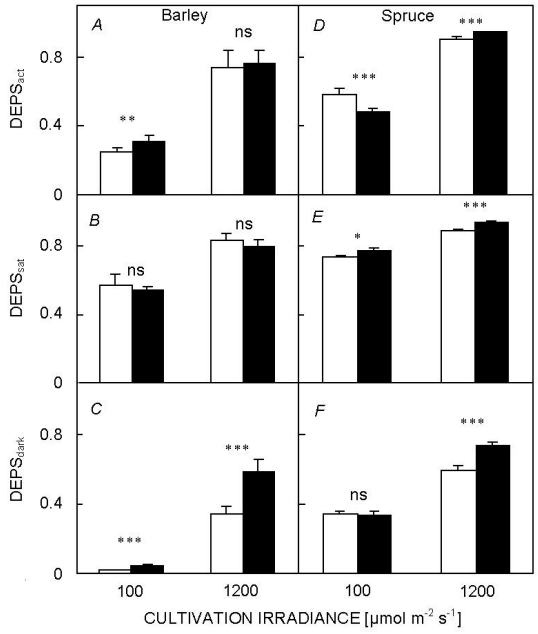

Fig. 2. The de-epoxidation state of the xanthophylls (DEPS) for barley (*A–C*) and spruce (*D–F*) cultivated at low and high irradiance (100 and 1 200 μmol m^{-2} s^{-1}) together with ambient (*empty bars*) or elevated (*full bars*) CO_2 concentration. DEPS was estimated on 1 h dark-adapted leaf segments ($DEPS_{dark}$; *C*, *F*), on leaf segments after 10 min exposure to the irradiance used during plant cultivation ($DEPS_{act}$; *A*, *D*), or to high irradiance of 1 870 (*B*) and 2 100 (*E*) μmol m^{-2} s^{-1} ($DEPS_{sat}$). The de-epoxidation of xanthophylls was estimated at ambient (*empty bars*) or photosynthesis-saturating [CO_2] (*full bars*). *n* = 10±SD (barley) or 6±SD (spruce). The statistical differences are presented as in Fig. 1.

$DEPS_{dark}$ in $^{Barley}EC_{1200}$ in comparison with $^{Barley}AC_{1200}$ (Fig. 2*C*). The considerably slowed-down epoxidation was also reported for plants experiencing HI stress alone (Jahns and Miehe 1996) or HI combined with other environmental stresses such as low temperatures (Adams and Demmig-Adams 1994, Ottander *et al.* 1995), nitrogen-deficiency (Verhoeven *et al.* 1997), *etc*.

In Norway spruce plants, the CO_2 enrichment notably affected the de-epoxidation of xanthophylls in contrast to barley (Fig. 2*D–F*). EC evoked an opposite response of the xanthophyll cycle characteristics in the low- and high-irradiance acclimated plants as demonstrated by decreased and increased de-epoxidation of V in $^{Spruce}EC_{100}$ and $^{Spruce}EC_{1200}$ (Fig. 2*D*). The V convertibility was enhanced by EC both for low- and particularly for high-irradiance spruce plants, whereas no effect was observed for barley plants (cf. *E* and *B* in Fig. 2). In addition, the extremely high ability to convert V, yielding almost 95 % Z+A in total VAZ, was established for $^{Spruce}EC_{1200}$. These plants were also characterised by the most marked reduction of Z and A epoxidation in darkness (Fig. 2*F*), when only 22 % of Z+A accumulated under the actual cultivation conditions (Fig. 2*D*) was subjected to conversion back to V during 1 h of dark-adaptation.

The higher ability to effectively de-epoxidise V to Z+A and increased V convertibility were confirmed for spruce plants under all regimes of acclimation in comparison with barley plants. Increased convertibility of V was established in spite of the fact that spruce needles revealed a lower relative amount of VAZ and lower Chl *a*/*b* ratio in $^{Spruce}EC_{1200}$ if compared with estimates for barley plants (Table 1). This seems to be in contradiction with the results of Gilmore (1997), where the higher convertibility of V is related to reduction of LHCs and enhanced VAZ size. The extremely strong V convertibility could be explained by different organisation of pigment-protein complexes in spruce photosynthetic apparatus allowing a higher accessibility of V de-epoxidase to V.

