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Shade effect alters leaf pigments and photosynthetic responses in Norway spruce (*Picea abies* L.) grown under field conditions

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Abstract

The contents of chlorophyll (Chl) and carotenoids (Car) per fresh mass were lower in shade needles than in sun needles. Ribulose-1,5-bisphosphate carboxylase (RuBPC) activity and contents of soluble proteins were also significantly lower in shade needles. In isolated thylakoids, a marked lower rate of whole chain and photosystem (PS) 2 activities were observed in shade needles. Smaller lower rate of PS1 activity was also observed in shade needles. The artificial exogenous electron donors, diphenyl carbazide (DPC) and NH_2OH, significantly restored the loss of PS2 activity in shade needles. Similar results were obtained when F_v/F_m was evaluated by Chl fluorescence measurements. The marked lower rate of PS2 activity in shade needles was due to the lower contents of 47, 33, 28–25, 23, and 17 kDa polypeptides. This conclusion was confirmed by immunological studies showing that the content of the 33 kDa protein of the water-splitting complex was diminished significantly in shade needles.

Additional key words: chlorophyll fluorescence; diphenyl carbazide; donor side; electron transport; NH_2OH; photosystems 1 and 2; polypeptides; ribulose-1,5-bisphosphate carboxylase.

Introduction

The structure of a forest stand canopy creates differences in solar irradiance within the canopy space. The acclimation of foliage to reduced irradiance in the lower crown layers has distinct anatomical and physiological consequences leading to photosynthetic characteristics different from those found in exposed, sunny parts of the canopy (Woodman 1971, Golovko *et al*. 2004). High values of silhouette *versus* total area ratio (Leverenz 1996) and specific needle area, low chlorophyll (Chl) contents on leaf area basis, low Chl *a*/*b* ratio (Boardman 1977, Björkman 1981, Marek *et al*. 1997, Senevirathna *et al*. 2003, Griffin *et al*. 2004), low RuBPC activity (Priwitzer *et al*. 1998), and low electron transport rate (Evans 1987) commonly occur in shade foliage compared to sun foliage.

Plants, when exposed to high or low irradiance during growth, react with a variety of adaptations, *i.e*. the formation of sun and shade leaves as well as sun and shade chloroplasts (Lichtenthaler 1981, Lichtenthaler *et al*. 1984). Sun leaves are generally described as requiring a higher photosynthetic photon flux density (PPFD) and having a higher saturated photosynthetic rate than corresponding shade leaves (Lichtenthaler 1981). However, the basis (Charles-Edwards and Ludwig 1975) on which the photosynthetic rate is expressed will make a difference in this comparison, because sun leaves are thicker than shade leaves (Björkman *et al*. 1973, McClenden and McMillen 1982). Thus, the pigment contents of sun leaves are higher on a leaf area unit but less on a fresh mass basis than those of shade leaves. Sun leaves with sun chloroplasts possess higher rates of photosynthesis on a leaf area and Chl basis than shade leaves with their low-irradiance chloroplasts (Lichtenthaler 1981, Lichtenthaler and Burkart 1999, Feng *et al*. 2004, Laisk *et al*. 2005).

Extensive research on sun-to-shade adaptations in leaves has demonstrated that low-irradiance grown plants adapt to the irradiance-limited growth conditions by

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Abbreviations: Car – carotenoid; Chl – chlorophyll; DCBQ – 2,6-dichloro-p-benzoquinone; DCPIP – 2,6-dichlorophenol indophenol; DPC – diphenyl carbazide; F_0 – minimal fluorescence; F_v – variable fluorescence; MV – methyl viologen; PAR – photosynthetic active radiation; PFD – photon flux density; PS – photosystem; RuBPC – ribulose-1,5-bisphosphate carboxylase; SDS-PAGE – sodium dodecylsulphate-polyacrylamide gel electrophoresis; SiMo – silicomolybdate.

