Thermotolerance of photosystem 2 of three alpine plant species under field conditions

V. BRAUN, O. BUCHNER, and G. NEUNER

Institute of Botany, University of Innsbruck, Sternwartestrasse 15, 6020 Innsbruck, Austria

Abstract

The species specific response of photosystem 2 (PS2) efficiency and its thermotolerance to diurnal and seasonal alterations in leaf temperature, irradiance, and water relations were investigated under alpine field conditions (1 950 m) and in response to an *in situ* long-term heat treatment (+3 K). Three plant species were compared using the naturally occurring microstratification of alpine environments, *i.e.* under contrasting leaf temperatures but under similar macroclimatic conditions. Thermotolerance of PS2 showed a high variability in all three species of up to 9.6 K. Diurnal changes (increases or even decreases) in PS2 thermotolerance occurred frequently with a maximum increase of +4.8 K in *Loiseleuria procumbens*. Increasing leaf temperatures and photosynthetic photon flux density influenced thermotolerance adjustments. Under long-term heating (+3 K) of *L. procumbens* canopies with infra-red lamps, the maxima of the critical (T_c) and the lethal (T_p) temperature of PS2 increased by at least 1 K. Thermotolerance of the leaf tissue (LT_{50}) increased significantly by +0.6 K. The effects of slight water stress on thermotolerance of PS2 were species specific. High temperature thresholds for photoinhibition were significantly different between species and increased by 9 K from the species in the coldest microhabitat to the species in the warmest. Experimental heating of *L. procumbens* canopies by +3 K caused a significant ($p>0.01$) upward shift of the high temperature threshold for photoinhibition by +3 K. Each species appeared to be very well adapted to the thermal conditions of its microhabitat as under the most frequently experienced daytime leaf temperatures no photoinhibition occurred. The observed fine scale thermal adjustment of PS2 in response to increased leaf temperatures shows the potential to optimise photosynthesis under varying environmental conditions as long as the upper thermal limits are not exceeded.

Additional key words: heat stress; high temperature; *Loiseleuria procumbens*; photoinhibition; *Rhododendron ferrugineum*; *Soldanella pusilla*; thermostability.

Introduction

Photosynthesis has a temperature optimum and distinct low and high temperature limits (for review, see Larcher 1994). The most heat sensitive part of photosynthesis is PS2 (Berry and Björkman 1980). Thermotolerance of PS2, as determined by chlorophyll (Chl) fluorescence measurements during constantly increasing leaf temperatures (Schreiber and Berry 1977), can be affected by different environmental factors. Leaf temperature, irradi-ance, and leaf water potential are capable of inducing long- or short-term adjustments (Weis and Berry 1988). These adjustments in thermotolerance of PS2 have mainly been studied in single factor experiments on potted plants under greenhouse and field conditions with few studies on plants under natural conditions (Seemann *et al.* 1986, Taub *et al.* 2000). In nature, heat stress is usually progressive and high temperatures are accompanied by high

irradiance. Heat stress is further aggravated during drought when stomatal closure prevents cooling by transpiration. Under field conditions the action of each environmental factor may, thus, be much less clear.

Changes in tissue heat tolerance, *i.e.* temperature at which 50 % of leaf tissue is heat damaged, can occur on 80 % of days at a rate of up to 4.7 K d^{-1} in *Silene acaulis*, a typical alpine cushion plant (Neuner *et al.* 2000, Buchner and Neuner 2001). As thermotolerance of PS2 usually corresponds with heat tolerance of the leaf tissue (Bilger *et al.* 1984) similar frequent thermal adjustments could be expected to occur for PS2. However, little is known about how frequently adjustments in thermotolerance of PS2 occur in nature. Particularly in alpine environments, short-term thermotolerance adjustments in PS2 could be ecologically significant. During days with clear sky, excess irradiation, potentially dry soil, and little wind, leaves overheat during the middle of the day with daily leaf temperature amplitudes of up to 50 K (Körner and Larcher 1988). Under such conditions tissue temperatures may come close to or even exceed heat tolerance of the leaves and can produce heat damage (Larcher and Wagner 1976, Gauslaa 1984, Neuner *et al.* 1999).

In alpine environments a strong microclimatic stratification caused by differences in microexposure and plant stature can be found (Cernusca 1976, Körner 1999). This peculiar climatic situation offers the opportunity to investigate high temperature thresholds for photoinhibition and thermotolerance of different plant species that grow in close proximity and are thus exposed to comparable macroclimatic conditions but to distinctly different leaf temperatures.

To test the thermal adaptation of PS2 to leaf temperature under natural conditions we measured PS2 efficiency and the frequency of adjustments of thermotolerance of PS2 *in situ* on leaves of three contrasting alpine plant species. PS2 measurements were conducted at least once a week, from predawn until sunset, throughout the main growing period to cover the wide range of naturally occurring environmental conditions. Leaf temperatures and irradiation climate were monitored together with water relations to get information on the environmental forces driving thermal adjustments in PS2 under alpine field conditions. To test the intra-specific response to elevated leaf temperatures with respect to PS2 efficiency we increased canopy leaf temperatures of *L. procumbens in situ* with infrared lamps. Leaf temperatures were increased by +3 K compared to a reference canopy. The temperature increase reflected an assumed global climatic change scenario with a predicted rise in global mean surface air temperature of 1.5-4.5 K by the middle of the 21st century (Gates 1993, Manabe 1998).

