Photosynthetic Capacity and PSII Efficiency of the Evergreen Alpine Cushion Plant Saxifraga paniculata during Winter at Different Altitudes

Authors: Hacker, J., and Neuner, G.

Source: Arctic, Antarctic, and Alpine Research, 38(2) : 198-205

Published By: Institute of Arctic and Alpine Research (INSTAAR), University of Colorado

URL: https://doi.org/10.1657/1523-0430(2006)38[198:PCAPEO]2.0.CO;2

Arctic, Antarctic, and Alpine Research, Vol. 38, No. 2, 2006, pp. 198–205

Photosynthetic Capacity and PSII Efficiency of the Evergreen Alpine Cushion Plant *Saxifraga paniculata* during Winter at Different Altitudes

J. Hacker† and*
*G. Neuner**

*Institute of Botany, University of
Innsbruck, Sternwartestrasse 15,
6020 Innsbruck, Austria.
†juergen.hacker@uibk.ac.at

Abstract

The effect on photosynthetic capacity, photochemical efficiency of photosystem II (PSII), and frost resistance to environmental changes during winter in the evergreen alpine cushion plant *Saxifraga paniculata* were investigated at different altitudes. *S. paniculata* was more resistant to cold induced photoinhibition during midwinter than other evergreen subalpine species. Photochemical efficiency (F_v/F_m) was only reduced to 0.62. Photosynthetic capacity was only reduced if weekly mean leaf temperatures dropped below +5°C and remained positive during the entire investigation period. Growth cessation was accompanied by low values of photosynthetic capacity to maintain photostasis, i.e., the balance between energy input through photochemistry and subsequent energy utilization through metabolism. Even in January after night temperatures down to −22°C, photosynthetic capacity still averaged $+4.0 \pm 0.8$ µmol CO_2 m^{-2} s^{-1}. Initial frost damage did not commence until leaf temperatures dropped below −27.6°C. This was sufficient to survive absolute air temperature minima. As an evergreen species, the sustained high efficiency of PSII in winter potentially enables *S. paniculata* to immediately utilize periods with moderate temperature conditions for photosynthesis, particularly at the end of winter when there is sufficient water on rocks due to melting snow.

Introduction

Evergreen plants of temperate alpine zones have to cope with the combined effects of low temperature and high irradiation on photosynthesis during winter (Adams III et al., 2004). Cold acclimation of photosynthesis can occur to overcome the combined effects of low temperature and high irradiation on evergreen leaves (Öquist and Huner, 2003; Adams III et al., 2004). Two divergent strategies of acclimative response of photosynthesis to winter conditions have been reported for conifers and winter cereals. Various other species-specific responses have been observed under varying environmental conditions (Adams III et al., 2002).

In conifers, photosynthetic capacity is strongly suppressed during winter (Savitch et al., 2002). In Scots pine (*Pinus sylvestris*) and snow gum (*Eucalyptus pauciflora*), the formation of special protein-pigment complexes has been observed, enabling high rates of nonphotochemical quenching (Ottander et al., 1995; Gilmore and Ball, 2000). The increased capacity for nonphotochemical quenching of excitation energy correlates with a decreased photochemical efficiency of photosystem II (PSII), i.e., decreased F_v/F_m values (Krivosheeva et al., 1996). Low F_v/F_m values have been measured in several temperate woody species during winter (Neuner et al., 1999a; Verhoeven et al., 1999; Matsubara et al., 2002).

In contrast to conifers, there is no sustained down-regulation of photosynthetic capacity in herbaceous plants such as winter cereals, as the plants continue to grow and develop under conditions of frost hardening in autumn. The difference between photosynthetic responses in conifers and winter cereals during cold acclimation is likely to be a result of differences in their sink capacities (Öquist and Huner, 2003). While sink capacity is strongly reduced in conifers because of growth cessation and dormancy, winter cereals maintain a strong sink capacity and continue to grow during cold acclimation (Savitch et al., 2002). This balance between energy absorbance and utilization through metabolism and growth is called photostasis (Huner et al., 2003). When the demand for photosynthetic assimilates is decreased, e.g., by environmental limits to growth, photosynthesis is down regulated.

During winter, photosynthetic CO_2 consumption in evergreen leaves can cease due to extracellular ice formation (Larcher, 2003), or it can be suppressed either by a reduced sink capacity (photostasis) or by low temperatures. In this situation the excitation energy produced in the temperature-insensitive photochemical processes cannot be dissipated via photosynthesis (Huner et al., 1998) and commonly results in light saturation and overexcitation. Evergreen plants have therefore evolved mechanisms of nonphotochemical quenching to regulate and protect the photosynthetic apparatus over winter (Demmig-Adams et al., 1999; Horton and Ruban, 1999).

In the alpine life zone many perennial plants (herbaceous and woody) are evergreen or produce their leaves before snow cover melts (Körner, 1999). Little is known about photosynthesis of evergreen alpine herbaceous plants during winter. Usually these plants are snow covered during the entire winter and therefore protected from both low temperatures and high irradiance. Nevertheless, on exposed ridges and steep rocky sites, the snow cover blows or slips away regularly during winter.

The evergreen herbaceous rosette plant *Saxifraga paniculata* occurs in snow-free areas during the winter. In contrast to woody plants *S. paniculata* does not depend on thawing of the soil to replace water lost through transpiration under frost drought conditions in winter. Melting snow water is a potential water source for *S. paniculata* as the species readily takes up water through leaf hydathodes (Taschler D., unpublished). Complete re-saturation takes place following severe dehydration within 12 h (Neuner et al., 1999b). The xerophytic leaf structure with a thick cuticle, additional wax layers, and a water storage tissue at the leaf base (Hegi, 1975) further helps to preserve water. During short thawing periods in fall, winter, and spring, photosynthesis of *S. paniculata* may not be adversely affected by drought-induced water shortage as is usually the case in woody plants.

We compared the photosynthetic capacity (overall CO_2 gas exchange and PSII efficiency) of *S. paniculata* during winter with

1523-0430/06 $7.00

those described for evergreen conifers and winter cereals. To detect the effect of temperature on photosynthesis and frost resistance, we investigated *S. paniculata* plants from midwinter until summer at two alpine and two lowland growing sites with significantly different microclimates.

Materials and Methods

PLANT MATERIAL AND EXPERIMENTAL SITES

Saxifraga paniculata Mill. is an evergreen acaulescent cushion-forming rosette plant commonly found in alpine rock habitats. Cushions of *S. paniculata* were collected from three natural sites between 2000 and 2400 m a.s.l. in the Central Alps, south of Innsbruck, Austria. Individual vegetative leaf rosettes were separated from the cushions and potted in plastic containers ($7 \times 7 \times 10$ cm) filled with potting soil mixed in equal parts with sand. The plants were cultivated under natural environmental conditions either in the Botanical Garden of the University of Innsbruck (600 m a.s.l.) or in the Alpine Garden on Mount Patscherkofel near Innsbruck (1950 m a.s.l.).

