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Effects of temperature and irradiance on quantum yield of PSII photochemistry and xanthophyll cycle in a tropical and a temperate species

A. DONGSANSUK, C. LÜTZ, and G. NEUNER+

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

Abstract

The effect of a wide range of temperatures (–15 and 60°C) in darkness or under strong irradiation [1,600 μmol(photon) m^{-2} s^{-1}] on quantum yield of photosystem II photochemistry and xanthophyll cycle pigments was investigated in a tropical fruit crop (*Musa* sp.) and a temperate spring flowering plant (*Allium ursinum* L.). In darkness within the nonlethal thermal window of *A. ursinum* (from –6.7 to 47.7°C; 54.5 K) and of *Musa* sp. (from –2.2°C to 49.5°C; 51.7 K) maximal quantum yield of PSII photochemistry (F_v/F_m) was fairly unaffected by temperature over more than 40 K. At low temperature F_v/F_m started to drop with ice nucleation but significantly only with initial frost injuries (temperature at 10% frost damage; LT_{10}). The critical high temperature threshold for PSII (T_c) was 43.8°C in *A. ursinum* and 44.7°C in *Musa* sp. Under strong irradiation, exposure to temperatures exceeding the growth ones but being still nonlethal caused photoinhibition in both species. Severity of photoinhibition increased with increasing distance to the growth temperature range. $\Delta F/F_m'$ revealed distinctly different optimum temperature ranges: 27–36°C for *Musa* sp. and 18–27°C for *A. ursinum* exceeding maximum growth temperature by 2–7 K. In both species only at temperatures > 30°C zeaxanthin increased and violaxanthin decreased significantly. At nonlethal low temperature relative amounts of xanthophylls remained unchanged. At temperatures > 40°C β-carotene increased significantly in both species. In *Musa* sp. lutein and neoxanthin were significantly increased at 45°C, in *A. ursinum* lutein remained unchanged, neoxanthin levels decreased in the supraoptimal temperature range. In darkness, F_v/F_m was highly temperature-insensitive in both species. Under strong irradiation, whenever growth temperature was exceeded, photoinhibition occurred with xanthophylls being changed only under supraoptimal temperature conditions as an antiradical defence mechanism.

Additional key words: *Allium ursinum*; β-carotene; freezing stress; heat; ice nucleation; lutein; *Musa* sp.; photosynthetic pigments.

Introduction

Chlorophyll (Chl) fluorescence studies have long been used as an indicator of functional changes of PSII photochemistry under temperature stress (Berry and Björkman 1980). Numerous studies have investigated the effect of certain temperatures in combination with various irradiation intensities (*e.g.* Lichtenthaler 1998, Adams and Demmig-Adams 2004). However, hardly any study except for Pospíšil *et al.* (1998) working with spring barley, has investigated the temperature response of quantum yield of PSII photochemistry in darkness within the growth temperature range and the whole nonlethal stress temperature range including both low- and high-temperature thermal limits of photosynthetic functions. Particularly in the low-temperature range, chilling-susceptible tropical species may show significant differences when compared to freezing-tolerant plants. While in the tropical species ice formation in the mesophyll exerts immediate damage, mesophyll cells of freezing-tolerant plants survive extracellular ice formation and consequent freeze dehydration down to a certain

Received 13 January 2012, *accepted* 5 September 2012.
+Corresponding author; fax: +43–512–50751099, phone: +43–512–51026, e-mail: gilbert.neuner@uibk.ac.at
Abbreviations: Chl *a*(*b*) – chlorophyll *a*(*b*); F_0 – minimal fluorescence in the dark-adapted state; F_0/T – temperature-dependent change in minimal fluorescence; F_m – maximal fluorescence in the dark-adapted state; F_m' – maximal fluorescence in the light-adapted state; F_s – steady state fluorescence; F_v – variable fluorescence; F_v/F_m – maximal quantum yield of PSII photochemistry; $\Delta F/F_m'$ – effective quantum yield of PSII photochemistry; LT_{10} (LT_{50}) – temperature at 10% (50%) heat or frost damage, respectively; PPFD – photosynthetic photon flux density; PSII – photosystem II; ROS – reactive oxygen species; T_c – critical high temperature threshold of the F_0/T curve calculated as the intersection of a horizontal line at the level of F_0 at 20°C and of a line fitted visually to the fast rising phase; T_p – peak temperature of the F_0/T curve.
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low freezing temperature. Both processes must have effects on functioning of PSII photochemistry.

Under heat-stress conditions natural sunlight can result either in the inactivation or oxidation of photosynthetic proteins in PSII (Huner *et al*. 1993, Baker 1996). Irradiation absorbed in excess under adverse temperatures can be dissipated to some extent as heat in the antenna pigment complexes (Demmig-Adams *et al.* 1995, Genty *et al.* 1990, Gilmore 1997), until a certain amount where the capacity of thermal dissipation is overrun and photooxidative damage occurs. Thermal dissipation of excessive excitation energy is closely related to the xanthophyll cycle activity. Temperature response under superimposed strong irradiation of PSII quantum yield and of the xanthophyll cycle activity has hardly been studied over the whole nonlethal temperature range of a species. The same is for other carotenoids such as β-carotene, lutein and neoxanthin that may additionally function to protect plants against photodamage.

Comparisons of photosynthetic performances of plants that are grown under natural field conditions in various biomes and in relation to plant distribution have a long tradition, aiming to compare and contrast photosynthetic properties in very different ecosystem with the same mechanistic base (for review *see* Woodward and Smith 1994). While the temperature response of photosynthetic CO_2 gas exchange is well assessed in this respect (Woodward and Kelly 1997), comparatively little information, except for the comparison of heat limits of PSII of alpine, temperate, and tropical plants (Smillie and Nott 1979), exists on the overall temperature response of primary processes of photosynthesis and photosynthetic pigments.