Materials and methods

Experimental site and plant species: The experimental site is situated at the timberline (1 950 m a.s.l.) on the NNW-exposed slope of the summit of Mt. Patscherkofel south of Innsbruck, Austria (47°12'N, 11°27'E). At this site three species with different morphological growth habit grow close to each other. Their particular growing sites differ greatly in exposure to wind and sun. A small population of 0.6-m tall *Rhododendron ferrugineum* L. shrubs grows in a sheltered, 0.8-m deep gully. About 5 m away a population of *Loiseleuria procumbens* (L.) Desv., a typical alpine dwarf shrub (0.05 m canopy height), can be found on an exposed ridge. In between these two sites a population of the herbaceous rosette plant *Soldanella pusilla* Baumg. inhabits a small, steep, north facing slope.

Microclimate measurements were conducted with a data logger (*CR10X Micrologger, Campbell Scientific*, Logan, USA). Photosynthetic photon flux density (PPFD) and air temperature (the sensors were mounted inside non-aspirated radiation shields; *URS1, Campbell Scientific*, Logan, USA) were measured 50 cm and 2 m above ground. Leaf temperatures were measured with type *T* 0.2-mm copper constantan fine-wire thermocouples. PPFD and temperatures were sampled every 30 s and averages were calculated every 30 min. The maximum diurnal increase in leaf temperature, Δd_{LT}, was calculated from daily minimum and maximum leaf temperatures.

Thermotolerance of PS2 was determined in accordance with the method of Schreiber and Berry (1977). Leaves were fixed at a distance of 1 cm to the fibre optic of the *PAM 101* fluorometer (*Walz*, Effeltrich, Germany). A copper-constantan thermocouple was attached to the lower leaf surface to record leaf temperature. Leaves and their attachments were then wrapped together in a heat-proof plastic bag and plunged into a thermostatic water bath. Basic Chl fluorescence (F_0) was recorded with the *PAM 101* fluorometer on these leaves. Leaf temperatures were then increased from 25 to 60 °C at a rate of 0.017 K s^{-1}. Using two fluorometers at each sampling time for each species or treatment gave two replicates. The critical temperature (T_c) and the lethal temperature (T_p) for PS2 were then determined as described by Schreiber and Berry (1977) and Bilger *et al.* (1984) (Fig. 1). Thermotolerance of PS2 was calculated from T_c and T_p as ($T_c + T_p$)/2. The summer variability of thermotolerance of PS2, Δst_{PS2}, was calculated as the difference between summer minimum and maximum values of thermotolerance of PS2. The maximum diurnal increase in thermotolerance of PS2, Δdt_{PS2}, was calculated for *L. procumbens* leaves as the difference between predawn and the afternoon value.

In situ **PS2 fluorescence**: Chl fluorescence of PS2 was measured *in situ* on leaves of the three species. After pre-darkening (20 min), potential efficiency of PS2, F_v/F_m, was recorded with the Plant Efficiency Analyser (*PEA*, *Hansatech*, King's Lynn, Norfolk, England). Day courses of PS2 efficiency were measured from predawn until sunset at least weekly on chosen days to represent the wide range of naturally occurring environmental conditions during the growing period (June to September 1999). Measurements of five randomly chosen leaves of each species and treatment were taken at least every 150 min. This experimental set-up allowed us to determine the efficiency of PS2 and calculate the potential frequency of temperature and PPFD stress situations for PS2 under natural field conditions.
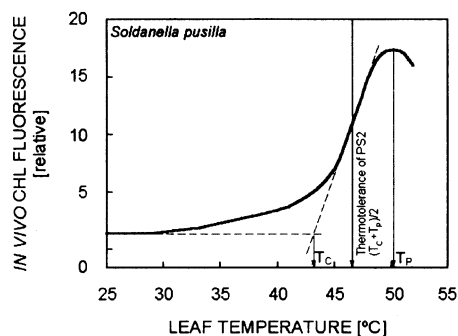

Fig. 1. Changes in *in vivo* chlorophyll (Chl) fluorescence of leaves of *S. pusilla* during controlled heating at a rate of 0.017 K s^{-1} (1 K min^{-1}). The thermotolerance of PS2 is determined as the mean of the critical (T_c) and the lethal (T_p) temperature.

Water relations: *In vivo* studies of thermotolerance of PS2 showed that decreasing water potentials could increase it (Seemann *et al.* 1986, Havaux 1992). Santarius (1973) reported that *in vitro* apparent thermal tolerance of thylakoids increased with increasing sugar concentration in the vacuole. Therefore, actual osmotic leaf water potentials, $\Psi_{0(act)}$, were measured together with the thermotolerance of PS2.

For determination of $\Psi_{0(act)}$, the samples were wrapped in aluminium foil after excision and immediately frozen in liquid nitrogen (Kikuta and Richter 1992). Water potentials were then measured on leaf discs (diameter 0.5 cm) of these freeze-killed leaves in calibrated *C-52* Sample Chambers (*Wescor*, Logan, USA) connected to a *HR-33T* Dew Point Microvoltmeter (*Wescor*, Logan, USA) that was operated in the hygrometric mode. The maximum diurnal decrease of $\Psi_{0(act)}$, $\Delta\Psi_{0(act)}$, was calculated as the difference between the predawn and the afternoon value.

In situ **long-term heat treatment**: During the whole investigation period, selected *L. procumbens* canopies were heated. For this *in situ* long-term heat treatment, four *L. procumbens* canopies which had exactly the same

orientation and exposure to wind and irradiation were chosen. One canopy remained untreated and was used as a reference. The other three canopies were heated so they were +3 K warmer than the reference canopy (Fig. 2).
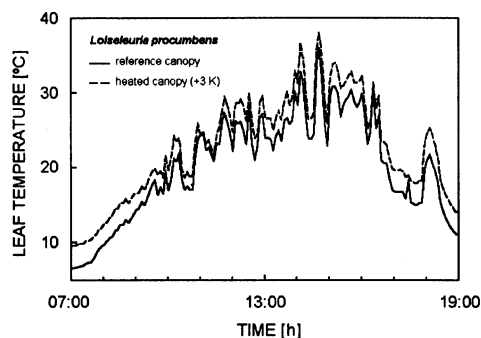

Fig. 2. Typical diurnal change in leaf temperature of *L. procumbens* with naturally occurring leaf temperature fluctuations measured on an untreated reference canopy (*continuous line*) and on canopies that were heated by +3 K with infra-red lamps (*hatched line*) at 1 950 m (a.s.l.) on Mt. Patscherkofel.

