Photosynth Res (2008) 95:87–99
DOI 10.1007/s11120-007-9238-1

REGULAR PAPER

Leaf age as a factor in anatomical and physiological acclimative responses of *Taxus baccata* L. needles to contrasting irradiance environments

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Received: 27 April 2007 / Accepted: 5 September 2007 / Published online: 22 September 2007

Abstract To evaluate the acclimative ability of current-year and previous-year needles of a shade tolerant conifer *Taxus baccata* L. to contrasting irradiance conditions, seedlings were raised under 27% solar irradiance and at 3 years of age they were transferred to an experimental garden and grown for one season under full irradiance (HL), 18% irradiance (ML) or 5% irradiance (LL). Whereas previous year needles did not change anatomically, current year needles in HL were thicker and had a thicker palisade and spongy mesophyll, and greater leaf mass per area than ML or LL needles. LL needles had greater nitrogen concentration than HL needles irrespective of age but only previous year LL needles also had an increased N per area content, thanks to their lack of reduction in LMA. Adjustment of chlorophyll and carotenoid content occurred in both needle age classes with LL and ML needles having much higher concentrations but, in current year needles, only slightly higher per area content than HL needles. Chlorophyll *a*/*b* ratio was not affected by age or irradiance. These modifications had no significant effect on photosynthetic capacities, which did not significantly differ between the age classes in HL or LL treatment and between treatments. On the other hand, high growth irradiance resulted in a greater photochemical yield, photochemical quenching, apparent electron transport rate and inducible non-photochemical quenching in needles formed in the current season. In previous year needles, however, only inducible NPQ was enhanced by high irradiance with other parameters remaining identical among treatments. To test sensitivity to photoinhibition, at the end of the summer plants from the three irradiance levels were transferred to a HL situation and F_v/F_M was determined over the following 18 days. Sensitivity to photoinhibition was negatively related to growth irradiance and previous year needles were less photoinhibited than current year needles. Thus, differences in acclimation ability between needle age classes were most pronounced at the level of anatomy and light reactions of photosynthesis, both of which showed almost no plasticity in previous year needles but were considerably modified by irradiance in current year needles.

Keywords Acclimation to irradiance · Photoinhibition · Sun/shade leaves · *Taxus baccata*

Abbreviations

ETR	Apparent electron transport rate (μmol $e^- m^{-2} s^{-1}$)
F_V/F_M	Maximum quantum yield of photosystem II photochemistry
F_M	Maximum fluorescence yield
F_M'	Maximum fluorescence in the light
F_0	Minimum fluorescence yield
F_S	Steady-state fluorescence
F_0'	Minimum fluorescence yield in light-adapted state

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HL	High light
ML	Medium light
LL	Low light
NPQ	Non-photochemical quenching of fluorescence
qP	Coefficient of photochemical quenching of chlorophyll fluorescence
PPFD	Photosynthetic photon flux density (μmol quanta m^{-2} s^{-1})
PNUE	Photosynthetic nitrogen use efficiency (μmol CO_2 mol N^{-1} s^{-1})
Φ_{PSII}	Quantum yield of PSII photochemistry

Introduction

Tree seedlings regenerating in the understorey experience much spatial and temporal heterogeneity of light conditions. Shifts in irradiance may occur as a result of gap formation in the canopy or a canopy closure (Canham et al. 1990; Naidu and DeLucia 1998). Local irradiance environment to which individual leaves are exposed also varies throughout the crown (Sack et al. 2006). To efficiently utilize the energy of light and at the same time to avoid photodamage to their light harvesting apparatus, trees must have a sufficient ability for acclimation.

Plants are able to respond to changes in irradiance by modifying structural and physiological traits of their leaves. Physiological acclimation to high light typically involves an increase in total nitrogen per area content, an increase in photoprotective compounds, enhanced dark respiration rate, increase in chlorophyll *a*/*b* ratio and decrease in the density of thylakoids and size of grana together leading to high light saturated CO_2 uptake rate, and high light compensation and saturation points (Björkman 1981; Givnish 1988). Anatomical features of sun leaves in comparison to shade leaves involve a thicker mesophyll, often composed of more cell layers and having a pronounced palisade layer, better-developed sclerenchymatic tissues and denser stomata (Givnish 1988; Eschrich et al. 1989; Hanba et al. 2002; Robakowski et al. 2004). The consequences of sun leaf anatomy for photosynthetic acclimation to high light reside in an increased packing of chloroplasts along with their metabolic machineries (including Rubisco) into a unit of leaf area (Murchie and Horton 1997; Terashima et al. 2001). Secondly, mesophyll cells of sun leaves have additional wall surfaces contacting the intercellular spaces that become available for the expansion of existing chloroplasts. This, together with greater stomatal density, decreases the internal resistance to CO_2 diffusion and allows to avoid low CO_2 concentration at fixation sites (Oguchi et al. 2003, 2005) thus preventing photooxidative stress. Thirdly, thicker mesophyll causes a change in vertical light penetration profile with palisade cells acting as fiberoptics at high irradiance intensities (Vogelmann and Martin 1993).

Anatomical changes in response to changed irradiance normally require production of new tissues, therefore, after leaves have fully expanded, anatomical modifications are only possible in the next foliar flush or even in the next growing season. Only a limited number of reports show anatomical changes in mature leaves in response to increased light (briefly reviewed by Oguchi et al. 2005) including, however, a single instance of induction of cell division in mature leaves of *Hedera helix* (Bauer and Thoni 1988). Whereas changes in nitrogen allocation, photoprotective compounds, pigment contents, and internal chloroplast organization are possible even in fully formed leaves (Kirchgeßner et al. 2003), it is questionable whether such modifications alone are sufficient for full expression of photosynthetic acclimation in the absence of anatomical adjustment (Frak et al. 2001; Oguchi et al. 2003). The acclimative capacity expressed by leaves developing under contrasting irradiances is typically greater than the capacity for acclimation exhibited by mature leaves transferred between the different irradiance environments (Sims and Pearcy 1992; Pearcy and Sims 1994; Naidu and DeLucia 1998). Ability for full photosynthetic acclimation of mature leaves has, however, been reported in *Abies amabilis* (Brooks et al. 1994), a tropical vine *Stigmaphyllon* (Avalos and Mulkey 1999) and in *Fraxinus americana* and *Quercus rubra* (Naidu and DeLucia 1998).

Evergreen needles of conifer plants offer a convenient system to uncouple the different processes associated with light acclimation. Conifer needles usually do not grow and do not undergo anatomical changes beyond the first season of life (except secondary growth in vascular tissues, Ewers 1982). Common yew *Taxus baccata* L. is a highly shade-tolerant tree able to regenerate and grow to maturity in heavy shade but is also tolerant of open conditions (Thomas and Polwart 2003). This wide ecological tolerance of *T. baccata* should be reflected in the plasticity of its structural and functional foliar traits. *T. baccata* possesses a typical dorsiventral leaf anatomy with well-defined palisade and spongy mesophyll. To evaluate the acclimative potential of previous year and current year needles of *T. baccata*, we exposed young *T. baccata* plants to three irradiance regimes for most part of the growing season (from bud break to maturity of new leaves). We examined anatomical differences among needles developed in the current season under the three irradiance treatments and confirmed the lack of anatomical adjustment of needles formed in the previous season. Subsequently, we compared the responses to growth irradiance of nitrogen, pigment content, photosynthetic rate, and chlorophyll fluorescence characteristics between the two needle age classes.