Hence, we investigated differences in the whole temperature response of quantum yield of PSII photochemistry, xanthophyll cycle activity and photosynthetic pigment content between a chilling susceptible tropical species and a freezing-tolerant temperate plant in darkness and under superimposed strong irradiation. Between –15 and 60°C the species-specific response was determined within its growth temperature range and by assessment of thermal limits within its nonlethal thermal window (stress temperature range) but also at temperatures exceeding thermal limits.

Materials and methods

Plants: *Allium ursinum* L. is a temperate spring geophyte growing in the understorey at the Arboretum of the Botanical Garden of the University of Innsbruck. *Musa* sp. grew in the tropical greenhouse of the Botanical Garden. *A. ursinum* grew in the natural soil (pH 6–7). *Musa* sp. was cultivated in the following mixture of substrates: 40% humus, 25% peat moss, 20% sand, and 15% lavalit (pH 6.5). In the tropical greenhouse air humidity was between 80 and 95% and in the field during sunny days approximately 60%. Growth temperature conditions differed significantly between the two species. While *Musa* sp. was exposed to a 30/20°C day/night temperature cycle in the tropical greenhouse, *A. ursinum* was exposed to the natural daily temperature fluctuations of air from a night mean minimum of 6.4 ± 3.9°C to a daytime mean maximum of 20.2 ± 5.0°C. During the investigation period mean daily PPFD maximum was 1,440 μmol(photon) m^{-2} s^{-1}. Irradiation between the two plant species was comparable because the growing sites are in close vicinity. The spectral transmittance of the roofing of the tropical greenhouse allows similar transmittance of all irradiation classes. During the experiments the leaves of *A. ursium* were also exposed to full sunlight. Plants were sufficiently supplied with water throughout the experimental period. Fully expanded leaves of *A. ursinum* were collected during blooming. Fully developed leaves of *Musa* sp. were taken from 11-month-old individuals. Leaves from *A. ursinum* and *Musa* sp. were collected in the morning (8.00–10.00 MET) from the beginning of April until the end of May of 2010 and 2011 immediately before the onset of measurements. After detachment leaves were transferred inside a temperature-controlled leaf chamber (20°C) to the laboratory.

Temperature treatment: For temperature treatment 5 leaves per species were placed in a temperature-controlled refrigerator (Hacker and Neuner 2007) on wetted paper towels in order to keep them fully turgid during measurements. Leaf temperature was either lowered starting at 25°C down to –10°C or increased from 20°C to 60°C at cooling and heating rates of 12 K h^{-1}, respectively. Five copper constant thermocouples (solder junction diameter: 0.3 mm) were used, each was attached to a single leaf on the lower leaf surface. Leaf temperature data were recorded every second on a data logger (*CR10X data logger, Campbell Scientific Instruments*, Logan, UT, USA). During the temperature treatment leaves were either kept in complete darkness or illuminated under saturating irradiation [PPFD: 1,600 μmol(photons) m^{-2} s^{-1}] using a lighting unit (*FL-460, Walz*, Effeltrich, Germany).

Chl fluorescence measurements: By employment of five PAM fluorometers (three *PAM 101, PAM-2000* and *Mini-PAM; Walz*, Effeltrich, Germany) 5 measurements could be taken during each temperature run. Chl fluorescence was measured on the upper leaf surfaces. The following fluorescence parameters were measured: minimal fluorescence in the dark-adapted state (F_0), steady-state fluorescence under an irradiation of 1,600 μmol(photon) m^{-2} s^{-1} and upon application of a saturating light pulse (F_s), the maximal fluorescence in the dark-adapted state (F_m), and the maximal fluorescence in the light-adapted state [1,600 μmol(photon) m^{-2} s^{-1}] (F_m').

Maximal quantum yield of PSII photochemistry, F_v/F_m (Eq. 1), and effective quantum yield of PSII photochemistry, $\Delta F/F_m'$ (Eq. 2), were calculated according to Schreiber (2004).

$$F_v/F_m = (F_m - F_0)/F_m \quad (1)$$
$$\Delta F/F_m' = (F_m' - F_s)/F_m' \quad (2)$$

Minimal and steady-state fluorescences (F_0, F_s) and during repeated (every 20 min) application of saturating light pulses, F_m and F_m', respectively, were recorded permanently on the leaves directly during the temperature exposure inside the temperature-controlled refrigerator either kept in darkness or illuminated. Chl fluorescence signals were continuously recorded at a measurement frequency of 1 s on a data logger (*CR10X, Campbell Scientific*, Logan, UT, USA).

In some experiments 5 leaves per species were exposed to decreasing or increasing temperatures as described above under saturating irradiation [PPFD: 1,600 µmol(photon) m^{-2} s^{-1}] but they were taken out of the temperature controlled refrigerator and remained in darkness for 30 min before determination of F_v/F_m.

For determination of F_0/T curves cooling and heating rates were also set to 12ºC h^{-1}. The critical high temperature threshold (T_c), where F_0 starts to increase during progressive heating, was assessed as described by Neuner and Pramsohler (2006).

Ice formation: Freezing exotherms were measured on the same leaves on which Chl fluorescence was recorded. Copper-constantan thermocouples (solder junction diameter: 0.3 mm) connected to a data logger (*CR10X data logger, Campbell Scientific Instruments*, Logan, UT, USA) were used to detect freezing exotherms. Ice nucleation temperatures were determined graphically from the temperature time plot.

