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Winter photoinhibition in needles of *Taxus baccata* seedlings acclimated to different light levels

P. ROBAKOWSKI[*,+] and T. WYKA[**]

University of Life Sciences in Poznań, Department of Forestry, ul. Wojska Polskiego 69,
60-625 Poznań, Poland[*]
Adam Mickiewicz University, Department of Biology, General Botany Laboratory, ul. Umultowska 69,
61-614 Poznań, Poland[**]

Abstract

Seasonal variability of maximum quantum yield of PSII photochemistry (F_v/F_m) was studied in needles of *Taxus baccata* seedlings acclimated to full light (HL, 100% solar irradiance), medium light (ML, 18% irradiance) or low light (LL, 5% irradiance). In HL plants, F_v/F_m was below 0.8 (*i.e.* state of photoinhibition) throughout the whole experimental period from November to May, with the greatest decline in January and February (when F_v/F_m value reached 0.37). In ML seedlings, significant declines of F_v/F_m occurred in January (with the lowest level at 0.666), whereas the decline in LL seedlings (down to 0.750) was not significant. Full recovery of F_v/F_m in HL seedlings was delayed until the end of May, in contrast to ML and LL seedlings. F_v/F_m was significantly correlated with daily mean (T_{mean}), maximal (T_{max}) and minimal (T_{min}) temperature and T_{min} was consistently the best predictor of F_v/F_m in HL and ML needles. Temperature averages obtained over 3 or 5 days prior to measurement were better predictors of F_v/F_m than 1- or 30-day averages. Thus our results indicate a strong light-dependent seasonal photoinhibition in needles of *T. baccata* as well as suggest a coupling of F_v/F_m to cumulative temperature from several preceding days. The dependence of sustained winter photoinhibition on light level to which the plants are acclimated was further demonstrated when plants from the three light environments were exposed to full daylight over single days in December, February and April and F_v/F_m was followed throughout the day to determine residual sensitivity of electron transport to ambient irradiance. In February, the treatment revealed a considerable midday increase in photoinhibition in ML plants, much less in HL (already down-regulated) and none in LL plants. This suggested a greater capacity for photosynthetic utilization of electrons in LL plants and a readiness for rapid induction of photoinhibition in ML plants. Further differences between plants acclimated to contrasting light regimes were revealed during springtime de-acclimation, when short term regeneration dynamics of F_v/F_m and the relaxation of nonphotochemical quenching (NPQ) indicated a stronger persistent thermal mechanism for energy dissipation in HL plants. The ability of *Taxus baccata* to sustain winter photoinhibition from autumn until late spring can be beneficial for protection against an excessive light occurring together with frosts but may also restrict photosynthetic carbon gain by this shade-tolerant species when growing in well illuminated sites.

Additional key words: acclimation to irradiance, chlorophyll *a* fluorescence, photoinhibition, photoprotection, *Taxus baccata*, winter hardening.

Introduction

Evergreen plants maintain their leaves throughout winter, thus extending the period of CO_2 uptake within a single season and reducing annual construction costs of photosynthetic organs (Givnish 2002). Such life strategy, however, is associated with the exposure of leaves to unfavorable conditions, such as mechanical stress from snow and ice, freezing temperatures, physiological drought, and excess irradiance (Adams *et al.* 2004).

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[+]Author for correspondence; fax: +48 61 848 77 34, e-mail: pierrot@up.poznan.pl

Abbreviations: DOY – days of year; F_0 – minimal fluorescence yield of the dark adapted sample, F_m – maximal fluorescence yield of dark adapted sample, F'_m – maximal fluorescence yield in light, F_v/F_m – maximum quantum yield of PSII photochemistry, NPQ – non-photochemical quenching of fluorescence, PPFD – photosynthetic photon flux density [μmol m^{-2} s^{-1}], T_{max} – daily maximal temperature [°C], T_{mean} – daily mean temperature [°C], T_{min} – daily minimal temperature [°C].

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In winter, when photosynthetic enzymes are inhibited by low temperature, CO_2 assimilation and assimilate utilization are reduced but light absorption still continues (Adams *et al*. 2004, Öquist and Huner 2003). Excessive energy absorbed by the leaves may damage the photosynthetic apparatus unless it is efficiently dissipated through photoprotective mechanisms which help to prevent permanent photoinhibitory damage (Adams *et al*. 2004, Niyogi 1999, Verhoeven *et al*. 1996) and to maintain photostasis (the balance between energy gain through photochemistry and its utilization through biochemical processes) during the winter (Öquist and Huner 2003).

In conifers, as in angiosperms, the dissipation of excessive energy is achieved by an increase in non-photochemical quenching of chlorophyll *a* fluorescence (NPQ) leading to a reduction in quantum efficiency of PSII (Adams and Demmig-Adams 1994, Verhoeven *et al*. 1996, Špunda *et al*. 1997). On the diurnal scale, this downregulation peaks at midday and relaxes in the evening (Ishida *et al*. 1999, Robakowski and Wyka 2004). In evergreen leaves, such as gymnosperms, the photoinhibitory quenching has been found to operate on a seasonal timescale, providing a sustained photoprotection over the winter period (Groom *et al*. 1991, Han *et al*. 2004, Demmig-Adams *et al*. 2006, Yamazaki *et al*. 2007, Porcar-Castell *et al*. 2008).

The induction and persistence of sustained winter photoinhibition is thought to result from the combined effect of light and low temperature (Öquist and Huner 2003). Many temperate evergreen woody species are highly shade-tolerant at least at young age (Niinemets and Valladares 2004). This association with shade may be advantageous if it allows them to avoid photoinhibition and maintain positive CO_2 uptake over longer periods (Jifon and Syversten 2003).

Under an evergreen canopy, shade conditions may persist also in winter. It is not clear to what extent the sustained winter photoinhibition occurs also in leaves of such shaded plants, although within the same canopy it is usually less pronounced in shaded leaves (Lundmark *et al*. 1998). The influence of low temperature may occur through a reduction in sink activity at various levels of carbon metabolism. Since temperature during winter in the temperate climatic zone is highly variable, it would be interesting to learn how close is the match between the extent of photoinhibition and the external temperature, and also which aspect of the temperature regime (mean, minimal or maximal temperatures) are the most closely related to F_v/F_m. A study by Nippert *et al*. (2004) found that in several Idaho conifers the temperature effect was species specific and it involved lags of several days to weeks. In their report, maximal winter temperature was the best predictor of F_v/F_m.