Materials and methods

Plant origin and cultivation

We used *Taxus baccata* seedlings obtained from seeds collected locally in Rokita Forest District (NW Poland) where a healthy self-seeding population exists. Plants were initially raised in a forest nursery in individual pots and maintained under protective netting transmitting 27% solar irradiance. During the winter they were covered by conifer branches for frost protection. In March 2006, when the plants were three years old, they were transferred to Adam Mickiewicz University Botanical Garden in Poznań and potted into 3 l pots filled with a horticultural substrate. Two weeks after establishment, and again 8 weeks later plants were each top-dressed with 5 g of slow release fertilizer (10–10–10 NPK). Watering to field capacity was conducted as needed.

Experimental treatments

On April 13, 2006 i.e., soon before bud break, plants were randomly distributed among nine plots, each assigned to one of the three treatments: full ambient irradiance (high light—HL), intermediate irradiance (mid light—ML), and low irradiance (low light—LL). Reduction in irradiance was accomplished by covering respective plots with one (ML) or two (LL) layers of shading cloth. Spectral properties of the shading cloth were described previously (Wyka et al. 2007). The relative reduction of irradiance was determined by simultaneously measuring the photosynthetic photon flux density (PPFD, 400–700 μmol m^{-2} s^{-1}) close to an upper branch of each plant and in an adjacent unshaded location using light meters (Spectrum Technologies, Inc., Plainfield, USA). By this method, the mean PPFD in relation to ambient was 17.8 ± 3.8% under ML and 5.4 ± 1.6% under LL (mean ± s.d. for $N = 50$ readings in each treatment).

Leaf morphology and anatomy

Needles were collected at the beginning of the experiment (2005 cohort; $N = 49$) and then in mid August (2005 and 2006 cohorts; $N = 49$ for each light level). Length and width was determined with calipers and a subset of $N = 23$ needles for each treatment and age class were fixed in Navashin's fixative and dehydrated in a graded ethanol series from 10 to 70% ethanol. Cross sections were hand-cut with a razor blade, stained with floroglucine, examined through a light microscope (Axioskop, Zeiss, Germany) and photographed using an attached Powershot G5 camera (Canon, Japan). Measurements were taken from digital images using LSM 510 Image Browser software (Zeiss, Germany). To produce illustrations, a subset of needles from HL and LL treatments were fixed in a solution of 2% glutaraldehyde and 2% paraformaldehyde in cacodylate buffer, embedded in Spurr's resin and sectioned on Ultra-cut S microtome (Leica-Reichert, Germany). Sections were stained with 0.5% toluidine blue and photographed as above.

Gas exchange

Photosynthetic rates were measured in laboratory using LCA 4 gas exchange system (ADC Ltd., Hoddesdon, UK). Prior to measurements, plants were maintained under low light intensity (PPFD = 30–50 μmol m^{-2} s^{-1}) to maintain photosynthetic induction. For each plant, a current season (2006) twig and a previous season (2005) twig were selected. Care was taken to use only twigs that were not shaded by other branches of the same plant. Any needles that might have been shaded by other needles of the same twig were removed. The 2005 twigs were prepared by clipping off any later growth at least 12 h before the measurement to reduce wound respiration and the wound was covered with a gas tight coat of silica gel. A custom-made lamp consisting of sets of interspersed blue and red diodes was used as a cool light source with intensity controlled at the power adapter. Light intensity at leaf surface for different lamp settings was determined with a LCA 4 light sensor. Measurement protocol involved exposing the twig to a series of four light levels: 0 μmol m^{-2} s^{-1} (for 12 min), 132 μmol m^{-2} s^{-1} (20 min), 275, and 386 μmol m^{-2} s^{-1} (12 min each). Automatic records of gas exchange parameters were taken at 30 s intervals, however, only data for the last 5 min of each interval were retained as they represented a steady state, as determined in preliminary trials. These values were then averaged to generate a single data point for each light level. Eight or nine plants were used for measurements for the HL and LL treatment. For logistic reasons the ML treatment was excluded from the gas exchange measurements. Leaf areas were measured using a scanner and the ImageJ software. Results were expressed on the basis of leaf area, and nitrogen content (i.e., as photosynthetic nitrogen use efficiency PNUE).

Chlorophyll fluorescence

On the days of photosynthesis measurements, chlorophyll *a* fluorescence was also determined in 2005 and 2006 needles of 15 seedlings from each treatment group. Measurements

were conducted using Fluorescence Monitoring System (FMS 2, Hansatech, Norfolk, UK) controlled by a PC. Prior to measurements, needles were maintained in a humid atmosphere in the dark at 22°C ambient temperature. Then, they were tightly arranged in a factory provided leaf clip. The fiberoptics encased in a light-tight chamber was inserted onto the leaf clip and the needles were exposed to modulated low intensity amber measuring light (PPFD = 0.05 μmol m^{-2} s^{-1}). After reading minimum fluorescence F_0, a saturating 0.7 s light pulse (PPFD = 15.3 mmol quanta m^{-2} s^{-1}) was delivered to induce a maximum fluorescence (F_M). Maximum quantum yield of PSII photochemistry was calculated by the instrument's software according to the formula: maximum quantum yield = F_V/F_M, where $F_V = F_M - F_0$ is variable fluorescence. Subsequently, to generate light response curves of PSII quantum yield (Φ_{PSII}), needles in the clip were illuminated with actinic light using an inbuilt halogen lamp. Up to 10 levels of actinic light were applied in the order of increasing intensity. After a steady-state fluorescence under actinic light (F_S) was reached (as observed on a computer screen), a 0.7 s saturating light pulse was delivered and maximum light-adapted fluorescence (F_M') was determined. Quantum yield of PSII was calculated by the inbuilt

Table 1 Results of ANOVA for morphometric traits, LMA, nitrogen, pigment contents and fluorescence parameters in current year and previous year needles from three irradiance treatments