increasing the photon-harvesting ability and alter chloroplast anatomy by increasing the amount of appressed regions in thylakoid membranes (Anderson 1986). Adjustments of the concentration of Chl *b*, light-harvesting complex (LHC) proteins, and additional changes in ribulose-1,5-bisphosphate carboxylase (RuBPC) and Q_B protein contents are mostly regulated in the shade adapted leaves by changes in gene expression at the transcriptional or post-transcriptional level (Senger and Bauer 1987). In shade, additional changes in leaf photosynthetic performance result from ageing or senescence of the leaves what has eloquently been described as orderly withdrawal of materials from the general economy of the plant (Woolhouse 1987). Thus, the loss of photosynthetic activity with ageing due to shade occurs in concert with Chl breakdown, observed as a yellowing of photosynthetic tissues, and the orchestrated degradation of chloroplast processes in a step-wise fashion, including membrane proteins, stromal enzymes, and loss of chloroplast integrity (Woolhouse 1987, Nedunchezhian *et al*. 1995, Senevirathna *et al*. 2003, Dzhibladze *et al*. 2005).

According to Boardman (1977) photosynthetic productivity of a leaf is primarily governed by its position in the plant canopy. It is therefore important to determine the changes in Chl content of the leaves as well as the relationship, if any, with different photosynthetic activities. The aim of this study was to distinguish the photosynthetic response in the sun and shade needles of tall Norway spruce (*Picea abies* L.) trees grown in a natural forest stand.

Materials and methods

Plants: The quantitative characteristics of sun and shade needles were investigated in 15-year-old Norway spruce (*Picea abies* L.) trees growing in a natural forest stand situated at Istituto Agrario di San Michele all' Adige, Italy. In order to simplify the experimental procedure, we classified the same needle age samples into two groups according to irradiance they received on the needle surface and the canopy position: shade needles were collected in the inner tree part under PAR of less than 50 μmol m^{-2} s^{-1}, whereas the sun needles were collected from well irradiated outer canopy position with a maximum PAR of 1 700 μmol m^{-2} s^{-1}. Daily maximum and minimum air temperatures were 29–33 and 15–17 °C, respectively. Needles were sampled early in the morning before they had experienced direct sunlight. The shade influenced not only the PAR microclimate but also the air and leaf temperature; the maximal leaf temperature on sun plants was 33 °C.

Photosynthetic pigments were quantified in crude acetone extracts. Needles were frozen in liquid nitrogen, lyophilised, pulverised with a mill, and extracted with 80 % (v/v) acetone. Chl and carotenoids were measured spectrophotometrically and their concentrations calculated using extinction coefficients given by Lichtenthaler (1987).

Modulated Chl fluorescence was measured on needles using a *PAM 2000* fluorometer (*Heinz Walz*, Effeltrich, FRG). F_0 was measured by switching on the modulated radiation to 0.6 kHz; PPFD at the needle surface was less than 0.1 μmol m^{-2} s^{-1}. F_m was measured at 20 kHz with a 1-s pulse of 6 000 μmol m^{-2} s^{-1} of "white light". Chl fluorescence on isolated thylakoid membranes at room temperature was measured with the same device. Measurements were done in 1 cm^3 reaction mixture containing 50 mM Tris-HCl, pH 7.5, 2 mM $MgCl_2$, 10 mM NaCl, 100 mM sucrose, and 10 μg of Chl-equivalent thylakoid membranes. The integrated measuring radiation was (480 nm) 0.15 μmol m^{-2} s^{-1} with a red actinic radiation (650 nm) of 100 μmol m^{-2} s^{-1}.

Activities of electron transport: Thylakoid membranes were isolated from the needles as described by Berthhold *et al*. (1981). Whole chain electron transport ($H_2O \rightarrow MV$) and partial reactions of photosynthetic electron transport mediated by PS2 ($H_2O \rightarrow DCBQ$; $H_2O \rightarrow SiMo$) and PS1 ($DCPIPH_2 \rightarrow MV$) were measured as described by Nedunchezhian *et al*. (1997). Thylakoids were suspended at 10 μg(Chl) cm^{-3} in the assay medium containing 20 mM Tris-HCl, pH 7.5, 10 mM NaCl, 5 mM $MgCl_2$, 5 mM NH_4Cl, and 100 mM sucrose supplemented with 0.5 mM DCBQ and 0.2 mM SiMo.

