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ABILITY OF SILVER FIR AND EUROPEAN BEECH SAPLINGS TO ACCLIMATE PHOTOCHEMICAL PROCESSES TO THE LIGHT ENVIRONMENT UNDER DIFFERENT CANOPIES OF TREES

ABSTRACT: Ability to acclimate photochemical processes by saplings of silver fir (*Abies alba* Mill.) and European beech (*Fagus sylvatica* L.) growing in contrasting light environments was investigated using chlorophyll *a* fluorescence. The leaves of saplings acclimated to irradiance under the open canopy of *Larix decidua* Mill. showed higher photosynthetic efficiency and more efficient photoprotective mechanism than those under the shade of the denser *Picea abies* Karst. canopy. Interspecific differences in quantum yield of *PS II* photochemistry, apparent electron transport rate, non-photochemical quenching and the values of the cardinal points of light response curves of these parameters resulted from the shade adaptation of leaves and leaf life-span. Acclimation to shade did not increase sensitivity to strong *PS II* downregulation induced by light patch of low intensity but predisposed both species to photoinhibition caused by high light stress. They were able to efficiently use light patches of low intensity, but after high light stress beech leaves from the *Larix* stand recovered more dynamically in quantum yield of *PS II* photochemistry than silver fir. It may give an advantage to beech in between-species competition for use of short-lasting and intensive sun patches.

KEY WORDS: acclimation to irradiance; chlorophyll *a* fluorescence, light patch, photoinhibition, species competition, sun patch

1. INTRODUCTION

At the young age, shade-tolerant forest trees grow in shade of mature trees' canopy. Ability to survive in the low light environment is genetically determined but may be modified by site conditions of growth (climate and soil) and between-species competition (Ellenberg *et al.* 1992, Brzeziecki 1995, Küppers *et al.* 1996). Under a trees canopy, dispersed light of low photosynthetic photon flux (*PPF*) and direct irradiance of high *PPF* as sunflecks provide energy used for photosynthesis by plants growing in the forest floor. Ability to use sunflecks for photosynthesis is regarded as an important adaptation of late-successional trees to the forest light environment (Kitao *et al.* 2000, Niinemets and Valladares 2004, Kitao *et al.* 2006, Shimizu *et al.* 2006). Sunflecks last from seconds up to (rarely) minutes and may provide from about 30 to 80% of light energy which is absorbed by leaves (Küppers *et al.* 1996).

Plants require light for photosynthesis but on the other hand, they need protection from excessive light energy (Niyogi 1999). In high irradiance, photosynthetic processes do not consume the total absorbed energy. To

balance absorption and use of energy, plants developed the photoprotective mechanism. Namely, they are able to adjust light-harvesting antennae size and assimilatory linear electron transport, dissipate excessive energy as heat in xanthophyll cycle, use alternative electron transport pathways (photorespiration, water – water cycle) and scavenge reactive oxygen species to avoid photodamage (Adams and Demmig-Adams 1994, Verhoeven *et al.* 1996, Niyogi 1999, Adams *et al.* 2004). In nature, however, photo-inactivation of *PS II* and its, connected with D1 turnover (see Appendix for explanation), repair occur under any light environment (Chow *et al.* 2005). Therefore, the continual synthesis of proteins must match the rate of damage to avoid photoinhibition resulting from the loss of functioning of *PS II* centres (Niyogi 1999). Nonetheless, under some circumstances, reversible photoinhibition (downregulation of *PS II* – see Appendix for explanation) can also play a photoprotective role (Adams *et al.* 2004). All photoprotective processes act in concert and they may determine competitive interactions between plants. This subject is investigated in our study.

Susceptibility to photoinhibition is an important limitation to photosynthetic carbon acquisition and conifer seedlings establishment under the photoinhibitory conditions e.g. at the upper treeline (Germino and Smith 1999, Johnson *et al.* 2004). Photoinhibitory selective pressure may play a significant role in the between-species competition. Species equipped with the efficiently functioning photoprotective mechanism, fast-recovering from *PS II* downregulation and fast-acclimating to different light environments have an advantage compared with the slowly-acclimating ones. Adaptation to varying irradiance conditions has been crucial in the evolutionary success of plants (Weiner 1999, Close and McArthur 2002).

In the earlier study, we found that silver fir saplings acclimated to the light environments created by the open canopy of European larch and the dense canopy of Norway spruce. The saplings growing in the *Larix* stand showed greater increments in height, greater CO₂ assimilation rates and changes

in leaf structure typical for high-light growing plants compared with those in the *Picea* stand. Young firs from the *Larix* stand have been better acclimated to higher irradiance (Robakowski *et al.* 2004).

The purpose of the current study was to compare the evergreen conifer silver fir and the deciduous European beech ability to acclimate their photochemical processes and photoprotective responses to the contrasting light environments under the open canopy of larch or under the close canopy of Norway spruce. Silver fir and European beech are extremely shade tolerant at the young age (Forestry Compendium 2005) and they often compete for light growing in the same sites. Moreover, the seedlings of both species have been used for the stands' transformation in the Karkonosze Mts., Southern Poland, therefore they were chosen for our study. We hypothesized that: (1) the saplings of silver fir and European beech growing in the low shade of *Larix* crowns have greater efficiency of photochemical processes compared to those growing in the strong shade of *Picea* crowns; (2) the saplings from the *Larix* stand, which were better acclimated to high irradiance than those from the *Picea* stand, should faster and more efficiently recover *PS II* quantum yield (see Appendix for explanation) after being exposed to light patch i.e. short-lasting (several minutes) illumination provided from an artificial source; (3) the long-lived leaves of *Abies alba* can show similar photochemical adaptation to the light environment compared with the seasonal leaves of *Fagus sylvatica* as both often occur in the same sites in strong shade.