Heating was conducted by infrared lamps (type *13713Z/98*, 1 000 W, *Phillips*, Vienna, Austria) using the method described by Nijs *et al.* (1996) that allows a controlled increase in plant canopy temperature. The lamps were equipped with long pass filters (type *RG 850*, *Schott*, Vienna, Austria) that cut off all wavelengths lower than 850 nm emitted by the lamp. These lamps were placed 50 cm above the canopy surface and were mounted to the north of the investigated plants to prevent shading of them. Canopy leaf temperatures were recorded with 5 thermocouples (type *T*, 0.2 mm copper-constantan, fine-wire thermocouples) every 0.125 s on a multiplexer (*AM25T*, *Campbell Scientific*, Logan, USA) connected to a data logger (*CR10X*, *Campbell Scientific*, Logan, USA). Mean leaf temperature of each heated canopy was then compared with the mean leaf temperature of the reference canopy. Depending on the result the lamps were turned on or off.

Statistical data analysis: To determine critical high temperature and PPFD thresholds for significant reductions in PS2 efficiency under field conditions, F_v/F_m, leaf temperature, and PPFD values were analysed using hierarchical cluster analyses (*SPSS* software, *SPSS*, Chicago, IL, USA). Mean values of leaf temperature and PPFD from 1.5 h before each particular fluorescence measurement were used. The significance of differences between the clusters obtained was then tested using Student's *t*-test (*SPSS*, Chicago, IL, USA). The high temperature and PPFD threshold for significant reductions of PS2 efficiency was determined as the mean value of the 95th percentile of the cluster with no reductions in PS2 efficiency and the 5th percentile of the cluster with significant reductions in PS2 efficiency.

The significance of differences between means of different PS2 thermotolerance parameters and between means of different osmotic leaf water potentials for different species were tested by analysis of variance and the Bonferroni test.

Results

Leaf temperature conditions: Even though the investigated plants grow in close proximity, leaf temperatures differed greatly between species (Fig. 3). This micro-stratification of leaf temperature climate is typical of alpine habitats (Körner 1999) and is mainly due to small-scale differences in exposure to irradiation and wind and differences in the morphological growth habit of each species. Leaf temperatures exceeded air temperature by an average of 5 K. *L. procumbens* reached the highest maximum daytime leaf temperature (38.5 °C). *R. ferrugineum* and *S. pusilla* experienced maximum leaf temperatures of 27.0 and of 25.5 °C, respectively (more than 10 K colder).
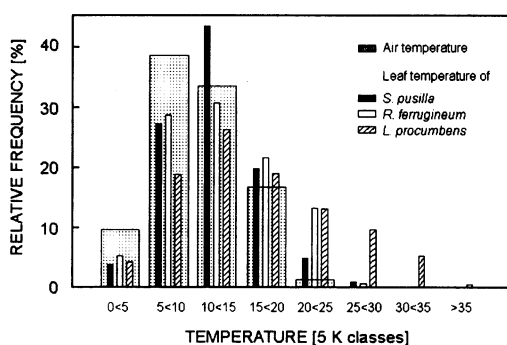

Fig. 3. Relative frequency [%] of daily air (2 m above the ground) and leaf temperature maxima of *L. procumbens*, *R. ferrugineum*, and *S. pusilla* in 5 K temperature classes. Leaf temperatures were measured from June until September 1999 on plants growing less than 5 m apart at 1 950 m (a.s.l.) on Mt. Patscherkofel. Differences in leaf temperatures reflect the effects of small scale differences in exposure and the growth habit peculiar to each species.

Thermotolerance of PS2: The summer means for thermotolerance of PS2 differed significantly (p<0.01) between *L. procumbens* and *S. pusilla* or *R. ferrugineum* (Table 1). The summer mean for T_p increased from the coldest to the warmest microhabitat, *i.e.* from *S. pusilla* to *L. procumbens*, by 4.4 K. Highest T_p values were recorded on *L. procumbens* leaves that were subjected to the long-term heat treatment. Summer means for heat tolerance of the leaf tissue, *i.e.* the temperature at which 50 % of the leaf tissue is heat damaged (LT_{50}), of *L. procumbens* increased significantly (p<0.01) under the long-term heat treatment by 0.6 K (from 49.4±0.1 to 50.0±0.1 °C; unpublished data of Buchner and Neuner). Summer means for heat tolerance of the leaf tissue of *L. procumbens* correspond well with that for thermotolerance of PS2. The summer means for thermotolerance of PS2, however, were not significantly different (p>0.01) between heated and reference plants. In 1999 leaf temperatures at the investigation site never increased to temperatures above 39.5-40 °C that could be critical for the functioning of PS2 (T_c). However, in earlier investigations (Cernusca 1976) and in 1998 at a different experimental site on Mt. Patscherkofel leaf temperatures of all three species came close to or even exceeded the lowest T_c value (values not shown).