Determination of heat and frost resistance of leaves: Temperature treatment for determination of foliar heat resistance was carried out in a temperature-controlled water bath using a thermostat (*CC1, Huber*, Offenburg, Germany). Samples were enclosed inside heat resistant polyethylene bags and plunged into water baths with preset temperatures between 30°C and 60°C. Samples remained exposed to target temperatures for 30 min. Temperature treatment for determination of frost resistance was conducted in computer-controlled commercial freezers as described in Neuner and Buchner (1999). Cooling and thawing below 0°C was conducted at rates of 4 K h^{-1}, and samples remained exposed to target temperatures for 4 h. Target temperatures in both, the heat and frost test, were chosen such as the highest temperature should cause no damage and the lowest temperature should kill the leaves. Difference between target temperatures usually did not exceed 2°C. Five leaves (*A. ursinum*) or leaf pieces (*Musa* sp.) per species and target temperature were used, *i.e.* in total about 40 leaf samples were taken for each frost and heat resistance test, respectively.

After temperature treatment the samples were kept on wet paper towels inside polyethylene bags. After one week, heat and frost damages were assessed by rating percentage damage to the leaf blades being clearly visible as brownish discolorations. Percentage damage was then plotted against the treatment temperature. A classical logistic function was fitted to the data using *P.Fit* (*Fig.P Software Corporation*, Durham, NC, USA). The temperature causing 10% damage (LT_{10}) was then calculated *via* the logistic function.

Pigment analysis: Leaves were exposed to decreasing or increasing temperatures under saturating irradiation [1,600 µmol(photon) m^{-2} s^{-1}] or in darkness as described above. Thirty leaf samples per species were used. After the target temperature (5°C steps from –5 to 50°C) had been reached, leaf samples were taken out of the temperature treatment chamber. Then 10 (*Musa* sp.) or 23 (*A. ursinum*) leaf discs (diameter: 1.4 cm), respectively were punched out from the treated leaves and immediately frozen in liquid nitrogen. Then samples were stored at –20ºC until analysis of leaf pigment started. Pigments were extracted with dimethylformamide (DMF). Analysis of the extracts was performed by high-pressure liquid chromatography (HPLC) as described by Wildi and Lütz (1996). The employed HPLC system was an *Agilent ChemStation 1,100* (*Agilent Technologies*, USA) with a binary solvent pump at a flow rate of 1 ml min^{-1} and a diode array detector (DAD) which drew chromatograms at 440 nm. The chromatographic column was a *LiChroSpher C18* (*Phenomenex*, Germany) reversed phase type, size 125 × 4 mm. The leaf pigments were identified by retention time and by absorption spectra.

Calculation of total pigment content: The total pigment amount was calculated by considering between the amount of pigments from the calculated peak area and total DMF volume which is the extracted amount of pigments based on leaf area.

Optimum temperature for $\Delta F/F_m'$ and statistical data analysis: The optimum temperature (T_{opt}) for effective quantum yield of PSII photochemistry was calculated by fitting a parabolic function (3) according to Battaglia *et al.* (1996) to the $\Delta F/F_m'(T)$ *vs*. temperature (T) data using *P.Fit* software (*Fig.P Software Corporation*, Durham, NC, USA).

$$\Delta F/F_m'(T) = \Delta F/F_{m'(opt)} - b[T - T_{(opt)}^2] \quad (3)$$

Statistical analyses were made using the statistical software *SPSS* (*SPSS*, Chicago, IL, USA). To determine an optimum temperature range for quantum yield of PSII photochemistry data were checked for significant difference from maximum values of F_v/F_m and $\Delta F/F_m'$ at T_{opt} by one-way *ANOVA* at $P<0.05$.

Results

Nonlethal thermal window: The species differed significantly with respect to the nonlethal temperature range (Fig. 1). In *A. ursinum* ice formed in leaves at mean at –1.1°C (± 0.1). Initial frost damage was observed not before –6.7°C (LT_{10}). Leaves of *A. ursinum* were ice-tolerant and able to survive extracellular freezing and freeze dehydration down to a certain critical freezing temperature. In *Musa* sp. ice nucleated under the experimental conditions at –2.2°C (± 0.3) causing incipient frost damage (–2.0°C; LT_{10}). High temperature limits were not much different. While *A. ursinum* leaves showed heat injury at 47.7°C (LT_{10}), leaves from *Musa* sp. got damaged by 49.5°C (LT_{10}). The total nonlethal thermal window ranged from –6.7 to 47.7°C (54.5 K) in *A. ursinum* and from –2.2°C to 49.5°C (51.7 K) in *Musa* sp.

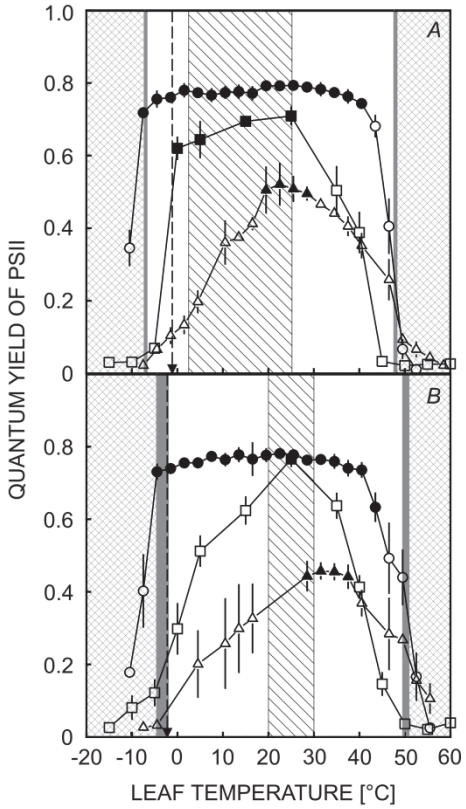

Fig. 1. Temperature response (–10°C and 60°C) of maximal quantum yield of PSII photochemistry [●,○ – F_v/F_m measured during temperature exposure in the darkness; ■,□ – F_v/F_m measured after 30-min dark adaptation after temperature exposure under 1,600 µmol(photon) m^{-2} s^{-1}] and effective quantum yield of PSII photochemistry [▲,Δ – $\Delta F/F_m'$ measured during temperature exposure under 1,600 µmol(photon) m^{-2} s^{-1}] determined on leaves of (*A*) *Allium ursinum* and (*B*) *Musa* sp. *Closed symbols* indicate optimum values. *Open symbols* show values exceeding the optimum range which was tested by *ANOVA* (P<0.05). The values are means ± SE (squares: n = 3; circles and triangles: n = 20). (*Hatched bar*: range of growth temperature; *grey bar*: damage between LT_{10} to LT_{50}; *cross hatched bar*: damage exceeding LT_{50}; *dashed line and arrow*: ice nucleation temperature).