2. MATERIALS AND METHODS

2.1. Plant material

Photochemical processes were studied in the most shade-tolerant European tree species: evergreen conifer silver fir and deciduous broadleaf European beech. Silver fir typically grows under moderately cool climatic conditions in lower zones of mountains (Brzeziecki 1995, Jaworski 1995). This late-successional species can grow for long in shade of mature trees canopy maintaining an ability to recover in growth under favourable

light conditions (Ellenberg *et al.* 1991, Jaworski 1995). Its long lived needles can function for 8–11 years (Forestry Compendium 2005). European beech often grows in the same stands as silver fir but has a larger scale of ecological requirements and geographical range. European beech, contrary to slowly-growing silver fir, combines strong shade-tolerance with a dynamic growth and it sheds leaves each autumn (Jaworski 1995, Kazda *et al.* 1998).

In 1996, seeds of silver fir and European beech collected from the mature trees growing in the Karkonosze Mountains were sown in a nursery “Jelenia Góra-Jagniątkow” (600 m a.s.l., 50.5°22’N, 15.5°15’E) in the Karkonosze National Park, Southern Poland. For three years, plants were cultivated in the substrate being a mixture of local forest soil and peat (7:3 v/v) without fertilization under growing tunnels covered with a green shading cloth (25% light transmittance). In March 1999, 400 root-balled seedlings were planted in five fenced wooded plots with monospecific stands dominated by different tree species: *Picea abies*, *Pinus sylvestris* L., *Larix decidua*, *Fagus sylvatica* and *Betula pendula* Roth.

2.2. Experimental conditions

The experimental plots were situated in the neighbourhood of the nursery. They were uniform with respect to the environmental characteristics (acid brown soil, altitude – about 600 m a.s.l., exposure – N or NNW) but they differed in the canopy structure (for the detailed description of the plots see Robakowski *et al.* 2004). Two plots characterised by contrasting light environments were chosen: the stand with a close canopy of Norway spruce and the stand with a more open canopy of European larch. The light conditions in the stands were determined by measurements of the canopy openness using photographs of the canopies. In August 2002, the pictures were taken in the network of the squares of 10 × 10 m using wide-angle lens with the focal length of 28 mm. Images covering the canopy projected area of each stand were then processed digitally using Photoshop 6.0 to obtain the percentage of an open area. The mean values of the *Picea* canopy

openness was $17 \pm 4\%$ and the *Larix* $45 \pm 7\%$ (the mean value \pm SD). In July 2005, the measurements of canopy openness were repeated using Lemon’s densiometer (Lemon 1957). The results were similar to those obtained from the canopy photographs. Canopy openness was highly correlated with relative values of photosynthetic photons flux (Robakowski *et al.* 2004)

2.3. Measurements of chlorophyll *a* fluorescence

At the end of July 2005, leaves from the current annual increments of silver fir (from the top verticil) and European beech saplings growing in the *Picea* and *Larix* stands were collected into plastic tubes with a moist filter paper to avoid a leaf drying and stomatal closure. On three occasions, leaves were removed from five randomly chosen *Abies alba* or five *Fagus sylvatica* saplings and transported to the laboratory located in the nursery. In total, the measurements were conducted on fifteen individuals from each species. Prior to fluorescence measurements, leaves were acclimated to dark for at least one hour (maximum for four hours) using leaf clips. Light response curves were generated using Fluorescence Monitoring System (FMS 2, Hansatech and Norfolk, UK) operating in an online mode. The fiberoptics encased in a light-tight chamber was inserted onto the leaf clip and the *Fagus* leaf or tightly arranged *Abies* needles were exposed to modulated measuring light of $0.05 \mu\text{mol m}^{-2} \text{s}^{-1}$. After reading minimum fluorescence yield F_0 , a saturating 0.7 s pulse of light (PPF = $15.300 \mu\text{mol m}^{-2} \text{s}^{-1}$) was switched on to provide a maximum fluorescence yield (F_m) (see Appendix for explanation). The measurements were taken at ambient temperature from about 19 to 20°C, which was monitored using a thermocouple installed in the leaf chamber. First, maximum quantum yield of *PS II* photochemistry (F_v/F_m) was determined. Then, to generate light response curves of *PS II* quantum yield photochemistry (Φ_{PSII}) (see Appendix), actinic light was provided to the leaf enclosed in the clip. Up to 10 levels of actinic light was applied in an increasing order, and for each level, after a stable steady state fluorescence (F_s) was reached, 0.7 s saturating pulse was