Under alpine field conditions frequent adjustments in thermotolerance of PS2 were observed. T_c as well as T_p showed a high variability of more than 8.0 K during the growing period in all three species. Diurnal increases in thermotolerance of PS2, of up to 4.8 K were observed in *L. procumbens* leaves in response to temperature increases over the day (see Table 1). For methodological reasons diurnal changes in thermotolerance of PS2 were studied only on *L. procumbens* leaves. Under field conditions, thermotolerance of PS2 either increased or decreased during the course of day (Fig. 4). Thermotolerance adjustments at a rate of 5 K d^{-1} were recorded. Both rising leaf temperatures and PPFD values appeared to increase the probability of an increase in thermotolerance of PS2. The total diurnal increase in thermotolerance of PS2 depended on the height of the predawn value that explains the scattering of data points in Fig. 4.
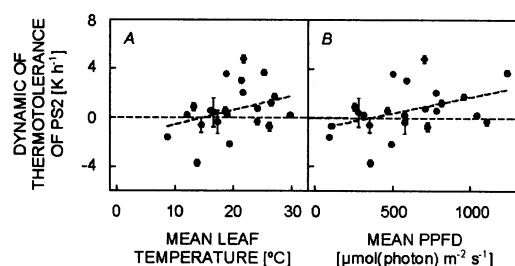

Fig. 4. Temporal dynamic of thermotolerance of PS2 [K h^{-1}] from predawn to afternoon in *L. procumbens* leaves from June until September 1999 compared to (*A*) mean leaf temperature and (*B*) mean PPFD between the times of the two thermotolerance measurements.

Water stress during the growing season of 1999 was not particularly severe but in all three species distinct variations of $\Psi_{0(act)}$ were observed (Table 2) and $\Psi_{0(act)}$ even exceeded actual osmotic water potentials reported for leaves of other alpine species (for review see Körner 1999). The heat treatment with infrared lamps had no significant effect (p>0.01) on leaf osmotic water potential

in *L. procumbens*. Shaw *et al.* (2000) reported similar observations. However, they found a species specific response with either no effect (*Pentaphylloides floribunda*) or a significant effect (*Artemisia tridentata*) of IR-heating (continuous irradiation at 22 W m^{-2}) on midday water potential. During periods with slight water stress as indicated by low $\Psi_{0(act)}$ values a high variation in thermotolerance of PS2 and the highest overall values were recorded on leaves of *R. ferrugineum* and *S. pusilla* (Fig. 5). For *L. procumbens* (untreated and heated +3 K) no distinct effect of the osmotic leaf water potential on thermotolerance of PS2 was observed during the growing period.

Table 1. Summer mean ±SE, minimum (min), and maximum (max) [°C] and summer, Δst_{PS2} and diurnal Δdt_{PS2} [K] variability of T_c, T_p, and thermotolerance of PS2 of leaves of *R. ferrugineum*, *S. pusilla*, and *L. procumbens* untreated or [x] subjected to a long-term heat treatment (+3 K). Maximum diurnal leaf temperature increase, Δd_{LT} [K] is also shown. The significance of differences between summer means of the different species and treatments is indicated by different capital letters and was tested by ANOVA and the Bonferroni test ($p < 0.01$) ($n = 99$ per species and treatment).

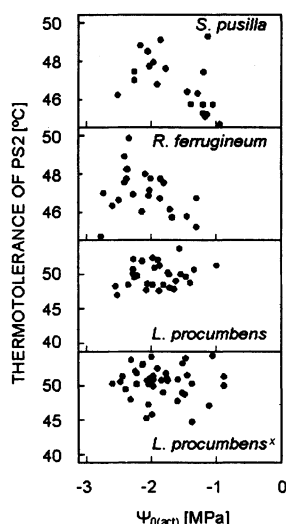|	Species	mean±SE	min – max	Δst_{PS2}	Δdt_{PS2}	Δd_{LT}
T_c	*S. pusilla*	43.6±0.3 [A]	39.5 – 49.0	9.5		23.6
	R. ferrugineum	43.9±0.2 [A]	39.8 – 47.8	8.0		20.1
	L. procumbens	46.4±0.3 [B]	40.0 – 52.3	12.3	5.2	36.4
	L. procumbens[x]	46.4±0.3 [B]	39.5 – 54.0	14.5	4.5	38.8
T_p	*S. pusilla*	49.2±0.3 [A]	45.5 – 54.0	8.5		
	R. ferrugineum	50.9±0.3 [B]	46.0 – 56.0	10.0		
	L. procumbens	53.6±0.2 [C]	48.0 – 57.5	9.5	4.6	
	L. procumbens[x]	54.0±0.1 [C]	49.0 – 58.5	9.5	2.9	
Thermotolerance of PS2 $(T_c + T_p)/2$	*S. pusilla*	46.3±0.3 [A]	44.0 – 49.3	5.3		
	R. ferrugineum	47.3±0.2 [A]	44.8 – 50.7	5.9		
	L. procumbens	50.0±0.3 [B]	44.6 – 53.8	9.2	4.8	
	L. procumbens[x]	50.2±0.2 [B]	44.8 – 54.4	9.6	3.7	

Fig. 5. Thermotolerance of PS2 [°C] at different actual osmotic leaf water potentials ($\Psi_{0(act)}$) measured on leaves of *S. pusilla*, *R. ferrugineum*, *L. procumbens*, and on leaves of *L. procumbens* ([x]) plants that were exposed to a controlled heat treatment (+3 K) from June until September 1999.

Leaf temperature and PPFD effects on PS2 efficiency: Significant changes in PS2 efficiency were observed in response to diurnal changes in environmental conditions (Fig. 6). High leaf temperature and PPFD thresholds for reductions of PS2 efficiency differed distinctly between

Table 2. Summer mean, minimum (min), and maximum (max) [MPa] and maximum (mean) diurnal decrease, $\Delta\Psi_{0(act)}$ [MPa] in leaves of *R. ferrugineum*, *S. pusilla*, and *L. procumbens* (untreated) or ([x]) subjected to a long-term heat treatment (+3 K). The significance of differences between summer means of the different species and treatments is indicated by different capital letters and was tested by ANOVA and the Bonferroni test ($p < 0.01$) ($n = 380$ per species and treatment).