DCPIP photoreduction was determined as the decrease in absorbance at 590 nm using a *Hitachi 557* spectrophotometer. The reaction mixture (3 cm^3) contained 20 mM Tris-HCl, pH 7.5, 5 mM $MgCl_2$, 10 mM NaCl, 100 mM sucrose, 0.1 mM DCPIP, and thylakoid membranes equivalent to 20 μg of Chl. Where mentioned, the concentrations of $MnCl_2$, DPC, and NH_2OH were 5.0, 0.5, and 5.0 mM, respectively.

SDS-PAGE: Thylakoid membranes were separated using the polyacrylamide gel system of Laemmli (1970), with following modifications. Gels consisted of a 12–18 % gradient of polyacrylamide containing 4 M urea. Samples of thylakoid membrane preparation were solubilised at 20 °C for 5 min in 2 % (m/v) SDS, 60 mM DTT, and 8 % sucrose using SDS-Chl ratio of 20 : 1. Electrophoresis was performed at 20 °C with constant current of 5 mA. Gels were stained in methanol/acetic acid/water (4 : 1 : 5, v/v/v) containing 0.1 % (m/v) Coomassie brilliant blue R and de-stained in methanol/acetic acid/water (4 : 1 : 5, v/v/v). Thylakoid membrane protein was estimated according to Lowry *et al.* (1951).

Immunological determination of thylakoid proteins: The relative contents of certain thylakoid proteins per Chl unit were determined immunologically by Western blotting. Thylakoids were solubilised in 5 % SDS, 15 % glycerol, 50 mM Tris-HCl, pH 6.8, and 2 % mercaptoethanol at room temperature for 30 min. The polypeptides were separated by SDS-PAGE as described above and proteins were then transferred to nitrocellulose by electroblotting for 3 h at 0.4 A, after saturation with 10 % milk powder in TBS buffer (pH 7.5). The first antibody in 1 % gelatine was allowed to react overnight at room temperature. After washing with TBS containing 0.05 % *Tween-20*, the secondary antibody [*Anti-Rabbit IgG* (whole molecule) biotin conjugate, *Sigma*, USA] was allowed to react in 1 % gelatine for 2 h. For detection of D1 protein a polyclonal antiserum against spinach D1 protein was used (kindly provided by Prof. I. Ohad, Jerusalem, Israel), and the antibody against the 33 kDa protein of the water-splitting system was a gift from Dr. Barbato, Padova, Italy. The densitometry analysis of Western blots was performed with a *Bio-Image* analyser (*Millipore Corporation*, Michigan, USA).

Soluble proteins were extracted by grinding needles (0.3–0.5 g fresh mass) in a mortar and pestle with 6 cm^3 of 100 mM Tris-HCl, pH 7.8 containing 15 mM $MgCl_2$, 1 mM EDTA, 10 mM 2-mercaptoethanol, and 10 mM PMSF in the presence of liquid nitrogen. Homogenate was filtered through nylon cloth. After centrifugation at 11 000×*g* for 10 min, the concentration of soluble proteins was determined in the supernatant according to Bradford (1976).

RuBPC activity: Needles were cut into small pieces and homogenized in a grinding medium of 50 mM Tris-HCl, pH 7.8, 10 mM $MgCl_2$, 5 mM DTT, and 0.25 mM EDTA. The extract was clarified by centrifugation at 10 000×*g* for 10 min. The clear supernatant was decanted slowly and used for RuBPC activity determination. RuBPC activity was measured as described by Nedunchezhian and Kulandaivelu (1991).