Trait	Error	Irradiance		Age		Irradiance × Age	
	d.f.	d.f.	*F*	d.f.	*F*	d.f.	*F*
Morphology							
Needle width	291	2	0.80 n.s.	1	143.3***	2	4.08*
Needle length	291	2	1.15 n.s.	1	323.4***	2	2.38 n.s.
Anatomy							
Wilks' Lambda		12; 242	7.21***	6; 121	28.1***	12; 242	4.11***
Midrib thickness	128	2	16.3**	1	50.4***	2	11.6***
Lamina thickness	127	2	41.5***	1	143.3***	2	18.02***
Palisade mesophyll thickness	128	2	37.7***	1	98.6***	2	12.2***
Spongy mesophyll thickness	127	2	28.2***	1	91.2***	2	12.2***
Palisade/spongy mes. Ratio	127	2	6.4**	1	13.3***	2	2.88 n.s.
Palisade cell length	127	2	16.9***	1	113.3**	2	15.28***
LMA	82	2	52.4***	1	163.6***	2	7.64***
Nitrogen per area	44	1	10.3**	1	40.1***	1	14.55***
Nitrogen per mass	45	1	194.0***	1	0.56 n.s.	1	3.35 n.s.
Pigments							
Wilks' Lambda		18; 148	16.8***	9; 74	35.2***	18; 148	7.39***
Carotenoids per area	82	2	14.1***	1	212.5***	2	8.53***
Carotenoids per mass	82	2	69.0***	1	17.6***	2	2.12 n.s.
Tot. chlorophyll per area	82	2	35.1***	1	246.3***	2	6.15**
Tot. chlorophyll per mass	82	2	133.2***	1	27.2***	2	3.52*
Chlorophyll *a* per area	82	2	35.5***	1	238.5***	2	5.73**
Chlorophyll *a* per mass	82	2	135.0***	1	22.7***	2	4.21*
Chlorophyll *b* per area	82	2	19.6***	1	169.2***	2	5.16**
Chlorophyll *b* per mass	82	2	66.0***	1	31.7***	2	0.68 n.s.
Chlorophyll *a*/*b* ratio	82	2	0.15 n.s.	1	14.1***	2	0.50 n.s.
Carot./chlorophyll ratio	82	2	62.4***	1	9.34***	2	7.33***
Fluorescence							
Wilks' Lambda		8; 98	11.2***	4; 49	12.5***	8; 98	2.03*
$\Phi_{PSII\ (343)}$	66	2	4.10*	1	0.90 n.s.	2	6.48**
$qP_{(343)}$	66	2	17.6***	1	1.77 n.s.	2	10.2***
ETR_{MAX}	52	2	4.34**	1	3.43 n.s.	2	4.77*
$NPQ_{(740)}$	66	2	14.6***	1	21.5***	2	1.99 n.s.

Additionally, MANOVA results (significance values for Wilks' Lambda) are given for anatomical traits, pigment contents and fluorescence parameters. Significance symbols as in Fig. 3

software as: $\Phi_{PSII} = (F'_M - F_S)/F_M'$ (Genty et al. 1989). For each actinic light level, non-photochemical quenching of fluorescence (NPQ) was calculated according to the formula: $NPQ = (F_M - F_M')/F_M'$ (Maxwell and Johnson 2000). During the fluorescence measurements conducted in the laboratory, air temperature varied from 22 to 23.5°C. Photochemical quenching was calculated at each actinic light level according to the formula: $qP = (F'_M - F_S)/(F_M' - F_0')$. Directly measured chlorophyll *a* fluorescence parameters F_0, F_M and F_M' were used to calculate minimum fluorescence yield in light-adapted state (F_0') defined by Oxborough and Baker (1997) as: $F'_0 = F_0/(F_v/F_M - F_0/F_M')$. For each light level, the apparent rates of photosynthetic electron transport (ETR) through PSII were calculated using the formula $ETR = \alpha \times \Phi_{PSII} \times PPFD \times 0.5$ (Maxwell and Johnson 2000). An assumption was made that the excitation energy is partitioned equally between the two photosystems (hence the factor 0.5, Maxwell and Johnson 2000). Leaf absorptance (α) was calculated for each species using needle chlorophyll content based on the experimental model of Evans (1993).

For statistical comparisons a single cardinal value was selected for each light-response curve. For Φ_{PSII} and qP the values at PPFD = 343 μmol m^{-2} s^{-1} (actinic but not photoinhibitory light) were used, for ETR a saturation curve was fitted and a maximal value calculated, whereas for NPQ the values at PPFD = 740 μmol m^{-2} s^{-1} were chosen.

Chemical analyses

Pigment contents were analyzed in leaf samples (40–50 mg FW) collected from twigs used for chlorophyll fluorescence measurements. Needles were cut into 2 mm pieces and incubated in 5 ml of 100% dimethylsulfamide (DMSO) saturated with $CaCO_3$ at 60°C in water bath until the solution became translucent (approximately 5 h). The absorbance of the extract was measured at 665, 648, and 470 nm. Chlorophyll *a*, *b*, and total carotenoid contents were calculated according to Barnes et al. (1992). Nitrogen

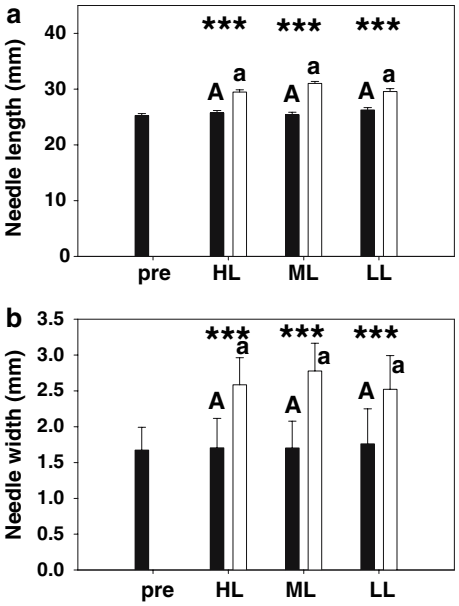

Fig. 1 Width and length of *Taxus baccata* needles exposed to three irradiance treatments (HL—high irradiance, ML—intermediate irradiance and LL—low irradiance) during the 2006 growing season. Means ± standard error are shown. Needles formed in 2005 are represented by black bars, and those formed in 2006 by white bars. Bars marked *pre* are for 2005 needles collected prior to experiment (March 2006). Shared letters indicate non-significant contrasts (capital letters are for comparisons among 2005 needles, and small letters are for 2006 needles). Asterisks refer to comparisons between age classes within each treatment group (***$P < 0.001$). ANOVA results are given in Table 1

