

Seasonal acclimation of photosystem II in *Pinus sylvestris*. II. Using the rate constants of sustained thermal energy dissipation and photochemistry to study the effect of the light environment

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Summary Photosynthesis in evergreen conifers is characterized by down-regulation in autumn and rapid up-regulation in spring. This seasonal pattern is largely driven by temperature, but the light environment also plays a role. In overwintering Scots pine (*Pinus sylvestris* L.) trees, PSII is less down-regulated and recovers faster from winter stress in shaded needles than in needles exposed to full sunlight. Because the effect of light on the seasonal acclimation of PSII has not been quantitatively studied under field conditions, we used the rate constants for sustained thermal energy dissipation and photochemistry to investigate the dynamics and kinetics of the seasonal acclimation of PSII in needles exposed to different light environments. We monitored chlorophyll fluorescence and needle pigment concentration during the winter and spring in Scots pine seedlings growing in the field in different shading treatments, and within the crowns of mature trees. The results indicated that differences in acclimation of PSII in overwintering Scots pine among needles exposed to different light environments can be chiefly attributed to sustained thermal dissipation. We also present field evidence that zeaxanthin-facilitated thermal dissipation and aggregation of thylakoid membrane proteins are key mechanisms in the regulation of sustained thermal dissipation in Scots pine trees in the field.

Keywords: chlorophyll fluorescence, Scots pine, spring recovery, zeaxanthin.

Introduction

Excitation pressure in photosystem II (PSII) increases when the rate of energy absorbed by the photosystem exceeds the rate of energy used by the dark reactions of photosynthesis. High excitation pressure may cause photooxidative damage to the thylakoid membrane (Huner et al. 1998). Boreal evergreens are subject to high excitation pressures during winter

and spring, when low temperatures limit carbon assimilation although light absorption continues. Therefore, boreal evergreens need seasonal acclimation mechanisms capable of adjusting both the rate of energy absorption and the energy partitioning in the light reactions of photosynthesis to the energy demands of the dark reactions (Öquist and Huner 2003).

An important acclimation mechanism modulates the fraction of absorbed light energy that is safely dissipated as thermal energy in PSII. This mechanism is largely regulated by the xanthophyll-cycle pigments (Demmig-Adams and Adams 1996, 2006). Excess light causes protons to accumulate in the thylakoid lumen, which in turn decreases luminal pH and promotes the de-epoxidation of violaxanthin to antheraxanthin and zeaxanthin. During summer, the reversible formation of zeaxanthin increases the thermal dissipation of excitation energy (Gilmore 1997, Müller et al. 2001). This flexible mechanism, which operates on a timescale of seconds to minutes (Krause and Weis 1991, Müller et al. 2001), is referred to variously as Δ pH-dependent NPQ (non-photochemical quenching), rapidly reversible NPQ, feedback de-excitation, or flexible dissipation (Demmig-Adams and Adams 2006). In contrast, when excess light persists over time, e.g., during boreal winter conditions, adjustments in the capacity of thermal dissipation are made on a much longer time-scale. Because this type of thermal dissipation is maintained in the dark, it has been termed sustained thermal energy dissipation (Demmig-Adams and Adams 2006). Sustained thermal dissipation during winter is a complex process that is thought to be regulated by the sustained retention of zeaxanthin as well as by the structural reorganization of protein complexes in the thylakoid membrane (Ottander et al. 1995, Verhoeven et al. 1998, Öquist and Huner 2003, Demmig-Adams and Adams 2006, Busch et al. 2007, Ensminger et al. 2008). Sustained thermal dissipation during winter has been observed in several species (Adams and Demmig-Adams 1994, Demmig-Adams 1998), includ-

ing Scots pine (*Pinus sylvestris* L.) (Ottander et al. 1995, Ensminger et al. 2004).

Another mechanism involved in the seasonal acclimation of PSII is represented by the capacity for repair of damaged PSII reaction centers (Ottander and Öquist 1991, Adams et al. 1994, Ottander et al. 1995, Matsubara and Chow 2004, Tyystjärvi 2008). Because damaged reaction centers cannot contribute to the photochemical process, it has been suggested that they represent a mechanism for regulating sustained thermal energy dissipation (Ivanov et al. 2002, Matsubara and Chow 2004). Finally, the seasonal acclimation of chlorophyll and carotenoids (Öquist et al. 1978, Ottander et al. 1995, Öquist and Huner 2003) modulates the rate of light absorption, and the capacity for thermal dissipation and protection against excess light, respectively.