Statistical analysis of the physiological responses was tested using a three-way analysis of variance (*ANOVA*) if the data met the assumptions of normality and homoscedasticity. Significant differences were determined by the Student's *t*-tests criterion. All the statistical procedures were performed with *SPSS 10.0 for Windows* (*SPSS*, Chicago, Illinois, USA).

Results

Leaf pigments: When expressed on a fresh mass basis, shade needles showed Chl and carotenoid (Car) values lower than the sun needles (Table 1). A reduction of 59 and 38 % was observed in shade needles for Chl and Car, respectively. The differences in total Chl content were associated with differences in Chl *a* and Chl *b*. The Chl *a*/*b* ratio was also markedly lower in shade needles (Table 1). In contrast to this, the Car/Chl ratio was higher in shade needles than in the sun ones (Table 1).

Table 1. Changes in leaf pigments, soluble proteins, and RuBPC of Norway spruce needles collected from sun and shade canopy positions. For abbreviations see the text. *Figures in parentheses* are percentage of sun needles. Means ± S.E.; $n = 5$, $^{***}p<0.001$, $^{**}p<0.01$.

Parameter	Sun	Shade
Chl *a* [g kg^{-1}(f.m.)]	1.10 ± 0.04	0.42 ± 0.01 (38)
Chl *b* [g kg^{-1}(f.m.)]	0.44 ± 0.01	0.21 ± 0.01 (47)
Chl *a*+*b* [g kg^{-1}(f.m.)]	1.54 ± 0.03	0.63 ± 0.03** (41)
Car [g kg^{-1}(f.m.)]	0.52 ± 0.02	0.32 ± 0.01** (62)
Chl *a*/*b*	2.50 ± 0.02	2.00 ± 0.01
Car/Chl	0.34 ± 0.01	0.52 ± 0.01
Soluble proteins [g kg^{-1}(f.m)]	28.20 ± 1.22	18.40 ± 0.64*** (35)
Soluble protein/Chl ratio	18.00 ± 0.51	9.60 ± 0.31** (62)
RuBPC [nmol(CO_2) kg^{-1}(protein) s^{-1}]	8.92 ± 0.31	5.53 ± 0.23*** (38)

Chl fluorescence and photosynthetic activities: To obtain information on PS2 activity, the ratio F_v/F_m, which reflects the quantum yield of PS2 photochemistry (Krause and Weis 1991), was determined *in vivo* using needles dark-adapted for 30 min. The effect of shade on the variable part of fluorescence was prominent (F_v) and showed no changes in F_0. The F_v and F_v/F_m were much lower in shade needles than in the sun needles (Fig. 1). F_v/F_m in the sun needles was 0.766 and the ratio was lower (0.583) in shade needles (Fig. 1).

When photosynthetic electron transport was studied using isolated thylakoids from sun and shade needles, the rate of $DCPIPH_2 \rightarrow MV$ (PS1) was about 5 % lower in shade needles as compared with sun needles (Fig. 2). The PS2 activities measured as $H_2O \rightarrow DCBQ$ and $H_2O \rightarrow$ SiMo were about 12 and 40 % lower in shade needles

than in sun needles (Fig. 2). A similar trend was also noticed for whole chain ($H_2O \rightarrow MV$) electron transport (Fig. 2).

To locate the possible site of inhibition in the PS2 reaction, we followed the DCPIP reduction supported by various exogenous electron donors in thylakoids of both types of the needles. Wydrzynski and Govindjee (1975) showed that $MnCl_2$, DPC, and NH_2OH could donate the electrons in the PS2 reaction. Fig. 3 shows the electron transport activity of PS2 in the presence and absence of the three above compounds. In the shade needles, the PS2 activity was reduced to about 42 % when water served as the electron donor. A similar trend was also found using $MnCl_2$ as donor. In contrast to this, a significant restoration of PS2 mediated DCPIP reduction was observed when NH_2OH and DPC were used electron donors in the shade needles (Fig. 3).