In this study, we investigated the occurrence of photoinhibition in needles of English yew (*Taxus baccata* L.) seedlings experimentally acclimated to three different light intensities and maintained in these respective microenvironments over winter. We asked: (*1*) whether photoinhibition occurs throughout winter in all experimental groups, (*2*) whether the extent of photoinhibition is related to ambient temperature (minimal, maximal or average) and solar irradiance prior to measurement, (*3*) whether shade acclimated leaves are more predisposed to photoinhibition upon exposure to full solar irradiance and (*4*) whether mechanisms of short-term down-regulation of PSII in high light- and shade-acclimated plants mirror those operating on a seasonal time scale?

Materials and methods

Plants: *T. baccata* L. is a highly shade-tolerant tree species (Ellenberg *et al*. 1992). Its seedlings survive in the heavy shade of mature yews and frequently grow under the dense crowns of *Tilia*, *Acer*, *Carpinus*, *Picea* and *Abies*, but also under more opened crowns of *Quercus* or *Pinus* or even in full sunlight (Iszkulo and Boratynski 2004, Thomas and Polwart 2003). *T. baccata* grows best in the oceanic climate with its high humidity and low-temperature amplitudes. In Europe, the ecological barriers limiting its distribution are low temperatures in the north, severe continental climate in eastern Poland, extended droughts in Turkey, and drought and high temperature in northern Africa.

T. baccata seedlings used in this study were obtained from seeds collected in Rokita Forest District (NW Poland, near Baltic Sea) where a natural population of *Taxus* grows mostly under the *Pinus* canopy. Plants were initially raised in a forest nursery in individual pots and maintained under a shading net transmitting around 27% of solar irradiance. During the winter, they were covered with conifer branches for frost protection. In March 2006, three-year-old seedlings were transferred to the Adam Mickiewicz University Botanical Garden in Poznań and potted into 7-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). Seedlings were watered up to the field capacity as needed.

The experimental design was described in Wyka *et al*. (2008). In brief, three light treatments were established: ambient irradiance (high light – HL, 100% of full light), moderate irradiance (mid light – ML, 17.8 ± 3.8% of full light), and low irradiance (low light – LL, 5.4 ± 1.6% of full light). The seedlings in ML and LL treatments were shaded using one or two layers of shading cloth, respectively. Spectral properties of the shading cloth are given in Wyka *et al*. (2007). On April 13, 2006, before bud break, 72 seedlings were randomly distributed among 9 plots, each assigned to one of the three treatments.

Plants remained under the respective light regimes throughout 2006 growing season and during the following winter and spring, when this study was performed.

Microclimate monitoring: Our experimental plots were situated 30 m from Adam Mickiewicz University Botanical Garden Weather Station, which supplied the meteorological parameters: daily mean, daily maximum and minimum temperatures, daily short-wave radiation and daily sunshine duration for the study period. Short-wave radiation (31–280 nm) intensity was measured with *CMP-3* thermopile pyranometer (*Kipp & Zonen*, Delft, The Netherlands). Daily sunshine duration was measured using the Campbell-Stokes burning heliograph (*Lambrecht GmbH*, Göttingen, Germany). The mean daily photosynthetic photon flux density (PPFD) (400–700 nm) was recorded at the University of Life Sciences Experimental Station in Rzecin, Poland (N 52° 45' 44", E 16° 18' 34") situated 70 km west of our experimental plots (*BF3H, Delta T*, Cambridge, UK). The PPFD data were kindly provided by dr. M. Urbaniak (University of Life Sciences in Poznan, Unit of Agrometeorology). Additionally, on days of hourly measurements of F_v/F_m the air temperature was measured *in situ* with a meteorological thermometer and PPFD with a quantum sensor (*Spectrum Technologies, Inc*., Plainfield, USA).

Chlorophyll *a* fluorescence: Maximum quantum yield of PSII photochemistry [$F_v/F_m = (F_m - F_0)/F_m$, where F_m is maximal fluorescence yield of dark adapted sample; F_0 – minimal fluorescence yield of the dark adapted sample] was measured on needles formed during 2006 season using the *Plant Efficiency Analyser* (*PEA, Hansatech*, King´s Lynn, UK) at weekly intervals from November 16, 2006 to May 24, 2007. The measurements were conducted *in situ* after a 60-min dark adaptation of needles using the factory-provided leaf clips, starting at 08:00. Needles were tightly arranged in a leaf clip and a pulse of red light was given at 3,200 μmol m^{-2} s^{-1} to induce F_m. F_0 was derived from the initial part of the fluorescence kinetics curve and F_v/F_m was calculated by the in-built software.

On selected days in December, February and April, three seedlings acclimated to each of the three light regimes were exposed to ambient light, and values of F_v/F_m were determined hourly following 20 min of dark adaptation. The measured values of F_v/F_m were normalized against the non-photoinhibited mean F_v/F_m obtained under optimal temperature conditions in August 2006. Maxwell and Johnson (2000) were followed for the nomenclature of the fluorescence parameters.

Recovery of PSII activity: In April 2007, needles were collected from HL and LL seedlings, wrapped in moist tissue paper, enclosed in Eppendorf tubes and transported to the laboratory. Recovery of F_v/F_m was studied from April 9 to 12 using the *Fluorescence Monitoring System 2* (*FMS 2, Hansatech*, King´s Lynn, UK) operating in an online mode. Prior to the measurements, needles were maintained in a humid atmosphere at 22°C in the dark for 1 h. Subsequently they were tightly arranged in a leaf clip. The fiberoptics encased in a light-tight chamber was inserted onto the leaf clip and the needles were exposed to a modulated low-intensity chamber measuring light (PPFD = 0.05 μmol m^{-2} s^{-1}). After reading F_0, a saturating 0.7-s light pulse [PPFD = 15.3 mmol(quantum) m^{-2} s^{-1}] was delivered to induce F_m. F_v/F_m was calculated by the instrument's software. Next, the needles were exposed to a 10-min. pulse of strong light (PPFD = 1340 μmol m^{-2} s^{-1} from the *FMS 2* halogen light source) and the dark recovery of F_v/F_m and the relaxation of NPQ were followed over a 19-min period by applying 0.7-s pulses of saturating light at selected time intervals. NPQ was calculated according to the formula: $NPQ = (F_m - F'_m)/F'_m$, where F'_m – maximal fluorescence yield in light at each time step) (Maxwell and Johnson 2000). During the fluorescence measurements conducted in the laboratory, air temperature was 22 to 23°C.