Maximum quantum yields of photochemistry, estimated by chlorophyll fluorescence as F_v/F_m , where $F_v = (F_m - F_o)$ and F_m and F_o are maximum and minimum chlorophyll fluorescence in dark-acclimated leaves, respectively, have been studied in Scots pine needles exposed to different light environments (Strand and Lundmark 1987, Ottander and Öquist 1991, Sveshnikov et al. 2006). Results support the hypothesis that acclimation in energy partitioning in PSII is regulated by excitation pressure (Huner et al. 1998, but see Tyystjärvi 2008). The light environment affects the seasonal acclimation of the pool of xanthophyll-cycle pigments and its de-epoxidation status (Demmig-Adams 1998, Adams et al. 2001, Sveshnikov et al. 2006), the fraction of damaged reaction centers in PSII (Karpinski et al. 1994, Langvall and Örlander 2001, Sveshnikov et al. 2006), and the kinetics of the recovery of PSII (Ottander and Öquist 1991, Strand and Lundmark 1995, Adams et al. 2001). However, it is not clear how the acclimation mechanisms in PSII interact under field conditions, and which mechanisms are responsible for the observed differences in dynamics and kinetics of recovery in PSII as measured in needles exposed to different light environments. In this study, we used the rate constants of sustained thermal dissipation (k_{NPQ}) and photochemistry (k_p) (Porcar-Castell et al. 2008) to investigate how seasonal acclimation of PSII is affected by the light environment under field conditions in the spring. We monitored chlorophyll fluorescence and needle pigment concentrations in the field in Scots pine seedlings growing under artificial shading and within the crowns of mature Scots pine trees during the winter and subsequent spring recovery.

Materials and methods

Shading experiment

Twenty seedlings were randomly selected in a 7-year-old planted Scots pine stand, located in the vicinity of the SMEAR II station (Station for Measuring Forest Ecosystem-Atmosphere Relations) in southern Finland (61°51' N, 24°17' E, 181 m elevation). Five seedlings were left uncovered (control), and the remainder were assigned to one of three shade treatments ($n = 5$) allowing 60, 30 or 10% of the incident light to

penetrate. Layers of gray net were arranged over a triangular pyramid over each tree. The shade net did not affect the spectral properties of the light. We installed the shade structures on October 30, 2002 and collected data from January 19 to June 10, 2003.

Photosynthetic active radiation (PAR) was measured at SMEAR II with a sensor (LI-190, Li-Cor, Lincoln, NE), and averaged to yield 30-min values. Air temperature was measured with ventilated Pt100 sensors shielded from direct radiation. In addition, from February 22 to May 22, a thermo-element, protected with a hood from direct radiation, was placed in each of the four shade treatments to examine the effect of shading on air temperature. During sunny days, noon air temperature was up to 2–3 °C lower in shade than in full sunlight. Because increasing winter temperatures and light have opposite effects on the excitation pressure (Huner et al. 1998), higher noon temperatures in the fully sunlit treatment should only decrease the observed differences in the down-regulation of PSII between treatment groups and not interfere with our analysis.

Once or twice a week, we measured chlorophyll fluorescence in situ, with one or two replicates per tree in each treatment ($n = 5$). Simultaneously, we collected needle samples from trees in each treatment and froze them immediately in liquid nitrogen for subsequent pigment analysis. We used current-year needles for all measurements.

Canopy profile measurements

Two 45-year-old Scots pine trees growing at SMEAR II were felled on each of April 3, May 6 and June 17, 2002. All trees were of dominant height and similar size (height 11.7–13.0 m, and diameter at breast height 11.2–14.6 cm), and were growing in a closed canopy. On each date, we measured chlorophyll fluorescence parameters in the felled trees at four heights, from three branches at each height, and collected pooled needle samples from the same branches for pigment analysis. The profile of PAR inside the canopy was measured with a series of photodiode sensors placed at different heights inside the tree crown and facing upward. These measurements were used to model the variation in PAR within the canopy according to Kolari et al. (2006).

Chlorophyll fluorescence data

We measured F_m , F_o and F_v/F_m (Krause and Weis 1991) with a pulse-modulated fluorescence system (FMS2, Hansatech, U.K.). All measurements were made around noon on needles that had been dark acclimated for 2 h with dark-acclimation leaf clips, with two pairs of needles per clip.

Estimation of the rate constants of sustained thermal energy dissipation and photochemistry

We estimated the rate constants of sustained thermal energy dissipation (k_{NPQ}) and photochemistry (k_p) relative to the sum of the rate constants of constitutive thermal energy dissipation (k_D) and fluorescence (k_f), assuming $k_D + k_f = 1$ (Porcar-Castell et al. 2008). The relative rate constants were estimated

based on F_o , F_m and light absorbance, according to Porcar-Castell et al. (2008):

$$k_{\text{NPQ}} = \left(\frac{F_{\text{ms}}}{F_m} \frac{Aa}{A_s a_s} - 1 \right) (k_f + k_D) \quad (1)$$

and

$$k_p = \left(\frac{F_{\text{ms}}}{F_o} - \frac{F_{\text{ms}}}{F_m} \right) \frac{Aa}{A_s a_s} (k_f + k_D) \quad (2)$$

where F_{ms} is a reference maximum chlorophyll fluorescence measured during the summer in the absence of down-regulated PSII, A and A_s are the light absorbances for each measuring date and for the summer reference, respectively, and a and a_s are the fractions of absorbed light captured by PSII for each date, and for the summer reference, respectively. We assumed that a and a_s remain equal to 0.5. Subsequently, A was estimated as:

$$A = 1 - 10^{-\varepsilon \text{Chl}} \quad (3)$$

where ε is the within-leaf light extinction coefficient and Chl is total leaf chlorophyll concentration ($\mu\text{mol m}^{-2}$ projected area). We used the approximation $\varepsilon = 0.0034 \text{ m}^2 \mu\text{mol}^{-1}$ for Scots pine needles (Porcar-Castell et al. 2008).