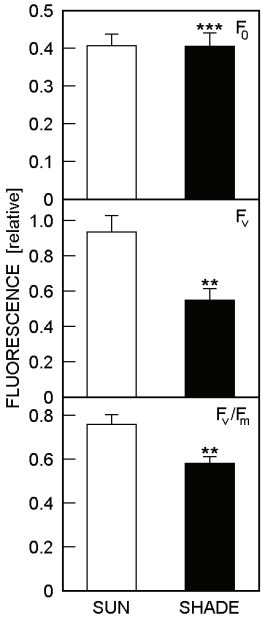

Fig. 1. Changes in the relative fluorescence emitted as minimal fluorescence (F_0), variable fluorescence (F_v), and the ratio of variable to maximum fluorescence (F_v/F_m) in sun and shade needles. Means ± S.E.; $n = 5$, $^{***}p<0.001$, $^{**}p<0.01$.

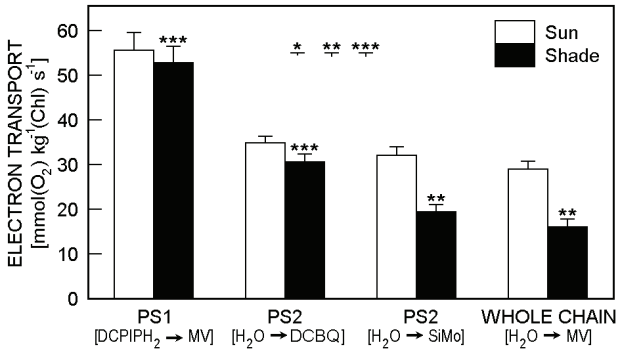

Fig. 2. Changes in the rates of whole chain ($H_2O \rightarrow MV$), PS2 ($H_2O \rightarrow DCBQ$; $H_2O \rightarrow SiMo$), and PS1 ($DCPIPH_2 \rightarrow MV$) electron transport activities in thylakoids isolated from sun and shade needles. Means ± S.E.; $n = 5$, $^{***}p<0.001$, $^{**}p<0.01$.

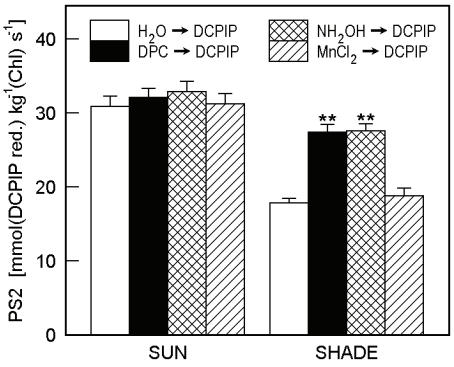

Fig. 3. Effect of various exogenous electron donors on PS2 activity ($H_2O \rightarrow DCPIP$) in thylakoid membranes isolated from sun and shade needles. Means ± S.E.; $n = 5$, $^{**}p<0.01$.

These results agree with measurements obtained by modulated Chl fluorescence with various exogenous electron donors used in the sun and shade thylakoids (Table 2). The addition of DPC and NH_2OH to the shade thylakoids induced a significant increase of variable fluorescence (F_v). The F_v/F_m ratio also increased from 0.555 to 0.680 and 0.673 for DPC and NH_2OH, respectively (Table 2).

Thylakoid membrane proteins: Since the changes in photosynthetic electron transport activities could be caused primarily by changes or reorganisation of thylakoid components, the thylakoid polypeptide profiles of the sun and shade needles were analysed by SDS-PAGE. A comparison of thylakoid polypeptides indicated a decrease in the amounts of 47, 33, 25, 23, and 17 kDa polypeptides in the shade needles (Fig. 4).

D1 and 33 kDa proteins tested by immunoblot: Shade induced inhibition of PS2 activity in the thylakoids was compared with changes in the relative contents of D1 and 33 kDa proteins as determined by Western blotting (Fig. 5) followed by quantification by the *Bio-Image* apparatus (Fig. 5). Decrease in the relative content of D1 protein in the shade needles was negligible (4 %) but that of 33 kDa protein was significant (48 %).