Temperature response of optimal quantum yield of PSII photochemistry: When leaves of *A. ursinum* and *Musa* sp. were exposed in darkness to temperatures within their nonlethal thermal window optimal quantum yield of PSII photochemistry, F_v/F_m, remained remarkably unchanged over a broad temperature range. There was no significant difference between the tropical and the temperate species except when they were exposed to extreme temperatures close to the killing temperature. Exposure to low temperature had hardly any effect on F_v/F_m in both species. A significant drop (P<0.05) was only found in the frost-killing temperature range. At temperatures lower than ice nucleation F_v/F_m was severely depressed in both species. While *Musa* sp. leaves were already frost-damaged, leaves of *A. ursinum* remained undamaged down to –6.7°C showing still a reversible reduction of F_v/F_m.

At high temperatures, but still within the nonlethal thermal window, F_v/F_m decreased above 40°C but in both species the decrease was significant (P<0.05) not before 45°C. Heat damage (LT_{10}) got visible at 2.7 up to 4.5 K higher temperatures, *i.e.* 47.7°C in *A. ursinum* and 49.5°C in *Musa* sp., respectively.

For measurement of F_0/T curves leaves of *A. ursinum* and *Musa* sp. were exposed to constantly increasing temperatures starting at 20°C up to 60°C. A sudden increase in F_0 revealed the critical high temperature threshold for PSII, T_c. In *A. ursinum* T_c was 43.8°C and in *Musa* sp. 44.7°C (Fig. 2). This was 4–5 K below LT_{10} (49.5°C in *Musa* sp., 47.7°C in *A. ursinum*).

When leaves of *A. ursinum* and *Musa* sp. were exposed to strong irradiation [1,600 µmol(photon) m^{-2} s^{-1}] at temperatures within the growing temperature range, no photoinhibition in *Musa* sp. or only minor

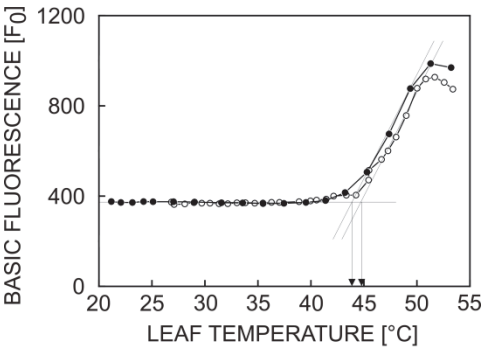

Fig. 2. The critical high temperature threshold (T_c) was investigated by the F_0/T technique. F_0 was recorded on leaves of (●) *Allium ursinum* and (○) *Musa* sp. during controlled heating at a rate of 12 K h^{-1} from 20 to 60ºC. The arrows indicate T_c. The data presented are mean values (n = 5).

Table 1. Comparison of total xanthophyll cycle pool size, Chl (*a*+*b*) content, the ratio between and Chl *a* to Chl *b* determined for leaves of *Musa* sp. and *Allium ursinum* within the optimum temperature range (means ± SE of *n* = 36).

Pigments	*A. ursinum* L.	*Musa* sp.
Xanthophyll cycle pool size [μmol m^{-2}]	12.3 ± 0.4	9.5 ± 0.2
Chl (*a*+*b*) [μmol m^{-2}]	236.2 ± 10.9	257.3 ± 7.6
Chl *a*/*b* [M ratio]	3.0 ± 0.04	3.5 ± 0.07