In September 2001 the plants were exposed to four different environments: (1) a frost-free greenhouse in the Botanical Garden of Innsbruck with a controlled minimum temperature of +5°C, (2) natural lowland environmental conditions in the Botanical Garden (600 m a.s.l.), (3) an alpine site on Mount Patscherkofel (1950 m a.s.l.) with secure snow cover from 10 November to 15 May, and (4) an alpine site at Mount Patscherkofel that was manually kept snow-free throughout the winter.

At all sites leaf temperatures and photosynthetic photon flux density (*PPFD*, μmol photons m^{-2} s^{-1}) were recorded on dataloggers (CR10; Campbell Scientific, Logan, UT, U.S.A.). Leaf temperatures were monitored using type T fine wire thermocouples (2×0.3 mm^2, Therm-Est, Maizieres-Les-Metz, France). *PPFD* was measured with Quantum sensors (Type QS2, Delta-T Devices, Burwell, U.K.). Leaf temperatures and *PPFD* were sampled every 30 s and averages were calculated every 30 min. In addition, daily maxima and minima for leaf temperature and *PPFD* were stored. The experiments lasted from October 2001 to June 2002. Microclimate data were collected continuously and physiological measurements, i.e., gas exchange, frost resistance, and *in vivo* chlorophyll fluorescence were carried out monthly.

PHOTOSYNTHETIC CAPACITY

Gas exchange was measured with a CO_2/H_2O-Porometer (Central unit CQP 130; probe PMK-10 type KA11; Heinz Walz, Effeltrich, Germany) and an NDIR-gas-analyzer (Binos 100, Leybold, Hanau, Germany). After removal of pots from the field, single leaf rosettes of *S. paniculata* including main and additional roots were detached from the cushion. Roots were immediately watered in small plastic vessels (0.7 mL). Vessels were sealed against water loss at the root to shoot transition zone. Old, senescent leaves were excised. The leaf rosette was cleaned with distilled water. Photosynthetic light response curves were measured (20°C leaf temperature, saturating air humidity, and ambient CO_2) on four single leaf rosettes from each of the four experimental sites. The first measurement was conducted on the darkened leaf rosette to get the rate of dark respiration. Afterward irradiation was increased in nine steps to a maximum of 2000 μmol photons m^{-2} s^{-1}. The different irradiation levels were achieved by placing neutral filters (transmission values 50% and 70%, Schott, Mainz, Germany) between the sample and the light source (halogen lamp, Philips Masterline, 20 W). The CO_2 gas exchange rates were sampled after 10 min adaptation time to each irradiation level. The CO_2 gas exchange rates were referenced to the projected leaf area. Projected leaf area was calculated from digital photographs of each leaf rosette using image analysis software (Optimas 6.5, Seattle, USA).

For the calculation of significant photosynthetic parameters, a nonrectangular hyperbola was fitted to the gas exchange data:

$$\Theta P^2 - (\phi I + P_{\max})P + \phi I P_{\max} = 0, \qquad (1)$$

where P is the rate of photosynthesis, I is the irradiance, ϕ is the maximum quantum yield, Θ is the convexity, and $P_{\max}$ is the light saturated rate of photosynthesis (Ögren and Evans, 1993; Leverenz, 1994; Lambers et al., 1998). The values of the gas exchange measurements and ϕ, calculated as the incline of the light response curve at the lowest level of irradiation, were inserted into the formula, prior to calculating dark respiration was added to each P-value to obtain positive values only. The parameters Θ and $P_{\max}$ were estimated using a regression analysis based on the Levenberg-Marquardt algorithm (SPSS, Chicago, IL, U.S.A.). The photosynthetic capacity P_{2000} was calculated using the model with an irradiation level of 2000 μmol photons m^{-2} s^{-1}. The light compensation point (*LCP*) is the value of irradiation at which the fitted curve crosses the ordinate.

IN VIVO *CHLOROPHYLL FLUORESCENCE*

In vivo chlorophyll fluorescence was measured with a Mini-PAM chlorophyll fluorometer system (Heinz Walz, Effeltrich, Germany). The fiberoptic probe of the fluorometer was centered above the investigated leaf rosette. After a dark adaptation period of 30 min, minimum fluorescence F_0 was determined. Maximum fluorescence was determined during a subsequent saturating light pulse with the duration of 800 ms. Potential efficiency of PSII (F_v/F_m) was calculated as $(F_m - F_0)/F_m$. Values presented are means of eight measurements per experimental site and date.

FROST RESISTANCE

Frost treatments were either conducted in field portable freezing chambers (BK-Elektronik, Natters, Austria) or computer controlled commercial freezers; both systems are described in Taschler and Neuner (2004). Cooling and thawing below 0°C was conducted at rates of 2 K h^{-1}, and samples remained exposed to target temperatures for 4 h. Six target temperatures were chosen with the highest temperature expected to cause no damage and the lowest temperature expected to kill all leaves. Thirty leaf rosettes from each of the four experimental sites were used in each frost resistance test. Old, senescent leaves were excised carefully before exposing leaf rosettes, including roots on wet paper towels, inside polyethylene bags.

As *S. paniculata* shows a clear discoloration if frost damaged (Sakai and Larcher, 1987), frost damage was visually assessed one week after the frost treatment (post frost test conditions: 20°C, 20 μmol photons m^{-2} s^{-1}). Digital photographs were taken after the frost test, and the percentage of leaf area damaged was calculated for each single leaf rosette using image analysis software (Optimas 6.5, Seattle, WA, U.S.A.). The percentage of leaf area damaged was plotted against the frost treatment temperature. A classic logistic function was fitted to the data using P.Fit (Fig.P Software Corporation, Durham, NC, U.S.A.). The temperature causing 50% frost damage (LT_{50}) was estimated from the fitted curve.

GROWTH

Formation and expansion of new leaves in the centre of the leaf rosette are signs of active growth of *S. paniculata* plants. Leaf tissue during the elongation phase is most frost susceptible both in woody and herbaceous plants (Taschler and Neuner, 2004). After cessation of