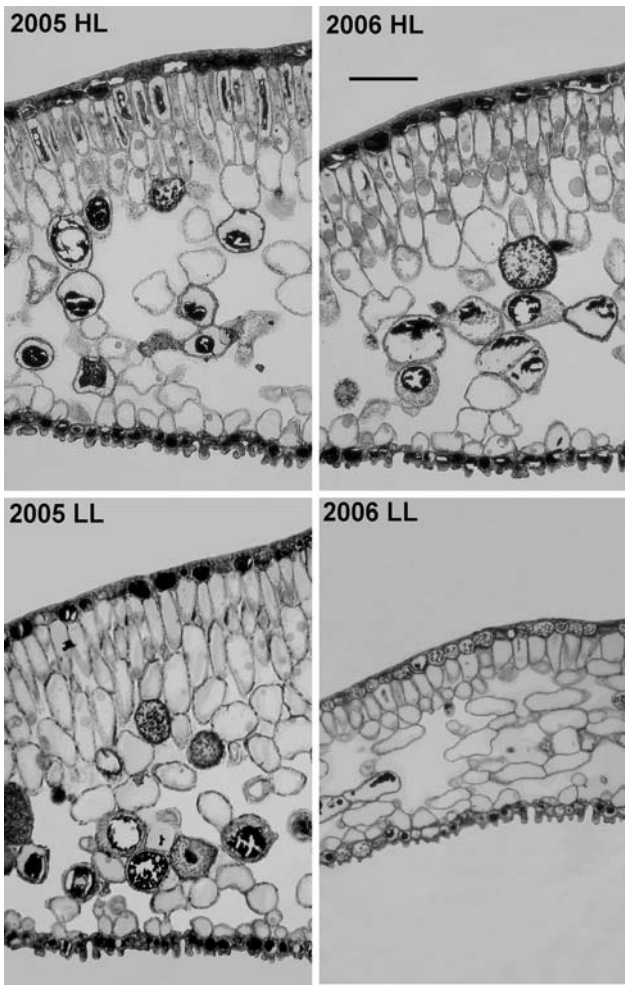

Fig. 2 Mesophyll structure of *Taxus baccata* needles formed in 2005 (**a**, **c**) or 2006 (**b**, **d**) growing seasons. During the 2006 season needles were exposed to high irradiance (HL; **a**, **b**), or low irradiance (LL, **c**, **d**). Bar is 100 μm. All photographs are at the same scale

was determined in leaves used for photosynthesis measurements. Plant material was oven dried for 3 days at 65°C and weighted. Nitrogen was analyzed spectrophotometrically following the Kjedahl digestion. LMA was computed from dry leaf biomass and projected area.

Photoinhibition

To evaluate the susceptibility of needles to photoinhibition, on September 16, 2006, six seedlings from each irradiance treatment were moved to a fully exposed area. Using Plant Efficiency Analyser (PEA, Hansatech, Norfolk, UK) maximum quantum yield of PSII (F_V/F_M) was measured on both 2005 and 2006 needles first prior to transfer (day 0) and then 1, 2, 3, 4, 11, and 18 days afterwards. Each time, leaves were dark-adapted overnight using factory-provided leaf clips, and measurements were conducted early in the following morning. The F_V/F_M was determined using a 1 s pulse of red light at PPFD = 4,000 μmol m^{-2} s^{-1}. Weather data for the experimental period was obtained from the AMU Botanical Garden weather station.

Statistical analysis

Anatomical measurements were log transformed. For sets of variables (anatomical traits, pigments, cardinal points of fluorescence curves) MANOVAs were conducted followed by ANOVAs for individual traits. For other traits univariate ANOVAs alone were applied. For analysis of the temporal variation in F_V/F_M after exposure of plants to full sun, repeated measures ANOVA was applied, with species and growth irradiance level as categorical variables and the seven measurement days as levels of repeated factor. Comparisons that were relevant for the current hypotheses were tested using preplanned contrasts or Tukey's test. Effects were considered significant when $P < 0.05$. All statistical analyses were conducted with Statistica 6.0 using the General Linear Model procedure.

Results

Structural traits

Needles produced in 2006 were longer and wider than the 2005 cohort (Table 1; Fig. 1a, b) although no differentiation was noted among treatments within either class. Likewise, the previous year (2005) needles showed no evidence of size increase in the course of the experiment. Dimensions of anatomical structures in current year needles were generally smaller then those of last year needles even in HL plants (thinner laminae and palisade layers and shorter palisade cell length; Figs. 2 and 3b, c, f).

None of the anatomical trait of previous year needles showed a significant differentiation among treatments (Figs. 2 and 3). In contrast, current year (2006) needles

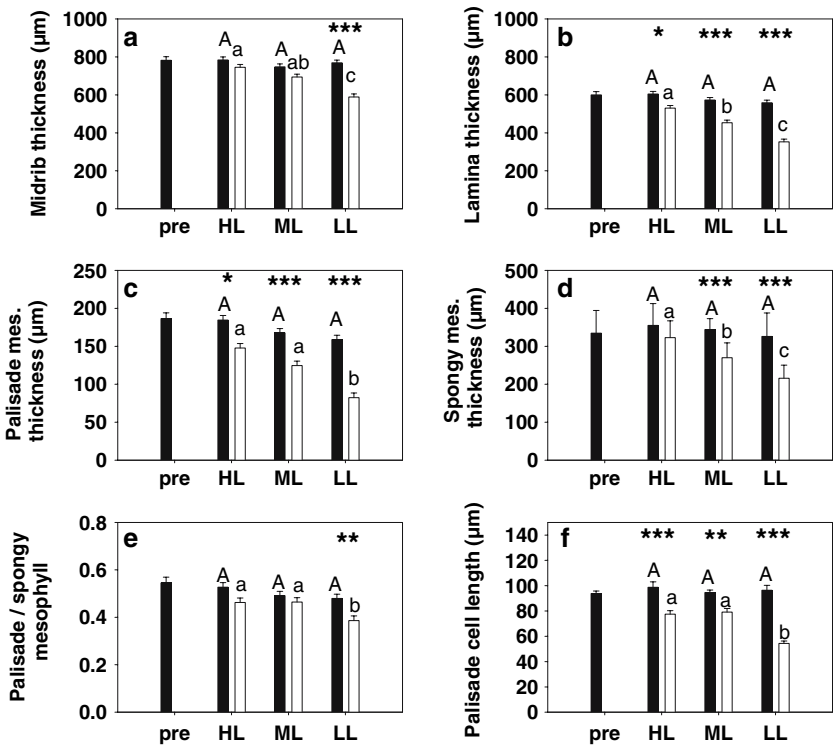

Fig. 3 Anatomical traits of *T. baccata* needles exposed to three irradiance treatments. Asterisks refer to comparisons between age classes within each treatment group (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). Other details and symbols as in Fig. 1. ANOVA results are given in Table 1

underwent a significant anatomical differentiation caused by the different irradiances (Table 1). Leaves produced under shade had significantly thinner midribs (Fig. 3a) and laminae (Figs. 2 and 3b). The reduction in lamina thickness could be explained by the reduction in thickness of both palisade (Fig. 3c) and spongy (Fig. 3d) layers, and the reduction in palisade cell length (Fig. 3f). The decreases were greater in palisade than in spongy mesophyll as shown by the reduced palisade to spongy mesophyll ratio in LL (Fig. 3e). The irradiance difference between ML and HL treatments was not sufficient to cause significant changes in most traits. Only lamina thickness and spongy mesophyll thickness were smaller in ML in comparison with HL plants (Fig. 3b, d).

These anatomical modifications of current year needles were reflected by reduced LMA in ML and LL needles (Fig. 4a). Also in LL needles from 2005, LMA was lower than in ML or HL needles (Fig. 4a) in spite of the absence of structural changes, suggesting differences in metabolite (e.g., starch) levels or wall features.