Species	$\Psi_{0(act)}$ mean±SE	min/max	$\Delta\Psi_{0(act)}$
S. pusilla	-1.6±0.03[A]	-3.3/-0.3	-1.0 (-0.6)
R. ferrugineum	-2.0±0.03[B]	-3.8/-0.4	-1.3 (-0.7)
L. procumbens	-1.8±0.04[C]	-3.4/-0.5	-1.4 (-0.7)
L. procumbens[x]	-1.9±0.03[C]	-4.0/-0.3	-1.2 (-0.6)

species. High temperature thresholds increased from the species in the coldest to the species in the warmest microhabitat by 9 K (Fig. 7). Significant reductions in PS2 efficiency were measured at leaf temperatures above 22 °C for L. procumbens, above 14 °C for R. ferrugineum and above 13 °C for S. pusilla. Such leaf temperatures occurred at the investigation site with a relative frequency of between 41 and 20 % depending on the species and coincided with irradiances higher than 900 μmol(photon) m^{-2} s^{-1} in all three species (values not shown). Thus, under such irradiances significant reductions in PS2 efficiency are highly probable.

In a controlled heat treatment experiment, leaf temperatures of *L. procumbens* were increased by exactly +3 K with reference to an untreated *L. procumbens* canopy throughout the whole growing period. These heated plants were used to study the effect of increased leaf tem-

peratures on PS2 activity. In response to the controlled heat treatment the high temperature threshold for reductions of PS2 efficiency of *L. procumbens* increased significantly ($p<0.01$) by + 3 K (see Fig. 7).

Discussion

Variability of thermotolerance of PS2 under alpine field conditions: Thermotolerance of PS2 (T_c and T_p) was in the range reported for various other plant species (Bilger *et al.* 1984, Havaux 1993, Valladares and Pearcy 1997). Thermotolerance of PS2 showed a high summer variability (of up to 9.6 K) in all three species and a high diurnal variability (of up to 4.8 K) in *L. procumbens*. This indicates frequent adjustments in thermotolerance of PS2 under alpine field conditions. Changes in heat tolerance

Fig. 6. Diurnal variation in PS2 efficiency (F_v/F_m) in leaves of *S. pusilla* in response to changes in leaf temperature measured at the investigation site at 1 950 m (a.s.l.) on Mt. Patscherkofel.

of the leaf tissue can occur as frequently and at similar diurnal rates in *S. acaulis* (up to 4.7 K d^{-1}) with leaf heating rates of up to $0.002\,8 \text{ K s}^{-1}$ (10 K h^{-1}) at leaf temperatures higher than 30 °C (Neuner *et al.* 2000, Buchner and Neuner 2001). The rapid diurnal adjustments in thermotolerance of PS2 observed *in situ* could be a particularly important adaptation to environmental conditions in alpine habitats where extreme amplitudes of daily leaf temperature can occur. Daily leaf temperature amplitudes are commonly 25 K and can be as high as 50 K (Körner and Larcher 1988). In the present investigation we recorded a maximum of diurnal leaf temperature increase of 38.8 K for *L. procumbens*. However, comparable rates of short-term adjustment in thermotolerance of PS2 are reported for several other plant species. A 5 K increase in thermotolerance of PS2 triggered by a slight change in leaf temperature within 0.5-1.0 h is reported for potato leaves (Havaux 1993). In *Heteromeles arbutifolia* a diurnal increase in thermotolerance of PS2 of less than 3 K in response to the natural diurnal increase in leaf temperature was observed (Valladares and Pearcy

1997). Although these observations are not directly comparable due to varying experimental set-ups they may indicate that the rapid adjustments in thermotolerance of PS2 are not peculiar to alpine plant species. However, as discussed earlier (Havaux 1993, Valladares and Pearcy 1997), dynamic as opposed to intrinsic thermotolerance is probably ecologically more significant particularly for alpine plant species.
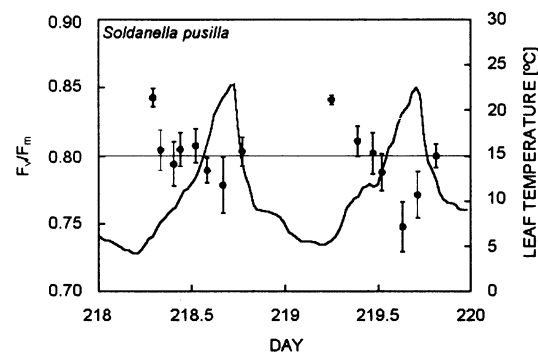
Leaf temperature and irradiation effects on thermotolerance of PS2: Under the natural growth conditions, leaf temperature and irradiance influenced changes in thermotolerance of PS2 in *L. procumbens*. Under the experimental long-term heat treatment where leaf temperatures were increased by +3 K and all other factors remained unchanged, an increase of maximum T_c and T_p and hence the thermotolerance of PS2 was observed. Summer means, however, were not significantly different. This is not surprising as heat hardening is not observed before critical high temperature thresholds are surpassed (Alexandrov 1977) and it can be expected that only on a few days heat hardening effective leaf temperature conditions occurred solely on the heated plots. Heated plants were, however, exposed for a longer time period to high temperatures that could explain the more distinct effect in maximum values. This leaf temperature effect under natural conditions corroborates earlier findings that long-term and short-term elevated leaf temperatures are able to increase thermotolerance of PS2 (Weis and Berry 1988, Havaux 1993, Königer *et al.* 1998). In nature, high leaf temperatures co-occur with high irradiances. We therefore cannot exclude effects of irradiance that also can affect thermotolerance of PS2 (Schreiber and Berry 1977, Weis 1982).