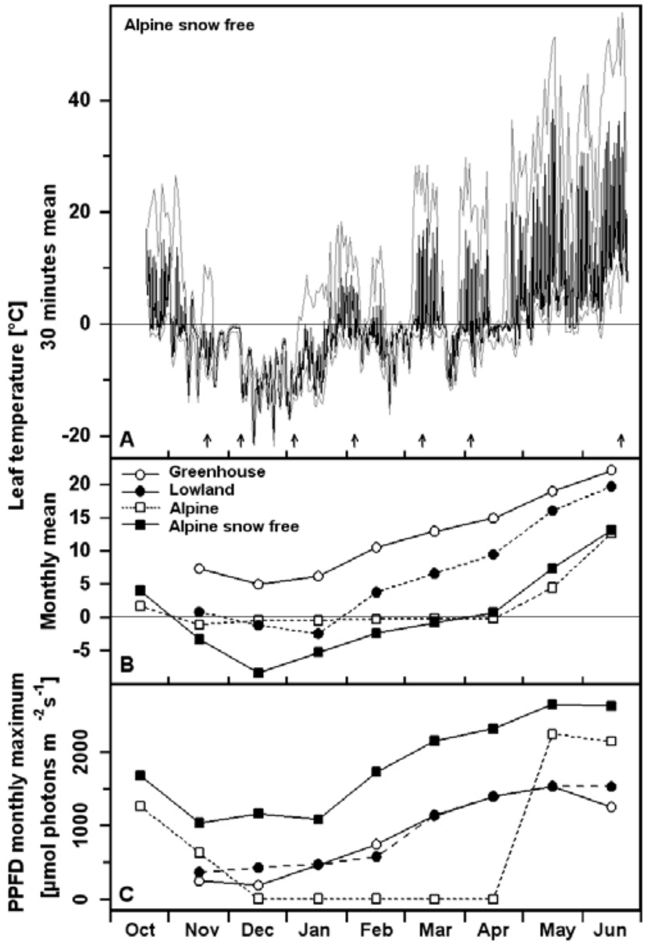

FIGURE 1. Microclimatic conditions at the four experimental sites from October 2001 to June 2002. (A) Leaf temperature at the alpine snow-free site. The black solid line represents the 30 min mean sampled every 30 s, gray solid lines represent daily minima and daily maxima, respectively. The arrows indicate the date of gas exchange, *in vivo* chlorophyll fluorescence, and frost resistance measurements. Regular night frosts occurred from October to April, and were particularly severe from November to January. (B) Monthly mean leaf temperatures and (C) monthly maximum of photosynthetic photon flux density (PPFD) (µmol photons m^{-2} s^{-1}) at the four experimental sites.

growth with full expansion of the new leaves, frost resistance is similar to that of old leaves. Hence, by determining frost resistance and frost damage patterns in the leaf rosette, it is possible to get information on whether or not new leaves are growing. Growth data presented in the results section were derived by this method.

STATISTICAL DATA ANALYSIS

Differences between means of photosynthetic capacity (P_{2000}), potential efficiency of PSII (F_v/F_m), and frost resistance (LT_{50}) were tested by analysis of variance followed by a Bonferroni multiple comparisons test ($P < 0.01$) using the statistical software SPSS (SPSS, Chicago, IL, U.S.A.).

Results

MICROCLIMATE AT THE EXPERIMENTAL SITES

At the snow-free alpine site, *S. paniculata* experienced an environment typical of a subalpine winter (Fig. 1A). Severe frosts down to –22°C occurred from October until April. For a period of nearly three months (November to January) leaf temperatures remained permanently below 0°C. Temperatures were much more moderate at the other three experimental sites (Fig. 1B). At the lowland site, frosts (down to –10°C) were only recorded between November and February. At the snow-covered alpine site, *S. paniculata* plants were exposed to leaf temperatures fluctuating between 0°C and –1.6°C. Snow cover also protected them from high irradiances (*PPFD*) (Fig. 1C). At the snow-free alpine site *PPFD* was higher than at other sites throughout the entire measurement period. At the lowland sites maximum irradiances were significantly lower due to shading by adjacent trees.

PSII EFFICIENCY

S. paniculata showed a high resistance to cold induced photoinhibition during the winter (Fig. 2A). Even in January after the severe frost period, only moderate photoinhibition, as indicated by F_v/F_m values higher than 0.62, was recorded at the alpine snow-free site. At the other three experimental sites, photochemical efficiency of PSII was not reduced significantly. Above 0°C photochemical efficiency of PSII was unaffected by changes in leaf temperatures and even under freezing conditions a high photochemical efficiency of PSII was maintained (see inset, Fig. 2B). In contrast to *S. paniculata*, other evergreen species from the same alpine environment showed pronounced photoinhibition at the same time (Fig. 2C). Only in the lichen *Cetraria islandica* were similar high F_v/F_m values recorded during midwinter. In the conifers *Juniperus communis*, *Picea abies*, and *Pinus cembra* and in the broadleaved evergreen angiosperms *Rhododendron ferrugineum* and *Vaccinium vitis-idea*, F_v/F_m ranged from 0.08 to 0.34 (see Fig. 2C), which indicates severe photoinhibition. As observed for *S. paniculata*, photoinhibition was less severe in leaves from individuals grown under more moderate environmental conditions at the lowland site.

PHOTOSYNTHETIC CAPACITY

The light responses for gross photosynthesis rate obtained in gas exchange measurements are shown in Figure 3. The most striking differences between plants from the four sites and in response to season were observed in the parameters P_{2000} and P_{max} (Table 1). In winter photosynthesis there was already light saturation at 500 µmol photons m^{-2} s^{-1} at all sites as indicated by similar values of P_{2000} and P_{max}. In plants from both lowland sites, photosynthesis declined at the highest irradiation values. In contrast to winter, photosynthesis in summer was not light saturated at 2000 µmol photons m^{-2} s^{-1}, particularly at the alpine snow-free site.

The photosynthetic capacity, P_{2000}, remained positive for *S. paniculata* during the entire investigation period at all four experimental sites (Fig. 4). Photosynthetic capacity of plants from the greenhouse, where nighttime leaf temperatures were not lower than +5°C, remained high (>11 µmol CO$_2$ m^{-2} s^{-1}) throughout the whole investigation period. At the alpine snow-free site, photosynthetic capacity did not reach the same high values for *S. paniculata* leaf rosettes until June. In January after night frosts down to –22°C at the snow-free alpine site, P_{2000} was still +4.0 ± 0.8 µmol CO$_2$ m^{-2} s^{-1}. These low rates for photosynthetic capacity were accompanied by only moderate photoinhibition as indicated by an F_v/F_m value higher than 0.62 ± 0.03. A similar P_{2000} value of +5.2 ± 1.6 µmol CO$_2$ m^{-2} s^{-1} was measured at the lowland site in January despite the more moderate frost conditions. At the lowland site, photosynthetic capacity had largely recovered to pre-winter values by February due to moderate late winter conditions with daily maximum leaf temperatures of up to 10°C and night temperatures not lower than –1°C. At the snow-free alpine site, the recovery of P_{2000} was delayed until April. At the snow covered