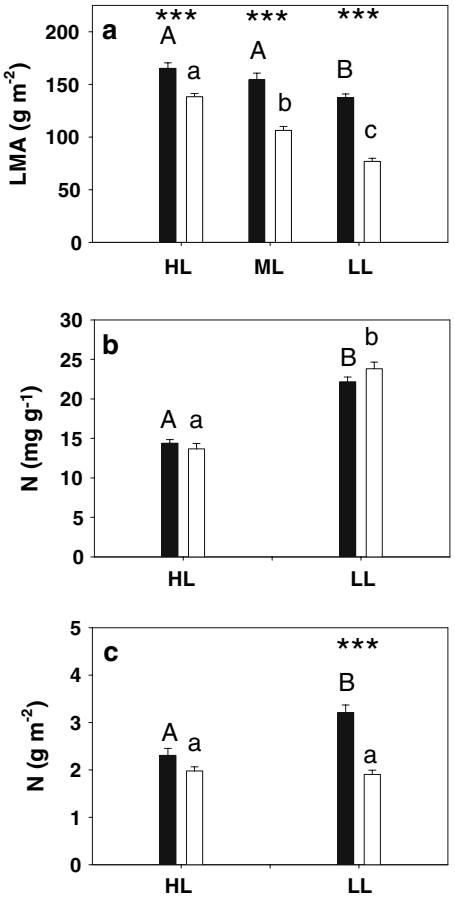

Fig. 4 Leaf mass-per area (**a**), dry mass-based nitrogen contents (**b**) and area-based nitrogen contents (**c**) in previous year (black bars) and current year (white bars) needles of *Taxus baccata* exposed to irradiance treatments. Nitrogen levels were not determined for the ML needles. Symbols as in Fig. 1. ANOVA results are given in Table 1

Nitrogen

The contents of nitrogen on dry weight basis were significantly greater in LL than in HL needles (ML needles were not sampled) and needles of both age classes revealed the same pattern (Table 1; Fig. 4b). On an area basis, however, the increase in nitrogen content in LL occurred only in 2005 needles (Fig. 4c).

Pigments

On area basis, carotenoids, total chlorophyll, chlorophyll *a*, and chlorophyll *b* in previous year needles reached greater levels in ML and LL plants in comparison to HL plants, with no difference between the two shading intensities (Table 1; Fig. 5a, c, e, g). In current year needles, however, only increases of total chlorophyll and chlorophyll *a* were significant (Fig. 5c, e). Pigment contents per area were always lower in 2006 needles. When comparisons on dry weight basis were made, the pattern of between treatment differences for 2005 needles was the same as for area-based values. In 2006 needles, however, the differences became magnified and the concentration of each pigment class was highest in LL followed by ML and HL needles (Fig. 5b, d, f, h). Shading treatments did not affect the chlorophyll *a*/*b* ratios (Fig. 5i), but did increase the chlorophyll/carotenoids ratio in both 2005 and 2006 needles (Fig. 5j).

Photosynthesis

Photosynthetic rates under our measurement conditions approached saturation at 386 μmol m^{-2} s^{-1} (Fig. 6a). Although no significant effect of treatment or age was found, the 2005 HL needles showed the highest photosynthetic rate at all light levels applied ($P < 0.06$ in comparison against 2005 LL). Photosynthetic nitrogen use efficiencies (PNUE) were significantly affected by needle age ($P < 0.01$ at PPFD = 275 and 386 μmol m^{-2} s^{-1}) and age × irradiance interaction ($P < 0.05$ at PPFD = 386 μmol m^{-2} s^{-1}) with lowest values in 2005 LL needles (Fig. 6b).

Chlorophyll fluorescence

Needles produced in 2005 showed little plasticity with regard to chlorophyll fluorescence parameters (Fig. 7). No significant differences among the three light exposure

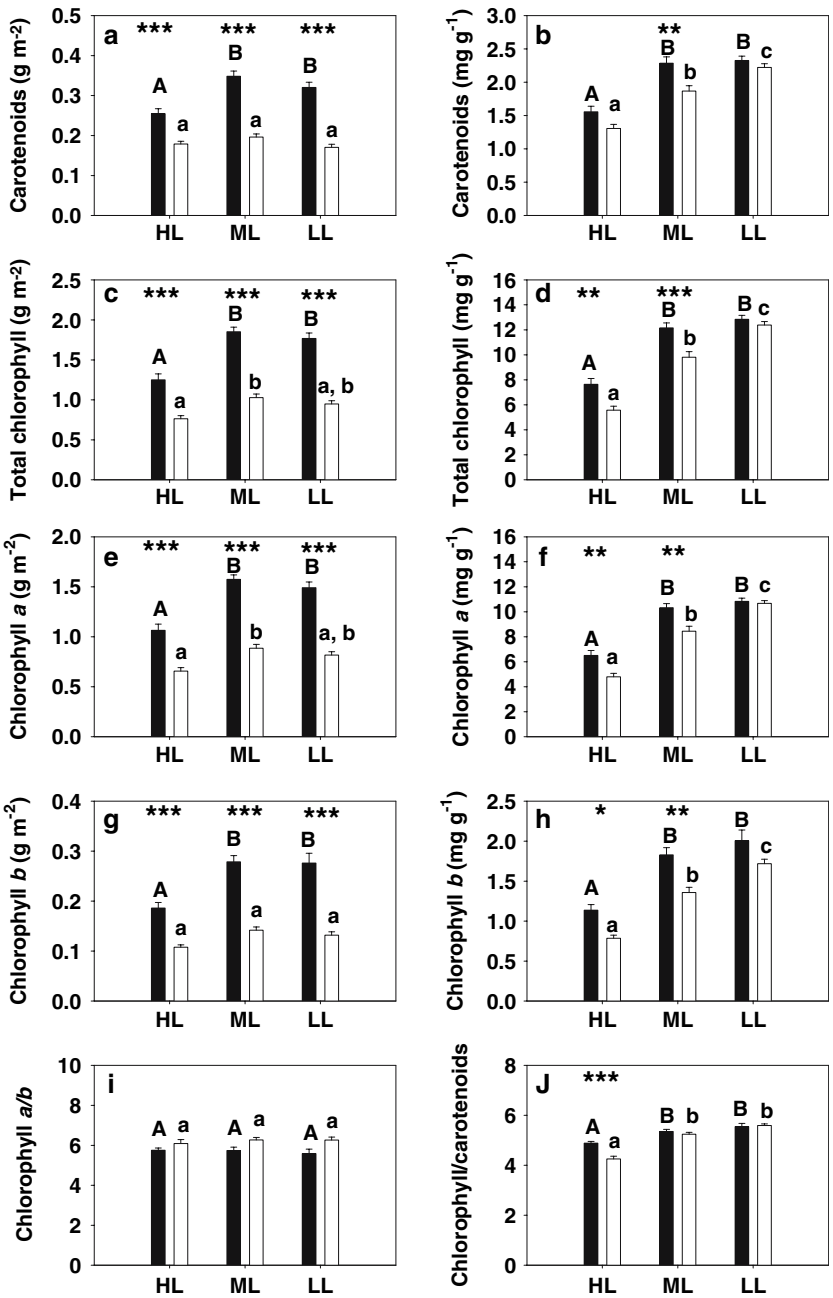

Fig. 5 Photosynthetic pigments in *T. baccata* needles exposed to three irradiance treatments. Panels **a**, **c**, **e**, **g** show area-based contents, and panels **b**, **d**, **f**, **h** show dry mass-based contents of, respectively, carotenoids, total chlorophyll, chlorophyll *a*, and chlorophyll *b*. Also shown are chlorophyll *a*/*b* ratio (**i**) and chlorophyll/carotenoid ratio (**j**). Other details and symbols as in Figs. 1 and 3