Non-significant difference between maximal PS2 photochemical efficiency was observed for $^{Barley}AC_{100}$ and $^{Barley}EC_{100}$ (Fig. 3*A*). In contrast to barley, signifi-

cantly higher F_v/F_m was found for $^{Spruce}EC_{100}$ in comparison with $^{Spruce}AC_{100}$ (Fig. 3*B*) supporting the positive effect of stimulated CO_2 assimilation for low-irradiance spruce plants. However, the concurrent effect of HI and EC during plant cultivation caused a pronounced suppression of F_v/F_m in photosynthetic apparatus of both barley and spruce in comparison with ambient [CO_2], but more pronounced reduction of F_v/F_m (by 17 %) was observed in spruce plants (Fig. 3*B*). In this case, the expressive retention of Z+A amount (Fig. 2*C,F*) induced by combined effect of HI and EC was accompanied by persistently low PS2 photochemical efficiency. Because F_v/F_m was determined within 1–8 h of darkness, the observed F_v/F_m decrease for EC_{1200} plants was associated with slowly reversible PS2 inactivation.

The pronounced acclimation depression of photosynthesis under EC of both plant species acclimated to HI was associated with decreased PS2 photochemical efficiency (Fig. 2*A,B*) in agreement with already published results (Peterson 1991, Marek and Kalina 1996, Kalina *et al.* 2000). In agreement with Špunda *et al.* (1998) and Hymus *et al.* (2001) our present results support a presumption that EC enhances sensitivity of photosynthetic apparatus of barley and especially of spruce plants cultivated at HI to photo-inactivation of PS2.

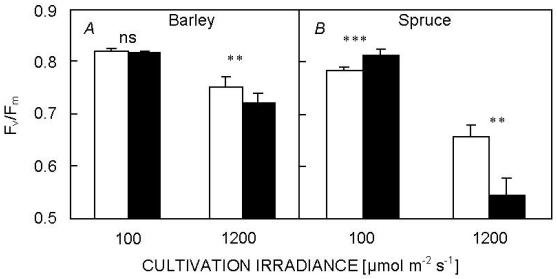

Fig. 3. The maximal photochemical efficiency of PS2 (F_v/F_m) of dark-adapted barley (*A*) and spruce (*B*) plants cultivated at low and high (100 and 1 200 µmol m^{-2} s^{-1}) irradiance together with ambient (*empty bars*) or elevated (*full bars*) CO_2 concentration. n = 20±SD (barley) or 6±SD (spruce). The statistical differences are presented as in Fig. 1.

The feedback-induced alternation of PS2 function under EC: The decline of photosynthetic activity reduces utilisation of absorbed PhAR in photochemical reactions and enhances demand on other de-excitation processes, particularly on non-radiative dissipation (Adams and Demmig-Adams 1994). The decreased PhAR utilisation in PS2 photochemical reactions (Fig. 3*A*) associated with acclimation depression of photosynthesis (Fig. 1*B*) for barley plants grown under EC induced an enhancement of non-radiative dissipation originating within LHCs (SV_0; Fig. 4*A*). As we have shown previously (Kurasová *et al.* 2002, 2003), the assessment of non-radiative dissipation by non-photochemical quenching of maximal fluorescence (NPQ) may be questionable due to possible underestimation of maximal fluorescence in systems with substantial Z+A amount persistent in the dark. Therefore, we suppose that NPQ is not a suitable probe for precise estimation of non-radiative dissipation in plants exposed to greater environmental stress and thus we used SV_0 and relative efficiency of non-radiative dissipation D (calculated as $1 - F_v'/F_m'$) in this study. The decreased PS2 photochemical efficiency observed in $^{Spruce}EC_{1200}$ (Fig. 3*B*) evoked enhancement of SV_0 by 18 % (Fig. 4*B*)

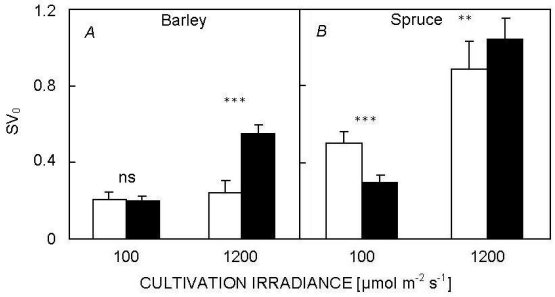

Fig. 4. The non-photochemical quenching of minimal (F_0) chlorophyll *a* fluorescence (SV_0) of barley (*A*) and spruce (*B*) plants cultivated at low and high irradiance (100 and 1 200 µmol m^{-2} s^{-1}) together with ambient (*empty bars*) or elevated (*full bars*) CO_2 concentration. The steady-state values of SV_0 were estimated at corresponding irradiance used during cultivation of plants and at ambient (*empty bars*) or photosynthesis saturating [CO_2] (*full bars*). n = 10±SD (barley) or 9±SD (spruce). The statistical differences are presented as in Fig. 1.