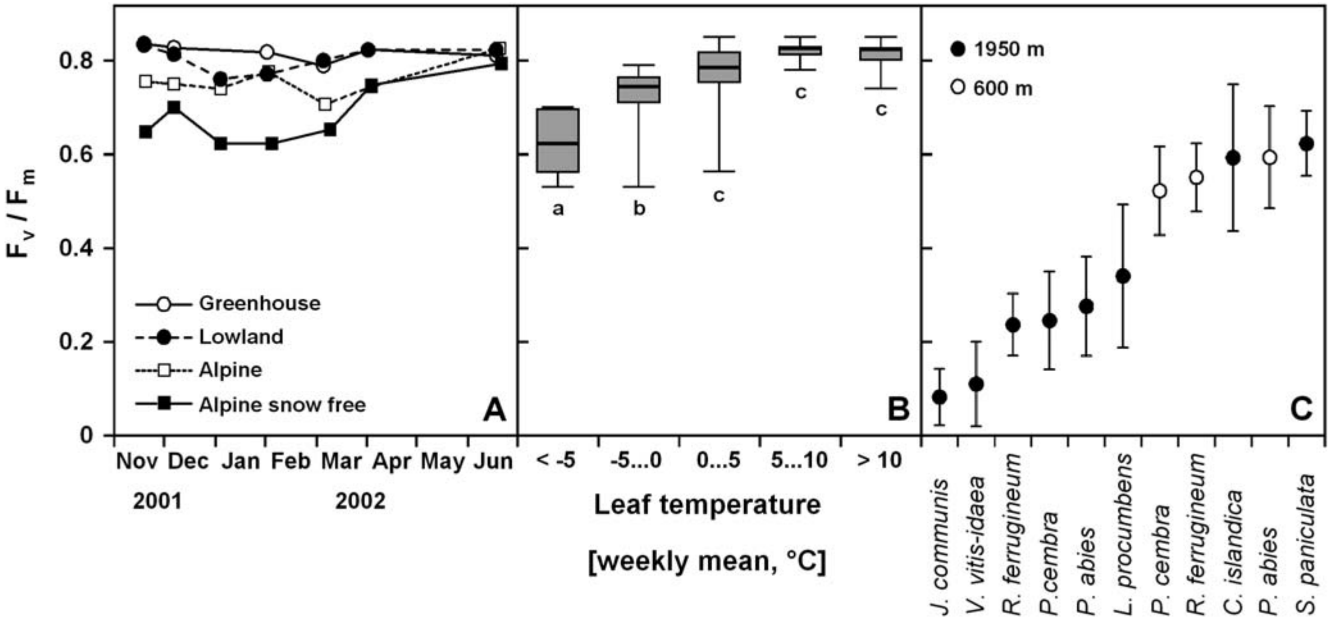

FIGURE 2. (A) Seasonal variation in potential efficiency of PSII (F_v/F_m) measured on leaf rosettes of *S. paniculata* at the four experimental sites. (Means ± SD, $n = 4$). (B) Effect of weekly mean leaf temperature on mean potential efficiency of PSII (F_v/F_m) measured on leaf rosettes of *S. paniculata* at the four experimental sites. Boxplots are illustrated by median, 25%-percentile, 75%-percentile, minimum, and maximum. Significant differences ($P \leq 0.01$) between mean F_v/F_m values are indicated by different letters and were tested with Duncan's multiple range test. (C) Potential efficiency of PSII (F_v/F_m) measured in January on leaves of different evergreen species (Conifers: *Juniperus communis*, *Picea abies*, *Pinus cembra*; broadleaved woody plants: *Loiseleuria procumbens*, *Rhododendron ferrugineum*, *Vaccinium vitis-idaea*; herbaceous plant *Saxifraga paniculata*; and the lichen *Cetraria islandica*) at the alpine site of Mount Patscherkofel (1950 m a.s.l) and in the Botanical Garden of Innsbruck (600 m a.s.l) ($n = 10$ for all species).

alpine site, P_{2000} did not recover fully until plants were snow-free in the middle of May.

At the three exposed sites, low temperature reduced photosynthetic capacity to different extents (Table 2). At the alpine snow-free site, photosynthetic capacity was reduced to 40% of its value relative to warmer periods. At the lowland site, the reduction in photosynthetic capacity was 53%. There was no significant difference ($P \leq 0.01$) between photosynthetic capacities at these two sites, which is surprising given a 1400 m altitudinal difference and a mean leaf temperature difference of 6.6°C during the entire measurement period.

A high photosynthetic capacity (>11 μmol CO_2 m^{-2} s^{-1}) in leaf rosettes of *S. paniculata* was associated with active growth (see Fig. 4). Active growth of new leaves appeared to coincide with weekly mean leaf temperatures above +5.3°C under long-day photoperiod (Fig. 5). Maximum photosynthetic capacity of leaves of *S. paniculata* was reduced at temperatures lower than +5.3°C, while photochemical efficiency of PSII was reduced only after temperatures dropped below 0°C. In greenhouse plants, growth ceased with short days in November and December despite mean leaf temperatures of between +6.9 and +8.2°C. Growth did not recover until February, despite a high photosynthetic capacity. In late winter at the lowland site, the onset of growth of new leaves lagged behind the recovery of photosynthesis by about three weeks.

FROST RESISTANCE

In contrast to photosynthetic capacity, frost resistance adjusted dynamically to the prevailing temperature conditions at all four experimental sites resulting in significant differences (Fig. 6A). At the snow-free alpine site, *S. paniculata* attained the highest frost resistance in December, with initial frost damage observed below –27.6°C. This was sufficient to protect *S. paniculata* from December's observed leaf temperature minimum of –22°C. Higher leaf temperatures at the other sites resulted in a lower, but also adequate, frost resistance. At the alpine site, exposure to constant temperatures around 0°C due to permanent snow cover (>100 cm) from January to April resulted in a constant low frost resistance (LT_{50}: –15.2°C ± 1.2). In contrast to photosynthesis, where there appears to be a threshold leaf temperature, LT_{50} data suggest frost resistance increases steadily with decreasing mean leaf temperature (Fig. 6B). A significant decrease in LT_{50} and thus cold acclimation occurs already between +10°C and +5°C.

Discussion

SUSTAINED HIGH EFFICIENCY OF PSII IN WINTER

When compared to other evergreen species of the alpine zone, *S. paniculata* exhibited a high potential efficiency of PSII during the entire winter, despite the occurrence of low temperatures down to –22°C. This high resistance to winter photoinhibition has also been reported for other herbaceous plants (*Malva neglecta*: Verhoeven et al., 1999; winter cereals: Savitch et al., 2002) where the lowering of the photochemical efficiency of PSII was reversible and observed only in response to very cold days. In contrast, evergreen woody plants showed a sustained down-regulation of PSII in the alpine life zone (Nagele, 1989) and in the lowlands (e.g., *Hedera helix*: Oberhuber and Bauer, 1991; *Pinus sylvestris*: Krivosheeva et al., 1996; Ottander et al., 1995; *P. ponderosa*: Verhoeven et al., 1999; *Amyema miquelii*: Matsubara et al., 2002; *P. cembra* and *P. mugo*: Lehner and Lütz, 2003).