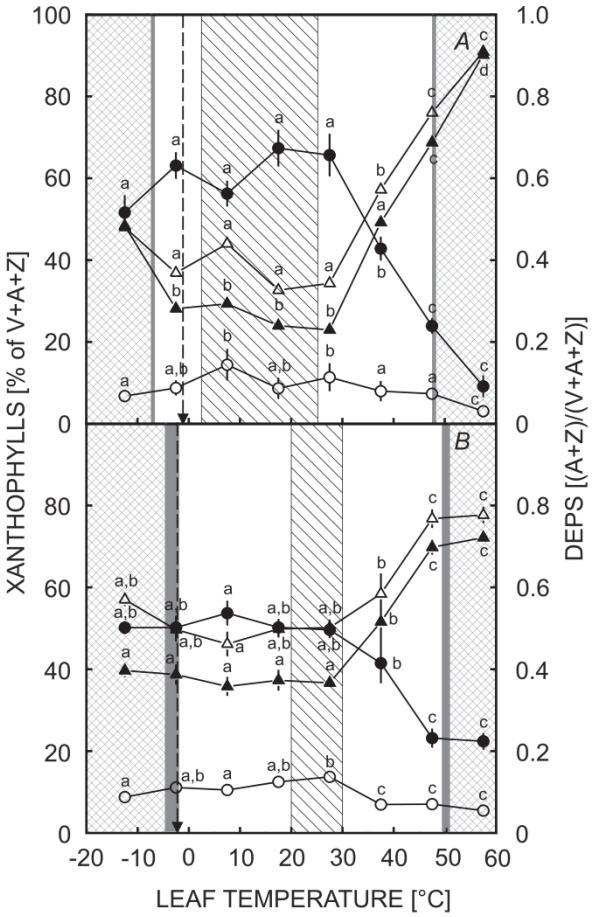

Fig. 3. Contents of the xanthophyll pigments (●) violaxanthin, (○) antheraxanthin, and (▲) zeaxanthin, and (Δ) de-epoxidation state activity determined on leaves of (*A*) *Allium ursinum* and (*B*) *Musa* sp. after exposure to various temperatures (–15ºC to 60ºC) during controlled cooling or heating at a rate of 12 K h^{-1} under strong irradiation [1,600 μmol(photon) m^{-2} s^{-1}]. Measurements were performed in 2010 and 2011. The values are means ± SE (n = 6). Significant differences between mean values are indicated by *different letters* (*ANOVA*, P<0.05). (*Hatched bar*: range of growth temperature; *grey bar*: damage between LT_{10} to LT_{50}; *cross hatched bar*: damage exceeding LT_{50}; *dashed line and arrow*: ice nucleation temperature).

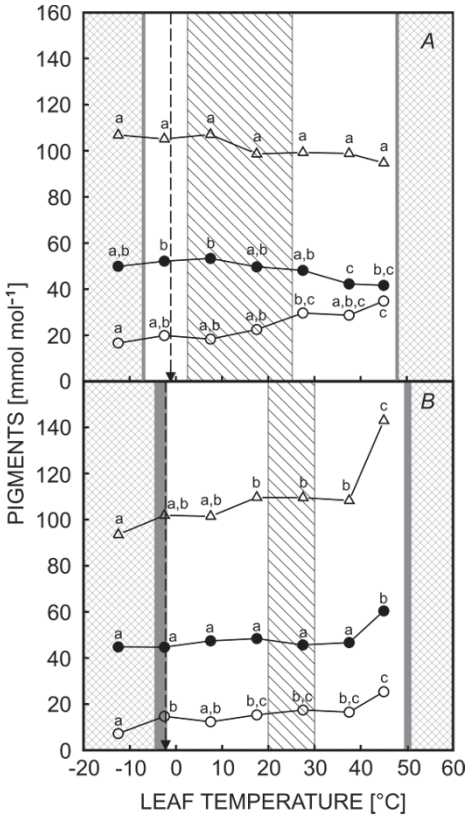

Fig. 4. Contents of (●) neoxanthin, (○) β-carotene and (Δ) lutein, based on Chl *a* and *b* (mmol mol^{-1}) determined on leaves of (*A*) *Allium ursinum* and (*B*) *Musa* sp. after exposure to various temperatures (–15ºC to 60ºC) during controlled cooling or heating at a rate of 12 K h^{-1} under strong irradiation [1,600 μmol(photon) m^{-2} s^{-1}]. Measurements were performed in 2010 and 2011. The values are means ± SE (n = 6). Significant differences between mean values are indicated by *different letters* (*ANOVA*, P<0.05). (*Hatched bar*: range of growth temperature; *grey bar*: damage between LT_{10} to LT_{50}; *cross hatched bar*: damage exceeding LT_{50}; *dashed line and arrow*: ice nucleation temperature).

photoinhibition in leaves of *A. ursinum* were recorded (Fig. 1). Exposure to temperatures exceeding the growth temperature range but being still within the nonlethal thermal window usually caused severe photoinhibition. Severity increased with increasing distance to the growth temperature.

Temperature response of effective quantum yield of PSII photochemistry: $\Delta F/F_m'$, showed a distinct optimum curve in response to temperature. For *A. ursinum* an optimum temperature of 25.1°C and for *Musa* sp. of 28.8°C was calculated by the fitted parabolic function. These optimum temperatures matched with daily maximum growth temperatures. $\Delta F/F_m'$ values did not change significantly within a temperature range of 18–27ºC in *A. ursinum* and 27–36ºC in *Musa* sp., respectively.

Pigments: Over the optimum temperature range, Chl *a* and Chl *b* amounts did not considerably change in both plants, with higher contents in average per leaf area in *Musa* sp. (Table 1). However, a drop of about 11% total Chls occurred when temperatures were increased from 37.5 to 47.5ºC in *Musa* sp. while Chl content did not change in *A. ursinum* between –12.5ºC and 47.5ºC.

Total xanthophyll cycle pool size was 9.5 μmol m^{-2} in leaves of *Musa* sp. and 12.3 μmol m^{-2} in *A. ursinum*. Significant changes in levels of pigments were only observed when the temperature treatment was conducted under strong irradiation but not in darkness (data not shown). In both species only when temperatures exceeded 30°C zeaxanthin increased and violaxanthin decreased significantly (Fig. 3). In the nonlethal low temperature range the relative amounts remained unchanged even in the tropical species that showed severe photoinhibition under these experimental conditions. The deepoxidation status in this cycle (DEPS) showed a sharp increase above 30°C.

β-carotene contents, based on Chl *a* and *b*, continuously increased from low to high temperature and were significantly (P>0.05) increased at 45°C in both species (Fig. 4). Lutein content remained unchanged in *A. ursinum* leaves but in *Musa* sp. was slightly decreased in the suboptimal temperature range and significantly (P>0.05) increased at 45°C. Neoxanthin was significantly (P>0.05) decreased in the supraoptimal temperature range in *A. ursinum*. The increased amounts for β -carotene, lutein and neoxanthin in leaves of *Musa* sp. at 45ºC reflected destruction of Chls which took place in this species at these temperatures (data not shown).