Biochemical data

Needle pigments were extracted in 100% acetone, buffered with NaHCO_3 , for 2 h at 4 °C in the dark. Pigments were separated by high-performance liquid chromatography (HPLC) with a reversed-phase C-18 column (Knauer, Berlin, Germany), as described by Ensminger et al. (2001).

Statistical analysis

Differences between samples were analyzed by a two-tailed Student's t -test. Dependencies between variables were analyzed by linear regression. The coefficient of determination (r^2) and the P -value associated with the slope coefficient were determined.

Results

Seasonal patterns in maximum quantum yield of PSII, and the rate constants of sustained thermal dissipation and photochemistry

The patterns of PAR and temperature were typical for the boreal conditions in southern Finland (Figure 1a). The maximum quantum yield of PSII was already down-regulated by the end of October, as indicated by the F_v/F_m values (Figure 1b). By January, the shading treatments had induced significant differences in F_v/F_m , with sun-exposed seedlings having lower F_v/F_m values than shaded seedlings (Figure 1b). Minimum F_v/F_m values were recorded between February and March, when mean temperatures remained well below zero and PAR

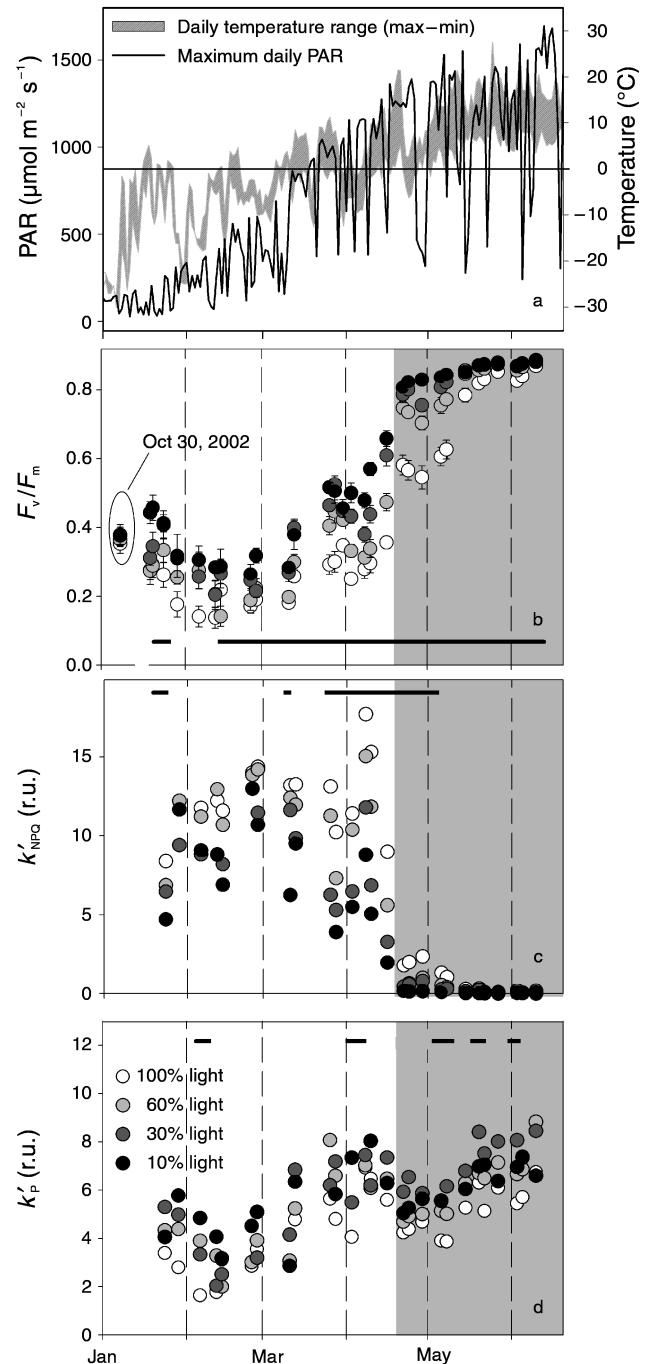


Figure 1. (a) Effects of shade treatments on seasonal changes in daily maximum photosynthetic active radiation (PAR) and the diurnal temperature range (maxima–minima). (b) Time series of maximum quantum yield of PSII measured as F_v/F_m (mean \pm SE; $n = 5$), reference values recorded before the experiment are presented inside the ellipses. (c, d) Time series of the rate constants of (c) sustained thermal dissipation (k'_{NPQ}) and (d) photochemistry (k'_p) estimated according to Equations 1 and 2. Horizontal black lines indicate significant differences between *Pinus sylvestris* seedlings in 100% and 10% of full sunlight ($P < 0.05$). White and gray areas indicate the periods before (winter) and after (summer) the shift in the type of thermal dissipation, respectively.

was increasing (Figure 1a). Recovery of F_v/F_m started concomitantly with increasing temperature in seedlings in all treatments, but summer values were attained faster in shaded seedlings than in sun-exposed seedlings (Figure 1b).