delivered and maximum fluorescence yield in light (F'_m) determined. Quantum yield of *PS II* was calculated by the built-in software as: $\Phi_{\text{PSII}} = (F'_m - F_s)/F'_m$ (Genty *et al.* 1989). At each value of actinic light, non-photochemical quenching of fluorescence (*NPQ*) and apparent rates of photosynthetic electron transport (*ETR*) (see Appendix) were calculated according to the formulae: $\text{NPQ} = (F_m - F'_m)/F'_m$; $\text{ETR} = 0.84 \times \Phi_{\text{PSII}} \times \text{PPF} \times 0.5$. The factor 0.5 accounts for the equal partitioning of the excitation energy between the two photosystems, whereas the factor 0.84 assumes that 16% of *PPF* is not absorbed by the photosystems (Maxwell and Johnson 2000). In many species, leaf absorptance is close to 0.84 and this factor has been often used for the calculation of *ETR* (White and Critchley 1999, Rascher *et al.* 2000, Lütge *et al.* 2002). Our unpublished results of the earlier study indicated that leaf absorptance calculated from chlorophyll concentration (Evans 1993) in *Abies alba* and *Fagus sylvatica* saplings did not significantly differ between the experimental stands. However, leaves acclimated to contrasting light environments might differ in absorptance (Close and Beadle 2006, Kitao *et al.* 2006) and we cannot exclude that it had taken place in the present experiment. Therefore, our values of *ETR* should be considered approximate.

Cardinal points of light response curves: maximum apparent rate of photosynthetic electron transport of *PS II* (ETR_{max}), quantum yield of *PSII* photochemistry at saturating *PPF* (Φ_{PPFsat}) and the saturating level of photosynthetic photon flux (PPF_{sat}) (see Appendix) were derived by fitting exponential function to the data according to the method described by (Rascher *et al.* 2000). Only the cardinal points obtained from highly statistically significant non-linear regressions with coefficient of determination $R^2 > 0.92$ at $P < 0.0001$ were used for further statistical analyses. Therefore the *ETR* values at high *PPF* of 740 and 1010 $\mu\text{mol m}^{-2} \text{s}^{-1}$, shown in Figure 1, were discarded for better fitting the exponential function.

2.4. Downregulation and recovery of *PS II* quantum yield photochemistry

Two experiments were conducted in order to observe downregulation and recovery

of *PS II* efficiency in silver fir and European beech leaves preliminary exposed to light patch of low or high intensity. Leaves collected from silver fir and European beech were dark acclimated and F_v/F_m was determined using the plant efficiency analyser (PEA, Hansatech, Norfolk, UK). Subsequently, a leaf of *Fagus* or three tightly arranged needles of *Abies* were put on a moist sponge to avoid a leaf drying and stomatal closure. In the first experiment, leaf was exposed to light patch of low *PPF* = 140 $\mu\text{mol m}^{-2} \text{s}^{-1}$ emitted by a diode lamp (LED Digital Lighting, SPOT, 4.1~4.4 W, Warszawa, PL) for 15 minutes to simulate a light patch in the *Picea* stand. During illumination, the air temperature close to leaf surface increased by about 0.1°C. Then, quantum yield of *PS II* was allowed to recover and Φ_{PSII} was recorded each 2 minutes in 9 steps. Recovery in *PS II* efficiency occurred at a very low *PPF* (below 5 $\mu\text{mol m}^{-2} \text{s}^{-1}$), which was monitored using a light sensor attached to the leaf chamber of FMS 2 and a lightmeter (Spectrum Technologies, Ltd.).

In the second experiment, once had F_v/F_m been determined, the leaf was exposed to light patch of saturating *PPF* = 1250 $\mu\text{mol m}^{-2} \text{s}^{-1}$ emitted by a halogen lamp (Tester, ICDR, 35 W, Wroclaw, PL) for 10 minutes to simulate a light patch of high intensity in the *Larix* stand. Subsequently, recovery in *PS II* efficiency was observed. A water filter was installed between the lamp and leaf surface to avoid an overheating of leaf tissue. However, the air temperature close to the leaf surface increased from 2 to 3°C when compared the values before and after illumination. A degree of recovery in *PS II* efficiency was expressed in the percentage of maximum quantum yield of *PS II*.

2.5. Statistical analysis

To compare light response curves of Φ_{PSII} , *NPQ* and *ETR* and the curves of F_v/F_m recovery, the analysis of covariance (ANCOVA in the general linear model) was applied ($P < 0.05$). The actinic light level or time of recovery in *PS II* efficiency was a continuous predictor variable; species and experimental plot (stand) were categorical predictor variables; measured parameters (Φ_{PSII} , *NPQ* and *ETR*) were dependent variables. The effect of measurements' day was

not statistically significant, hence it was not in the ANCOVA design. To linearize the model, the values of actinic light or time of recovery and the corresponding values of measured parameters were ln-transformed. First, homogeneity of slopes was checked to test whether the continuous and categorical predictors interacted in influencing the responses, and thus, whether the traditional ANCOVA design or the separate slope design was appropriate for modelling the effects of predictors. When the interaction was not statistically significant, the traditional ANCOVA was applied and followed by Tukey's *a posteriori* test. If not, a separate-slope design was applied with Tukey's test ($P < 0.05$). The detailed description of the mathematical models used in our study can be found in StatSoft, Inc. (2004). The analysis of variance (ANOVA) was carried out to compare the mean values of Φ_{PSII} , NPQ and ETR at the chosen value of actinic light ($531 \mu\text{mol m}^{-2} \text{s}^{-1}$) and the cardinal points of light response curves among the species and the experimental stands ($P < 0.05$). The statistical analyses were effected with Statistica 7.1.