Discussion

Low temperature in darkness: F_v/F_m was nearly unaffected by temperature within the nonlethal thermal window of both species. Particularly, low temperature had no effect until ice nucleation. Ice nucleation caused immediate damage (LT_{10}) to the tropical *Musa* sp. Upon nonlethal ice formation in leaves usually after a time lag of 20–30 min an increase of F_0 can be recorded (Neuner and Pramsohler 2006, Hacker *et al.* 2008) which is corroborated by our results obtained for *A. ursinum*. This increase of F_0 may be caused by blocking of the reaction centre of PSII either at the donor side by inactivation of the oxygen evolving complexes or at the acceptor side by inhibition of Q_A-Q_B electron transport or limitations by mobile diffusion limited transport molecules such as plastoquinones (Pospíšil *et al*. 1998, Mishra *et al*. 2011).

A similar insensitivity of F_v/F_m against low temperature was reported for salt stressed tropical *Vigna ungiculata* (Larcher *et al.* 1990), down to about –4°C in *Arabidopsis thaliana* accessions (Mishra *et al.* 2011), down to –5°C in *Fagus sylvatica* and *Betula pendula* (Wittmann and Pfanz 2007) and down to –10°C in spring barley (Pospíšil *et al.* 1998).

High temperature in darkness: In contrast to low temperatures high temperatures close to the thermal limit had a direct effect on PSII photochemistry. Temperatures between about 40°C and LT_{10} (47.7 and 49.5°C, respectively) caused an increase of F_0 in both species (T_c: 43.8 and 44.7°C, respectively). This only slight difference in high temperature tolerance between a tropical species and a temperate one is in accordance to the results of Smillie and Nott (1979): while mean T_c in temperate species (n = 10) was 44.1 ± 0.6°C, it was 46.0 ± 0.6°C in tropical species (n = 10). Similar low T_c values as in *Musa* sp. were found in the chilling susceptible cucumber and pepper (<45°C). The high temperature strain in natural environments of plants can be quite similar in different biomes of the world (Körner 2003). Additionally, T_c is a highly dynamic parameter. It can be changed within short periods of time due to environmental changes (Braun *et al.* 2002) and it can be influenced by experimental settings such as the heating rate (Frolec *et al.* 2008) which can make comparisons difficult.

The F_v/F_m decline was caused by increasing F_0 (according to Krause *et al.* 2010) and spring barley (Kouřil *et al.* 2004). The high temperature decline of F_v/F_m was observed above 40ºC in both species and for both the decrease was significant (P<0.05) not before 45ºC. This corroborates observations for tropical species where a heat-induced reduction of F_v/F_m occurred > 40ºC (Salvucci and Crafts-Brander 2004) or even > 47ºC (Krause *et al.* 2010). But it is in contrast to reports for temperate species where F_v/F_m dropped already at temperatures > 30 to 35ºC (Salvucci and Crafts-Brander 2004, Wittmann and Pfanz 2007). In winter wheat grown at 15ºC or 25ºC, respectively, F_v/F_m sharply decreased at temperatures above 35ºC whereas plants at 35ºC showed unchanged values till 45ºC, which indicated a strong effect of growth temperature on heat stability of PSII (Yamasaki *et al.* 2002). This could explain our results for the temperate *A. ursinum*.

The increase of F_0 in the supraoptimal temperature range was a complex process (Ducruet *et al.* 2007, Kouřil *et al.* 2004) and could principally be caused by blockage of the PSII reaction center either by inactivation of oxygen-evolving complex (OEC) (Havaux 1993), a limitation of electron donation to PSII (Laasch 1987, Santarius and Weis 1988) or acceptor side inhibition of Q_A-Q_B electron transport (Havaux 1993). However, acceptor side inhibition as shown only recently (Takahashi and Murata 2008) seems to have no effects on the rate of photodamage. Initially the heat induced blockage was reversible as only at 4–5 K higher temperatures heat damage was observed. T_p of the F_0/T curve (51.8 and 52.5°C, respectively) matched with an F_v/F_m

close to 0.0 which corroborates results obtained on spring barley (Pospíšil *et al.* 1998). The irreversible step lies in between of T_c and T_p being in accordance to findings of Braun *et al.* (2002).

Photoinhibition could be seen as a balance between the rate of photodamage to PSII and the rate of repair (Takahashi and Murata 2008). Under moderate heat it was shown that the repair of PSII could be blocked. The synthesis of PSII proteins (D1 translation) could be suppressed by ROS that readily form under adverse environmental conditions (Takahashi and Murata 2008).

Strong irradiation superimposed to temperature treatment

Optimum temperature range: Optimum temperatures for $\Delta F/F_m'$ matched with daily maximum air temperatures (25.1 and 28.8°C, respectively). Severe photoinhibition was intimately observed upon exposure to temperatures within the nonlethal thermal window but exceeding the growth temperature range. Optimum temperatures for $\Delta F/F_m'$ ranged from 18 to 27ºC in *A. ursinum* and from 27 to 36ºC in *Musa* sp. which exceeds maximum air temperatures by 2 and 6 K, respectively. In an alpine field study it has been shown that leaf temperatures most frequently experienced by a plant matched with the optimum temperatures for $\Delta F/F_m'$ (Braun and Neuner 2005).

Low suboptimal temperatures: The higher susceptibility to photoinhibition of the tropical species at temperatures below 20°C came nicely clear by significantly lower F_v/F_m values at chilling temperatures (10–0°C). This became particularly evident around 0°C (F_v/F_m: 0.3 *Musa* sp., > 0.6 *A. ursinum*).

