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Response of photosynthesis to radiation and intercellular CO_2 concentration in sun and shade shoots of Norway spruce

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Abstract

Functional differentiation of assimilation activity of sun *versus* shade foliage was analysed in a Norway spruce monoculture stand (age 15 years). The investigated stand density (leaf area index 8.6) and crown structure led to variation in the photosynthetically active photon flux density (PPFD) within the crowns of the sampled trees. At the saturating PPFD, the maximum rate of CO_2 uptake (P_{Nmax}) of exposed shoots (E-shoots) was 1.7 times that of the shaded shoots (S-shoots). The apparent quantum yield (α) of E-shoots was 0.9 times that of the S-shoots. A lower ability to use excess energy at high PPFD in photosynthesis was observed in the S-layer. The CO_2- and PPFD-saturated rate of CO_2 uptake (P_{Nsat}) of the E-shoots was 1.12 times and the carboxylation efficiency (τ) 1.6 times that of the S-shoots. The CO_2-saturated rate of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) carboxylation (V_{Cmax}) and of actual electron transport (J_{amax}) in the S-needles amounted to 89 and 95 % of V_{Cmax} and J_{amax} in the E-needles. Thus, in addition to the irradiation conditions and thus limitation by low J_a, the important limitation of

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Abbreviations: C_i at C_a - intercellular CO_2 at the ambient CO_2; Chl - chlorophyll; Δg_s - initial slope of g_s-PPFD response curve [mmol mol^{-1}]; g_{smax} - PPFD-saturated g_s [mmol(CO_2) m^{-2} s^{-1}]; ΔJ_a - 433initial slope of the J_a-C_i response curve [electron mol^{-1}(quantum)]; J_{amax} - C_i-saturated J_a [µmol m^{-2} s^{-1}]; LAI - leaf area index; LHC - light-harvesting complex; NAMR [cm^2 g^{-1}] needle area per mass ratio; P_{Nmax} - PPFD-saturated P_N [µmol(CO_2) m^{-2} s^{-1}]; P_{Nsat} - C_i- and PPFD-saturated P_N [µmol(CO_2) m^{-2} s^{-1}]; PPFD - photosynthetic photon flux density; R_D - rate of dark respiration [µmol(CO_2) m^{-2} s^{-1}]; R_D' - day respiration [µmol(CO_2) m^{-2} s^{-1}]; R_S - rate of CO_2 evolution into CO_2-free air in the light [µmol(CO_2) m^{-2} s^{-1}]; RuBPCO - ribulose-1,5-bisphosphate carboxylase/oxygenase; ΔV_C - initial slope of V_C-C_i response curve [µmol mol^{-1}]; V_{Cmax} - C_i saturated V_C [µmol m^{-2} s^{-1}]; α - apparent quantum yield [mol(CO_2) mol^{-1}(quantum)]; Γ^* - compensation C_i excluding photorespiration [µmol mol^{-1}]; Γ_C - compensation C_i [µmol mol^{-1}]; Γ_I - compensation PPFD [µmol(quantum) m^{-2} s^{-1}]; τ - carboxylation efficiency [mol(CO_2) m^{-2} s^{-1}].

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photosynthesis in shade needles is due to carboxylation. This limitation of photosynthesis is accompanied by lower stomatal conductance.

Additional key words: apparent quantum yield; carboxylation efficiency; compensation irradiance; dark respiration; electron transport rate; intercellular CO_2 concentration; needle area/mass ratio; *Picea abies*; shade/sun foliage; stomatal conductance.

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). High values of silhouette *versus* total area ratio (Leverenz 1996) and specific needle area, low chlorophyll (Chl) content on leaf area basis, low Chl *a/b* ratio (Boardman 1977, Lewandowska *et al.* 1977, Björkman 1981, Marek *et al.* 1997), low dark respiration (R_D) rate (Pearcy and Sims 1994), low CO_2 uptake rate (Marek *et al.* 1989), low ribulose-1,5-bisphosphate carboxylase activity (Priwitzer *et al.* 1998), and low electron transport rate (Evans 1987) commonly occur in shade foliage compared to sun foliage.

To identify the constraints on the CO_2 uptake of shaded foliage, further investigations are needed to evaluate the importance of the electron transport and carboxylation related limitations, and the contribution of stomatal conductance (g_s) and R_D to these limitations (Pearcy and Sims 1994). In a natural forest stand, net photosynthetic rate (P_N) within the crown space is mainly controlled by irradiance, which supplies energy for the regeneration of ribulose-1,5-bisphosphate (RuBP). The role of nitrogen in key photosynthetic enzymes, particularly RuBPCO, also affects P_N. Furthermore, plant water status and temperature have to be considered because of their respective influence on the stomatal regulation of gas exchange of foliage and the enzyme activity, respectively. Finally, the concentration of CO_2 also affects P_N because it is the substrate for carboxylation reactions. Thus, a comparison of photosynthetic performance between E- and S-foliage in varying environmental conditions may help to identify their physiological differences and the main limitations to assimilation. The identification of differences in the photosynthetic performance between E- and S-foliage within a canopy under varying environmental conditions is also of great importance to the prediction of carbon and energy balances of the forest trees in the field.