3. RESULTS

3.1. Light response curves of chlorophyll *a* fluorescence

Acclimation of photochemistry in leaf of the investigated species to the light environments of the *Picea* and *Larix* stands has been demonstrated using light response curves of Φ_{PSII} , NPQ and ETR and their cardinal points: F_v/F_m , $\Phi_{\text{PPF}_{\text{sat}}}$, PPF_{sat} and ETR_{max} (Fig. 1, 2). When the light response curves were compared with the use of ANCOVA, the species showed statistically significant differences (Appendix and Table 1). Φ_{PSII} and NPQ at the same level of actinic light ($531 \mu\text{mol m}^{-2} \text{s}^{-1}$) differed between the stands within each species except for Φ_{PSII} and ETR in *Fagus* (Fig. 1A, B, C, D). The light curves of ETR differed between the experimental stands for both species (Fig. 1E, F). The curves of NPQ and ETR began to differ conspicuously when PPF of actinic light exceeded $200 \mu\text{mol m}^{-2} \text{s}^{-1}$. At the same level of actinic light, NPQ and ETR were

higher in leaf of the saplings growing under the *Larix* canopy.

F_v/F_m was slightly but statistically significantly higher in silver fir (in the *Larix* stand: 0.823 ± 0.011 , in the *Picea* stand: 0.826 ± 0.001) than in European beech (in the *Larix* stand: 0.812 ± 0.001 , in the *Picea* stand: 0.809 ± 0.001) and its values indicated that there was not photoinhibition ($F_v/F_m > 0.8$) in the leaves of both species (Fig. 2A) (Lüttge *et al.* 2002, Lepeduš *et al.* 2005). $\Phi_{\text{PPF}_{\text{sat}}}$ did not significantly change between the species and between the saplings acclimated to irradiance conditions of the stands within each species (Fig. 2B). ETR_{max} and PPF_{sat} were greater in fir compared with beech (Fig. 2C, D). Under the *Larix* canopy, in fir and beech leaves both parameters attained higher values than under the *Picea* canopy (Fig. 2C, D).

3.2. Downregulation of PS II and recovery following light patch

After exposure of fir needles to the 15-min. light patch of low intensity, F_v/F_m decreased to 89 and 87% of the maximum value in the saplings from the *Picea* and *Larix* stand. In leaf of beech, F_v/F_m decreased to 86 and 78%, respectively. Both species fully recovered PS II efficiency within several minutes but only in case of European beech, the recovery was faster in the saplings acclimated to shade of *Picea* crowns (Fig. 3A, B).

Halogen light of high intensity induced a severe photoinhibition in leaf of both investigated species. In the silver fir needles, F_v/F_m decreased to 31 and 45% in the saplings from the *Picea* and *Larix* stand. In the beech leaves to 35 and 49%, respectively. Any investigated species did not fully recover in PS II efficiency within 20 min. (Fig. 3C, D). In the fir needles, a dynamic recovery was observed only in the first two minutes and then the reduced PS II efficiency was sustained at about 41% in the needles from the *Picea* stand and 60% in the needles from the *Larix* stand. Contrary, recovery in the *Fagus* leaves showed increasing trend with time to 60 and 80%, respectively. For the statistical confirmation of differences between the species and stands in response to light patches, see the results of ANCOVA in Appendix and Table 1.

Table 1. The results of analyses of covariance (ANCOVA). Continuous predictor was PPF or time of recovery of *PS II* efficiency, species and stand were categorical predictor variables, measured parameters (Φ_{PSII} , NPQ and *ETR*) were dependent variables. The differences between the mean values are statistically significant when $P < 0.05$. For further details see “Statistical analysis” and Fig. 1, 3. Explanations of the terms and abbreviations – see Appendix.

Effect	Degrees of freedom	Sums of squares	Mean Sum of squares	F	P
ANCOVA; dependent variable: Φ_{PSII}					
PPF	1	11.32733	11.32733	1133.913	0.000
Species	1	0.10809	0.10809	10.820	0.001
Stand	1	0.03303	0.03303	3.306	0.070
Species×Stand	1	0.00114	0.00114	0.115	0.735
Error	409	4.08574	0.00999		
Total	413	15.46917			
Separate-slopes model; dependent variable: NPQ					
Species×PPF×Stand	4	700.016	175.0040	246.4747	0.000
Species	1	3.629	3.6286	5.1104	0.024
Stand	1	1.381	1.3806	1.9444	0.164
Species×Stand	1	0.034	0.0343	0.0483	0.826
Error	409	288.272	0.7100		
Total	413	1034.507			
ANCOVA; dependent variable: <i>ETR</i>					
PPF	1	408.0936	408.0936	4119.017	0.000
Species	1	1.5338	1.5338	15.481	0.000
Stand	1	1.0286	1.0286	10.382	0.001
Species×Stand	1	0.0665	0.0665	0.671	0.413
Error	409	40.5219	0.0991		
Total	413	459.6747			
Halogen light; ANCOVA; dependent variable: Φ_{PSII}					
Recovery time	1	4.5897	4.5897	47.829	0.000
Species	1	2.3548	2.3548	24.540	0.000
Stand	1	13.3499	13.3499	139.119	0.000
Species×Stand	1	0.3498	0.3498	3.645	0.0571
Error	346	33.2023	0.0960		
Total	350	54.9218			
Diode light; separate-slopes model; dependent variable: Φ_{PSII}					
Species×Stand×Time	4	0.587	0.147	43.0	0.000
Species	1	0.073	0.073	21.4	0.000
Stand	1	0.083	0.083	24.3	0.000
Species×Stand	1	0.005	0.005	1.4	0.236
Error	343	1.170	0.003		
Total	350	1.981			