In both species at suboptimal temperatures activity of de-epoxidation and conversion of V to A and Z were found to be unaffected as values remained unchanged as compared to the optimum temperature range. Under low winter temperatures the xanthophyll cycle seems to be impeded (Lehner and Lütz 2003). Similarly, in over wintering leaves nocturnally cold-sustained retention of zeaxanthin and antheraxanthin was reported that relaxed upon rewarming during daytime (Adams and Demmig-Adams 2004). Barth and Krause (1999) reported enhanced PSII inhibition *in vivo* at low temperatures in chilling-sensitive and chilling-tolerant plants which was partly explained by a reduced function of the xanthophyll cycle. Our results support these findings at least for xanthophylls as they remained unchanged at temperatures lower than 30°C in both species. A lower susceptibility to photoinhibition at 4°C of *Spinacia oleracea* as compared to tropical species was associated with a faster de-epoxidation of violaxanthin, a larger pool size of xanthophyll cycle pigments and a higher content of β-carotene (Barth and Krause 1999). *A. ursinum* had also a larger pool size of xanthophyll cycle pigments and a slightly higher content of β-carotene than *Musa* sp.

Barth and Krause (1999) observed at 4°C a reduced activity of the antioxidative scavenging system in chilling-susceptible plants. This would explain increased levels of ROS at low temperature that in turn could suppress the synthesis of PSII proteins, inhibiting the rate of PSII repair increasing photoinhibition (Takahashi and Murata 2008).

High supraoptimal temperatures: Under strong irradiation in both species at supraoptimal temperatures an increased activity of de-epoxidation and conversion of V to A and Z were found which corroborates recent observations on rice plants (Yin *et al.* 2010). While in the darkness no changes were observed (data not shown), there is an evidence that very low irradiation intensities of only 12 μmol(photon) m^{-2} s^{-1} can be sufficient for a marked stimulation of the V to Z conversion under heat (Ilík *et al.* 2010). Under heat stress inhibition of the violaxanthin de-epoxidase in rice plants resulted in an increase of the sensitivity of PSII to photoinhibition corroborating the photoprotective function of the xanthophyll cycle during high-temperature exposure (Yin *et al.* 2010). But similarly to cold also during moderate heat it has been additionally shown that the repair of PSII through suppression of the synthesis of PSII proteins (D1 translation) can be inhibited by ROS (Takahashi and Murata 2008).

At the temperatures slightly higher than 40°C F_v/F_m values were quite equal to $\Delta F/F_m'$. At temperatures higher than T_c, where PSII gets reversibly blocked until damage (LT_{10}), F_v/F_m values after a dark adaptation of 30 min were even lower than the $\Delta F/F_m'$ values of the respective temperature indicating processes further reducing F_v/F_m during dark adaptation and the initial stage of recovery.

The increasing amounts of lutein with increasing temperature in *Musa* sp. as opposed to *A. ursinum* could be a sign of increasing turnover activities in the carotenoid metabolism as a protective strategy which was obviously not required in *A. ursinum*. However, these changes could not avoid Chl destruction at the highest temperature treatment in *Musa* sp.

Under strong irradiation photoinhibition occurred whenever growth temperature was exceeded. Xanthophylls changed only under supraoptimal temperature conditions but quite similarly in both species. The other investigated pigments revealed species-specific responses. In darkness PSII efficiency was highly temperature-insensitive in both species as quite similar changes with only a few K difference were observed in the tropical *vs*. the temperate plant.

References

Adams, W.W., III, Demmig-Adams, B.: Chlorophyll fluorescence as a tool to monitor plant response to the environment. – In: Papageorgiou, G.C., Govindjee (ed.) Chlorophyll *a* Fluorescence. A Probe of Photosynthesis. Pp. 583-604. Springer, Dordrecht 2004.

Baker, N.R.: Photosynthesis and the Environment. – Kluwer Academic Publisher, Dordrecht 1996.

Barth, C., Krause, G.H.: Inhibition of photosystems I and II in chilling-sensitive and chilling-tolerant plants under light and low-temperature stress. – Zeitschr. Naturforsch. **54c**: 645-657, 1999.

Battaglia, M., Beadle, C., Loughhead, S.: Photosynthetic temperature response of *Eucalyptus globulus* and *Eucalyptus nitens*. – Tree Physiol. **16**: 81-89, 1996.

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

Braun, V., Buchner, O., Neuner, G.: Thermotolerance of PS II of three alpine plant species under field conditions. – Photosynthetica **40**: 587-595, 2002.

Braun, V., Neuner, G.: Response of effective quantum yield of photosystem 2 to *in situ* temperature in three alpine plants. – Photosynthetica **42**: 607-613, 2005.

Demmig-Adams, B., Adams, W.W., Logan, B.A., Verhoeven, A.S.: Xanthophyll cycle-dependent energy dissipation and flexible photosystem II efficiency in plants acclimated to light stress. – Aust. J. Plant Physiol. **22**: 249-260, 1995.

Ducruet, J.M., Peeva, V., Havaux, M.: Chlorophyll thermofluorescence and thermoluminescence as complementary tools for the study of temperature stress in plants. – Photosynth. Res. **93**: 159-171, 2007.

Frolec, J., Ilík, P., Krachňák, P., Sušila, J., Nauš.: Irreversible changes in barley leaf chlorophyll fluorescence detected by the fluorescence temperature curve in a linear heating/cooling regime. – Photosynthetica **46**: 537-546, 2008.

Genty, B., Harbinson, J., Briantais, J.M., Baker, N.R.: The relationship between non-photochemical quenching of chlorophyll fluorescence and the rate of photosystem 2 photochemistry in leaves. – Photosynth. Res. **25**: 249-257, 1990.

Gilmore, A.M.: Mechanistic aspects of xanthophyll cycle-dependent photoprotection in higher plant chloroplast and leaves. – Physiol. Plant. **99**: 197-209, 1997.

Hacker, J., Neuner, G.: Ice propagation in plants visualized at the tissue level by IDTA (infrared differential thermal analysis). –Tree Physiol. **27**: 1661-1670, 2007.