Conifers remain in a sustained quenching mode during winter that enables high rates of nonphotochemical dissipation of excess irradiation (Öquist and Huner, 2003). The response of evergreen species in winter also depends on the severity of the environment. Species with strong down-regulation of photosynthesis at sun-exposed sites show no down-regulation at shade sites or even an up-regulation of photosynthesis (Adams III et al., 2004). This is corroborated by our

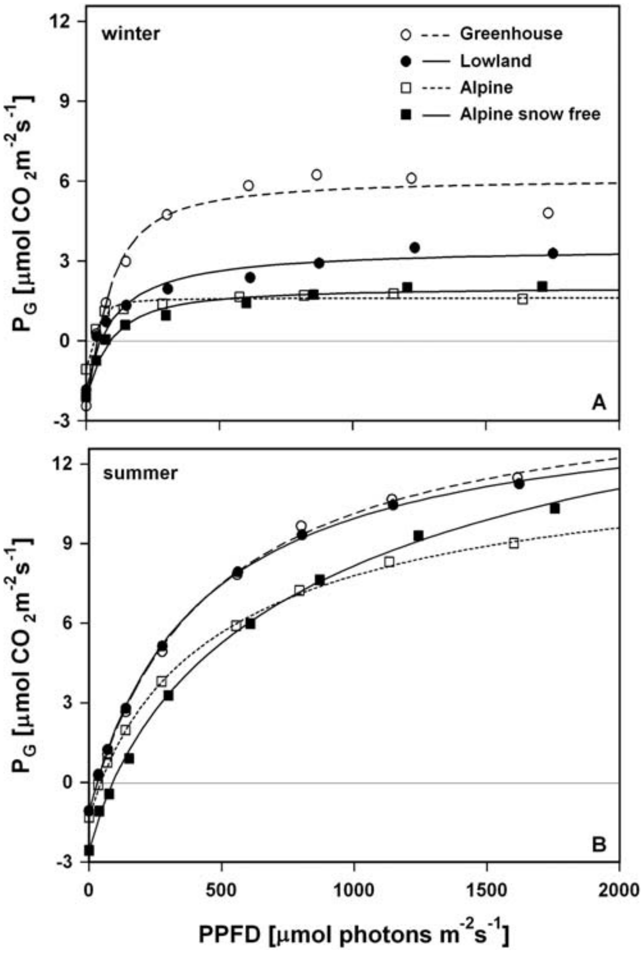

FIGURE 3. Light response curves of the gross rate of photosynthesis P_G at the four experimental sites in winter (A, January) and summer (B, June) obtained by fitting a nonrectangular hyperbola to the gas exchange data with the formula $\Theta P^2 - (\phi I + P_{max})P + \phi I P_{max} = 0$ (see section "Material"). Each figure contains gas exchange values (mean of four single data) and the associated calculated light response curve for each site. Parameters of the gas exchange measurements and the light response curves are summarized in Table 1.

finding of a lower amount of photoinhibition at the lowland site than at the alpine site.

As *S. paniculata* shows only a low ability to quench excess energy via photochemical dissipation in winter, as shown by a nearly unaltered F_v/F_m value, other photoprotective mechanisms must exist to avoid photodamage.

Plants have developed a variety of protective systems to prevent overexcitation (Adams III et al., 2004). Reducing the amount of irradiation that reaches the chlorophyll by altering of the angle of leaves is observed in members of the genus *Rhododendron*. Other approaches include aligning the chloroplasts along the cell walls or reflecting irradiation with waxes, pigments, or leaf hairs of the epidermis. Down-regulation of photosynthetic capacity includes a degradation of PSII core proteins and the oxygen-evolving complex. These processes, resulting in photoinhibition, can be seen as photoprotective mechanisms. The xanthophyll cycle plays a key role in quenching excess energy (Demmig-Adams et al., 1999). Besides an increase in the pool of xanthophyll cycle carotenoids, the carotenoids can be sustained in the quenching forms of zeaxanthin and antheraxanthin for long-term photo protection (Adams III et al., 2004).

TABLE 1

Gas exchange parameters and the light response curves at the four experimental sites measured in winter (W) and summer (S). Dark respiration (R_d) [μmol CO$_2$ m^{-2} s^{-1}], maximum quantum yield (Φ), light compensation point (LCP) [μmol photons m^{-2} s^{-1}], photosynthetic capacity (P_{2000}) [μmol CO$_2$ m^{-2} s^{-1}], and light saturated rate of photosynthesis (P_{max}) [calculated value μmol CO$_2$ m^{-2} s^{-1}]. Differences between mean values in winter and summer were tested for each site by *t*-test and are indicated by stars (*$P \leq 0.05$, **$P \leq 0.01$).

Experimental site	R_d		Φ		LCP		P_{2000}		P_{max}	
	W	S	W	S	W	S	W	S	W	S
Greenhouse	2.4	1.1	0.071*	0.037*	39	31	8.4*	13.3*	8.6*	16.4*
Lowland	1.8	1.1	0.052	0.039	53	31	5.1**	12.9**	5.3**	15.7**
Alpine	1.1	1.3	0.041	0.035	31	44	2.7**	10.9**	2.7**	13.9**
Alpine snow-free	2.1	2.6	0.035	0.038	92	90	4.0**	13.6**	4.1**	20.7**

In summer, several alpine herbaceous plant species (*Geum montanum*, *Ranunculus glacialis*, *Soldanella alpina*) showed a high resistance to photoinhibition realized by divergent strategies (Streb et al., 1997). A common feature of the three alpine species was the high resistance of PSII and catalase against photo-inactivation, even when repair by new protein synthesis was blocked. The chloroplasts of the alpine plants additionally contained much higher levels of ascorbate and glutathione which is in contrast to several lowland plants (*Primula veris*, *Ranunculus acris*, *Taraxacum officinalis*; Streb et al., 1997).

At the snow-free alpine site, *S. paniculata* showed increased coloration in the lower leaf epidermal layer, which was achieved by formation of anthocyanins that are typically responsible for coloration in most vascular plants (Gould, 2004). The absorption band of anthocyanins is between 500 and 600 nm with a maximum near 550 nm (Merzlyak et al., 2000; Pietrini and Massacci, 1998) and overlaps with the absorption band of chlorophyll b and thus protects leaves from excess irradiation. Anthocyanins may prevent photoinhibition (Chalker-Scott, 1999; Steyn et al., 2002; Close and Beadle, 2003; Gould, 2004) particularly during leaf expansion, autumnal leaf senescence, and abiotic stress.

An important photoprotective mechanism for *S. paniculata* is the hydronastic movement of leaves resulting in closure of the leaf rosette and hence shading of the leaves in the rosette center by the outermost leaves. This mechanism has been reported to occur in response to summer drought in *S. paniculata* (Neuner et al., 1999b). The lower leaf surface of outermost, exposed leaves shows a more pronounced photoinhibition than their upper leaf surface or the shaded and protected innermost leaves. The occurrence of anthocyanins in the lower epidermal layer of outermost leaves combined with leaf rosette closure during frost drought may be an important photoprotective mechanism for *S. paniculata* during winter.