Data analyses: Mean values of F_v/F_m were compared between the light treatments within the seasonal or daily time scale using *MANOVA* (treating values for individual days as separate variables) followed by *Tukey*'s tests at *P*<0.05.

To determine the relationship between F_v/F_m and air temperature, the equation $F_v/F_m = [b+a\times\log(T+10)]^{1/3}$ (Nippert *et al.* 2004) was fitted to the data. The parameter "*a*" was estimated using the Levenberg-Marquardt (Stanisz 2007) method. The minimum of this function was forced to be the minimal value of F_v/F_m measured in seedlings from a particular treatment group. Coefficients of determination (r^2) were subsequently tested. The fit of the function was evaluated for moving averages of three measures of temperature (daily mean, minimum and maximum) calculated for 1, 3, 5 or 30 days preceding the fluorescence measurement.

The differences in dynamics of recovery of F_v/F_m and the NPQ relaxation between HL and LL needles were tested using *MANOVA* (*P*<0.05) only for the 120-s data followed by analysis of contrasts between the treatments for each dependent variable. All statistical analyses were conducted with *Statistica 8.0* (*Statsoft, Inc*., Tulsa, Oklahoma, USA).

Results

Weather conditions: In winter 2006/07 negative daily mean temperatures occurred first at the end of December, then in second half of January and at the end of February (Fig. 1*A*). Negative temperature minima first occurred in the beginning of December and then at the end of December, on 10 days in January, 16 days in February, 4 days in March and 2 days in April. The lowest minimum temperature for the whole experimental period

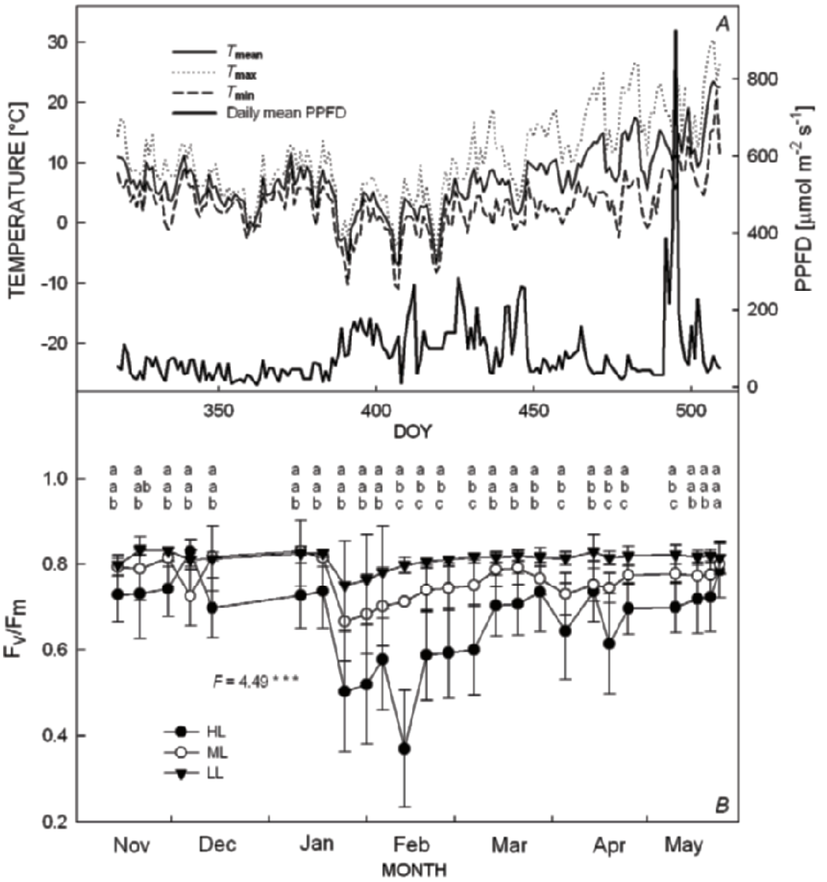

Fig. 1. *A*: Daily mean (T_{mean}), minimal (T_{min}) and maximal (T_{max}) temperature and daily mean PPFD at 2 m above ground level from November 16, 2006 (DOY – 318) to May 24, 2007 (DOY – 509). *B*: Time course of F_v/F_m (mean ± SD) from 16 November 2006 to 24 May 2007 in needles of *Taxus baccata* seedlings acclimated to high light (100% of full irradiance), mid light (18%) or low light (5%). Asterisks indicate statistically significant differences between the light treatments (***$P<0.001$) according to *MANOVA* ($P<0.05$). The same letters indicate that there are not statistically significant differences between the light treatments in *post-hoc Tukey*'s test ($P<0.05$; $n = 5$). DOY – days of year.

Table 1. Determination coefficients (r^2) for regression of the mean F_v/F_m (n = 5) against moving averages of meteorological parameters calculated over 1, 3, 5 or 30-day time windows. Regression model was $F_v/F_m = [b + a \times \log(T+10)]^{1/3}$ and the minimum of the function was forced to be the minimal value of F_v/F_m measured during the season in seedlings from a particular light environment (HL – high, ML – medium and LL – low light intensity). ** $P<0.01$, *** $P<0.001$, n = 24, n – the number of pairs of values. T_{mean}, T_{max}, and T_{min} – daily mean, maximal, and minimal temperature, respectively.