The photosynthetic differences between E- and S-foliage were investigated in Norway spruce trees (*Picea abies* [L.] Karst.) because spruce has special internal crown irradiation conditions which significantly vary vertically. A comparison was made between exposed and shade acclimated shoots under multiple variations of photosynthetically active photon flux density (PPFD) and intercellular carbon dioxide concentration (C_i). Using a biochemical model for C_3 photosynthesis, the electron transport capacity and carboxylation capacity were calculated and related to g_s to determine which one is more limiting to P_N in E- *versus* S-foliage.

Materials and methods

Plants and experimental site: The stand was an even-aged (15 years, mean stand height 4.5 m) plantation of Norway spruce (*Picea abies* [L.] Karst.). It is part of the Experimental Ecological Study Site Bílý Kříž, which is situated in the Beskydy Mts. (49°33'N, 18°32'E, 943 m a.s.l., NE Moravia) in the Czech Republic. A detailed description of the experimental site was provided by Kratochvílová *et al.* (1989). The experimental plot (0.5 ha) with a high density of trees (2650 trees per ha, on a projected needle area basis LAI = 8.6 - unpublished results from autumn 1995) was used for this study. The LAI was estimated during overcast days using a technique based on the relation between diffuse solar radiation inside the canopy and the structure of the canopy (Lang and Xiang 1986). Measurements were carried out using the canopy analyzer *LI-2000* (*Li-Cor*, Lincoln, USA).

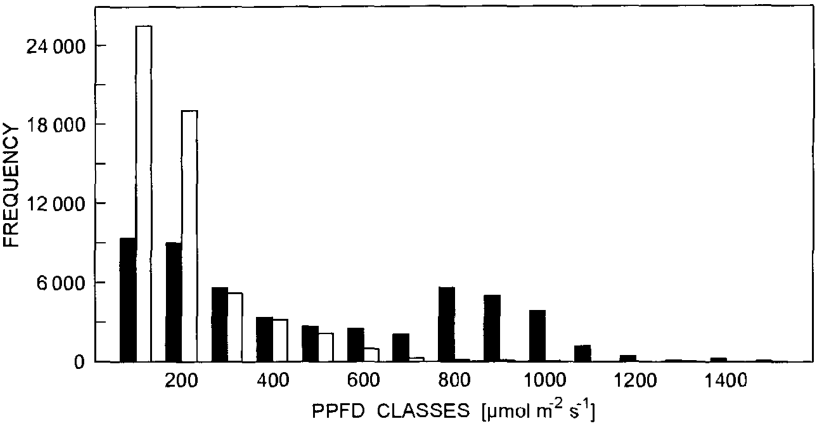

Fig. 1. Day-time frequency of the incident photosynthetically active radiation in the exposed and shaded crown layers during the period May-September 1995. *Open areas*: exposed crown layer; *closed areas*: shaded crown layer.

The canopy was divided into two crown layers according to the vertical distribution of PPFD (Fig. 1). Because of the height of the investigated stand, only exposed (E) and shaded (S) crown layers were identified within the canopy. The exposed canopy layer occupied 25 % and shaded one 65 % of the canopy height (determined from the top of the canopy).

A special linear holder system of laboratory-made sensors (separated by 10 cm) which were based on a photocell (*BPW-21*, 400-700 nm, *ELFA AB*, Solna, Sweden), was used for simultaneous measurement of the vertical PPFD within the E- and S-crown layers. The sensors were cosine-corrected, and the maximum sensitivity of the photocell was of 550 nm. The standard sensors (*LI-180, Li-Cor*, Lincoln, USA) were located on the sensor holders in each middle of canopy layers. Possible differences in sensors sensitivity for photons at all wavelengths in the individual canopy layers were accounted for in a calibration routine (non-linear regression between the raw V output of *BPW-21* and *LI-180* sensors, once per month). The sensors were supported on booms 15 m long and oriented East-West, *i.e.*, transversally through the plot. Thus, the measurements represented a horizontal cross-section within the crown layers at the 75 and 10 % of the stand height. One reference quantum sensor (*LI-180, Li-Cor*, Lincoln, USA) was placed in the plot, 4 m above the canopy on a steel meteorological mast. Measurements of incident and penetrating PPFD into the individual crown layers were made at 30-min intervals, and values were automatically recorded, averaged, and stored by a data-logger (*Delta-T Devices*, Cambridge, England).