groups were found with respect to Φ_{PSII}, qP, and ETR_{MAX} (Fig. 7a, c, e). Only the non-photochemical quenching parameter NPQ reached greater values in response to light in HL when compared to LL plants (Fig. 7g). In contrast, the 2006 needles showed significant acclimation in all fluorescence variables studied with comparisons between HL and LL plants being always significant and ML plants remaining intermediate for at least a part of the PPFD range tested (Fig. 7b, d, f, h). Thus, 2006 needles from HL plants displayed a greater ability to utilize increasing light intensity (higher Φ_{PSII}, qP, and ETR_{MAX}) and a greater ability for protective down-regulation of PSII (NPQ parameter) compared to needles from plants grown at lower light levels.

Photoinhibition

Weather during the period of exposure to HL was hot and sunny with maximal temperatures often exceeding 30°C and sky remaining cloudless except on days 6, 8, and 9 (Fig. 8a). Both shading treatment and needle age had a significant influence on the performance of PSII in a high irradiance situation, and variability over the measurement

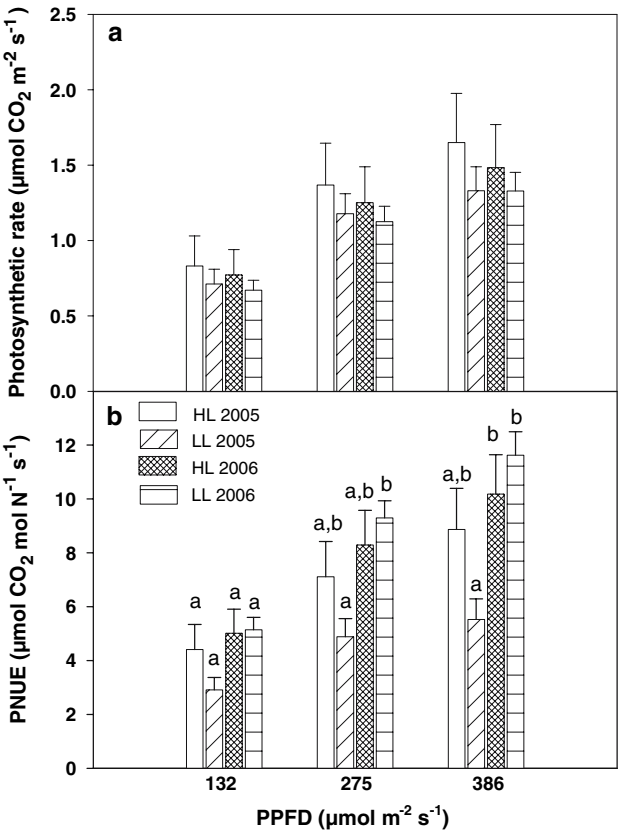

Fig. 6 Photosynthetic responses to light of previous year (2005) and current year (2006) *T. baccata* needles exposed during the 2006 season to two contrasting irradiance regimes (high irradiance HL and low irradiance LL). (**a**) Leaf area-based net CO_2 uptake (ANOVA effects were not significant and thus no pairwise testing was conducted). (**b**) Photosynthetic nitrogen use efficiency (shared letters indicate lack of significant differences between means within each PPFD level as determined by Tukey's test)

period was also significant (Table 2). A decline in F_V/F_M occurred in both age classes of needles from ML and LL plants but initially only in 2006 needles of HL plants (Fig. 8b, c, d). The declines were strongest in LL plants, followed by ML plants, with only a slight reduction in HL plants. Within each irradiance group, greater declines of F_V/F_M were found in 2006 needles. Both ML and LL needles remained photoinhibited throughout the experimental period although in LL needles a partial recovery was noted on day 4 (Fig. 8c).

Discussion

Taxus baccata is an extremely shade-tolerant species and yet it has a wide amplitude of light tolerance (Thomas and Polwart 2003). We showed that young *Taxus* plants are capable of generating large anatomical modifications to their leaf structure similar to many other plant species (Givnish 1988) but in contrast to the limited anatomical responsiveness of other conifer seedlings (Wyka et al. 2007). Based on data in Fig. 2, plasticity indices [PI = (max – min)/max); Valladares et al. 2000] for mesophyll traits in current year needles in *Taxus* were above 0.3 (in comparison between 5% and 100% irradiance) whereas under similar experimental conditions, these indices were between 0.04 and 0.17 for another shade tolerant conifer (*Abies alba*) and a moderately shade tolerant *Picea abies* (Wyka et al. 2007). Nevertheless, anatomical changes in *Taxus* were induced only in current year needles, i.e., those that developed under contrasting irradiances.

The aim of our experiment was to test if acclimative ability of previous year needles to ambient light is smaller than that of current year needles. This certainly was not the case with nitrogen allocation. Within each irradiance regime, needles from both age classes had similar concentrations of nitrogen. Unexpectedly, nitrogen concentration was greater in LL than in HL needles, which is inconsistent with a well-known trend (Lei and Lechowicz 1998; Valladares et al. 2000; Hättenschwiler 2001; Meir et al. 2002). The likely explanation for this deviation is that N accumulation might have resulted from a nutritional imbalance caused by growth inhibition under LL. Although some shade tolerant species under low light may accumulate nitrogen in compounds that enhance their light harvesting capacity (Niinemets 1997), much of nitrogen in LL needles might have simply represented a surplus pool. This conclusion is strengthened by the fact, that 2005 LL needles also had the lowest PNUE suggesting that the additional nitrogen was probably not allocated to photosynthesis limiting components.