Meteorological parameter	HL 1 d	3 d	5 d	30 d	ML 1 d	3 d	5 d	30 d	LL 1 d	3 d	5 d	30 d
T_{mean} [ºC]	0.44***	0.53***	0.57***	0.41***	0.39***	0.47***	0.43***	0.30***	0.50***	0.59***	0.52***	0.30***
T_{min} [ºC]	0.50***	0.61***	0.74***	0.43***	0.49***	0.52***	0.49***	0.32***	0.54***	0.58***	0.51***	0.18***
T_{max} [ºC]	0.42***	0.49***	0.46***	0.40***	0.37***	0.43***	0.40***	0.29***	0.51***	0.60***	0.54***	0.35***
Daily mean PPFD [μmol m^{-2} s^{-1}]	n.s.	n.s.	n.s.	0.33**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Short-wave radiation [W m^{-2}]	0.39***	0.51***	0.51***	0.49***	n.s.	0.33***	0.32***	0.30***	0.34***	0.54***	0.54***	0.50***
Daily sunshine duration [h]	n.s.	0.58***	0.56***	0.47***	n.s.	0.28***	0.23***	0.27***	n.s.	0.49***	0.45***	0.47***

(–10.9°C) occurred on February 11, 2007 (DOY 42) when the mean daily temperature fell to –7.1°C. The daily mean temperature was positively correlated with the daily mean intensity of short-wave radiation ($r^2 = 0.71$, $P<0.001$) and with daily sunshine duration ($r^2 = 0.65$, $P<0.001$) but not with daily mean PPFD ($r^2 = 0.002$). Generally, during winter the weather was cloudy except for several days in February.

Seasonal course of F_v/F_m: From the beginning of the study, F_v/F_m was generally lowest in needles of HL seedlings (Fig. 1*B*). It decreased especially sharply with falling temperature in mid-January and February and started to recover from around the beginning of March. The lowest level of F_v/F_m (0.37) in HL seedlings occurred in February (DOY 44) when the temperature values on the previous day were $T_{mean} = 1.0$°C, $T_{max} = 3.6$°C, and $T_{min} = –3.1$°C. Minima of F_v/F_m in both ML and LL seedlings (0.666 and 0.750, respectively) were noted on January 25 (DOY 25) when the temperature values on previous day were $T_{mean} = –2.8$°C, $T_{max} = –2.0$°C, and $T_{min} = –3.9$°C. Full recovery of F_v/F_m to the summer level in ML and LL leaves took place in March while in HL leaves full recovery was observed as late as in the end of May.

The Nippert *et al.*'s (2004) equation adequately reflected the relationship between the temperature and F_v/F_m (Table 1, Fig. 2). Within each light environment, F_v/F_m was positively correlated with air temperature, but there was no obvious effect of seedlings' light environment on the strength of this relationship, other than the occurrence of the strongest determination coefficient ($r^2 = 0.74$) in HL seedlings (Table 1). The strength and statistical significance of this correlation depended, however, on the specific temperature parameter (daily mean, maximum or minimum temperature) and the width of time window prior to measurement used to calculate the temperature moving average (Table 1). Of the three temperature measures studied, daily minimum temperatures were the best predictors of F_v/F_m in HL and ML plants but not in LL plants. The closest fit in all light treatments was achieved when temperatures were averaged over 3 or 5 day time-windows. The F_v/F_m ratio was weakly positively correlated with daily sunshine duration and short-wave radiation, but not with PPFD, except for the 30-day moving average in HL plants (Table 1).

Diurnal variation of F_v/F_m in full light: Compared with the values determined under optimal temperature conditions in August 2006, F_v/F_m in needles of HL seedlings was significantly lower throughout the day in mid-February, but not in mid-December or at the end of April (Fig. 3*B*,*C*,*D*). In mid-February, weaker, although also significant lowering of F_v/F_m was detected in ML needles, but not in LL ones. On February measurement date F_v/F_m in HL and ML needles exhibited a clear diurnal course with a morning peak followed by a midday

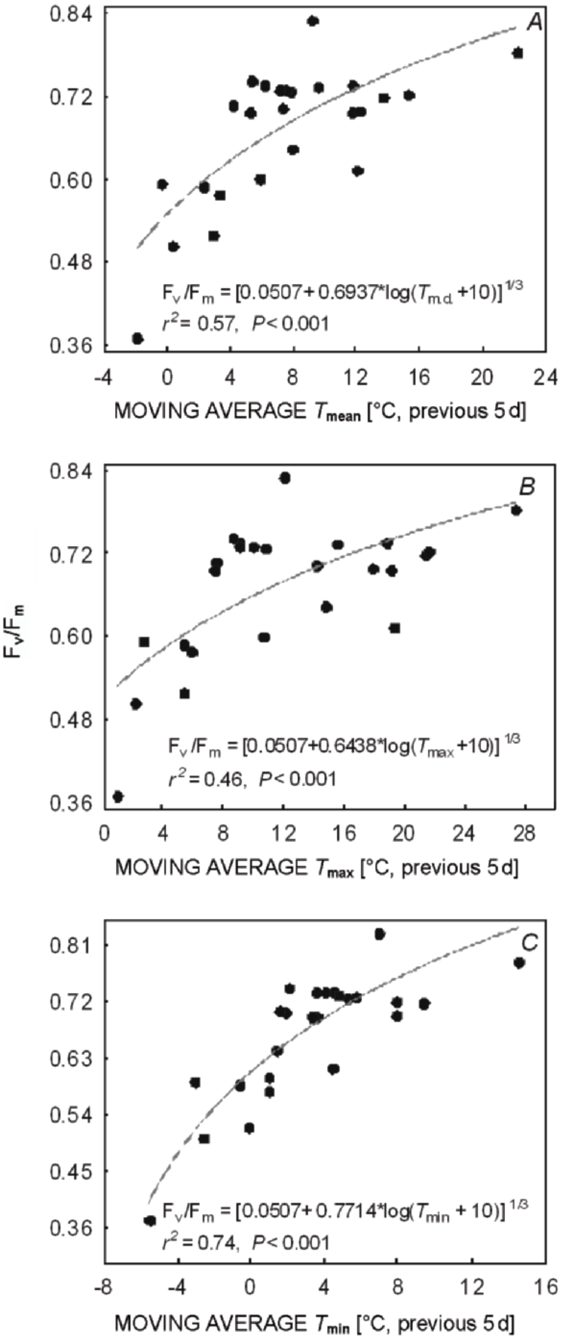

Fig. 2. Non-linear regression between daily mean (T_{mean}) (*A*), daily maximum (T_{max}) (*B*), and daily minimum (T_{min}) (*C*) temperatures averaged over the latest 5 days (moving averages) and the mean values of maximum quantum yield of PSII (F_v/F_m) ($n = 5$) determined in needles of *Taxus baccata* seedlings acclimated to high light (HL). Regression model was $F_v/F_m = [b + a \times \log(T + 10)]^{1/3}$ and the minimum of the function was forced to be the minimal value of F_v/F_m measured during the season in seedlings from a particular light environment. Regression equations, coefficients of determination (r^2) with associated probabilities (P) obtained from the analysis of variance for regression are given ($n = 24$, n – the pairs of values).

decline. In contrast, in mid-December, no diurnal variability was apparent in any group whereas in mid-April, after a morning peak, there was a notable variability of F_v/F_m in all treatment groups, especially in ML needles (Fig. 3*D*).