Osmotic water potential effects on thermotolerance of PS2: The influence of water stress on thermotolerance of PS2 under alpine field conditions and for the plant species investigated was not clear. Under slight water stress, thermotolerance of PS2 was highly variable and the highest overall values for thermotolerance of PS2 were found in leaves of *R. ferrugineum* and *S. pusilla*. In leaves of *L. procumbens* no distinct response to the observed osmotic leaf water potential deficits was observed. In several other species dehydration (Seemann *et al.* 1986, Havaux 1992, Epron 1997, Lu and Zhang 1999) and also osmotic stresses such as salinity (Larcher *et al.* 1990)

increased thermotolerance of PS2. On the other hand, drought-preconditioned cedar seedlings were able to keep their increased PS2 thermotolerance over a long time period even when drought stress was removed (Ladjal et al. 2000). Similar observations were reported by Valladares and Pearcy (1997): an increased thermotolerance of PS2 was maintained for more than one month after re-watering *H. arbutifolia* plants. This could explain why we observed partly a similar high thermotolerance of PS2 in leaves of *R. ferrugineum* and *S. pusilla* at both high and low osmotic leaf water potentials. Our results could indicate the potential existence of plant functional types. Our results for *L. procumbens* could also indicate that water stress was not particularly severe, although, $\Psi_{0(act)}$ values measured during the 1999 summer dropped below the lowest actual osmotic water potentials reported for other alpine species (for review, see Larcher 1977, Körner 1999).
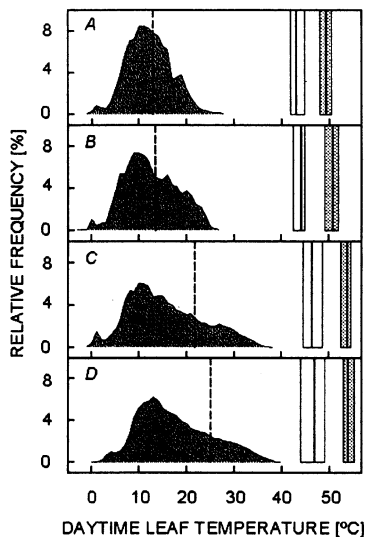

Fig. 7. Relative frequency [%] of leaf temperatures (*grey shaded aerogramm*; 1 K temperature classes) measured during daytime hours [PPFD $\geq$ 30 $\mu mol(photon)$ m^{-2} s^{-1}] from June until September 1999 at the investigation site at 1 950 m (a.s.l.) on Mt. Patscherkofel on leaves of (*A*) *S. pusilla*, (*B*) *R. ferrugineum*, (*C*) *L. procumbens* untreated and (*D*) *L. procumbens* exposed to a controlled heat treatment (+3 K). The high temperature thresholds for photoinhibition were determined as the mean value of the 95th percentile of the cluster with no reductions in PS2 efficiency and the 5th percentile of the cluster with significant reductions in PS2 efficiency and are indicated by a scattered line. The range of thermal limits for PS2 efficiency are indicated by *light grey bars* (T_c) and *dark grey bars* (T_p). (Bars range from the 25th to the 75th percentile; median value is given by a *thick solid black line*.)

High temperature and PPFD thresholds for photoinhibition: The reversible reductions in PS2 efficiency observed above the species specific high temperature and PPFD threshold result from excess photon absorption (Demmig-Adams and Adams 1992, Long et al. 1994)

exacerbated by high leaf temperatures (Gamon and Pearcy 1990, Valladares and Pearcy 1997, Königer et al. 1998). In nature, high leaf temperatures and high irradiances co-occur and particularly in alpine environments leaf temperatures are strongly driven by irradiation. Therefore, the observed reversible reductions in PS2 efficiency must be considered to be the result of both factors. The complete reversibility of the reductions in PS2 efficiency over night or even before sunset (values not shown) indicates that they were due to thermal energy dissipation from the antenna system of PS2 associated with the xanthophyll cycle (Demmig-Adams and Adams 1992). Significant reductions in PS2 efficiency occurred at irradiances higher than 900 $\mu mol(photon)$ $m^{-2} s^{-1}$ that is above the saturation irradiance for carbon gain of leaves of *L. procumbens* [385-545 $\mu mol(photon)$ m^{-2} s^{-1}: Grabherr 1977] and *R. ferrugineum* [600-800 μmol (photon) m^{-2} s^{-1}: Siegwolf and Cernusca 1984]. At these irradiances the rate of CO_2 uptake is no longer limited by photochemical processes but rather by enzymatic ones and the supply of CO_2. Reductions in PS2 efficiency in this supra-optimum environmental range are thus of limited importance with respect to carbon gain.

Our results further indicate that, at least for *L. procumbens* under the long-term heat treatment (+3 K), species readily adjust PS2 efficiency thermally to the leaf temperature climate peculiar to a growing site. This is important as within the distribution boundaries of each species a variety of microenvironmental conditions may occur. Thermal adjustments of PS2 efficiency evidently play an important role in nature. Such thermal adjustments including alterations in thermotolerance of PS2 may be achieved by different protective mechanisms. These mechanisms include xanthophyll cycle carotenoids and their influence on thylakoid membrane fluidity (Havaux and Tardy 1996, Havaux 1998), emission of the hydrocarbon isoprene (Sharkey and Singaas 1995), protective soluble compounds in the chloroplast stroma (Santarius 1973, Seemann et al. 1986), and the level of unsaturation of thylakoid membrane fatty acids and its influence on membrane heat stability (Thomas et al. 1986, Murakami et al. 2000, Alfonso et al. 2001). A potential role for chloroplast heat shock proteins, particularly low molecular mass heat shock proteins, is also under discussion (Heckathorn et al. 1998, Preczewski et al. 2000, Härndahl and Sundby 2001).