Gas exchange measurements: Three sample trees belonging to the dominant layer of the stand on the plot were used for investigation of P_N. On each sample tree three sample shoots were chosen in the middle position of the E- and S-crown layer, respectively. The evaluation of P_N was based on results obtained from 1-year-old shoots located in the S/SW parts of the crown. At the end of growing season the shoots were removed and the projected area of needles was estimated using a leaf area meter (*LI-3000A, Li-Cor*, Lincoln, USA), then the dry mass of needles was obtained. Values of gas exchange measurements were then recalculated on the basis of the estimated projected needle area. Furthermore, on the area and mass bases, the needle/mass ratio was estimated.

The relationship between P_N and intercellular carbon dioxide concentration (C_i), P_N-C_i, was estimated using a closed system (*LI-6200* Portable Photosynthesis System, *Li-Cor*, Lincoln, USA). A special laboratory-made gas-exchange chamber (volume 270 cm^3) connected to a standard *LI-6200* chamber housing system was used. The P_N-C_i relation was obtained under standardized conditions in the gas exchange chamber: needle temperature (20±2 °C), relative air humidity (55±3 %), CO_2 concentration (intervals from 0 to 1400 $\mu mol\ mol^{-1}$), and PPFD (1200 $\mu mol\ m^{-2}\ s^{-1}$). The estimation of leaks (*Li-Cor* application note #103) showed reasonable leak-proof [0.6 $\mu mol(CO_2)\ mol^{-1}\ s^{-1}$] of the used closed system. A special laboratory-made artificial light source (two 100 W lamps with two thermal filters and fans, maximum PPFD of 1300 $\mu mol\ m^{-2}\ s^{-1}$) was mounted perpendicularly to the top of the gas-exchange chamber. The radiation source provided homogeneous irradiation (±20 %) onto the 6×3 cm plane within the chamber. The estimation of the P_N-C_i relationship started at saturated CO_2 concentration [1500 $\mu mol(CO_2)\ m^{-2}\ s^{-1}$] in the chamber. The pre-determined set of nine CO_2 concentrations (*ca.* 1200, 900, 700, 500, 350, 150, 100, 50, and 20 $\mu mol\ mol^{-1}$) in the gas-exchange chamber was used.

The relationship between P_N and PPFD was estimated using an open system (*CIRAS-1, PP-Systems*, Hitchin, Herts, UK). This relationship was estimated in the gas-exchange chamber at a CO_2 concentration of 340 $\mu mol\ mol^{-1}$. The standard gas exchange chamber (the Parkinson conifer chamber) was connected to the above mentioned artificial radiation source. The P_N-PPFD response curve was measured under a predetermined set of PPFD (0, 20, 50, 100, 200, 500, 800, and 1200 μmol m^{-2} s^{-1}). The PPFDs were provided using metal screens mounted between the radiation source and the top of the chamber. The conditions within the assimilation chamber were similar to those for the P_N-C_i relationship. The P_N measurements at each PPFD were recorded within an average 15-min interval. The same chamber conditions and protocol of measurements were used to estimate the relationship between stomatal conductance for CO_2 (g_s) and PPFD.

The ratio of $1 - P_{Na}/P_{Np}$ was used as a measure of the proportion of photons which are not utilised in photosynthesis (Demmig and Winter 1988). Potential assimilation rate (P_{Np}) was calculated from the initial slope of the P_N-PPFD response curve, assuming a constant apparent quantum yield (α) at all used PPFDs. Actual assimilation rate (P_{Na}) is P_N at a given PPFD commonly decreasing below P_{Np} with increasing PPFD. Thus, the increase of PPFD is responsible for an increase in the portion of absorbed radiation energy which is excessive.

Estimation of the Chl content in needles: Shoots for pigment analysis removed between 07:00 and 08:00 h from the S/SW-exposed part of the E- and S-crown layers were transported to the laboratory in dark. The shoots (five per each variant) were kept moist at a temperature close to 0 °C. In the laboratory all needles were removed from the shoots and a sample for analysis contained a mixture of all needles from different parts of the excised shoots. Each mixed sample (E- and S-variants) of needles was divided into five subsamples. The content of pigments was calculated on a needle area basis. The projected needle area of each subsample was estimated using a leaf area meter (*LI-3000A, Li-Cor*) and the subsamples were ground in an 80 % acetone solution in darkness, at room temperature with a small amount of $MgCO_3$. To achieve absorbances within the range of 0.4-0.8 [relative], a volume of 15 to 25 cm^3 was made up. The content of Chls and total carotenoids was determined spectrophotometrically using a spectral resolution of 2 nm (*Specord M400, Carl Zeiss*, Jena, Germany) in accordance with Lichtenthaler (1987).