The difference in courses of F_v/F_m between December and February measurement days, when mean temperatures were similar, could be attributed to the higher PPFD levels in mid-February (although cloud cover was complete on both days) and also to the fact that prior to the measurement in February there occurred several days with mean temperatures below freezing (Figs. 1*A*, 3*A*).

Recovery of PSII after high-light exposure: In April 2007, winter photoinhibition expressed as a decrease in F_v/F_m *in situ* was still significant in HL and ML seedlings, but not in LL seedlings (Fig. 1*B*). At that time the sensitivity of the photosynthetic apparatus in HL and LL seedlings to high light stress was tested in the laboratory. F_v/F_m measured in dark-adapted needles before the application of light stress was lower in HL compared to LL seedlings (Fig. 4*A*). Two phases of recovery of the PSII function were apparent: a fast recovery phase with a quick rise in F_v/F_m and relaxation of NPQ taking place within 20 s of restoring darkness, and a slow recovery phase lasting up to 20 min (Fig. 4*A*). Recovery of F_v/F_m was faster in LL needles (achieving 80% of the original value during the fast phase), than in HL needles (53% during the fast phase). NPQ relaxation was faster in LL needles compared to HL ones.

Discussion

T. baccata maintains its photosynthetic apparatus during winter, similarly to many other evergreen conifers (Adams *et al.* 2004, Han *et al.* 2004, Öquist and Hunert 2003). To prevent an energetic imbalance and associated cellular damage that could result from the reduced activity of assimilate sinks, plants activate mechanisms leading to reduced photosystem activity (Adams *et al.* 2004, Groom *et al.* 1991). In our experimental plants a sustained winter decrease of F_v/F_m (below the 0.8 level) was detected, in line with this tendency. This decrease occurred in HL seedlings throughout the whole experimental period from November to May, in ML seedlings mostly from January till April and in LL seedlings only in mid-winter. The steepest declines took place in January, with the onset of sub-zero temperatures. In previous studies, *Cryptomeria japonica, Abies alba*, *Abies mariesii*, *Picea abies*, *Pinus mugo* and *Pinus sylvestris* showed very similar seasonal courses of F_v/F_m with the strongest photoinhibition leaves occurring in coldest months (Han *et al.* 2003, 2004, Robakowski 2005, Yamazaki *et al.* 2007, Porcar-Castell 2008). This seasonal variability suggests a key role of temperature in inducing the photoinhibition although there is little information on whether this effect is only indirect (*i.e.* through a negative feedback from inactive temperature-sensitive energy sinks), or also direct, *e.g.* through low-temperature dependent gene expression (Öquist and Hunert 2003) or membrane modifications. The role of temperature in the down-regulation of F_v/F_m is illustrated by calculated correlations with moving temperature averages, and especially by the fact that the strongest determinant of F_v/F_m was minimum temperature (Table 1). Moreover, the effect of temperature on F_v/F_m appears to be cumulative over at least several days, as shown by the improved determination coefficient when 3- or 5-day moving averages were used instead of just previous day temperatures. A similar conclusion was reached by Nippert *et al.* (2004) who identified time lags of several days to two months between the occurrence of low temperature and response of F_v/F_m in conifers, with species-specific differences. Temperature effects lasting several days were reported also for *Picea rubens* (Lawson *et al.* 2000). In *T. baccata* the prolonged effect of temperature is further shown by the fact that F_v/F_m was lower in the spring than in the autumn, even thought springtime temperatures were higher.

Our results also clearly show an interaction of temperature with the light environment, as indicated by the stronger and longer-lasting declines of F_v/F_m in HL plants compared to ML and LL plants. This pattern was only poorly reflected by differences in coefficients of determination in Table 1 probably because of the greater variability of the HL data leading to decreased determination coefficients. Nevertheless, plants exposed to the lowest light level experienced a significant down-regulation of PSII only during the coldest period of winter. Thus, in the shade, *Taxus* seedlings retained nearly full photochemical efficiency for most of the winter. The link between F_v/F_m and the photosynthetic capacity based on gas exchange measurements is not tight, but generally, a decrease in F_v/F_m is associated with a lowering of photosynthetic rate (Genty *et al.* 1989, Maxwell and Johnson 2000, Ögren 1988), especially in overwintering evergreens (Demmig-Adams *et al.* 2006). Therefore, if other components of the photosynthetic apparatus also remain in the state of readiness in shaded *Taxus* seedlings, this low degree of sustained photoinhibition in winter might allow some CO_2 uptake during periods of warmer weather thus counterbalancing the costs of occupying a shady site. This conclusion is strengthened by the fact that in HL plants full recovery of F_v/F_m did not take place until May, suggesting an ecological disadvantage associated with growth in full light. The retention of lower F_v/F_m into spring months in HL plants might reflect well the need for protection of plants from late frosts that are usually more severe in exposed situations. Alternatively, the lowering of F_v/F_m followed by a slow recovery could have been caused by