In comparison to sun leaves, shade leaves typically have a greater per area chlorophyll contents that increases their light harvesting ability (Murchie and Horton 1997; Demmig-Adams 1998). Such differentiation in *Taxus* occurred not only for chlorophyll but also with respect to bulk carotenoids. The functional significance of the latter is, however, less clear because carotenoids take part in light harvesting but also protect the photosynthetic apparatus from excessive light (Havaux and Kloppstech 2001; Kirchgeβner et al. 2003). For sun/shade acclimation, the adjustment of carotenoid composition may be more relevant than the size of total carotenoid pool (Demmig-Adams 1998). Tissue concentrations of carotenoids and chlorophylls in LL were identical (and higher than in HL) between the two age classes although, at least for chlorophylls, the span between HL and LL was relatively smaller in 2005 needles. This suggests a lower acclimative ability of previous year tissues. Changes in chlorophyll content are at least to some extent dependent on the cell's ability to regulate the number of chloroplasts per cell but also on ultrastructural modifications such as formation of more

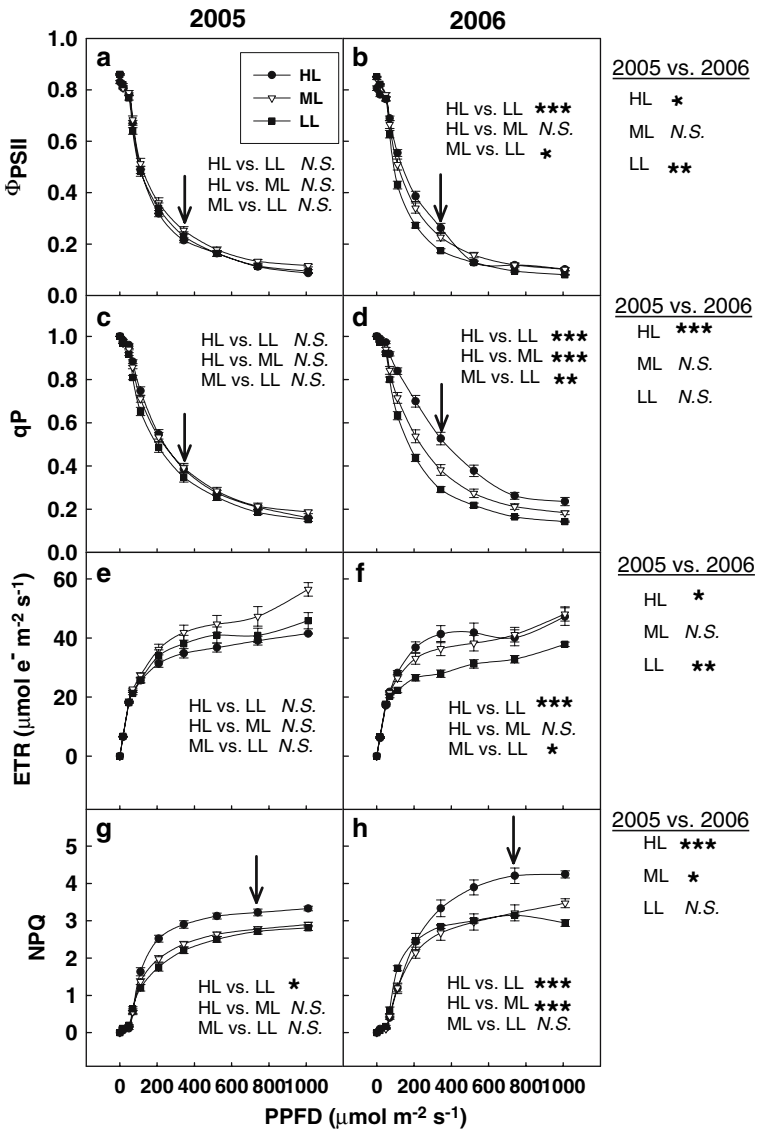

Fig. 7 Responses of chlorophyll *a* fluorescence traits to light (PPFD) in previous year (left column) and current year (right column) needles of *T. baccata* exposed to three irradiance regimes (HL—high irradiance, circles; ML—intermediate irradiance, triangles; LL—low irradiance, squares). Points indicate means, and whiskers are standard errors. Traits are: photochemical efficiency of PSII (Φ_{PSII}; **a**, **b**), photochemical quenching (qP; **c**, **d**), apparent electron transport rate (ETR; **e**, **f**) and non-photochemical quenching (NPQ; **g**, **h**). Statistical comparisons for Φ_{PSII} and qP were conducted only at PPFD = 343 μmol m^{-2} s^{-1}, for NPQ only at PPFD = 740 μmol m^{-2} s^{-1} (arrows) whereas for ETR maxima of fitted curves were compared (see Materials and methods). Within each panel, results of independent contrasts for pairwise comparisons between treatments for a given age class are shown. Contrasts between age classes within each treatment are given on right-hand side of the figure (n.s., non-significant; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$). ANOVA results are given in Table 1

grana and more thylakoids per granum in shade leaves (Lichtenthaler et al. 1981; Weston et al. 2000), both less likely in older needles. However, because 2006 needles became adjusted with respect to LMA, whereas 2005 needles had a relatively stable LMA, the differences in pigment contents per area among irradiance groups turned out to be more pronounced in 2005 needles.

The 2005 needles were formed under intermediate light conditions (closest to ML treatment). When these needles are used as a reference, no accumulation of pigments occurred in LL needles and a degradation of pigments occurred in HL. Evidence for ability of mature leaves to accumulate chlorophyll is ambiguous (but see Brooks et al. 1994; Tognetti et al. 1998) whereas chlorophyll degradation is a well-investigated (often senescence-related) process (Hendry et al. 1987; Thomas 1997). Degradation of chlorophyll may also be triggered by shading (Pons and de Jong-van Berkel 2004) which represents a counter-acclimative response. Assuming the occurrence of chlorophyll and carotenoid degradation in 2005 HL needles, this process was more effective in diversifying pigment contents per area between irradiance treatments than the more finely tuned plastic regulation of both structural and biochemical traits in 2006 needles (Fig. 6 a, c, e, g). Our results provide no evidence that mature *Taxus* needles could accumulate chlorophyll in response to decline in irradiance such as shown for *Abies amabilis* (Brooks et al. 1994). However, seasonal variation of pigments has been recorded in mature needles of conifers, including evidence of chlorophyll accumulation (Kirchge*ß*ner et al. 2003).

The most characteristic feature of sun leaves is their greater photosynthetic rate in comparison with shade leaves (Björkman 1981; Givnish 1988). Surprisingly in