Modelling of assimilation characteristics: The sets of values for both types of response curves (P_N-PPFD, P_N-C_i) were fitted using a special self-made program *FOTOS* (Pirochtová and Marek 1991). The *FOTOS* program is a non-linear regression of the set of C_i values and corresponding P_N which give the maximum rates of CO_2 uptake, CO_2 compensation concentration, R_D, initial slope of P_N-PPFD and P_N-C_i response curves. An indirect evaluation of some parameters of the biochemistry of CO_2 assimilation was based on a C_3 photosynthesis model (Caemmerer and Farquhar 1981). The calculations of the electron transport rate (J_a) and the rate of RuBPCO carboxylation (V_C) were based on in the field measured values of "day respiration" (R_D') and the CO_2 compensation concentration Γ^*, estimated under conditions excluding photorespiration. Values of R_D' and Γ^* were estimated using gas exchange technique according to Brooks and Farquhar (1985). The initial linear sections of P_N-C_i curves used for R_D' and Γ^* estimations were measured at three PPFD's (50, 100, 200 µmol m^{-2} s^{-1}), CO_2 concentration intervals between 50-300 µmol mol^{-1}, and at a leaf temperature of 20 °C. The point where measured initial sections of P_N-C_i curves intersect each other determines the value of R_D' (corresponding rate of CO_2 efflux) and Γ^* (corresponding CO_2 concentration). If the values of R_D', Γ^*, C_i, and corresponding P_N are known, then V_C can be determined (Brooks and Farquhar 1985). Actual electron transport rate (J_a) for each C_i and corresponding P_N were calculated on the basis of obtained V_C according to Caemmerer and Farquhar (1981). Maximum values of the electron transport rate (J_{amax}) and the rate of RuBPCO carboxylation (V_{Cmax}) were calculated from the relationship between J_a, V_C, and C_i using a non-linear regression program (*Statgraphics* program package).

Statistical processing: Each variant (E- and S-layer) was represented by three shoots on one sample tree, and three sample trees were used. Thus, each crown layer was represented by nine shoots. Chl content of each variant was represented by five samples. Means of values obtained were compared using *F*- and *t*-tests (*EXCEL* program package). The coincidence of sampled sets was taken as the zero hypothesis. The coefficient of determination (r^2) for each fitted curve (*i.e.*, P_N-C_i, P_N-PPFD relation) was used to determine solution acceptability. Only solutions with the values of r^2 higher than 0.95 were used.

Results

Radiation conditions of individual crowns layers: The E- and S-crown layers within the chosen stand canopy differed in irradiance (Fig. 1). The assimilation apparatus of needles in the S-layer was exposed to low irradiance during the whole period of the investigated growing season (May-September) which is an important environmental factor leading to the acclimation of foliage to shade. The needles in the E-layer were exposed to a wide range of irradiances typical to the sunny conditions within the canopy. These differences were obvious under sunny conditions but less so under diffuse irradiance (results not shown).

PPFD response of P_N was higher for E-shoots than for the shaded ones (Fig. 2*A*). At the saturating PPFD, the maximum rate of CO_2 uptake (P_{Nmax}) in the E-shoots was 1.7 times that of the S-shoots. The initial slope (α) representing apparent quantum yield of E-shoots was 0.87 times that of the S-shoots. The compensation irradiance (Γ_I) of the E-shoots was 1.46 times that of the S-shoots (Table 1). These results were statistically significant ($p = 0.05$). R_D was significantly higher for the E-needles (Table 1).

The PPFD-saturated value of stomatal conductance (g_{smax}) was significantly higher ($p = 0.01$) in the E-needles (Table 1). Compared to the S-layer, the reactivity of stomata to the changes of PPFD-Δg_s, calculated as the linear initial slope of the g_s-PPFD response, was 2.49 times greater in the E-needles (Table 1, Fig. 4).

The shape and achieved values of the $1 - P_{Na}/P_p$ ratio related to incident PPFD (Fig. 2*B*) indicated a rapid saturation of the assimilation apparatus of shaded needles by radiation energy. This rapid saturation is responsible for the lower ability to use excess energy of high PPFD during photosynthesis.