Conclusion: PS2 of all three species appears well adapted to the leaf temperatures peculiar to each growing site. *L. procumbens* at the investigation site has the potential for thermal adjustments of PS2 that would be sufficient to adapt PS2 thermally to the predicted temperature rise. However, this result may not hold true for warmer growing sites at distribution boundaries of the species. In nature, thermotolerance of PS2 is adjusted frequently at highly dynamic rates that appear appropriate to prevent

PS2 heat damage. The predicted temperature increase may thus not pose a severe problem with respect to PS2 functioning as long as high leaf temperature extremes, that are also anticipated to increase in frequency with global climate change (Wagner 1996), do not exceed rates of change and the upper thermal limits of PS2.

References

Alexandrov, V.Y.: Cells, Molecules and Temperature. Conformational Flexibility of Macromolecules and Ecological Adaptation. – Springer, Berlin – Heidelberg – New York 1977.

Alfonso, M., Yruela, I., Almarcegui, S., Torrado, E., Perez, M.A., Picorel, R.: Unusual tolerance to high temperatures in a new herbicide-resistant D1 mutant from *Glycine max* (L.) Merr. cell cultures deficient in fatty acid desaturation. – Planta 212: 573-582, 2001.

Berry, J., Björkman, O.: Photosynthetic response and adaptation to temperature in higher plants. – Annu. Rev. Plant Physiol. 31: 491-543, 1980.

Bilger, H.-W., Schreiber, U., Lange, O.L.: Determination of leaf heat resistance: comparative investigation of chlorophyll fluorescence changes and tissue necrosis methods. – Oecologia 63: 256-262, 1984.

Buchner, O., Neuner, G.: Determination of heat tolerance: A new equipment for field measurements. – J. appl. Bot. 75: 130-137, 2001.

Cernusca, A.: Bestandesstruktur, Bioklima und Energiehaushalt von alpinen Zwergstrauchbeständen. – Oecol. Plant. 11: 71-102, 1976.

Demmig-Adams, B., Adams, W.W., III: Photoprotection and other responses of plants to high light stress. – Annu. Rev. Plant Physiol. Plant mol. Biol. 43: 599-626, 1992.

Epron, D.: Effects of drought on photosynthesis and on the thermotolerance of photosystem II in seedlings of cedar (*Cedrus atlantica* and *C. libani*). – J. exp. Bot. 48: 1835-1841, 1997.

Gamon, J.A., Pearcy, R.W.: Photoinhibition in *Vitis californica*. The role of temperature during high-light treatment. – Plant Physiol. 92: 487-494, 1990.

Gates, G.M.: Climate Change and its Biological Consequences. – Sinauer, Sunderland 1993.

Gauslaa, Y.: Heat resistance and energy budget in different Scandinavian plants. – Holarct. Ecol. 7: 1-78, 1984.

Grabhherr, G.: Der CO_2-Gaswechsel des immergrünen Zwergstrauches *Loiseleuria procumbens* (L.) Desv. in Abhängigkeit von Strahlung, Temperatur, Wasserstreß und phänologischem Zustand. – Photosynthetica 11: 302-310, 1977.

Härndahl, U., Sundby, C.: Does the chloroplast small heat shock protein protect photosystem II during heat stress in vitro? – Physiol. Plant. 111: 273-275, 2001.

Havaux, M.: Stress tolerance of photosystem II *in vivo*. Antagonistic effects of water, heat, and photoinhibition stresses. – Plant Physiol. 100: 424-432, 1992.

Havaux, M.: Rapid photosynthetic adaptation to heat stress triggered in potato leaves by moderately elevated temperatures. – Plant Cell Environ. 16: 461-467, 1993.

Havaux, M.: Carotenoids as membrane stabilizers in chloroplasts. – Trends Plant Sci. 3: 147-151, 1998.

Havaux, M., Tardy, F.: Temperature-dependent adjustment of the thermal stability of photosystem II *in vivo*: possible involvement of xanthophyll-cycle pigments. – Planta 198: 324-333, 1996.

Heckathorn, S.A., Downs, C.A., Sharkey, T.D., Coleman, J.S.: The small, methionine-rich chloroplast heat-shock protein protects photosystem II electron transport during heat stress. – Plant Physiol. 116: 439-444, 1998.

Kikuta, S.B., Richter, H.: Leaf discs or press sap? A comparison of techniques for the termination of osmotic potentials in freeze-thawed leaf material. – J. exp. Bot. 43: 1039-1044, 1992.

Königer, M., Harris, G.C., Pearcy, R.W.: Interaction between photon flux density and elevated temperatures on photoinhibition in *Alocasia macrorrhiza*. – Planta 205: 214-222, 1998.

Körner, C.: Alpine Plant Life. Functional Plant Ecology of High Mountain Ecosystems. – Springer, Berlin 1999.

Körner, C., Larcher, W.: Plant life in cold climates. – In: Long, S.P., Woodward, F.I. (ed.): Plants and Temperature. Pp. 25-57. The Company of Biologists, Cambridge 1988.

Ladjal, M., Epron, D., Ducrey, M.: Effects of drought preconditioning on thermotolerance of photosystem II and susceptibility of photosynthesis to heat stress in cedar seedlings. – Tree Physiol. 20: 1235-1241, 2000.

Larcher, W.: Ergebnisse des IBP-Projekts "Zwergstrauchheide Patscherkofel". – Sitzungsber. österr. Akad. Wiss., math.-naturwiss. Kl., Abt. I 186: 301-371, 1977.

Larcher, W.: Photosynthesis as a tool for indicating temperature stress events. – In: Schulze, E.-D., Caldwell, M.M. (ed.): Ecophysiology of Photosynthesis. Pp. 261-277. Springer-Verlag, Berlin 1994.