The relative rate constant of sustained thermal dissipation (k'_{NPQ}) ranged between 5 and 18 (relative units) during winter, and decreased drastically in seedlings in all treatment during mid-April, remaining low thereafter (Figure 1c). Throughout the experiment, sun-exposed seedlings tended to have higher k'_{NPQ} than the more shaded seedlings, although differences were not always significant (Figure 1c). The seedlings receiving 10% of incident light reached maximum k'_{NPQ} values at the end of January and February, when maximum daily temperatures were -20 and -10 °C, respectively. In contrast, the control seedlings—which received 100% of incident light—reached maximum k'_{NPQ} values during early April, when sunny days were combined with below-zero temperatures (Figure 1a).

The relative rate constant of photochemistry (k'_p) showed a gradual increase after reaching a minimum in mid-February (Figure 1d). Winter k'_p was 50–75% lower than summer k'_p . Shading did not lead to continued differences in k'_p between treatment groups (Figure 1d). When maximum temperatures exceeded 20 °C during mid-April, k'_p reached summer values. However, k'_p decreased again in seedlings in all treatments after a cold spell (Figure 1a), recovering once more during May. We divided the data into two sets (Figures 1b–d), before and after the observed decrease in k'_{NPQ} around April 16, to test the hypothesis that a shift in the type of thermal energy dissipation occurs during this period (i.e., from sustained to rapidly reversible thermal dissipation).

Seasonal patterns in pigment concentration

Artificial shading affected the degree of de-epoxidation of the xanthophyll-cycle pigments, whereas seasonal changes in temperature affected the cycle's pattern of variation (Figures 1a and 2a). In contrast, the pool of xanthophyll-cycle pigments—violaxanthin + antheraxanthin + zeaxanthin—expressed on a mole chlorophyll basis (VAZ/Chl), total chlorophyll concentration (Chl a+b), as well as Chl a/b, remained fairly constant until April, when differences emerged between treatment groups (Figures 2b–d) as temperatures increased (Figure 1a). The VAZ/Chl pool increased during April in seedlings exposed to 100 and 60% of incident light but decreased in seedlings in the other treatments. During May, the VAZ/Chl decreased in seedlings in all treatments. Total chlorophyll concentration (Chl a+b) also increased during April (Figure 2c). The largest increase occurred in seedlings receiving 10 and 30% of incident light. In contrast to Chl a+b concentration, a small but steady decrease in Chl a/b was apparent from April to May in seedlings in all treatments (Figure 2d).

Correlations between variables

The sustained rate of thermal dissipation correlated with the de-epoxidation status of the xanthophyll-cycle pigments (DEPS) in seedlings in all treatments ($P < 0.01$) during the winter, i.e., before the shift in the type of thermal energy dissipation.