Table 1. Values of selected parameters of P_N-PPFD response curve for exposed (E) and shaded (S) canopy layers. P_{Nmax} - PPFD saturated P_N [μmol(CO_2) m^{-2} s^{-1}]; Γ_I - compensation PPFD [μmol(quantum) m^{-2} s^{-1}]; R_D - rate of dark respiration [μmol(CO_2) m^{-2} s^{-1}]; α - apparent quantum yield [mol(CO_2) mol^{-1}(quantum)]; Δg_s - initial slope of g_s-PPFD response curve [mmol mol^{-1}]; g_{smax} - PPFD saturated g_s [mmol(CO_2) m^{-2} s^{-1}]; NAMR [cm^2 g^{-1}] needle area/mass ratio. Three measured shoots in each crown layer, 3 measurements on each shoot, and 3 sample trees ($n = 27$). Means ± standard deviations. *,** levels of significance (0.05, 0.01).

Variant	P_{Nmax}	Γ_I	α	R_D	Δg_s	g_{smax}	NAMR
E	4.01±0.44**	19.95±1.59*	0.038±0.010**	0.76±0.06*	1.12	562	78.12±5.41**
S	2.34±0.21**	13.63±1.21*	0.043±0.020**	0.37±0.05*	0.45	267	38.41±3.84**

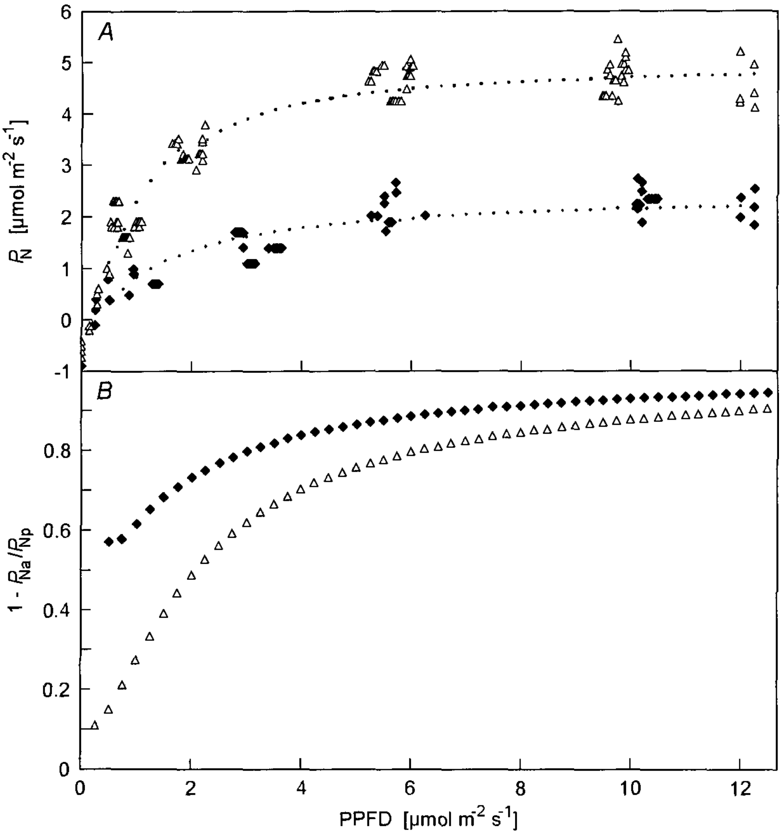

Fig. 2. Relationship between the rate of CO_2 uptake of shoots (P_N) and incident (*A*) or in photosynthesis used ($1 - P_{Na}/P_{Np}$) (*B*) photosynthetically active photon flux density (PPFD) in August 1995 for shoots with one-year-old needles from the exposed (Δ) and shaded (◆) crown layers. Conditions of measurements: temperature of needles 20±2 °C; relative air humidity 55±3 %; CO_2 concentration 340±10 μmol mol^{-1}. Three measured shoots in each crown layer, 3 measurements on each shoot, 3 sample trees.

The Chl (a+b) content was 0.42±0.03 g m^{-2} in exposed needles and 0.39±0.02 g

m^{-2} in the shaded ones. The difference was significant ($p = 0.05$). However, the content of Chl *a* was not significantly different between E- and S-needles (0.32±0.02 g m^{-2} compared to 0.28±0.01 g m^{-2}). Chl *b* content was higher in S-needles (0.11± 0.01 g m^{-2}) than in E-needles (0.09±0.01 g m^{-2}). The Chl *a/b* ratio was 3.28±0.08 for E-needles while 2.77±0.04 for S-needles. These differences were statistically significant ($p =$ 0.05).