Larcher, W., Wagner, J.: Temperaturgrenzen der CO_2-Aufnahme und Temperaturresistenz der Blätter von Gebirgspflanzen im vegetationsaktiven Zustand. – Oecol. Plant. 11: 361-374, 1976.

Larcher, W., Wagner, J., Thammathaworn, A.: Effects of superimposed temperature stress on *in vivo* chlorophyll fluorescence of *Vigna unguiculata* under saline stress. – J. Plant Physiol. 136: 92-102, 1990.

Long, S.P., Humphries, S., Falkowski, P.G.: Photoinhibition of photosynthesis in nature. – Annu. Rev. Plant Physiol. Plant mol. Biol. 45: 633-662, 1994.

Lu, C., Zhang, J.: Effects of water stress on photosystem II photochemistry and its thermostability in wheat plants. – J. exp. Bot. 50: 1199-1206, 1999.

Manabe, S.: Study of global warming by GFDL climate models. – Ambio 27: 182-186, 1998.

Murakami, Y., Tsuyama, M., Kobayashi, Y., Kodama, H., Iba, K.: Trienoic fatty acids and plant tolerance of high temperature. – Science 287: 476-479, 2000.

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

Neuner, G., Buchner, O., Braun, V.: Short term changes in heat tolerance in the alpine cushion plant *Silene acaulis ssp. excapa* [All.] J. Braun at different altitudes. – Plant Biol. 2: 677-683, 2000.

Nijs, I., Kockelberg, F., Teughels, H., Blum, H., Hendrey, G.,

Impens, I.: Free air temperatures increase (FATI): a new tool to study global warming effects on plants in the field. – Plant Cell Environ. **19**: 495-502, 1996.

Preczewski, P.J., Heckathorn, S.A., Downs, C.A., Coleman, J.S.: Photosynthetic thermotolerance is quantitatively and positively correlated with production of specific heat-shock proteins among nine genotypes of *Lycopersicon* (tomato). – Photosynthetica **38**: 127-134, 2000.

Santarius, K.A.: The protective effect of sugars on chloroplast membranes during temperature and water stress and its relationship to frost, desiccation and heat resistance. – Planta **113**: 105-114, 1973.

Schreiber, U., Berry, J.A.: Heat-induced changes of chlorophyll fluorescence in intact leaves correlated with damage of the photosynthetic apparatus. – Planta **136**: 233-238, 1977.

Seemann, J.R., Downton, W.J.S., Berry, J.A.: Temperature and leaf osmotic potential as factors in the acclimation of photosynthesis to high temperature in desert plants. – Plant Physiol. **80**: 926-930, 1986.

Sharkey, T.D., Singaas, E.L.: Why plants emit isoprene. – Nature **374**: 769, 1995.

Shaw, M.R., Loik, M.E, Harte, J.: Gas exchange and water relations of two Rocky Mountain shrub species exposed to a climate change manipulation. – Plant Ecol. **146**: 197-206, 2000.

Siegwolf, R., Cernusca, A.: CO$_2$-Gaswechsel von *Rhododendron ferrugineum* an der alpinen Waldgrenze. – Verhandl. Ges. Ökol. **12**: 111-121, 1984.

Taub, D.R., Seemann, J.R., Coleman, J.S.: Growth in elevated CO$_2$ protects photosynthesis against high-temperature damage. – Plant Cell Environ. **23**: 649-656, 2000.

Thomas, P.G., Dominy, P.J., Vigh, L., Mansourian, A.R., Quinn, P.J., Williams, W.P.: Increased thermal stability of pigment-protein complexes of pea thylakoids following catalytic hydrogenation of membrane lipids. – Biochim. biophys. Acta **849**: 131-140, 1986.

Valladares, F., Pearcy, R.W.: Interactions between water stress, sun-shade acclimation, heat tolerance and photoinhibition in the sclerophyll *Heteromeles arbutifolia*. – Plant Cell Environ. **20**: 25-36, 1997.

Wagner, D.: Scenarios of extreme temperature events. – Climatic Change **33**: 385-407, 1996.

Weis, E.: The influence of metal cations and pH on the heat sensitivity of photosynthetic oxygen evolution and chlorophyll fluorescence in spinach chloroplasts. – Planta **154**: 41-47, 1982.

Weis, E., Berry, J.A.: Plants and high temperature stress. – In: Long, S.P., Woodward, F.I. (ed.): Plants and Temperature. Pp. 329-346. Company of Biologists, Cambridge 1988.

BOOK REVIEW

After 13 years, a revised and expanded second edition of this book appeared in the INRA series "Du labo au terrain". Five scientists working in the INRA, French National Institute of Agronomic Research, wrote it in French. The volume is divided into three parts. The 1st part deals with root system of plants grown in pots and containers, describes the effects of volume limitation on root growth, and gives particular constraints for irrigation, fertilisation, and mineral nutrition. The use of cultivated plants, and differences in cultivation systems follow.

The second part is devoted to culture installation (choice of materials, preparations of substrates, disinfecting of substrates, *etc.*). The third part of the volume summarises the growing praxis, mainly irrigation and fertilisation.

42 tables and 64 figures, mainly graphs, help the reader in understanding the text, even if it is written in French. Over 50 colour photographs are presented on eight plates. The attached list of references presents more than 200 items. There is a useful glossary. The book is supplemented also with subject and plant indexes.

Although the volume is specialised on plant culture in pots and containers, the readers of Photosynthetica can find here interesting information for experimental praxis, models, and tools.

The book, interesting for plant scientists and many other specialists, is available from INRA Editions, Route de St Cyr, F-78026 Versailles Cedex, France.

J. ČATSKÝ (*Praha*)