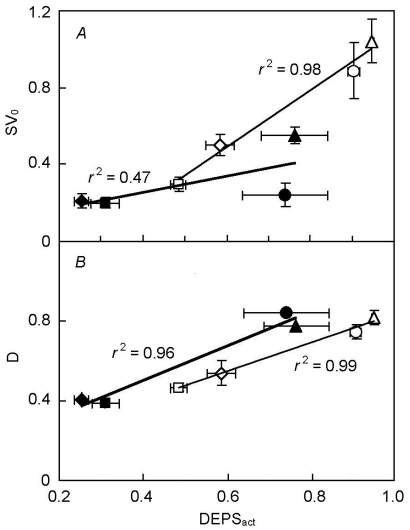

Fig. 5. The relationships between de-epoxidation state of the xanthophylls ($DEPS_{act}$) and non-photochemical quenching of Chl *a* fluorescence (SV_0; *A*) and relative efficiency of non-radiative dissipation (D calculated as $1 - F_v'/F_m'$; *B*) for barley (*full symbols*) and spruce (*empty symbols*) plants. The values were obtained on plants cultivated at low irradiance (100 µmol m^{-2} s^{-1}) and ambient (*diamonds*) or elevated (*squares*) [CO_2], and at high irradiance (1 200 µmol m^{-2} s^{-1}) and ambient (*circles*) or elevated (*triangles*) [CO_2] and from Figs. 2*A*,*D* and 4. The linear regressions of values are represented by *thin* (spruce) and *broad* (barley) lines.

and corresponding increase of V conversion to Z+A in comparison with $^{Spruce}AC_{1200}$ (Fig. 2*D*). On the contrary, the low-irradiance spruce plants revealed decreased non-radiative dissipation under EC (Fig. 4*B*) in agreement with increased photosynthetic activity and photochemical efficiency (Figs. 1*C* and 3*B*) and with decreased de-epoxidation state of xanthophylls (Fig. 2*D*). To better understand the role of the xanthophyll cycle in regulation of PS2 photochemistry in spruce and barley, we draw the dependencies of SV_0 and D on xanthophyll de-epoxidation (Fig. 5*A,B*). As shown in Fig. 5*A*, satisfactory linear dependence of SV_0 on the de-epoxidation of xanthophylls was obtained in spruce in contrast to barley. Thus, the non-radiative dissipation within LHCs does not directly correlate with the xanthophyll de-epoxidation in barley plants. On the contrary, in both plant species the relative efficiency of non-radiative dissipation (D) very well correlated with the de-epoxidation state of xanthophylls (Fig. 5*B*). The high absolute D values estimated in $^{Barley}AC_{1200}$ and $^{Barley}EC_{1200}$ predicate marked non-radiative dissipation within inactivated PS2 RCs, and the correlation between D and the content of de-epoxidized xanthophylls should be taken with care.

In addition, analysis of SV_0 showed that both low- and high-irradiance spruce plants revealed higher non-radiative dissipation of absorbed PhAR than barley plants (cf. absolute values of SV_0 in Fig. 4*A,B*). The measurements of dependence of Chl *a* fluorescence on time (Fig. 6*A–D*) also showed that non-radiative dissipation of absorbed PhAR was both more efficient and rapidly induced within the photosynthetic apparatus of Norway spruce in comparison with spring barley. The rapid induction of non-photochemical quenching of Chl *a* fluorescence was the most pronounced for $^{Spruce}EC_{1200}$, when already at 23 s after switching on strong irradiation (1 200 μmol m^{-2} s^{-1}) the fluorescence was quenched below minimal fluorescence (F_0) level (Fig. 6*D*). The induction of non-photochemical quenching for $^{Barley}EC_{1200}$ was considerably slower and the decrease below F_0 occurred at 145 s of the same irradiation (Fig. 6*D*).