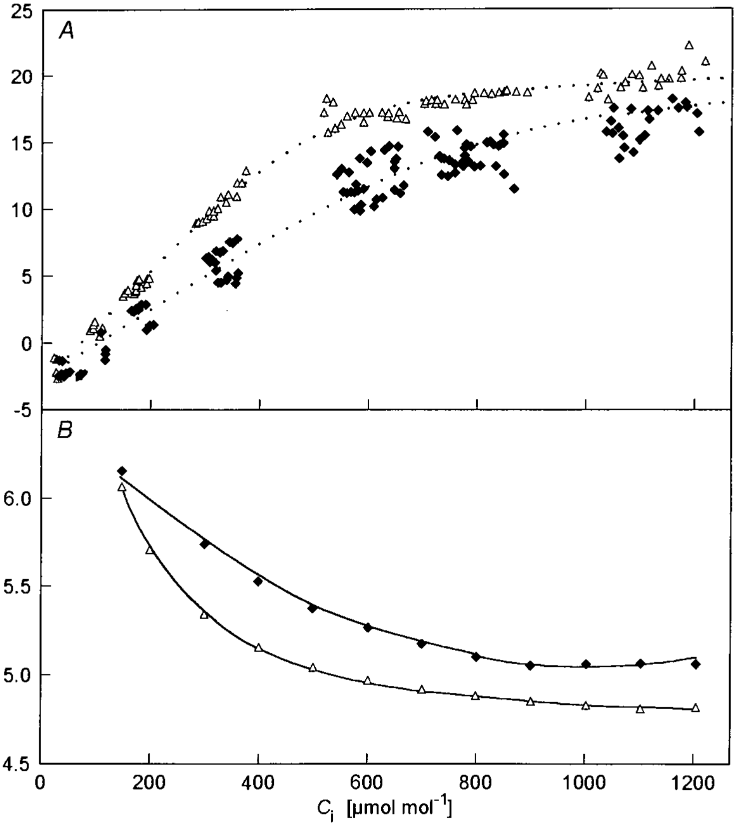

Fig. 3. Relationship between the rate of CO_2 uptake of shoots, P_N (*A*) or calculated ratio of actual electron transport rate, J_a, to calculated rate of carboxylation (V_C) (*B*) and intercellular carbon dioxide concentration (C_i) in August 1995 for shoots with one-year-old needles from the exposed (Δ) and shaded (♦) crown layer. Conditions of measurements: temperature of needles 20±2 °C; relative air humidity 55±3 %; CO_2 concentration 1200 µmol mol^{-1}.

CO_2 response of P_N differed for E- and S-shoots (Fig. 3*A*, Table 2). The CO_2 and PPFD saturated rate (P_{Nsat}) of the E-shoots was 1.12 times that of the S-shoots. The obtained values of P_N for C_i at ambient CO_2 (350 µmol mol^{-1}) can be used for comparison of gas exchange systems used for the measurements of the P_N-PPFD relation (open system) and the P_N-C_i relation (closed system). The value obtained from P_N-C_i relation was 4.21 µmol(CO_2) m^{-2} s^{-1} for E-shoots and 2.42 µmol(CO_2) m^{-2} s^{-1} for S-shoots (Fig. 3*A*, C_i values from Table 1). PPFD-saturated P_N obtained

Table 2. Values of selected parameters of P_N-C_i response curve for exposed (E) and shaded (S) canopy layers. P_{Nsat} - C_i and PPFD saturated P_N [µmol(CO_2) m^{-2} s^{-1}]; Γ_C - compensation C_i [µmol mol^{-1}]; τ - carboxylation efficiency [mol(CO_2) m^{-2} s^{-1}]; R_S - rate of CO_2 evolution into CO_2-free air in the light [µmol(CO_2) m^{-2} s^{-1}]; Γ^* - compensation C_i excluding photorespiration [µmol mol^{-1}]; R_D' - day respiration rate [µmol(CO_2) m^{-2} s^{-1}]; C_i at C_a - intercellular CO_2 at the ambient CO_2; ΔJ_a - initial slope of the J_a-C_i response curve [electron mol^{-1}(quantum)]; J_{amax} - C_i-saturated J_a [µmol m^{-2} s^{-1}]; ΔV_C - initial slope of V_C-C_i response curve [µmol mol^{-1}]; V_{Cmax} - C_i-saturated V_C [µmol m^{-2} s^{-1}]. Three measured shoots in each crown layer, 3 measurements on each shoot, and 3 sample trees. *,** levels of significance (0.05, 0.01).

Variant	P_{Nsat}	Γ_C	τ	R_S	Γ^*	R_D'
E	20.25±0.89*	70.07±1.08**	0.044±0.004*	3.05±0.15*	38.40±2.45**	0.45±0.11*
S	18.08±1.41*	99.21±2.94**	0.026±0.005*	2.62±0.44*	77.80±3.05**	0.36±0.27*
	C_i at C_a	ΔJ_a	J_{amax}	ΔV_C	V_{Cmax}	
E	211	0.21	99.84	0.09	25.42	
S	186	0.19	91.05	0.06	22.61	

from the P_N-PPFD relation was 4.01 µmol(CO_2) m^{-2} s^{-1} for E-shoots and 2.34 µmol(CO_2) m^{-2} s^{-1} for S-shoots (Table 1). Thus both gas exchange systems used gave comparable results.