PHOTOSYNTHETIC CAPACITY

The winter photosynthetic capacity of *S. paniculata* during periods with sub-freezing temperatures (40% of frost-free periods) is unique compared to many evergreen woody subalpine species. *Pinus cembra* and *Rhododendron ferrugineum* from the same environment (Mount Patscherkofel, 1950 m a.s.l.) did not show a positive net photosynthesis rate from December until March even 12 h after detachment when the samples had been exposed to optimal external conditions (Tranquillini and Machl-Ebner, 1971). After 24 h only 11% of the summer value was reached. In contrast to *S. paniculata*, no CO$_2$ uptake was measured under winter conditions at the alpine timberline in evergreen conifers (for review, see Havranek and Tranquillini, 1995). The same species may have photosynthetic rates of up to 50%

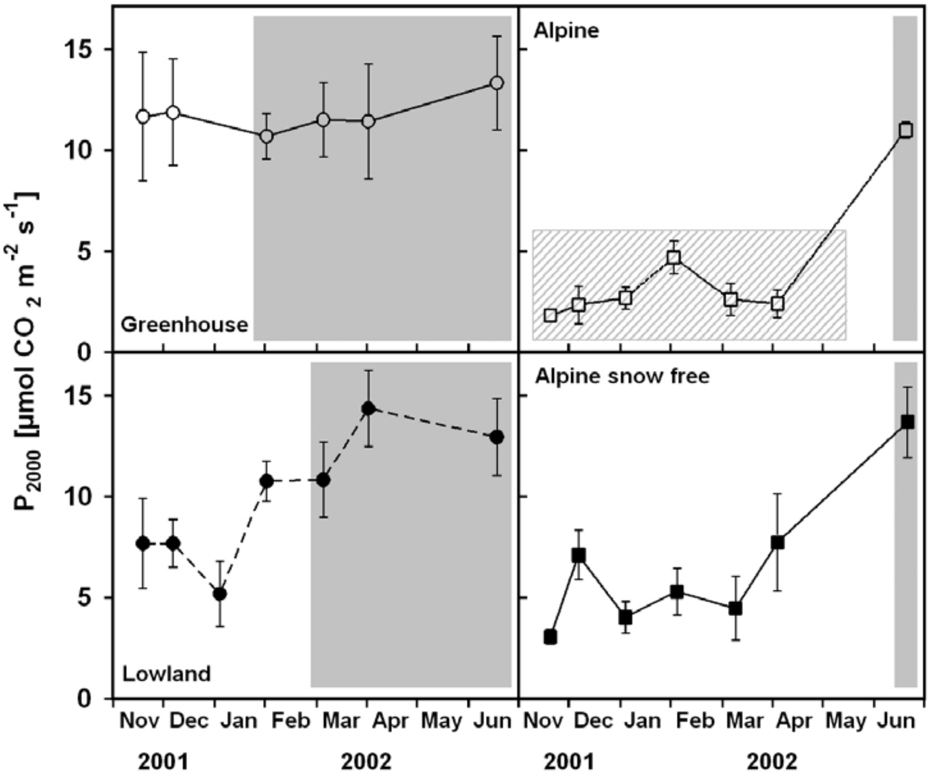

FIGURE 4. Seasonal variation in photosynthetic capacity P_{2000} (µmol CO$_2$ m^{-2} s^{-1}) measured on leaf rosettes of *S. paniculata* at the four experimental sites. Periods with active growth of new leaves are shown by light gray bars. The light gray shaded area indicates periods with complete snow cover. High P_{2000} was usually accompanied by active growth.

of summer values when investigated under warmer lowland conditions (600 m a.s.l.; Körner and Perterer, 1988).

The lowering of the photosynthetic capacity observed during winter at all sites cannot solely be explained by a down-regulation of the primary photochemistry. Reduced photosynthetic capacity is likely to be a combination of the aftereffects of freezing events (Bauer et al., 1992) and a down-regulation to reach photostasis (Huner et al., 2003; Öquist et al., 2003). As shown in Figure 4, photosynthetic capacity correlates well with the occurrence of active growth. During periods without growth, the demand for assimilates is reduced resulting in a down-regulation of photosynthesis. High P_{2000} without growth occurred at the greenhouse site, indicating the existence of alternate sinks. This may include allocation of carbon in belowground organs (Crawford, 1989) and the preformation of organ primordia, which is common in alpine and arctic plants (Meloche and Diggle, 2003).

The onset of growth of new leaves is under the control of both temperature and photoperiod. In November and December under

TABLE 2

Mean photosynthetic capacity (P_{2000}) during periods with monthly mean temperatures lower than 0°C compared to mean P_{2000} during periods with monthly mean temperatures higher than 0°C. A ratio of mean P_{2000} for these two periods was calculated, describing the reduction in the photosynthetic capacity in winter. Different letters indicate significant differences ($P \leq 0.01$) between mean values of P_{2000} (tested with Duncan's multiple range test).

	Temperature < 0°C		Temperature > 0°C		
Experimental site	Period	P_{2000} (µmol CO$_2$ m^{-2} s^{-1} ± SD)	Period	P_{2000} (µmol CO$_2$ m^{-2} s^{-1} ± SD)	Ratio (%)
Greenhouse	—	—	Nov–June	11.8 ± 2.2^{a}	—
Lowland	Dec, Jan	6.4 ± 1.9^{b}	Nov, Feb–June	12.1 ± 2.3^{a}	53
Alpine	Nov–Apr	2.8 ± 1.1^{c}	May, June	$11.0 \pm 0.4^{*a}$	26*
Alpine snow-free	Nov–Mar	$4.8 \pm 1.7^{b,c}$	Apr-June	$12.2 \pm 2.8^{\ a}$	40

* Only values for June are available.

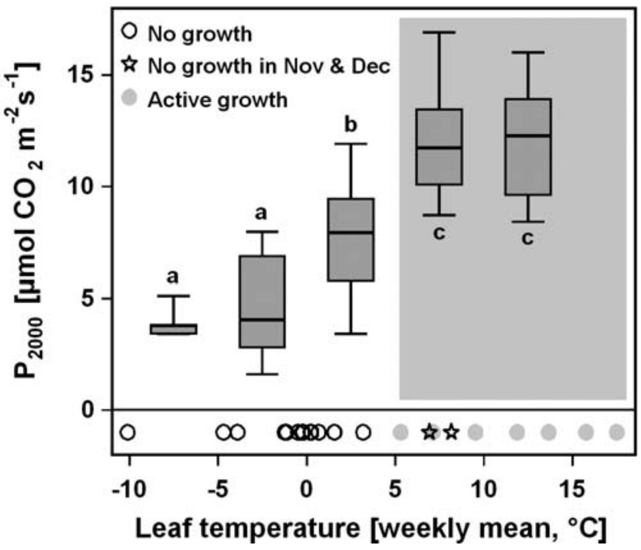

FIGURE 5. Effect of weekly mean leaf temperature on mean photosynthetic capacity P_{2000} (µmol CO$_2$ m^{-2} s^{-1}) measured on leaf rosettes of *S. paniculata* at the four experimental sites. Boxplots are illustrated by median, 25%-percentile, 75%-percentile, minimum, and maximum. Significant differences ($P \leq 0.01$) between mean P_{2000} values are indicated by different letters and were tested for with Duncan's multiple range test. Periods with active growth are indicated by a light gray shaded bar and were restricted to periods with weekly mean leaf temperatures higher than +5.3°C. During short days in November and December, growth ceased in the greenhouse despite moderate temperatures (+6.9 and +8.2°C).