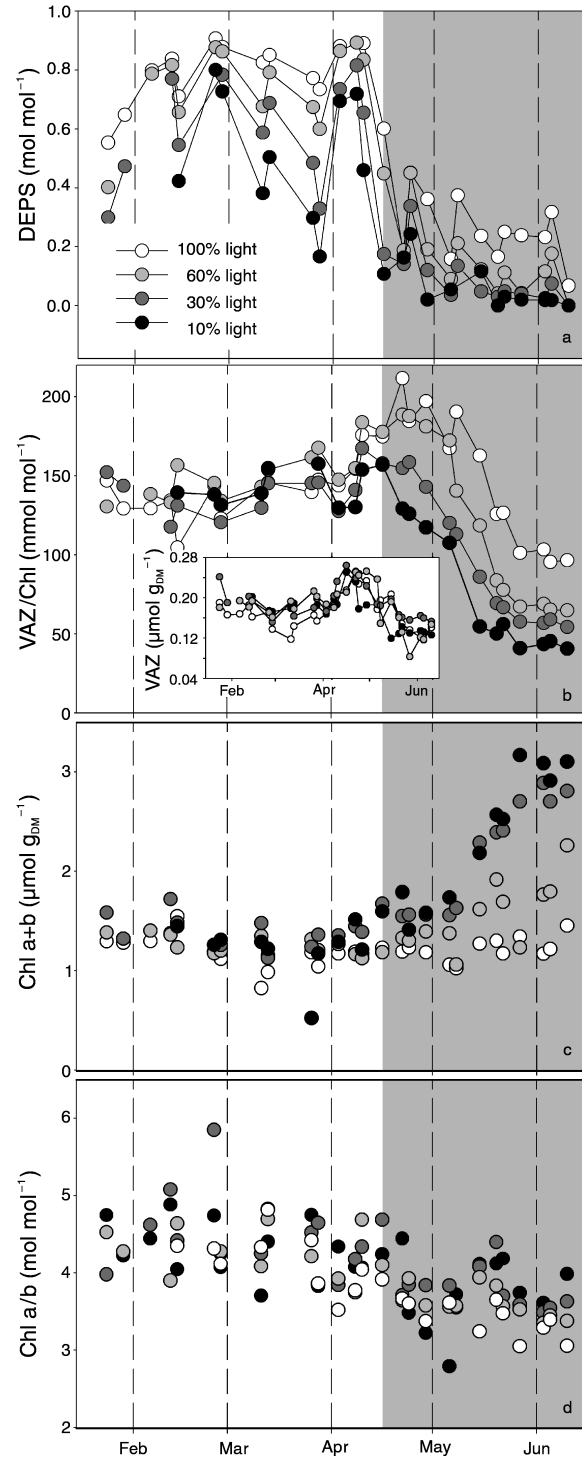


Figure 2. Effects of shade treatments on seasonal changes in pigment concentrations. (a) De-epoxidation status of the xanthophyll-cycle pigments (DEPS), (b) pool size of pigments from the xanthophyll cycle: violaxanthin, antheraxanthin and zeaxanthin on a chlorophyll basis (VAZ/Chl) and on a leaf dry mass basis (inset), (c) total chlorophyll concentration (Chl a+b) on a dry mass basis and (d) the ratio of chlorophyll a to chlorophyll b (Chl a/b). Each value represents the measurements from a pooled needle sample obtained from five *Pinus sylvestris* trees per treatment. White and gray areas indicate the periods before (winter) and after (summer) the shift in the type of thermal dissipation, respectively.

pation (Figures 3a–d). In contrast, during the summer, changes in DEPS had little effect on k'_{NPQ} (Figures 3a–d). Furthermore, k'_{NPQ} was inversely correlated with the prevailing temperatures during the winter only in the most shaded seedlings (receiving 10 % of incident light) (Figure 3h). In seedlings in the other treatments, the correlation between k'_{NPQ} and temperature decreased with increasing incident light and was never significant (Figures 3e–g).

Kinetics of the acclimation process in response to temperature

Regression models between k'_{NPQ} or k'_p and temperature were used to evaluate the temperature response of k'_{NPQ} and k'_p (Figure 4). Models were estimated with different running mean pe-

riods for air temperature. Subsequently, the proportion of variation explained by the model (r^2) was used as an indicator to analyze the response of the rate constant to the specific averaging period. The number of days used in the running mean that resulted in maximum r^2 indicated the speed at which k'_{NPQ} or k'_p adjusted to temperature. During the winter, k'_{NPQ} responded faster to changes in temperature in the seedlings receiving 10% of full sunlight than in seedlings in the other treatments. During the summer, k'_{NPQ} responded faster to variations in temperature and r^2 increased in seedlings in all treatments compared with the winter.

During the winter, k'_p responded more slowly to changes in temperature than k'_{NPQ} in seedlings in all treatments. Maximum r^2 values were obtained when using more than a 14-day