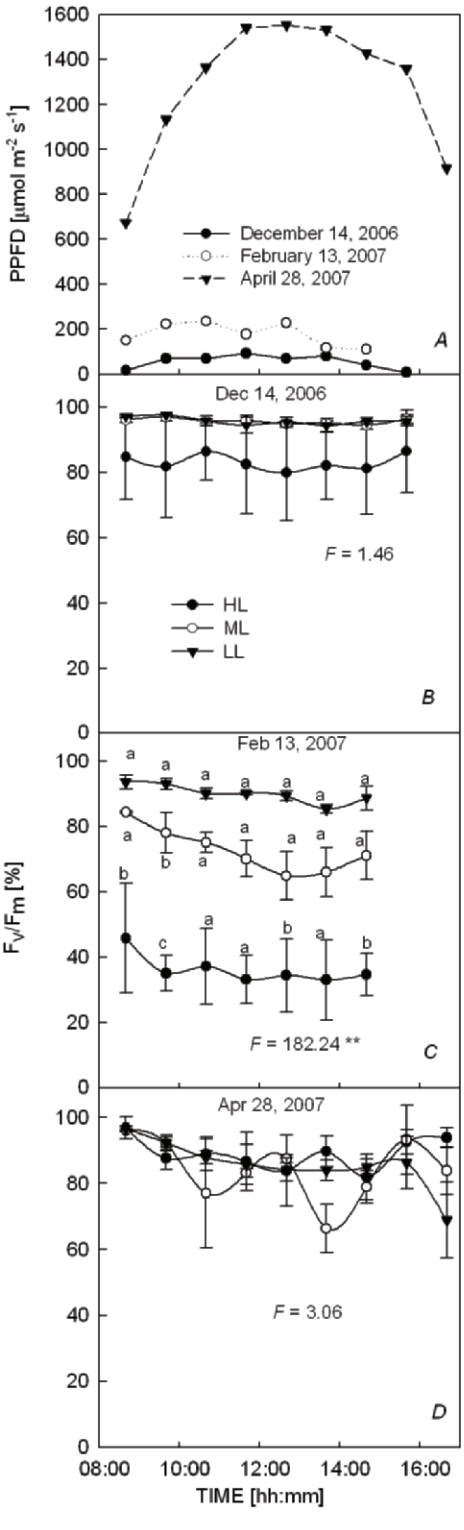

Fig. 3. Daily courses of PPFD (*A*) and F_v/F_m in needles of *Taxus baccata* seedlings acclimated to high light (HL, 100% of full irradiance), mid light (ML, 18%) or low light (LL, 5%) determined on three occasions: in December (*B*) in February (*C*) and in April (*D*). Measured values of F_v/F_m were standardized against mean F_v/F_m values obtained under optimal temperature conditions in August (HL – 0.807±0.031, ML – 0.844±0.012, LL – 0.852±0.006) and mean relative values (%) ± SD (n = 3) are plotted. Mean photosynthetic photon flux densities (PPFD) and temperature intervals on each day were: (*B*) 55 ± 29 µmol m^{-2} s^{-1}, 7.8 – 9.2°C; (*C*) 178 ± 50 µmol m^{-2} s^{-1}, 4.5 – 7.5°C; (*D*) 1210 ± 350 µmol m^{-2} s^{-1}, 12 – 24°C. *MANOVA* followed by *Tukey*'s test was applied to each day's data (** P<0.01). In *B* data points sharing the same letter within each curve are not significantly different.

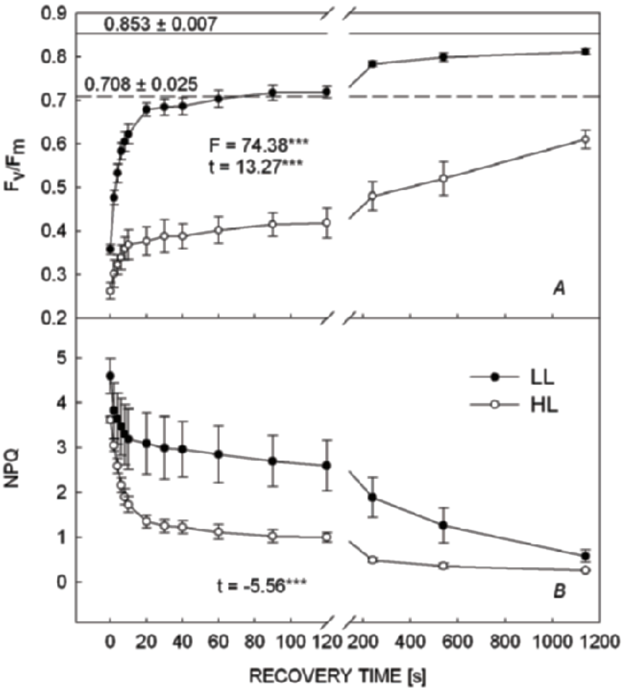

Fig. 4. Recovery of F_v/F_m (*A*), and relaxation of non-photochemical quenching (NPQ) (*B*) in needles of *Taxus baccata* L. seedlings acclimated to high light (HL, 100% of full irradiance) or low light (LL, 5%) after a 10-min exposure to halogen light at 1340 µmol m^{-2} s^{-1}. PSII quantum yield recovery was measured in 15 steps at 0, 2, 4, 6, 8, 10, 20, 30, 40, 60, 90, 120, 240, 540, and 1140 s after switching off the halogen lamp. The recovery test was conducted from April 9 to 12, 2007. Horizontal lines in *A* show the mean values (± SD) of F_v/F_m (n = 5) measured in dark-adapted needles prior to the light exposure (LL – continuous line, HL – dashed line). The *F* – value accompanied by asterisks (*A*) indicates statistically significant differences between treatments according to *MANOVA* (P<0.05) after 120 s of recovery. *MANOVA* was followed by the analysis of contrasts between the treatments for each dependent variable.

photooxidative damage to molecular components of PSII antennae or reaction centers, especially of the D1 protein, (Yokthongwattana and Melis 2006) as well as degradation of thylakoid membranes (Havaux and Niyogi 1999). Contrasting views of photoinhibitory downregulation of PSII are often expressed, ranging from entirely adaptive interpretations (Demmig-Adams *et al.* 2006) to ones emphasizing photodamage (Yokthongwattana and Melis 2006). As suggested by Demmig-Adams *et al.* (2006), this distinction is to some extent a matter of semantics, because oxidative damage may well be incorporated into a wider plant-protective strategy. Ultimately, a cost and benefit analysis in an ecological context may be required before the value of photoinhibition to plants is fully appreciated. Additional mechanisms of protection from excess light during winter may operate in *Taxus* needles. A reddish needle coloration was observed in January, February and March and was more pronounced in HL than in ML and LL seedlings suggesting an accumulation of rhodoxanthin, a xanthophyll pigment involved in photoprotection in *Cryptomeria* (Han *et al.* 2004) and known to occur in *Taxus* (Parmar *et al.* 1999). Likewise, increased winter concentrations of antioxidant compounds were detected in needles of *Taxus* × *media* (Verhoeven *et al.* 2005). Transient degradation of chlorophyll has also been documented in overwintering evergreen leaves (Öquist and Huner 2003, Han *et al.* 2004, Miyazawa and Kikuzawa 2005). Overall, it appears that *Taxus* needles were adequately protected from winter-related damage, although we cannot exclude that a more severe combination of frost and irradiance might have induced a deeper and irreversible photoinhibition, leading to their death as documented *e.g.* in exposed *Abies mariesii* needles from treeline in Japanese Alps (Yamazaki *et al.* 2007).