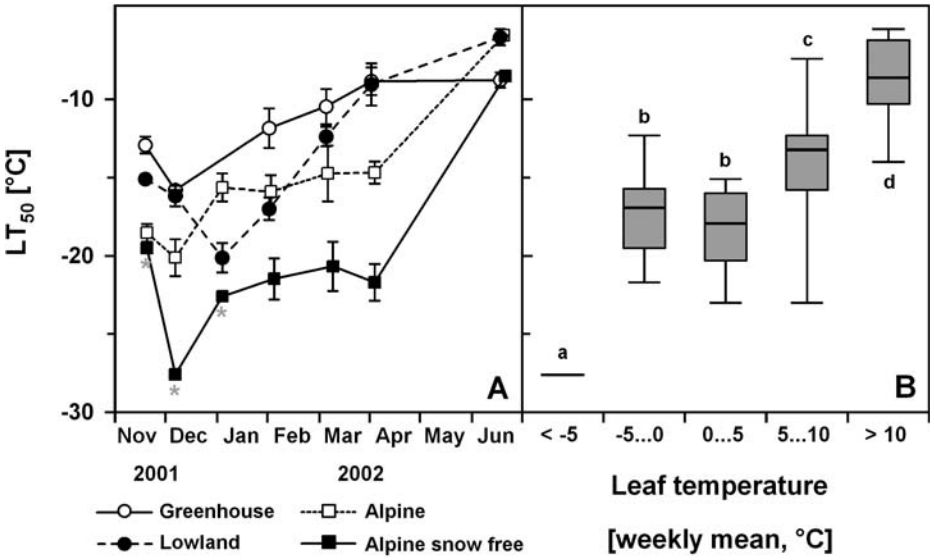

FIGURE 6. (A) Seasonal variation in frost resistance LT_{50} (°C) measured on leaf rosettes of *S. paniculata* at the four experimental sites (means ± SD, $n = 4$). Stars indicate a lower LT_{50} at the alpine snow-free site; the data points represent the minimum temperature during the frost treatment. (B) Effect of weekly mean leaf temperature on mean frost resistance measured on leaf rosettes of *S. paniculata* at the four experimental sites. Boxplots are illustrated by median, 25%-percentile, 75%-percentile, minimum, and maximum. Significant differences ($P \leq 0.01$) between mean LT_{50} values are indicated by different letters and were tested with Duncan's multiple range test.

moderate temperatures (up to +8.2°C), growth ceased. In February day length had increased sufficiently to enable growth of new leaves. In March temperatures higher than +5.3°C initiated growth. Insensitivity of growth to temperature and photoperiod after snow melt has also been reported for two other species of the genus *Saxifraga*, i.e., *S. oppositifolia* and *S. seguiri* (Keller and Körner, 2003).

COLD ACCLIMATION

The strongest response to temperature of all measured parameters detected was for frost resistance. At every site the attained frost resistance was sufficient to survive the prevailing low temperatures. The frost resistance of *S. paniculata* adjusted dynamically to the prevailing temperatures as acclimative processes are metabolically costly (Xin and Browse, 2000). Higher frost resistance was attained only when *S. paniculata* was exposed to low temperatures that induced frost hardening (Sakai and Larcher, 1987). In herbaceous species low temperatures alone can initiate frost hardening, but hardening is not necessarily linked to dormancy. In contrast, in woody plants growth ceases and dormancy occurs in response to a shortened photoperiod, and in a second phase an increase in frost resistance is induced by low temperatures (Sakai and Larcher, 1987; Pearce, 1999).

ECOLOGICAL SIGNIFICANCE OF BEING AN ALPINE EVERGREEN

One of the most important constraints on alpine plant growth is the shortness of the growing season, during which plants have to complete their life cycle and accumulate enough reserves for winter survival (Körner, 1999). In alpine rock habitats, the growing season may be further shortened by drought, with shallow substrates on rocks drying out regularly during warm and rainless periods in summer (Körner, 1994). *S. paniculata* has developed a special avoidance mechanism involving reversible leaf rosette closure to shade the physiologically active leaves with old leaves (Neuner et al., 1999b), to prevent damage and sustaining aftereffects on photosynthesis.

S. paniculata can potentially use periods with moderate temperatures because of the high resistance of PSII to photoinhibition and its ability to reach maximum photosynthetic capacity by +5°C. These temperatures typically occur at the beginning and end of the growing season and may occur even below melting snow. Given that there is enough irradiation, and water supply is provided by melting snow, *S. paniculata* could potentially start photosynthesizing even when it is still covered with snow, as reported for arctic plants (Starr and Oberbauer, 2003).

Acknowledgments

This study was financially supported by the Austrian Science fund (FWF project P14524-BOT). We wish to dedicate this article to Prof. Walter Larcher on the occasion of his 75th birthday.

References Cited

Adams III, W. W., Demmig-Adams, B., Rosenstiel, T. N., Brightwell, A. K., and Ebbert, V., 2002: Photosynthesis and photoprotection in overwintering plants. *Plant Biology,* 4: 545–557.

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

Bauer, H., Nagele, M., Comploj, M., Galler, V., Mair, M., and Unterpertinger, E., 1992: Photosynthesis after freezing stress in plants with various degrees of freezing tolerance. *Photosynthetica,* 27: 627–635.

Chalker-Scott, L., 1999: Environmental significance of anthocyanins in plant stress responses. *Photochemistry and Photobiology,* 70: 1–9.

Close, D. C., and Beadle, C. L., 2003: The ecophysiology of foliar anthocyanin. *Botanical Review,* 69: 149–161.

Crawford, R., 1989: *Studies in Plant Survival. Ecological Case Histories of Plant Adaptation to Adversity.* Oxford, London: Blackwell Scientific Publisher, 296 pp.

Demmig-Adams, B., Adams III, W. W., Ebbert, V., and Logan, B. A., 1999: Ecophysiology of the xanthophyll cycle. *In* Frank, H. A., Young, A. J., Britton, G., and Cogdell, R. J. (eds.), *Advances in Photosynthesis. The Photochemistry of Carotenoids. Vol. 8.* Dordrecht: Kluwer Academic Publishers, 245–269.

Gilmore, A. M., and Ball, M. C., 2000: Protection and storage of chlorophyll in overwintering evergreens. *Proceedings of the*

National Academy of Sciences of the United States of America, 97: 11098–11101.

Gould, K. S., 2004: Nature's Swiss army knife: the diverse protective roles of anthocyanins in leaves. *Journal of Biomedicine and Biotechnology,* 5: 314–320.

Havranek, W. M., and Tranquillini, W., 1995: Physiological processes during winter dormancy and their ecological significance. *In* Smith, W. K., and Hinckley, T. M. (eds.), *Ecophysiology of Coniferous Forests.* San Diego: Academic Press, 95–124.

Hegi, G., 1975: *Illustrierte Flora von Mitteleuropa.* Band IV, 2. Teil, Teilband A, bearbeitet von Huber H. Verlag Paul Parey, Berlin.

Horton, P., and Ruban, A. V., 1999: Regulation of the structure and function of the light harvesting complexes of photosystem II by the xanthophyll cycle. *In* Frank, H. A., Young, A. J., Britton, G., and Cogdell, R. J. (eds.), *Advances in Photosynthesis. The Photochemistry of Carotenoids. Vol. 8.* Dordrecht: Kluwer Academic Publishers, 272–291.