RuBPC activity and soluble proteins: When the enzyme activity in crude needle extracts was expressed on a protein basis, significantly less RuBPC activity was observed in shade needles (by 38 %) than in sun needles (Table 1). A similar result was also noticed for soluble proteins in shade needles (Table 1).

Discussion

In our experiment, the contents of Chl and Car were significantly lower in shade needles in comparison with sun needles. Both Chl *a* and Chl *b* contents were lower and shade probably also enhanced the chlorophyllase activity in needle tissues. An increase in the Car/Chl ratio and a decrease in the Chl *a*/*b* ratio in shade needles has been observed before (Björkman *et al*. 1972, Marini and Marini 1983, Feng *et al*. 2004, Baig *et al*. 2005). Because all Chl is non-covalently attached to either reaction centre or LHC (Green 1988), the differences in Chl *a*/*b* indicated that shade affected the distribution of Chl between the Chl-protein complexes. Also an increase of Car/Chl is due to the relatively faster decrease of Chl than Car. These differences in pigment ratios between sun and shade were described before. They are due to the high-irradiance adaptation response of the photosynthetic pigment apparatus of sun needles with much less LHCP2 and more reaction centres on a total Chl basis compared to shade needles which exhibit higher and broader grana thylakoid antenna (Lichtenthaler *et al*. 1982, 2000, Laisk *et al*. 2005).

Table 2. Changes in the relative fluorescence emitted as minimal fluorescence (F_0), variable fluorescence (F_v), and the ratio of variable to maximum fluorescence (F_v/F_m) in thylakoids isolated from sun and shade needles with or without exogenous electron donors. Concentrations of $MnCl_2$, DPC, and NH_2OH were 5.0, 0.5, and 5.0 mM, respectively (mean ± S.E.; $n = 5$, $^{***}p < 0.001$, $^{**}p < 0.01$).

	Addition	F_0	F_v	F_v/F_m
Sun	None	1.60 ± 0.04	4.00 ± 0.15	0.714 ± 0.040
	DPC	1.60 ± 0.03	4.20 ± 0.13	0.724 ± 0.030**
	NH_2OH	1.60 ± 0.03	1.60 ± 0.14	0.719 ± 0.030**
	$MnCl_2$	1.60 ± 0.04	1.60 ± 0.14	0.714 ± 0.020
Shade	None	1.60 ± 0.04	2.00 ± 0.07	0.555 ± 0.020
	DPC	1.60 ± 0.05	3.40 ± 0.13	0.680 ± 0.020**
	NH_2OH	1.60 ± 0.04	3.30 ± 0.10	0.673 ± 0.030**
	$MnCl_2$	1.60 ± 0.03	2.20 ± 0.06	0.578 ± 0.020

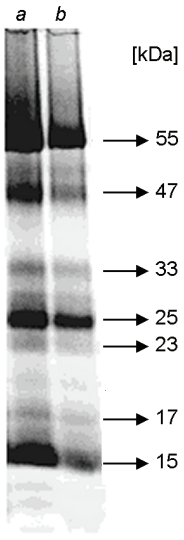

Fig. 4. Coomassie blue stained polypeptide profiles of thylakoid membranes isolated from sun (*a*) and shade (*b*) needles. Gel lanes were loaded with equal amounts of protein (100 μg).

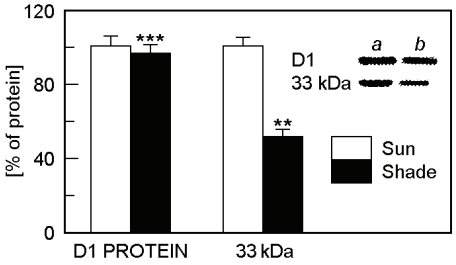

Fig. 5. Degradation of the D1 and 33 kDa proteins in thylakoids of sun (*a*) and shade (*b*) needles. Each lane was loaded equal amounts (5 μg) of chlorophyll. *Histogram*: *Bio-Image* densitometric evaluation. *Inset*: Western-blot. Means ± S.E.; $n = 5$, $^{***}p<0.001$, $^{**}p<0.01$.