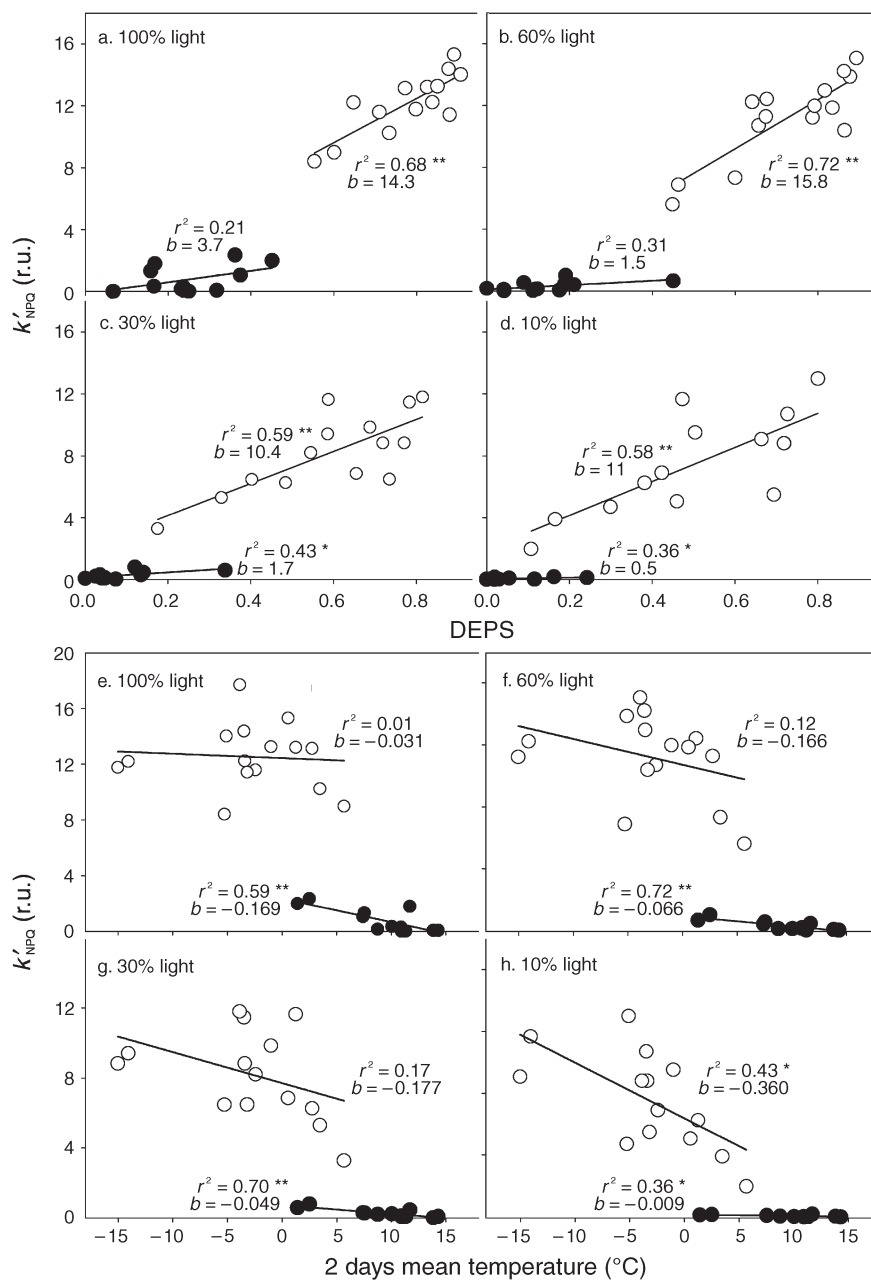


Figure 3. Linear regression models between (a–d) the relative rate constant of sustained thermal dissipation (k'_{NPQ}) and the de-epoxidation status of the xanthophyll-cycle pigments (DEPS), and (e–h) between k'_{NPQ} and the mean temperature of the last 2 days for *Pinus sylvestris* seedlings in each shade treatment. Data are divided into before (winter; ○) and after (summer; ●) the shift in the type of thermal dissipation. Significance levels are indicated as: *, $P < 0.05$; and **, $P < 0.01$.

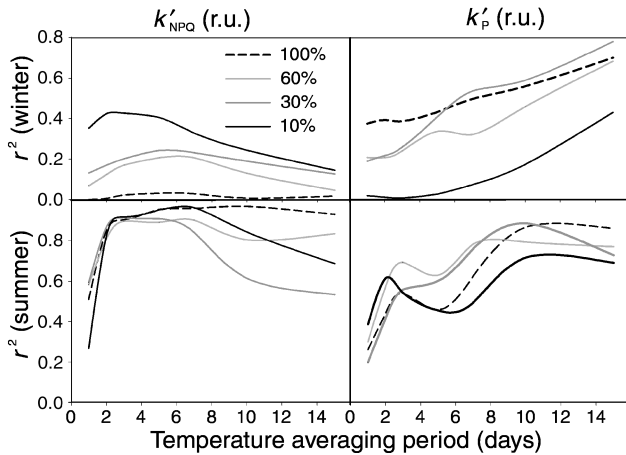


Figure 4. Proportion of explained variation (r^2) associated with the linear regression model predicting the rate constants of sustained thermal dissipation (k'_{NPQ}) and photochemistry (k'_p) (y) from the air temperature history (x), i.e., $y = a + bx$. Temperatures were averaged over periods from one day to two weeks. Data are divided into two sets, before (winter) and after (summer) the shift in the type of thermal dissipation. The length of the temperature averaging period, for which maximum r^2 is obtained, reflects the kinetics of the responses of k'_{NPQ} and k'_p to temperature.

temperature running average. During the summer, the kinetics of the temperature response of k'_p increased in seedlings in all treatments.

Seasonal acclimation within the canopy

Irradiance within the canopy of the Scots pine stand ranged from 30% of incident light in the lower part of the canopy, to 100% of the incident light at the top of the canopy (Figure 5a). Maximum quantum yield of PSII recovered during the spring at all sampling heights (Figure 5b). Generally, F_v/F_m increased from the top to the lower part of the canopy. In early April, lower and upper parts of the canopy had lower F_v/F_m values than the middle canopy ($P < 0.05$). Both the de-epoxidation status of the xanthophyll-cycle pigments (DEPS) and the total pool of xanthophyll-cycle pigments (VAZ/Chl) were highest in April. Furthermore, there was a clear within-canopy gradient in DEPS and VAZ/Chl in April, with concentrations increasing toward the top of the canopy. This gradient disappeared later in May and June (Figures 5c and 5d). During the spring, total chlorophyll concentrations in needles increased at all sampling heights. In addition, within-canopy chlorophyll concentrations increased from the top to the lower part of the canopy (Figure 5e), whereas the chlorophyll a/b ratio decreased from the top to the lower part of the canopy (Figure 5f).