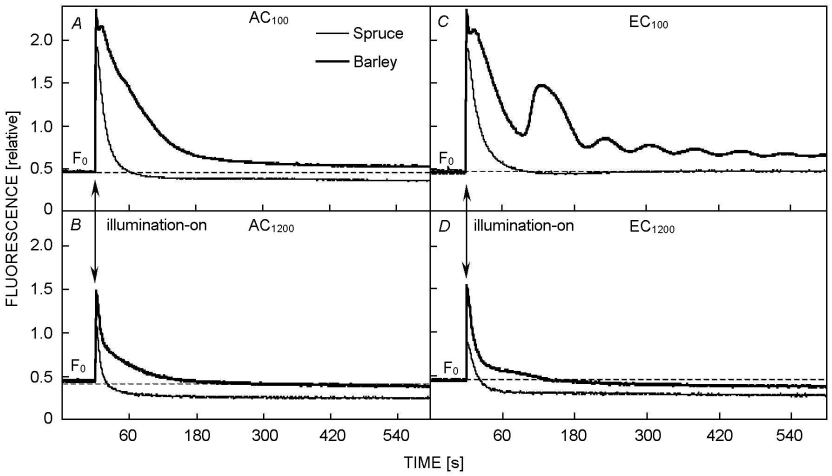

Fig. 6. A slow phase of the Chl *a* fluorescence induction curves for barley (*broad line*) and spruce (*thin line*) plants cultivated at low and high irradiance (100 and 1 200 μmol m^{-2} s^{-1}) together with ambient (*A*, *B*) or elevated (*C*, *D*) CO_2 concentration. The curves were measured under corresponding irradiance used during cultivation and corresponding [CO_2] (*A*, *B*) or photosynthesis-saturating [CO_2] (*C*, *D*). For better legibility of the slow phase fluorescence induction curves, the relative Chl *a* fluorescence was normalised to 0.45 at ` level. The representative curves from ten measurements are presented.

The expressive retention of Z+A amount induced by combined effect of HI and EC on spruce photosynthetic apparatus (Fig. 2*F*) was accompanied by low PS2 photochemical efficiency (Fig. 3*B*) and also by permanent non-radiative energy dissipation that may be either Z+A-dependent or Z+A-independent. The permanency of non-radiative energy dissipation was confirmed by its relaxation kinetics after switching radiation off and following the period of darkness (Fig. 7). As shown, the low-irradiance spruce plants exhibited rapid relaxation kinetic of both D (Fig. 7*A*) and SV_0 (Fig. 7*B*). After 17 min of darkness a full relaxation of SV_0 was achieved. On the contrary, the HI spruce plants revealed markedly slowed relaxation of both fluorescence parameters and the lowest extent of relaxation was estimated for the $^{Spruce}EC_{1200}$ (Fig. 7). These plants even after 23 min of darkness exhibited only 28 and 50 % relaxation of D and SV_0, respectively. Hence in spruce plants cultivated under HI and particularly under HI in combination with EC the long-term fluorescence quenching originating both in photo-inactivated PS2 RCs (Fig. 7*A*) and light-harvesting complexes (Fig. 7*B*) is induced. Ottander *et al*. (1995) saw the success of evergreens (Scots pine) under combined effect of cold climate and HI in ability to preserve LHCs pool in a highly quenched state that dissipates absorbed energy non-radiatively due to elevated and retained levels of Z+A. We observed a similar manifestation of the co-occurring effect of EC and HI on marked retention of Z+A (Fig. 2*F*) and their engagement in non-radiative dissipation of absorbed PhAR for spruce plants

(Fig. 4*B*). Despite of finding that EC sensitises photosynthetic apparatus of Norway spruce to PS2 photo-inactivation (Fig. 3*B*), we suppose that the effective non-radiative dissipation of excitation energy within LHCs is a key feature of protection and main strategy of acclimation in the Norway spruce plants.

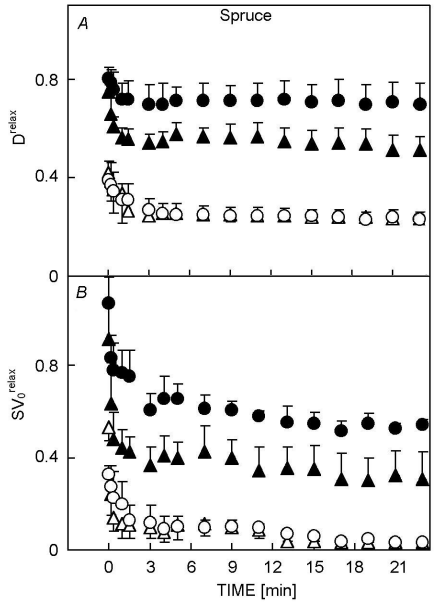

Fig. 7. A kinetic of relaxation relative efficiency of non-radiative dissipation (D^{relax}; *A*) and non-photochemical quenching of Chl *a* fluorescence (SV_0^{relax}; *B*) measured on spruce shoots during a 23 min period of darkness after 10 min irradiation by cultivation irradiance. The values were obtained on spruce plants cultivated at low (100 μmol m^{-2} s^{-1}; *empty symbols*) or high irradiance (1 200 μmol m^{-2} s^{-1}; *full symbols*) together with ambient (*triangles*) or elevated (*circles*) CO_2 concentration. The measurements were provided under cultivation irradiance and ambient (AC_{100} and AC_{1200}) or photosynthesis-saturating [CO_2] (EC_{100} and EC_{1200}). The fluorescence parameters at time 0 s correspond to steady-state values after 10 min of irradiation. n = 5±SD.