Chl fluorescence induction curves, reflecting photosynthesis and electron transport, have characteristic patterns, which undergo changes when the photosynthetic system becomes impaired. They can therefore be used as indicators of damage (Govindjee and Papageorgiou 1971). The sun needles showed a high PS2 activity, measured as the F_v/F_m ratio, while shade leaves showed the lowest F_v/F_m ratio (Senevirathna *et al*. 2003, Griffin *et al*. 2004). The extent of variable fluorescence (F_v) was markedly lower in the shade leaves without affecting the F_0 level. Reduction in variable fluorescence yield, as often shown, indicates impairment of PS2 activity, particularly at the donor site (Allakhverdiev *et al*. 1987, Šetlík *et al*. 1990).

Analysis of various electron transport activities measured by using electron acceptors in thylakoids isolated from sun and shade needles, showed a lower activity of the whole chain electron transport activity of shade needles as compared to sun needles; only a marginal effect on PS1 mediated reactions was noticed. Hence the shade must have action site(s) in the PS2 reaction. Similar lower rates of PS2 activity have been reported in low-irradiance grown plants of *Atriplex* (Boardman *et al*. 1975) and *Picea* (Lewandowska and Jarvis 1977). An analysis of electron transport in the thylakoids isolated from shade needles showed that O_2 evolution was significantly lower when SiMo was used as electron acceptor, but this decline was not significant when the electron acceptor was DCBQ. Since DCBQ accepts the electrons directly from Q_A^- (Cao and Govindjee 1990), the rates measured represent the true rate of photochemistry by PS2, uninfluenced by the PQ pool. This proved that shade effect induced changes on the donor side of PS2 in Norway spruce.

In order to locate the possible site of shade induced inhibition, we measured PS2 mediated DCPIP reduction in the presence of various artificial exogenous electron donors acting at the oxidizing side of PS2. Among the artificial electron donors tested, DPC and NH_2OH were most effective in increasing the PS2 activity in the shade needles. These results were also confirmed by measurement of modulated Chl fluorescence in isolated thylakoids. After addition of DPC and NH_2OH to thylakoids from shade needles, a marked increase in the level of variable fluorescence occurred. These results indicate that shade needles had changes on the donor side of PS2, perhaps close to the DPC donation side. The present results agree with the findings that the water-oxdising system is sensitive to ageing (Misra and Biswal 1982, Nedunchezhian *et al*. 1995, 1996).

The most likely explanation for the lower PS2 activity in shade needles is that the related protein(s) is(are) affected because they are exposed at the thylakoid surface (Seidler 1994). The extrinsic proteins of 33, 23, and 17 kDa associated with the lumen surface of the thylakoid membranes are required for optimal functioning of the oxygen evolving machinery (Millner *et al*. 1987, Enami *et al.* 1994). Removal of the 33 and 23 kDa proteins from PS2 membrane preparations by treatments with $CaCl_2$ or NaCl (Enami *et al.* 1994) results in strong inhibition of O_2 evolution. The lower contents of 33, 23, and 17 kDa polypeptides accompanied the observed lower PS2 activity in shade needles. From the results we confirm that the significant lower contents of 33, 23, and 17 kDa polypeptides could be one of the reasons for significantly lower O_2 evolution capacity in shade needles. Thylakoid stacking, energy distribution, and damage to the LHC have multiple effects on the photosynthetic system. In our experiment a significant lower content of LHCP2 (28–25 kDa) polypeptides was observed in shade needles. This could be one of the reasons for the observed marked lower PS2 activity in shade needles. As shown by the corresponding Western blots, a marginally lower content of the D1 protein occurs in shade needles. This lower content of the D1 protein was accompanied by a significantly lower content of the 33 kDa protein of the water-splitting system, showing that the whole PS2 was present at low levels in shade needles.