Hacker, J., Spindelböck, J., Neuner, G.: Mesophyll freezing and effects of freeze dehydration visualized by simultaneous measurement of IDTA and differential imaging chlorophyll fluorescence. – Plant Cell Environ. **31**: 1725-1733, 2008.

Havaux, M.: Characterization of thermal damage to the photosynthetic electron transport system in potato leaves. – Plant Sci. **94**: 19-33, 1993.

Huner, N.P.A., Öquist, G., Hurry, V.M., *et al.*: Photosynthesis, photoinhibition and low temperature acclimation in cold tolerant plants. – Photosynth. Res. **37**: 19-39, 1993.

Ilík, P., Kotabová, E., Špundová, M. *et al*.: Low-light-induced violaxanthin de-epoxidation in shortly preheated leaves: Uncoupling from ΔpH-dependent nonphotochemical quenching. – Photochem. Photobiol. **86**: 722-726, 2010.

Körner, C.: Alpine Plant Life. Functional Plant Ecology of High Mountain Ecosystems. – Springer, Berlin 2003.

Kouřil, R., Lazár, D., Ilík, P. *et al*.: High-temperature induced chlorophyll fluorescence rise in plants at 40-50°C: experimental and theoretical approach. – Photosyn. Res. **81**: 49-66, 2004.

Krause, G.H., Winter, K., Krause, B., *et al*.: High-temperature tolerance of a tropical tree, *Ficus insipida*: methodological reassessment and climate change considerations. – Func. Plant Biol. **37**: 890-900, 2010.

Laasch, H.: Non-photochemical quenching of chlorophyll a fluorescence in isolated chloroplasts under conditions of stressed photosynthesis. – Planta **171**: 220-226, 1987.

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.

Lehner, G., Lütz, C.: Photosynthetic functions of cembran pines and dwarf pines during winter at timberline as regulated by different temperatures, snow cover and light. – J. Plant Physiol. **160**: 153-166, 2003.

Lichtenthaler, H.K.: Stress of life: from molecules to man. – Ann. NY Acad. Sci. **851**: 187-198, 1998.

Mishra, A., Mishra, K.B., Hörmiller, I.I., Heyer, A.G., Nedbal, L.: Chlorophyll fluorescence emission as a reporter on cold tolerance in *Arabidopsis thaliana* accessions. – Plant Signaling & Behaviour **6**: 301-310, 2011.

Neuner, G., Buchner, O.: Assessment of foliar frost damage: a comparison of in vivo chlorophyll fluorescence with other viability tests. – J. Appl. Bot. **73**: 50-54, 1999.

Neuner, G., Pramsohler, M.: Freezing and high temperature thresholds of photosystem 2 compared to ice nucleation, frost and heat damage in evergreen subalpine plants. – Physiol. Plant. **126**:196-204, 2006.

Pospíšil, P., Skotnica, J., Nauš, J.: Low and high temperature dependence of minimum F_o and maximum Fm chlorophyll fluorescence *in vivo*. – Biochim. Biophys. Acta **1363**: 95-98, 1998.

Salvucci, M.E., Crafts-Brander, S.J.: Relationship between the heat tolerance of photosynthesis and thermal stability of Rubisco activase in plants from contrasting thermal environments. – Plant Physiol. **134**: 1460-1470, 2004.

Santarius, K.A., Weis, E.: Heat stress and membranes. – In: Harwood, J.L., Walton, T.J. (ed.) – Plant Membranes – Structure, Assembly and Function. Pp. 97-112, Biochem. Soc., London 1988.

Schreiber, U.: Pulse-amplitude-modulation (PAM) fluorometry and saturation pulse method: An overview. – In: Papageorgiou, G. C., Govindjee (ed.) Chlorophyll *a* Fluorescence. A Signature of Photosynthesis. Advances in Photosynthesis and Respiration, Vol 19. Pp. 279-319. Springer, Dordrecht 2004.

Smillie, R.M., Nott, R.: Heat injury in leaves of alpine, temperate and tropical plants. – Aust. J. Plant Physiol. **6**: 135-141, 1979.

Takahashi, S., Murata, N.: How do environmental stresses accelerate photoinhibition? –Trends in Plant Sci. **13**: 178-182, 2008.

Wildi, B., Lütz C.: Antioxidant composition of selected high alpine plant species from different altitudes. – Plant Cell Environ. **19**: 138-146, 1996.

Wittmann, C., Pfanz, H.: Temperature dependency of bark photosynthesis in beech (*Fagus sylvatica* L.) and birch

(*Betula pendula* Roth.) trees. – J. Exp. Bot. **58**: 4293-4306, 2007.

Woodward, F.I., Kelly, C.K.: Plant functional types: towards a definition by environmental constraints. – In: Smith, T.M., Shugart, H.H.,Woodward, F.I. (ed.): Plant Functional Types - Their Relevance to Ecosystem Properties and Global Change. Pp. 47-65. Cambridge University Press, Cambridge 1997.

Woodward, F.I., Smith, T.M.: Predictions and measurements of the maximum photosynthetic rate, A_{max}, at the global scale. – In: Schulze E.-D., Caldwell M.M. (ed.): Ecophysiology of Photosynthesis. Pp. 491-509. Ecological Studies 100, Springer, Berlin 1994.

Yamasaki, T., Yamakawa, T., Yamane *et al*.: Temperature acclimation of photosynthesis and related changes in photosystem II electron transport in winter-wheat. – Plant Physiol. **128**: 1087-1097, 2002.

Yin, Y., Li, S., Liao, W., Lu, Q., Wen, X., Lu, C.: Photosystem II photochemistry, photoinhibition, and the xanthophyll cycle in heat-stressed rice leaves. – J. Plant Physiol. **167**: 959-966, 2010.