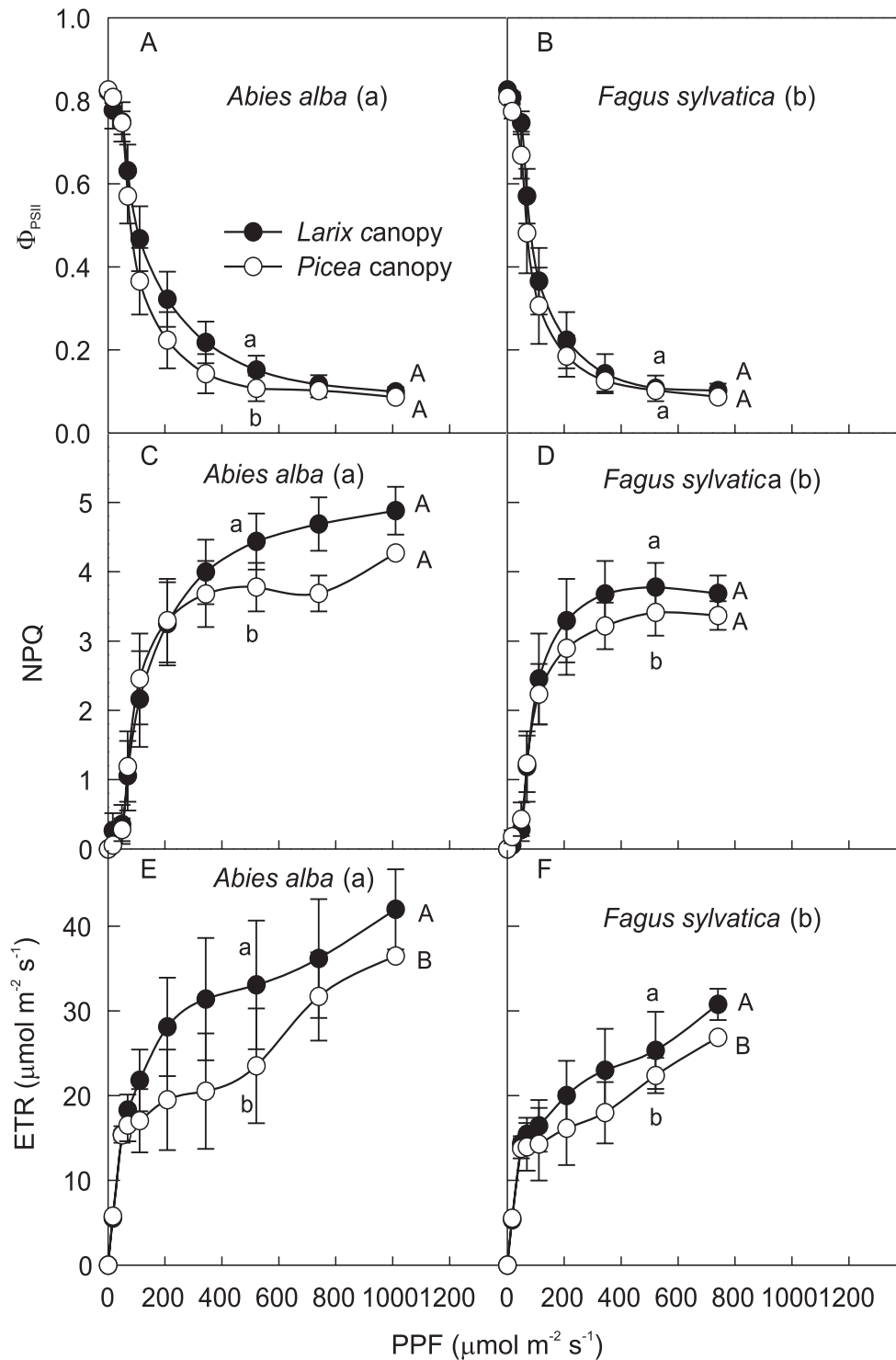


Fig. 1. Light response curves of Φ_{PSII} , NPQ and ETR (means \pm SD, $n = 8-10$) vs. different values of PPFs in leaves of *Abies alba* (panels A, C, E for Φ_{PSII} , NPQ and ETR, respectively) and *Fagus sylvatica* (panels B, D, F) acclimated to irradiance under *Larix* or *Picea* canopy. F_v/F_m was measured in dark adapted leaves at PPF = 0. A two-way ANCOVA followed by Tukey's *post-hoc* test for the interaction between the species and stand at $P < 0.05$ were used to compare the mean values of the parameters between the investigated species (small letters in parenthesis) and between the stands for each species (capital letters). Additionally, two-way ANOVA and Tukey's test were applied to compare the mean values of the parameters at PPF = 531 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (small letters). Abbreviations of symbols and the ANCOVA results – see Appendix and Table 1.