The needles grown under the shade had relatively low contents of soluble proteins and soluble protein/Chl ratio (Table 1) that concurs with similar report observed in *Erythrina variegata* (Muthuchelian *et al*. 1989) and soybean leaves (Burkey *et al*. 1997). The content of soluble proteins was markedly lower in the shade needles that might have been due to the lower synthesis of RuBPC, the major soluble protein of assimilation tissue, since a lower RuBPC activity was observed in shade needles. The carboxylating enzyme RuBPC is not fully activated at low irradiance and thus the degree of activation may regulate the flux of carbon through the photosynthetic pathway (Usuda *et al*. 1985).

Our results demonstrate that needles collected from Norway spruce grown under sun and shade have different leaf pigment composition, contents of ribulose-1,5-bisphosphate carboxylase and soluble proteins, and photosynthetic activities of the thylakoids. In addition, the shade induced changes on the donor side of PS2 in Norway spruce needles. This is due to (*1*) a marked inhibition of PS2 activity when SiMo is used as an electron acceptor, (*2*) artificial exogenous donors DPC and NH_2OH significantly increase the PS2 activity, (*3*) the contents of 33 and 23 kDa polypeptides are reduced, and (*4*) the content of 33 kDa protein is significantly lower by the immunological studies in the shade needles than in the sun ones.

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Pessarakli, M. (ed.): **Handbook of Photosynthesis. 2nd Ed.** – Tailor & Francis, Boca Raton – London – New York – Singapore 2005. ISBN 0-8247-5839-0. 928 pp., USD 159.95, GBP 92.00.

First edition of this manual was certainly successful and this is why after eight years a new edition appeared. On the first sight it differs from the first edition not only by a change of cover colour (a darker tone of green), but also by larger volume dimensions (220×285 mm *versus* 180×260 mm). The second edition is not a revised edition, but has some characteristics of a new book.

The first edition contained 63 chapters in XIV parts, but the new one contains only 46 chapters in XIV sections that have similar titles. The chapters are supplemented with full references to the respective papers. There are 27 to 271 references per chapter, mostly to papers published in the last fifteen years. In the 1st Ed. only short references were presented and such manner remained in some chapters (chapter 34) that were transferred without change from the first edition (last reference in this case is from the year 1995). This is strange, because during eight years new facts were certainly found in all analysed fields and hence these chapters appear as old-fashioned. I do not think that abstracts should be cited in manuals of this type. On the other hand, the chapters and topics contained only in the first edition are not cited here. There is also a change in style of the chapters – they are induced by contents preceding the text. Some figures and schemes were newly produced, some were overtaken from the first edition.

Who wrote the chapters? Most authors, some well known and some less known, are from the U.S.A. (21), followed by India (9), Argentina (6), Bulgaria, Hungary, Japan, and Slovakia (5 each), France and Pakistan (4 each), Canada, Germany, and the U.K. (3 each), China, Czech Republic, the Netherlands, and Poland (2 each), and Israel and Spain (1 each). Thirty four of these authors wrote chapters to both editions, 49 are new comers.

There are almost no new topics in this book and thus the reader only sees that some topics are missing (chlororespiration, effects of leaf age, plants cultivated *in vitro*, stomata patchiness, midday depression, photosynthetic bacteria, *etc*.). Some chapters have different numbers in both editions (*e.g*. chapters 1 and 9, 7 and 11).

I do not think that publishing this book under the same title was a good solution. It will certainly lead to a mess in references. If a book appears in a new edition, the main lay-out should remain the same or the title should be changed (at least to, say, New Handbook of Photosynthesis).

What are the positive sides of this edition? First, larger letter size and the two-column setting help in reading. The index is detailed enough. Some new chapters bring interesting information. Hence I believe that this second edition will again find its readers.

Z. ŠESTÁK (*Praha*)