Discussion

Our objective was to determine the mechanisms responsible for the observed differences in dynamics and kinetics of acclimation of PSII among needles exposed to diverse light envi-

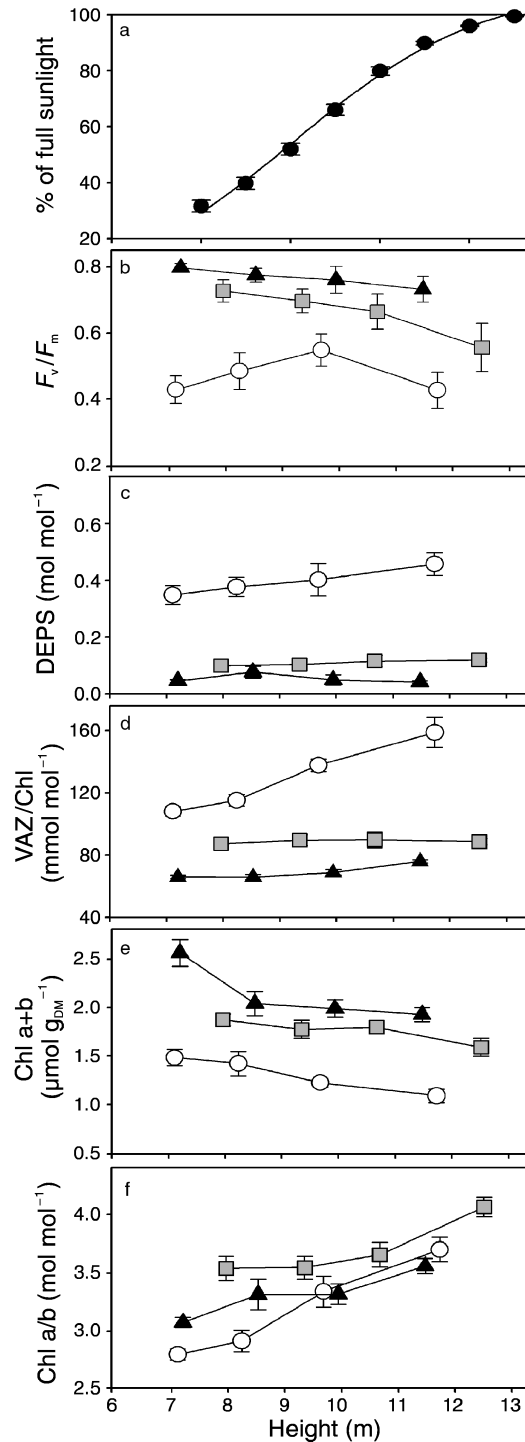


Figure 5. Canopy profiles of the light environment and photosynthetic properties on April 3 (○), May 6 (■) and June 17 (▲) during recovery of photosynthesis in 45-year-old *Pinus sylvestris* trees. (a) The proportions of full sunlight at different heights inside the canopy were estimated for three periods during March, April and May (mean \pm SE, $n = 3$) according to Kolarik et al. (2006), (b) values of maximum quantum yields of photochemistry (F_v/F_m), (c) de-epoxidation status of the xanthophylls (DEPS), (d) pool of xanthophyll-cycle pigments expressed on a chlorophyll basis (VAZ/Chl), (e) total chlorophyll concentration (Chl a+b) and (f) the ratio of chlorophyll a to chlorophyll b (Chl a/b). Values represent the mean \pm SE obtained from two trees and three branches in each tree ($n = 6$).

ronments. Adjustments in thermal dissipation explained most of the differences, and the mechanisms underlying these adjustments appeared to be largely controlled by zeaxanthin and structural changes in the thylakoid membrane.

The maximum quantum yield of photochemistry, estimated as F_v/F_m , was lower in needles of seedlings and trees exposed to high light environments (Figures 1b and 5b) than in more shaded needles. This pattern supports the anticipated acclimation of energy partitioning in response to increased excitation pressure (Huner et al. 1998), and is consistent with previous observations in boreal Scots pine (Strand and Lundmark 1987, Ottander and Öquist 1991, Sveshnikov et al. 2006). The finding that F_v/F_m was as low in the lower part of the canopy as in the upper part of the canopy during April, when the ground was still covered with snow (Figure 5b), is likely related to the increased albedo (data not shown) affecting the irradiance in the lower part of the canopy. Recovery of F_v/F_m during the spring was faster in shaded needles than in sun-exposed needles (Figures 1b and 5b), as reported earlier for conifers (Ottander and Öquist 1991, Adams et al. 2001).

Modulation of light absorption by the light environment

The concentration of needle chlorophyll increased with the degree of shading (Figures 2c and 5e), indicating an increase in overall needle light absorption capacity, which is typical for acclimation to low light environments (Demmig-Adams 1998, Walters 2005). Furthermore, the chlorophyll a/b ratio decreased with the degree of shading in the canopy (Figure 5f), indicating an increase in the number of peripheral light-harvesting complexes, because these are the main binding sites of chlorophyll b (Demmig-Adams 1998, Walters 2005). Overall, these results are consistent with an increase in cross-sectional light absorption per PSII in response to shade.