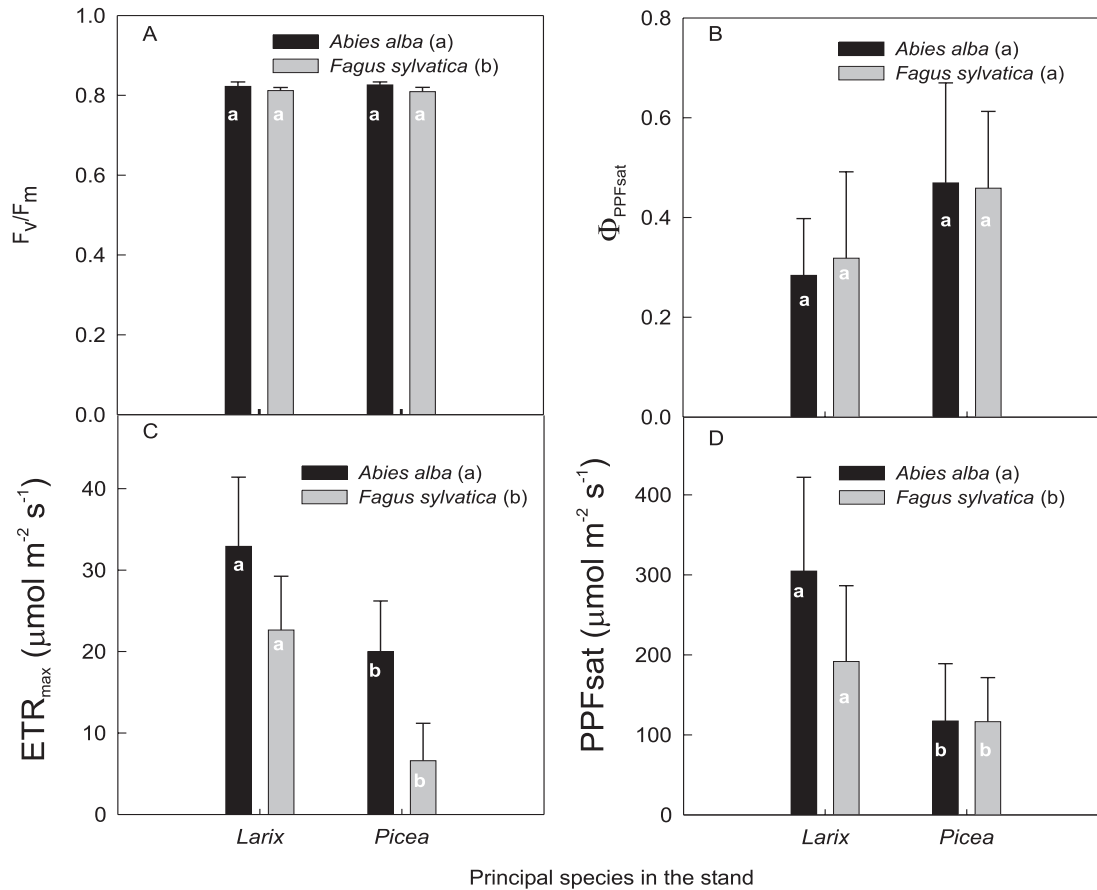


Fig. 2. Mean values (\pm SD) of F_v/F_m (A), $\Phi_{PPF_{sat}}$ (B), ETR_{max} (C) and PPF_{sat} (D). The values of the cardinal points were calculated from the light response curves of the fluorescence parameters measured in leaves of *Abies alba* and *Fagus sylvatica* saplings growing under the canopies of *Larix decidua* or *Picea abies*. Different letters in columns of the histograms indicate that the mean values are statistically significantly different according to two-way ANOVA ($n = 8-10$, $P < 0.05$).

4. DISCUSSION

4.1. Photochemical acclimation to the contrasting light environments

The photosynthetic apparatus of the evergreen conifer silver fir and deciduous broadleaf European beech acclimated to strong shade of the Norway spruce or low shade of the European larch canopy. Acclimation to the contrasting light environments consisted in adjusting performance of photochemical processes in shade and photo-protective mechanism against excess light energy provided within sun patches. Generally, both species showed similar tendency in responses to irradiance. The photosynthetic

performance of saplings assessed as ETR and ETR_{max} (for abbreviations see Appendix) was lower in shade under the denser *Picea* canopy compared with the photosynthetic performance of saplings under the open *Larix* canopy (Fig. 1, 2). Φ_{PSII} at higher values of actinic light ($> 200 \mu\text{mol m}^{-2} \text{s}^{-1}$) was greater in low shade acclimated fir needles (Fig. 1A). Needles acclimation to low shade under the open *Larix* canopy reduced photoinhibitory effect of high light, inversely to strong shade acclimation under the close *Picea* canopy (Fig. 3). Moreover, fir and beech leaves under the *Picea* canopy showed low values of PPF_{sat} which can also be regarded as a typical physiological adjustment to shade. In accordance with our results, acclimation of European