An interesting insight into the consequences of sustained photoinhibition is provided by the diurnal courses of F_v/F_m determined under ambient light and temperature, especially in mid-February (Fig. 3*B*) when there was a large diversification between treatment groups with regard to the degree of photoinhibition. On that relatively mild and cloudy day, LL plants, when exposed to ambient light, exhibited no photoinhibition and ML plants showed photoinhibition that was almost entirely reversible. Since even the most light-sensitive LL plants showed no drop in F_v/F_m, flexible changes of F_v/F_m in ML plants could be considered as regulatory and photoprotective. A moderate amount of shade in winter may therefore be conducive to retention of both photochemical capacity and fast-relaxing photoprotective mechanisms that are both lacking in overwintering HL plants. Moreover, the decline of F_v/F_m appeared in excess of that which is required for actual photoprotection (as judged by the performance of LL plants) unless it was required by a more extensive and prolonged shut-down of other components of the photosynthetic pathway.

Further differences in mechanisms of photoinhibition between HL and LL plants could be observed at the end of the study period when winter photoinhibition had largely, but not entirely, relaxed. Although the daily course of F_v/F_m on a sunny day in April (Fig. 3*C*) suggested similar sensitivity to light among treatments (*see* also Gamper *et al.* 2000), the study of recovery dynamics over 19 min following application of high-light stress (Fig. 4) revealed a profound differentiation between HL and LL plants. Recovery responses in LL needles were much more dynamic in agreement with a greater state of readiness of the photosynthetic apparatus suggested earlier. Based on these differences in relaxation of fluorescence quenching, we suggest a relatively greater involvement of the fast-relaxing mechanism of NPQ in LL needles, and a greater role of the slow relaxing mechanisms in HL needles (Müller *et al.* 2001, Demmig-Adams *et al.* 2006). Similarly, a greater engagement of thermal dissipation mechanisms was responsible for the slower recovery of F_v/F_m after winter in sun needles compared to shade ones of *Pinus sylvestris* (Porcar-Castell *et al.* 2008).

The adaptability of *T. baccata* to conditions of both deep-shade and well-illuminated open sites can be summarized as differences in the seasonal plasticity index of F_v/F_m measured as PI = [(max – min)/max] (Valladares *et al.* 2000), where "max" is the optimal value of F_v/F_m determined in August and "min" is the minimum F_v/F_m measured in winter. PI differed between the light environments and was 0.55 in HL, 0.225 in ML, and 0.128 in LL. Calculated for the species across light environments in winter (*i.e.* based on LL and HL seedlings), PI was 0.507. Although these values refer to just a single trait, they illustrate a remarkable capacity for seasonal (*i.e.* temperature-dependent, but also irradiance-dependent) down-regulation of PSII in *Taxus*. This contrasts with the much lower declines of F_v/F_m in response to change from LL to HL conditions during summer, recorded previously in *T. baccata* (Wyka *et al.* 2008) or in another shade-tolerant conifer *Abies alba* and a moderately shade-tolerant *Picea abies* (Wyka *et al.* 2007). The strength of temperature-dependent winter photoinhibition in *Taxus* and other conifers suggests that photooxidative stress associated with winter conditions might have played a role in determining the evolution and current ecological and geographic distribution of these plants. It may also constitute a factor limiting photosynthetic productivity of some gymnosperms in well-illuminated sites (Niinemets and Valladares 2004, Nippert *et al.* 2004).

References

Adams, W.W., III, Zarter, C.R., Ebbert, V., Demmig-Adams, B.: Photoprotective strategies of overwintering evergreens. – BioScience **54**: 41-49, 2004.

Adams, W.W., III, Demmig-Adams, B.: Carotenoid composition and down regulation of photosystem II in 3 conifer species during the winter. – Physiol. Plant. **92**: 451-458, 1994.

Adams, W.W., III, Zarter, C.R., Mueh, K.E., Amiard, V., Demmig-Adams, B.: Energy dissipation and photoinhibition. – In: Demmig Adams B., Adams, W.W., III, Mattoo, A.K. (ed.): Photoprotection, Photoinhibition, Gene Regulation and Environment. Pp. 49-64. Springer, Dodrecht 2006.

Demmig-Adams, B., Ebbert, V., Zarter, C.R., Adams, W.W., III: Characteristics and species-dependent employment of flexible versus sustained thermal dissipation and photoinhibition. – In: Demmig-Adams, B., Adams, W.W., III, Mattoo, A.K. (ed.): Photoprotection, Photoinhibition, Gene Regulation and Environment. Pp. 39-48. Springer, Dodrecht 2006.

Ellenberg, H., Weber, H.E., Düll, R., Wirth, V., Werner, W.,Paulißen, D.: Zeigerwerte von Pflanzen in Mitteleuropa. – Scripta Geobot. **18**: 1-258, 1992. [In German.]

Gamper, R., Mayr, S., Bauer, H.: Similar susceptibility to excess irradiance in sun and shade acclimated saplings of Norway spruce [*Picea abies* (L.) Karst.] and stone pine (*Pinus cembra* L.). – Photosynthetica **38**: 373-378, 2000.

Genty, B., Briantais, J-M., Baker, N.R.: The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. – Biochim. Biophys. Acta. **990**: 87-92, 1989.

Givnish, T.J.: Adaptive significance of evergreen vs. deciduous leaves: Solving the triple paradox. – Silva Fennica **36**: 703-743, 2002.

Groom, Q.J., Baker, N.R., Long, S.P.: Photoinhibition of holly (*Ilex aquifolium*) in the field during the winter. – Physiol. Plant. **83**: 585-590, 1991.

Han, Q., Shinohara, K., Kakubari, Y., Mukai, Y.: Photoprotective role of rhodoxanthin during cold acclimation in *Cryptomeria japonica*. – Plant, Cell and Environ. **26:** 715-723, 2003.

Han, Q.M., Katahata, S., Kakubari, Y., Mukai, Y.: Seasonal changes in the xanthophyll cycle and antioxidants in sun-exposed and shaded parts of the crown of *Cryptomeria japonica* in relation to rhodoxanthin accumulation during cold acclimation. – Tree Physiol. **24**: 609-616, 2004.