The carboxylation efficiency (τ) was 1.7 times higher for E-shoots than S-shoots. Differences between E- and S-needles were statistically significant ($p = 0.01$). Values of Γ and Γ^* were higher for S-shoots than E-shoots ($p = 0.01$, Table 2). Obtained values of C_i at ambient 350 µmol(CO_2) mol^{-1} indicate more significant stomatal limitations of P_N in S-shoots (Table 1).

The CO_2-saturated rate of RuBPCO carboxylation (V_{Cmax}) in S-needles amounted to 89 % of the E-needles. However, the sensitivity of V_C to changing C_i-ΔV_C, calculated as the linear initial slope of the V_C-PPFD (values not shown) was higher in the S-needles (Table 2). The change of J_a with changing C_i-ΔJ_a, calculated as the linear initial slope of the J_a-C_i relation (values not shown) and the value of CO_2-saturated actual electron transport rate J_{amax} were higher for E-needles in comparison to the S-needles (Table 2). Needle area/mass ratio was significantly higher for E- than S-needles (Table 1).

Discussion

Results of measurements on selected clear summer days in August 1995 are presented. This part of growing season was selected due to the inherent completion of ontogeny of the needles and the stability of their physiological activity (Marek *et al.* 1997). The needles of 1-year-old shoots were used because of their important contribution to the photosynthetic production of the whole crown (Zimmermann *et al.* 1988). Furthermore, the use of 1-year-old needles minimizes the effects of needle ontogeny during the growing season (Troeng and Linder 1982), typical for the current year needles.

Acclimation of an photosynthetic apparatus of plants to the sun/shade conditions is related to distinct leaf structural changes (Pearcy and Sims 1994), such as an increase in the number of mesophyll cells per unit of leaf area and relative internal leaf area (Nobel 1976) in sun plants. These differences were confirmed by obtained values of the SLA for E- and S-shoots (Table 1). A lower Chl *a/b* ratio, lower electron transport capacity (J_{amax}), lower carboxylation capacity (V_{Cmax}), and generally lower P_N expressed per projected needle area were found in S-needles. These characteristics can be regarded as distinct properties of shade acclimation (Sims and Pearcy 1989, Zhang *et al.* 1995). The lower P_N in the S-needles than in the E-needles of Norway spruce may be partly due to the lower electron transport (Table 2), which is a consequence of reduced amount of electron carriers per unit of leaf area (Boardman 1977). A lower value of the electron transport rate and the rate of carboxylation (V_C) in shade plants of *Phaseolus vulgaris* has been reported by Caemmerer and Farquhar (1981).

The Chl *a+b* content per unit of leaf area in E-needles was 7.7 % higher than in the S-needles. However, this lower Chl content in S-needles was connected with the 14.6 % increase of the quantum yield compared to the E-needles. An increase in the quantum yield related to increasing Chl content above 0.40 g m^{-2} has been reported as a general phenomenon for C_3-species (Björkman 1981, Leverenz 1996). However, our findings do not fully confirm such phenomenon. The higher α obtained in S-needles indicates a larger amount of light-harvesting complex (LHC) per leaf area. It is supported by the obtained Chl *a/b* ratio which was lower in S-needles. Lower Chl *a/b* manifests an increase in the relative amount of LHC system with respect to the core complexes of especially PS2 and partly PS1 (Björkman 1981, Lam *et al.* 1984, Gnojek 1992). Increased amount of LHC is efficient especially in the capture of low PPFD. The different ability of sun/shade needles to utilize excessive photons was observed (Fig. 2*B*). The assimilatory apparatus of S-needles had a lower capacity to utilize photons compared to the E-needles. Therefore, a large portion of incident solar energy absorbed by S-needles must have been dissipated.

The obtained greater difference between C_a and C_i for the S-shoots is mainly an expression of the higher stomatal closure and resulting lower g_s at all PPFD's used (Fig. 4). This indicates that the CO_2 transport co-limits the CO_2 uptake rate to higher extent in S-needles than in the E-needles under the same irradiation.