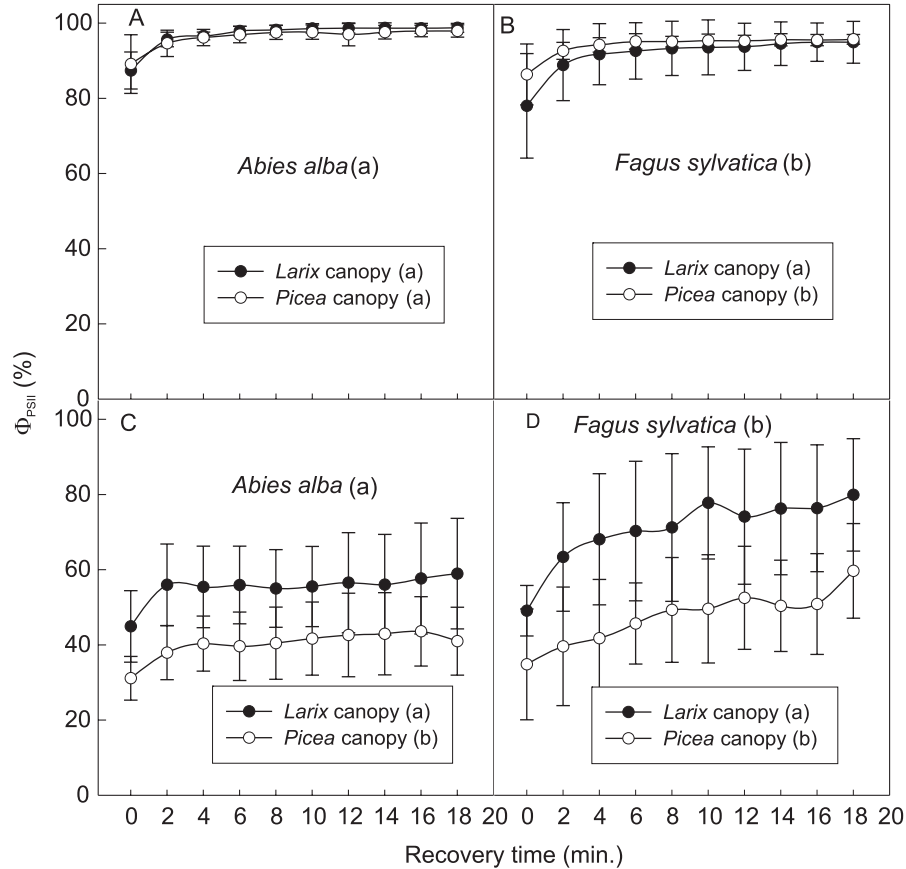


Fig. 3. Time course of recovery of quantum yield of photosystem II photochemistry in leaves of *Abies alba* and *Fagus sylvatica* saplings growing under *Picea* or *Larix* canopies after a 15-min. illumination with the diode light of $PPF = 140 \mu\text{mol m}^{-2} \text{s}^{-1}$ (A, B) or after a 10-min. illumination with the halogen light of $PPF = 1250 \mu\text{mol m}^{-2} \text{s}^{-1}$ (C, D). The different letters indicate statistically significant differences between the species according to ANCOVA results and between the stands within the species as shown by ANCOVA and Tukey's test at $P < 0.05$. The ANCOVA results are given in Appendix and Table 1.

beech saplings to stronger shade in the control conditions resulted in more drastic decrease in F_v/F_m after exposure to full sunlight (Valladares *et al.* 2002). Under field conditions, higher vulnerability to photoinhibition was observed in willow leaves developed in shade and exposed to full sunlight by trimming of the stand as compared to peripheral leaves (Ögren 1988).

Thermal energy dissipation in the silver fir and European beech saplings was more efficient in leaves growing under the *Larix* than under the *Picea* canopy as demonstrated by higher NPQ (Fig. 1). Higher photosynthetic performance that was an important "sink" of energy together with effective dissipation of excess energy as heat indicated

that the saplings growing in the *Larix* stand were better acclimated to use sun patches for photosynthesis. It is consistent with the results indicating that in deep shade the greater ETR capacity could contribute to mitigate photoinhibition and improved growth potential when a gap event occurs (Kitao *et al.* 2006).

The higher mean values of the parameters describing photochemical efficiency and NPQ in silver fir compared with European beech might reflect interspecific differences. Interestingly, even F_v/F_m attaining similar values in many species (about 0.83), was statistically significantly higher in silver fir than in European beech. It suggested that long-lived fir needles may be characterised by higher

photochemical yield of PS II. In understory plants of twelve shade-tolerant rain-forest species, photoinhibition was greater in those with short-lived leaves than with long-lived leaves, which might be the result of a lower yield of PS II (Lovelock *et al.* 1998). On the other hand, differences between our species might result from morphological and anatomical adaptation of leaves to the light environment. Thick epidermis covered with a waxes layer of the silver fir needles more efficiently attenuated high light penetration into palisade parenchyma. The leaves of European beech have not such morphological protection. The strategy developed by this species consist in that the canopy light gradient is paralleled by plastic changes in foliar structure and chemistry and the fact that this species sheds leaves each year (Fleck *et al.* 2003). In contrast, silver fir needles with a long life-span have to acclimate to changing light conditions (Aussenac 1973, Grassi and Bagnaresi 2001). Leaves of long life-span are characterised by lower photosynthetic capacity than those of short life-span (Niinemets 1997, Reich *et al.* 1998). However, in our experiment, at the end of July, long-lived needles of silver fir were able to sustain higher photochemical performance (expressed as *ETR*) than more quickly ageing seasonal European beech leaves.