Havaux, M., Niyogi K.K.: The violaxanthin cycle protects plants from photooxidative damage by more than one mechanism. – Proc. Natl. Acad. Sci. USA **96**: 8762-8767, 1999.

Ishida, A., Nakano,T., Matsumoto, Y., Sakoda, M., Ang, H.L.: Diurnal changes in leaf gas exchange and chlorophyll fluorescence in tropical tree species with contrasting light requirements. – Ecol. Res. 14: 77-88, 1999.

Iszkulo, G., Boratynski, A.: Interaction between canopy tree species and European yew *Taxus baccata* (*Taxaceae*). – Pol. J. Ecol. **52**: 523-531, 2004.

Jifon, J.L., Syversten, J.P.: Moderate shade can increase net gas exchange and reduce photoinhibition in citrus leaves. – Tree Physiol. **23**: 719-719, 2003.

Lawson, S.T., Perkins, T.D., Adams, G.T.: Winter-time patterns of chlorophyll fluorescence in red spruce (*Picea rubens* Sarg.). – J. Sustainable Forest **10**: 149-153, 2000.

Lundmark, T., Bergh, J., Strand, M., Koppel, A.: Seasonal variation of maximum photochemical efficiency in boreal Norway spruce stands. – Trees **13**: 63-67, 1998.

Maxwell, K., Johnson, G.N.: Chlorophyll fluorescence - a practical guide. – J. Exp. Bot. **51**: 659-668, 2000.

Miyazawa, Y. Kikuzawa K.: Winter photosynthesis by saplings of evergreen broad-leaved trees in a deciduous temperate forest. – New Phytol. **165**: 857-866, 2005.

Müller, P., Li, X.-P., Niyogi, K.K.: Non-photochemical quenching. A response to excess light energy. – Plant Physiol. **125**: 1558-1566, 2001.

Niinemets, U., Valladares, F.: Photosynthetic acclimation to simultaneous and interacting environmental stresses along natural light gradients: Optimality and constraints. – Plant Biol. 6: 254-268, 2004.

Nippert, J.B., Duursma, R.A., Marshall, J.D.: Seasonal variation in photosynthetic capacity of montane conifers. – Functional Ecol. **18**: 876-886, 2004.

Niyogi, K.K.: Photoprotection revisited: Genetic and molecular approaches. – Annu. Rev. Plant Physiol. Plant Mol. Biol. **50**: 333-359, 1999.

Ögren, E.: Photoinhibition of photosynthesis in willow leaves under field conditions. – Planta **175**: 229-236, 1988.

Öquist, G., Huner, N.P.A.: Photosynthesis of overwintering evergreen plants. – Annu. Rev. Plant Biol. **54**: 329-355, 2003.

Parmar, V.S., Jha, A., Bisht, K.S., Taneja, P., Singh, S.K., Kumar, A., Poonam, Jain R, Olsen, C.E.: Constituents of yew trees. – Phytochem. **50**: 1267-1304, 1999.

Porcar-Castell, A., Juurola, E., Ensminger, I., Berninger, F., Hari, P., Nikinmaa, E.: Seasonal acclimation of photosystem II in *Pinus sylvestris*. II. Using the rate constants of sustained thermal enegy dissipation and photochemistry to study the effect of light environment. – Tree Physiol. **28:** 1483-1491, 2008.

Robakowski, P.: Susceptibility to low-temperature photoinhibition in three conifers differing in successional status. – Tree Physiol. **25**: 1151-1160, 2005.

Robakowski, P., Wyka, T.: Down-regulation of PSII in needles of silver fir (*Abies alba* Mill.) seedlings growing under the canopy of European larch and Norway spruce. – Zeszyty Postepow Nauk Agronomicznych **496**: 421-431, 2004.

Stanisz, A.: [Course of statistics with the application of Statistica PL using examples from medicine. Linear and non-linear models]. – Statsoft Polska **2**: 178-184, 2007. [In Polish.]

Špunda, V., Kalina, J., Marek, M.V., Nauš, J.: Regulation of photochemical efficiency of photosystem 2 in Norway spruce at the beginning of winter and in the following spring. – Photosynthetica **33**: 91-102, 1997.

Thomas, A.P., Polwart, A.: *Taxus baccata* L. – J. Ecol. **91**: 489-524, 2003.

Verhoeven, A.S., Adams W. W., III, Demming-Adams, B.: Close relationship between the state of xanthophyll cycle pigments and photosystem II efficiency during recovery from winter stress. – Physiol. Plant. **96**: 567-576, 1996.

Verhoeven, A.S., Swanberg, A., Thao, M., Whiteman, J.: Seasonal changes in leaf antioxidant systems and xanhophyll cycle characteristic in *Taxus*×*media* growing in sun and shade environments. – Physiol. Plant. **123**: 428-434, 2005.

Valladares, F., Martinez-Ferri E., Balaguer, L., Perez-Corona, E., Manrique, E.: Low leaf-level response to light and nutrients in Mediterranean evergreen oaks: a conservative resource-use strategy? – New Phytol. **148**: 79-91, 2000.

Wyka, T., Robakowski, P., Żytkowiak, R.: Leaf acclimation to contrasting irradiance in juvenile trees differing in shade tolerance. – Tree Physiol. **27**: 1293-1306, 2007.

Wyka, T., Robakowski, P., Żytkowiak, R.: Leaf age as a factor in anatomical and physiological acclimative responses of *Taxus baccata* L. needles to contrasting irradiance environments. – Photosynth. Res. **95**: 87-99, 2008.

Yamazaki, J.-Y. Tsuchiya, S., Nagano, S., Maruta, E. Photoprotective mechanisms against winter stresses in the needles of *Abies mariesii* grown at the tree line on Mt. Norikura in Central Japan. – Photosynthetica **45**: 547-554, 2007.

Yokthongwattana, K., Melis, A.: Photoinhibition and recovery in oxygenic photosynthesis: Mechanism of a photosystem II damage and repair cycle. – In: .Govindjee (ed.): Photoprotection, Photoinhibition, Gene Regulation, and Environment, Advances in Photosynthesis and Respiration. Vol. 21, pp. 175–191. Springer, Dordrecht 2006.