Huner, N. P. A., Öquist, G., and Sarhan, F., 1998: Energy balance and acclimation to light and cold. *Trends in Plant Science,* 3: 224–230.

Huner, N. P. A., Öquist, G., and Melis, A., 2003: Photostasis in plants, green algae and cyanobacteria: the role of light harvesting antenna complexes. *In* Green, B. R., and Parson, W. W. (eds.), *Advances in Photosynthesis and Respiration. Light-Harvesting Antennas in Photosynthesis. Vol. 13.* Dordrecht: Kluwer Academic Publisher, 402–421.

Keller, F., and Körner, C., 2003: The role of photoperiodism in alpine plant development. *Arctic, Antarctic, and Alpine Research,* 35: 361–368.

Körner, C., 1994: Impact of atmospheric changes on high mountain vegetation. *In* Beniston, M. (ed.), *Mountain environments in changing climates.* London: Routledge, 155–166.

Körner, C., 1999: *Alpine Plant Life. Functional Plant Ecology of High Mountains Ecosystems.* Berlin: Springer Verlag, 338 pp.

Körner, C., and Perterer, J., 1988: Nehmen immergrüne Waldbäume im Winter Schadgase auf? *Bericht der Gesellschaft für Strahlen–Umweltforschung (GSP), ARGE-ALP Symposium Garmisch,* 1–15.

Krivosheeva, A., Tao, D., Ottander, C., Wingsle, G., Dube, S. L., and Öquist, G., 1996: Cold acclimation and photoinhibition of photosynthesis in Scots pine. *Planta,* 200: 296–305.

Lambers, H., Chapin III, F., and Pons, T., 1998: *Plant Physiology Ecology.* New York: Springer Verlag, 540 pp.

Larcher, W., 2003: *Physiological Plant Ecology. Ecophysiology and Stress Physiology of Functional Groups.* Berlin: Springer Verlag, 513 pp.

Lehner, G., and Lütz, C., 2003: Photosynthetic functions of Cembran pines and Dwarf pines during winter at timberline as regulated by different temperatures, snowcover and light. *Journal of Plant Physiology,* 160: 153–166.

Leverenz, J. W., 1994: Factors determining the nature of the light dosage response curve of leaves. *In* Baker, N., and Bowyer, J. (eds.), *Photoinhibition of Photosynthesis.* Oxford: Bios Scientific Publishers, 239–254.

Matsubara, S., Gilmore, A. M., Ball, M. C., Anderson, J. M., and Osmond, C. B., 2002: Sustained downregulation of photosystem II in mistletoes during winter depression of photosynthesis. *Functional Plant Biology,* 29: 1157–1169.

Meloche, C. G., and Diggle, P. K., 2003: The pattern of carbon allocation supporting growth of preformed shoot primordia in *Acomastylis rossii* (Rosaceae). *American Journal of Botany,* 90: 1313–1320.

Merzlyak, M. N., and Chivkunova, O. B., 2000: Light-stress-induced pigment changes and evidence for anthocyanin photoprotection in apples. *Journal of Photochemistry and Photobiology B–Biology,* 55: 155–163.

Nagele, M., 1989: *Winterliche Veränderungen der Photosyntheseaktivität ausgewählter Holzpflanzen.* Ph.D. thesis. University of Innsbruck, Austria, 148 pp.

Neuner, G., Ambach, D., and Aichner, K., 1999a: Impact of snowcover on photoinhibition and winter desiccation in evergreen *Rhododendron ferrugineum* leaves during subalpine winter. *Tree Physiology,* 19: 725–732.

Neuner, G., Braun, V., Buchner, O., and Taschler, D., 1999b: Leaf rosette closure in the alpine rock species *Saxifraga paniculata* Mill.: significance for survival of drought and heat under high irradiation. *Plant, Cell and Environment,* 22: 1539–1548.

Oberhuber, W., and Bauer, H., 1991: Photoinhibition of photosynthesis under natural conditions in ivy (*Hedera helix* L.) growing in an understory of deciduous trees. *Planta,* 185: 545–553.

Ögren, E., and Evans, J., 1993: Photosynthetic light-response curves. 1. The influence of CO_2 partial pressure and leaf inversion. *Planta,* 189: 182–190.

Öquist, G., and Huner, N. P. A., 2003: Photosynthesis of overwintering evergreen plants. *Annual Review of Plant Biology,* 54: 329–355.

Ottander, C., Campbell, D., and Öquist, G., 1995: Seasonal changes in photosystem II organisation and pigment composition in *Pinus sylvestris. Planta,* 197: 176–183.

Pearce, R. S.; 1999: Molecular analysis of acclimation to cold. *Plant Growth Regulation,* 29: 47–76.

Pietrini, F., and Massacci, A., 1998: Leaf anthocyanin content changes in *Zea Mays* L. grown at low temperature: significance for the relationship between the quantum yield of PSII and the apparent quantum yield of CO_2 assimilation. *Photosynthesis Research,* 58: 213–219.

Sakai, A., and Larcher, W., 1987: *Frost Survival of Plants. Responses and Adaptation to Freezing Stress. Ecological studies, vol. 62.* Berlin: Springer Verlag, 321 pp.

Savitch, L. V., Leonardos, E. D., Krol, M., Jansson, S., Grodzinski, B., Huner, N. P. A., and Öquist, G., 2002: Two different strategies for light utilization in photosynthesis in relation to growth and cold acclimation. *Plant, Cell and Environment,* 25: 761–771.

Starr, G., and Oberbauer, S. F., 2003: Photosynthesis of arctic evergreens under snow: implications for tundra ecosystem carbon balance. *Ecology,* 84: 1415–1420.

Steyn, W. J., Wand, S. J. E., Holcroft, D. M., and Jacobs, G., 2002: Anthocyanins in vegetative tissues: a proposed unified function in photoprotection. *New Phytologist,* 155: 349–361.

Streb, P., Feierabend, J., and Bligny, R., 1997: Resistance to photoinhibition of photosystem II and catalase and antioxidative protection in high mountain plants. *Plant, Cell and Environment,* 20: 1030–1040.

Taschler, D., and Neuner, G., 2004: Summer frost resistance and freezing patterns measured in situ in leaves of major alpine plant growth forms in relation to their upper distribution boundary. *Plant, Cell and Environment,* 27: 737–746.

Tranquillini, W., and Machl-Ebner, I., 1971: Über den Einfluß von Wärme auf das Photosynthesevermögen der Zirbe (*Pinus cembra* L.) und der Alpenrose (*Rhododendron ferrugineum* L.) im Winter. *Report Kevo Subarctic Research Station,* 8: 158–166.

Verhoeven, A. S., Adams III, W. W., and Demmig-Adams, B., 1999: The xanthophyll cycle and acclimation of *Pinus ponderosa* and *Malva neglecta* to winter stress. *Oecologia,* 118: 277–287.

Xin, Z., and Browse, J., 2000: Cold comfort farm: the acclimation of plants to freezing temperatures. *Plant, Cell and Environment,* 23: 893–902.

Revised ms submitted September 2005