4.2. Sensitivity to photoinhibition and recovery in PS II quantum yield efficiency

Light patch of low intensity did not cause photoinhibition but only transient PS II downregulation which was slightly deeper in European beech than in silver fir. Moreover, in low shade-acclimated leaves of beech PS II downregulation was deeper and recovery in PS II efficiency lower compared to high shade-acclimated leaves (Fig. 3b) which indicated that acclimation to shade may reduce sensitivity to PS II downregulation caused by sun patch of low intensity in leaves. Similarly, the daily reversible photoinhibition in *Citrus paradisi* Macfayden and *Citrus sinensis* (L) Osbeck leaves was less pronounced in the shaded leaves than in the sunlit ones (Jifon and Syversten 2003). Higher values of PS II quantum yield efficiency in shade leaves than in sun leaves may result from a greater

relative nitrogen allocation to light harvesting complex and an increase in quantity of appressed grana membranes enriched in PS II centres in shade leaves (Hikosaka and Terashima 1995, 1996, D. Kierzkowski *et al.* – unpublished). However, after exposure to high light stress, both investigated species showed strong decline in F_v/F_m indicating photoinhibition. Differences in the magnitude of photoinhibition and dynamic of recovery in PS II efficiency between saplings from the *Picea* and *Larix* stands were more conspicuous compared with the response to light patch of low intensity (Fig. 3). Remarkably, the saplings growing under the *Larix* canopy were less sensitive to photoinhibition. Recovery in PS II efficiency was not complete neither in fir nor in beech. The latter showed more dynamic recovery and its mean relative Φ_{PSII} attained higher value after 20 min. independently of the stand where saplings were grown (Fig. 3C, D). We did not expect such a result because in our earlier study, after exposure to full sun irradiance, *Fagus* acclimated to strong shade under a green net (5% of total irradiance) in the control conditions responded by stronger photoinhibition and lower recovery in F_v/F_m compared with *Abies* (Wyka *et al.*, in press). In the present experiment, the response to light stress might result from the fact that the investigated individuals had been growing in the heterogeneous light environment under the trees canopy and they were acclimated to different micro-site conditions (Niinemets and Valladares 2004). On the other hand, at the end of July, seasonal leaves of *Fagus* might have higher concentration of phenolic compounds and xanthophylls which increased their tolerance to photoinhibition (Close and McArthur 2002).

4.3. Ecological implications

At the young age, silver fir and European beech occur in the same niche and compete for light. In this competition beech often has an advantage because its growth is more dynamic than that of fir. However, the latter survives and sustains high vitality despite growing at a very low light level for decennia. When light conditions become more favourable, silver fir is able to make up for

lost increments growing more dynamically (Grassi and Bagnaresi 2001, Jaworski 1995). In our study, both species showed physiological plasticity that allows them to use light for photosynthesis in the close *Picea* stand or in the open *Larix* stand. Beech leaves were characterised by more dynamic recovery after exposure to light patch of high intensity. Nevertheless, at time of our experiment, fir leaves had higher photochemical efficiency than more quickly ageing beech leaves, which may compensate to some extent its lower ability to recover rapidly after high light stress.

Interspecific differences in photochemical acclimation to the light environment, sensitivity to photoinhibition and ability to fast recovery in *PS II* efficiency after light stress may decide on the species success in the between-species competition. These differences mainly resulted from the fact that silver fir and European beech belong to different groups: evergreen conifers with long-lived needles and deciduous broad-leaf trees shedding leaves each year. Silver fir has an ability to extensively acclimate its photosynthetic apparatus to changing environmental conditions within several years. On the contrary, European beech can acclimate intensively its leaves within one season and avoids severe stresses by shedding and producing a new set of leaves. These strategies of acclimation to light were shown for pioneer shade-intolerant *Pinus sylvestris* and *Salix dasyclados* Willd (Hjelm and Ögren 2004). However, our results indicated that sensitivity to photoinhibition strongly depended on species, but also on light conditions of growth under the mature trees canopy.

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APPENDIX

Explanations of the terms and abbreviations used in the text. For definitions and further details see Genty *et al.* (1989), Maxwell and Johnson (2000) and Rascher *et al.* (2000).

Symbol	Units	Explanations
ETR	$\mu\text{mol m}^{-2} \text{s}^{-1}$	Apparent electron transport rate
ETR_{max}	$\mu\text{mol m}^{-2} \text{s}^{-1}$	Maximum value of ETR
F_v/F_m	–	Maximum quantum yield of photosystem II photochemistry measured (in dark adapted leaf)
F_m	–	Maximum fluorescence yield
F'_m	–	Maximum fluorescence yield in the light
F_0	–	Minimum fluorescence yield
F_s	–	Steady-state fluorescence yield
NPQ	–	Non-photochemical quenching of fluorescence
PPF	$\mu\text{mol m}^{-2} \text{s}^{-1}$	Photosynthetic photon flux
PPF_{sat}	$\mu\text{mol m}^{-2} \text{s}^{-1}$	Saturating level of photosynthetic photon flux
$PS II$	–	Photosystem II where light is absorbed and an electron derived from the splitting of water is transferred to the photosynthetic electron-transport chain.
Φ_{PSII}	–	Quantum yield of $PS II$ photochemistry measured in photosynthetic (actinic) light
Φ_{PPFsat}	–	Quantum yield of $PS II$ photochemistry at the saturating level of PPF
$PS II$ downregulation	–	Reductions in $PS II$ efficiency measured with chlorophyll <i>a</i> fluorescence (F_v/F_m). For example $PS II$ downregulation may occur in leaf exposed to high irradiance in midday. In contrast to photo-inhibition, this phenomenon is not permanent and may serve to protect photosynthetic apparatus.
Photoinhibition	–	Photoinhibition results from increased nonphotochemical processes and is detected as a decrease in F_v/F_m below 0.8 following exposure of leaves to bright light. It is related to damage to the D1 protein of $PS II$ caused by excessive energy.