Effect of light on the capacity of sustained thermal dissipation

Differences in F_v/F_m between shaded and sun-exposed seedlings were largely a result of differences in the capacity for sustained thermal dissipation (Figures 1b and 1c). During the winter, the rate constant of sustained thermal dissipation correlated with the de-epoxidation status of the xanthophyll-cycle pigments (DEPS) in seedlings in all treatments (Figures 3a–d), suggesting that zeaxanthin and the xanthophyll cycle play a key role in modulating sustained thermal dissipation capacity in PSII in overwintering Scots pine. These results are consistent with previous observations during the winter that associate higher zeaxanthin values with lower F_v/F_m (Adams and Demmig-Adams 1994, Adams et al. 1994, Verhoeven et al. 1996). In contrast, during the summer, most of the thermal dissipation rapidly reversed upon dark acclimation, explaining the large decrease in k'_{NPQ} (Figure 1c) and lower correlation with DEPS (Figures 3a–d) (note that DEPS was estimated from light-acclimated samples engaged in both sustained and rapidly reversible thermal dissipation). The drastic reduction in k'_{NPQ} in overwintering Scots pine demonstrates the shift between sustained and rapidly reversible thermal dissipation

during mid-April, which is likely induced by the structural reorganization or aggregation of protein complexes in the thylakoid membrane. Aggregation of protein complexes during winter may facilitate the sustained de-epoxidation of xanthophylls and participate in sustained thermal dissipation in PSII (Verhoeven et al. 1999, Demmig-Adams and Adams 2006, Busch et al. 2007), protecting the thylakoid membrane from excess light and subsequent photooxidation during cold winter days (Ottander et al. 1995, Gilmore and Ball 2000, Busch et al. 2007).

Effect of light on photochemical capacity

Only to a limited extent could differences in F_v/F_m between shaded and exposed seedlings be attributed to differences in the photochemical capacity (Figures 1b and 1d). Reduced photochemical capacity during winter has been related to damage to PSII reaction centers (Ottander and Öquist 1991, Adams et al. 1994, Ottander et al. 1995, Ensminger et al. 2004). In addition, a higher proportion of damaged reaction centers has been reported in sun-exposed needles than in shaded needles of boreal conifers (Karpinski et al. 1994, Langvall and Örlander 2001, Sveshnikov et al. 2006). However, our results did not conclusively demonstrate how the light environment affected the photochemical capacity of overwintering Scots pine, because k'_p (Figure 1d) was not consistently higher in seedlings in 10% of full sunlight than in seedlings in full sunlight. Thermoluminescence studies on Scots pine growing under controlled conditions (Ivanov et al. 2002) indicated that, after trees acclimate to the cold, the difference in redox potential between the primary and secondary quinone electron acceptors, Q_A and Q_B , decreases, thereby decreasing the probability of electron transport. This process may result in enhanced thermal dissipation by inactive reaction centers in overwintering Scots pine (Ivanov et al. 2002, Sveshnikov et al. 2006). Our results were inconsistent with these findings because the capacity for thermal dissipation, denoted by k'_{NPQ} , did not follow the inverse pattern of k'_p (Figure 1) that would be expected if an increase in the fraction of inactive reaction centers (indicated by low k'_p) were enhancing thermal dissipation.

Effect of light on the acclimation kinetics of PSII

During the winter, shaded seedlings responded faster to changes in temperature than sun-exposed seedlings (2 versus 5–7 days) (Figure 4), indicating that light influenced the kinetics of thermal dissipation. In contrast, the acclimation of k'_p responded very slowly to changes in temperature in seedlings in all treatments. These results indicate that differences in the kinetics of recovery of F_v/F_m between shaded needles and sun-exposed needles (Figure 1b) can be explained by differences in the kinetics of acclimation of thermal dissipation capacity. Faster kinetics of acclimation of thermal dissipation capacity in shaded leaves than in sun-exposed leaves have been observed (Demmig-Adams 1998, Verhoeven et al. 1998, Demmig-Adams and Adams 2006) and attributed to structural adjustments in the thylakoid membrane. We propose that seasonal differences in the kinetics of acclimation of k'_{NPQ} to temperature were caused by the shift between sustained and rapidly

idly reversible forms of thermal dissipation. We attribute this shift to the de-aggregation of protein complexes in PSII (Verhoeven et al. 1998, Öquist and Huner 2003, Demmig-Adams and Adams 2006, Busch et al. 2007, Ensminger et al. 2008). Consequently, differences in the temperature response of k'_{NPQ} among needles exposed to different light environments during the winter could be ascribed to the degree of aggregation of the thylakoid membrane proteins. Accordingly, needles exposed to higher irradiances would have a higher degree of aggregation and a slower response of k'_{NPQ} to temperature.

In conclusion, differences in how needles from field-grown overwintering Scots pine exposed to different light environments down-regulate PSII are mainly explained by differences in sustained thermal dissipation. Sustained thermal dissipation in overwintering Scots pine is largely controlled by a zeaxanthin-facilitated mechanism but also likely by aggregation of thylakoid membrane proteins. Furthermore, the light environment affects how sustained thermal dissipation responds to temperature: the kinetics of the response result in different rates of recovery of F_v/F_m between shaded and sun-exposed needles of field-grown field Scots pine. At the tree level, these mechanisms could increase the physiological flexibility of the seasonal acclimation of photosynthetic capacity, with a fraction of needles remaining more active and capable of rapid recovery of photosynthesis during occasional warm spells during spring.

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