In conclusion, our study confirmed that for both barley and Norway spruce a pronounced acclimation depression of CO_2 assimilation might develop even after 10-d cultivation under EC (Fig. 1*B*,*D*). Moreover, the reduced photosynthetic capacity under HI was accompanied with the reduced Chl content (Table 1), prolonged inhibition of PS2 photochemical efficiency (Fig. 3), retention of de-epoxidised xanthophylls in darkness (Fig. 2*C*,*F*) together with enhanced demand on non-radiative dissipation of absorbed PhAR within LHCs estimated as SV_0 (Fig. 4). We speculate that the found down-regulation of PS2 photochemistry is a consequence of feedback regulation of utilisation of absorbed PhAR due to sink-limitation of photosynthetic carbon reduction, rather than a direct effect of EC on PS2 function and composition.

On contrast to spring barley, different acclimation response to EC was established for low- and high-irradiance grown spruce plants. The stimulation of CO_2 assimilation in spruce plants grown under low irradiance and EC (Fig. 1*C*) was accompanied with increased Chl content (Table 1), significantly improved PS2 photochemical efficiency (Fig. 3*B*), and reduced demand on the non-radiative dissipation of absorbed PhAR demonstrated by reduced de-epoxidation of xanthophylls (Fig. 2*D*) and SV_0 (Fig. 4*B*). These characteristics correspond with the enhanced capacity of the PhAR utilisation within the PS2 photochemical reactions induced by a stimulation of CO_2 assimilation. Thus, these findings confirmed our previous results on Norway spruce acclimated to EC under field conditions. In this study, the sun acclimated needles were more susceptible to acclimation depression, whereas in the shaded ones photosynthesis was stimulated (Kalina *et al.* 2001, Marek *et al.* 2002). To better understand the development of individual manifestations of the positive and/or negative response to EC a further study of detailed kinetics of the acclimation to CO_2 enrichment is necessary.

Our results on the acclimation of spring barley and Norway spruce to the excess excitation pressure represented by HI alone or in combination with EC also confirmed the hypothesis of Huner *et al.* (1993, 1998). We established that crop species respond to high excitation pressure particularly by enhancement of photosynthetic activity (compare Fig. 1*A* and 1*C*), whereas evergreens posses an extensive capacity to cope with excess excitation energy *via* increased efficiency of non-radiative dissipation within LHCs (compare Fig. 4*B* and 4*A*). The view about dependency of the V convertibility on the size of xanthophyll pool and in opposite relation to the LHCs amount is generally accepted (Gilmore 1997). On the contrary, the Norway spruce revealed higher V convertibility than spring barley under all cultivation regimes (Fig. 2) in our study, despite of the same or even considerably smaller xanthophyll pool (Table 1). We suppose that a better accessibility of violaxanthin de-epoxidase to V in spruce plants is related to the different organisation of the pigment-protein complexes that is related to the different acclimation strategy of spruce photosynthetic apparatus to environmental stresses. Accordingly, both the non-photochemical fluorescence quenching of F_0 (SV_0) and the portion of absorbed PhAR dissipated as heat (D) were linearly correlated to the de-epoxidation state of the xanthophylls ($DEPS_{act}$) for the Norway spruce needles exposed to the corresponding cultivation conditions, but not for barley (Fig. 5*A*). This indicates a key role of the zeaxanthin and antheraxanthin mediated non-radiative dissipation within LHCs in prevention from the PS2 over-reduction under excess excitation pressure of Norway spruce represented by HI alone or by the combined action of HI and EC. Moreover, the spruce photosynthetic apparatus acclimated to HI alone and particularly to both HI and EC is capable to appreciably speed up the induction of non-radiative dissipation of absorbed

PhAR in comparison with barley (Fig. 6*B,D*) and considerably stabilise the non-radiative dissipation within LHCs in the darkness (Fig. 7). We suggest that this specific feature of Norway spruce photosynthetic apparatus is mediated by permanent accumulation of zeaxanthin and antheraxanthin (Fig. 2*F*) and confirms the capability of the efficient down-regulation of excitation energy transfer within LHCs in relation to the proposed specificity of pigment-protein organisation.

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