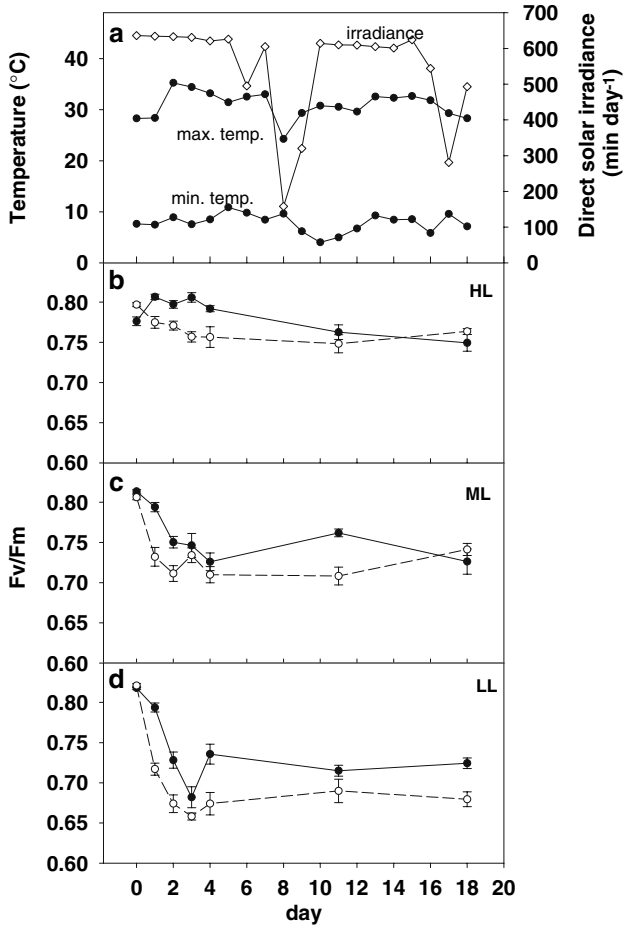

Fig. 8 Exposure of *Taxus baccata* plants to full irradiance. (**a**) Weather data showing daily minimal and maximal air temperature and duration of direct solar irradiance. (**b**–**d**) Dynamics of F_V/F_M in needles acclimated during the 2006 season to HL (**b**), ML (**c**) or LL (**d**). Closed symbols—2005 needles, open symbols—2006 needles

Table 2 Results of repeated measures ANOVA for the effect of growth irradiance, needle age and number of days after exposure to full light (day, the repeated factor) on F_v/F_M

Source	d.f.	*F*	*P*
Irradiance	2	9.59	0.000
Needle age	1	7.95	0.001
Irradiance × age	2	0.56	0.574
Error	29		
Day (rep. factor)	6	13.4	0.000
Day × irradiance	12	3.15	0.000
Day × age	6	1.94	0.077
Day × irradiance × age	12	0.47	0.932
Error	174		

T. baccata this difference did not develop to any significant degree (only in previous year needles did photosynthetic capacity appear slightly higher in HL than in LL). A similar finding was reported for *T. brevifolia* (Mitchell 1998) indicating a conservative homeostatic behavior within the genus rather than an experimental coincidence. This conservative pattern of photosynthetic responses is also in line with the typical ecological strategy of slow growing plants from unproductive habitats (Valladares et al. 2000; Thomas and Polwart 2003). As proposed by Björkman (1981), photosynthetic capacities are more limited by Rubisco than by light phase components, therefore, in the absence of N accumulation in HL needles in *Taxus*, no increase in photosynthetic potential over LL plants should be expected. Rates measured by us were on the low side perhaps suggesting also strong stomatal limitation or high internal resistance, especially relevant in the thick 2005 and 2006 HL needles (Warren and Adams 2004). Correspondingly, PNUE values for *Taxus* were among the lowest reported for seed plants (Poorter and Evans 1998; Reich 1998).

In contrast to photosynthetic rate, modifications of PSII photochemistry and electron transport properties in young needles of *Taxus* were consistent with the typical sun/shade diversification pattern of leaves (Tognetti et al. 1998; Bailey et al. 2004; Einhorn et al. 2004). Thus, sun leaves had a higher photochemical yield (at least at intermediate, non-photoinhibitory light range), maintained a larger fraction of open reaction centers under illumination, and had greater potential for electron transport and a greater capacity for inducible NPQ. Significant differences between treatments were induced, however, only in 2006 needles (except for the greater NPQ capacity in 2005 HL needles). This low photochemical plasticity of second year needles contrasts with the well studied strong seasonal adjustments of evergreen leaves (Adams and Demmig-Adams 1994) that regulate vulnerability of those needles to photoinhibition. Nevertheless, HL needles from both age classes appeared to be better photoprotected than needles from the other two treatments judging both from NPQ inducibility and behavior of F_V/F_M under prolonged exposure to high light. In addition, 2006 needles were always more photoinhibited then 2005 needles from the same irradiance treatment. The better tolerance to light stress of 2005 needles might be related to their greater ETR capacity (Grassi and Bagnaresi 2001; Robakowski et al. 2004) or carotene content (Havaux and Kloppstech 2001), along with other chemical or structural traits of the mesophyll and epidermis not analyzed here, such as anthocyanins (Smillie and Hetherington 1999), flavonoids (Havaux and Kloppstech 2001) or epidermal thickness (DeLucia et al. 1992).

Under non-photorespiratory conditions, the electron transport should be linearly related to CO_2 reduction rate with stoichiometry of 4 electrons for each CO_2 molecule (Tsuyama et al. 2003). This ratio is increased by photorespiration as well as by other potential electron sinks, such

as nitrogen reduction, cyclic flow around PSI, and the Mehler reaction (Polle 1996; Makino et al. 2002; Cornic and Fresneau 2002). In *Taxus*, deviations from this theoretical ratio were large, as photosynthesis (corrected for dark respiration—data not shown) accounted for only 15–20% of the estimated electron flow through PSII (Figs. 6a and 7e, f). Apart from the possible alternative electron flow pathways, we suspect that an additional reason for this discrepancy might have been the vertical stratification of chloroplast physiological characteristics across the leaf profile. Fluorescence signal has been shown to be derived mostly from the uppermost layer of the mesophyll where the chloroplasts are adapted to the highest light level, i.e., maintain a higher light-adapted Φ_{PSII} than chloroplasts from the deeper, more shaded leaf layers (Maxwell and Johnson 2000; Tsuyama et al. 2003). Integration of the electron flow for the whole leaf profile, based on fluorescence characteristics of the upper layer, thus must lead to an overestimation of this value, especially in thick leaves such as those of *Taxus*. This cautions against using fluorescence parameters as a proxy for photosynthesis (Maxwell and Johnson 2000) but does not preclude the use of apparent ETR as a sensitive indicator of leaf acclimation to light (Fig. 6f).

The general picture of acclimative ability of *Taxus* needles that emerges from the above comparisons shows that previous year needles, although destined to live for another 2–6 years before falling (Thomas and Polwart 2003) showed no acclimative ability in their size or mesophyll structure but were able to adjust their chlorophyll and carotene concentration on transfer to HL. Previous year needles were unable to modify their photochemical and electron transport characteristics except for NPQ (that changed less than in current year needles). In spite of this low plasticity, second year needles had a lower vulnerability to photoinhibition. Light environment experienced by older needles is not entirely unpredictable. Under typical circumstances they should be subject to a progressively increased self-shading. It would be interesting to know if an ontogenetic component of shade acclimation operates in this and other shade tolerant conifer species.

Acknowledgements We thank Mr. T. Szeszycki of Rokita Forest District for donating *Taxus* seedlings and Adam Mickiewicz University Botanical Garden for allowing the use of their growing space. The study was financed by Adam Mickiewicz University and August Cieszkowski Agricultural University Interdisciplinary Research Grant 512-00-056.

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