The higher value of R_D in E-shoots (Table 1) could be an expression of higher energy expenditures connected to both the growth and maintenance, due to greater assimilation (Sims and Pearcy 1991). The portion of the R_D in the P_{Nmax} was lower in the S-shoots, indicating a higher efficiency of the assimilates' utilisation (Masarovičová 1984). The ratio of the rate of day respiration (R_D', Table 2) to R_D (Table 1) represents the relative amount of CO_2 evolution which is not suppressed by irradiation and varies considerably within different plant species (Kent *et al.* 1992). The values of R_D'/R_D ratio amounted to 0.58 and 0.98 for the E- and S-shoots, respectively. These values are similar to those published by Kirschbaum and

Farquhar (1984) for the tree species *Eucalyptus pauciflora*. Thus higher R_D'/R_D ratio obtained for S-shoots indicates higher suppression of CO_2 evolution. This is in accordance with the results of Zhang *et al.* (1995) obtained for sun and shade leaves within a canopy of a tropical forest.

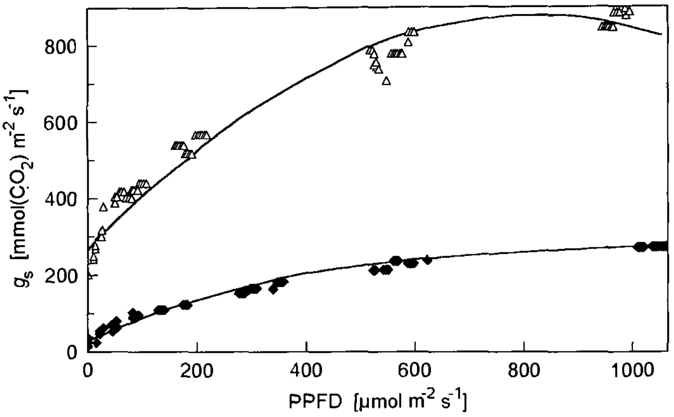

Fig. 4. Relationship between the stomatal conductance for CO_2 (g_s) and incident photosynthetically active photon flux density (PPFD) in August 1995 for shoots with one-year-old needles from the exposed (Δ) and shaded (♦) crown layer.

Zhang *et al.* (1995) present the ratio of J_a/V_C, which indicates the relative limitations of electron transport *versus* carboxylase activity. This ratio varies for different plant species and growth conditions. A greater ratio was found with increasing growth irradiance in *Pisum sativum* (Evans 1987), while a lower one was reported for sun plants of *Phaseolus vulgaris* (Caemmerer and Farquhar 1981). The S-shoots exhibited a higher J_a/V_C ratio during the entire interval of investigated C_i (Fig. 3*B*), which is close to results of Caemmerer and Farquhar (1981). A higher J_a/V_C in the shade crown layer indicates that the lower assimilation rate in the S-shoots resulted from important co-limitation caused by lower carboxylation, rather than co-limitation by lower RuBP regeneration associated with electron transport. Moreover, obtained values of J_a/V_C were inversely correlated to the nitrogen content of needles (15.8 for E- and 13.4 g kg^{-1}(d.m.) for S-needles, values from August 1995, Hrdlička 1996). The carboxylation capacity and Chl content are strongly related to the N content of the photosynthetic apparatus (Farquhar *et al.* 1980, Linder 1980). Thus, the carboxylation in S-shoots is significantly limited because the needle nitrogen content in August was 16 % lower in comparison to the E-shoots (Hrdlička 1996).

The obtained value of P_{Nmax} for the S-shoots was 58 % for the E-shoots, and this difference for the value of P_{Nsat} amounted to 89 %. The α in the S-shoots was 14.6 % higher and the τ amounted to 60 % in the E-shoots. It is generally accepted that the saturated part of the P_N-PPFD response curve represents RuBPCO-limited assimilation and the saturated part of the P_N-C_i response curve represents the RuBP-regeneration limitation of assimilation (Long and Hällgren 1993). Obtained E- *versus* S-shoots differences were greater and distinctive especially for the P_{Nmax}. This finding is supported by the significant lower carboxylation efficiency, *i.e.*, the measure of RuBPCO carboxylation efficiency (Farquhar *et al.* 1980, Brooks and

Farquhar 1985), estimated in the S-shoots compared to the E-shoots. Moreover, the obtained lower carboxylation rate and thus the RuBPCO activity in the S-needles is an expression of their low nitrogen content.

In conclusion, P_N was higher in exposed sunny foliage than in shaded foliage. The E-needles exhibited a higher P_N, higher capacity for electron transport, higher RuBPCO activity, and higher g_s compared to the S-needles. Our results indicate that the primary limitation of photosynthesis in shade needles is due to carboxylation. This primary limitation of P_N is accompanied by low electron transport rate and low stomatal conductance. Thus, co-limitation of the assimilation in shade needles can be ranged in the following order: carboxylation rate - electron transport - stomatal conductance.

Some degree of compensation from this limitation of photosynthesis could be found in the lower dark respiration rate and the lower portion of the respiration on the assimilation capacity. This results in some improvement in the shade assimilatory apparatus efficiency of photosynthesis.

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