The effect of heat on photosynthesis, dark respiration and cellular ultrastructure of the arctic-alpine psychrophyte *Ranunculus glacialis*

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Abstract

Effects of high temperatures on the leaves of *Ranunculus glacialis* were studied in plants taken from sites located between 2400-2550 m in the Central Alps. Changes in CO_2 exchange rates, *in vivo* chlorophyll fluorescence, and cellular ultrastructure were investigated during and after an experimental heat exposure. The earliest heat stress effect was inactivation of the net photosynthetic rate at 38-39 °C. Between 40-42 °C, disorders appeared in the photosynthetic apparatus and in the tonoplast. Heat shock granules were observed at 42 °C in chloroplasts, and at 44 °C also in mitochondria. In this temperature range, the dark respiration rate was reversibly enhanced, and an increased number of polyribosomes indicated repair after the primary injury. Above 44 °C, the degradation progress entered the phase of chronic impairment leading to irreversible damage at 45-46 °C. An unusually wide temperature range from the start of reversible photosynthetic inhibition to incipient necrosis indicated a pronounced heat sensitivity, particularly in cellular functions, of this arctic-alpine species.

Additional key words: CO_2 exchange; chlorophyll fluorescence *in vivo;* chloroplasts; heat shock granules; stress criteria; thermosensitivity.

Introduction

The terrestrial vascular plants tolerate heat in the range of about 45-65 °C for short periods of half up to one hour (Larcher 1994). Arctic and alpine herbaceous plants

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This paper is dedicated to Zdeněk Šesták on the occasion of his 65th birthday.
Abbreviations: Chl = chlorophyll; F_0 = basic Chl fluorescence (initial level); HLC = heat limit for CO_2 uptake; HSG = heat shock granules; LT = lethal temperature; pCO_2 = partial pressure of CO_2 in the air; PPFD = photosynthetic photon flux density; P_N = net photosynthetic rate; R_D = dark respiration rate; SLA = projected leaf area per unit leaf dry mass.
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are particularly susceptible to high temperatures (Pisek *et al.* 1968, Larcher and Wagner 1976, Denko *et al.* 1981, Gauslaa 1984). One of the most heat-sensitive species among arctic and alpine plants is *R. glacialis*, which is distributed at high altitudes in European mountains and in the Arctic up to 78° North. In the Alps, the species grows mainly between 2200 to 3200 m a.s.l. (Backhuys 1968), but it still can be found as high as 4270 m (Grabherr *et al.* 1995). At altitudes of 2600-3000 m, the mean air temperature of the warmest month (July) is 4-5 °C (Körner and Diemer 1987). The mean maximum canopy temperature recorded in July on sunlit sites at 3100 m was 12 °C, though leaf temperatures of *R. glacialis* may rise to 30 °C or even 40 °C for some minutes during strong irradiation and in the absence of wind (Moser *et al.* 1977). In the lower altitudes, where summer canopy temperatures may reach as much as 40-45 °C on drier sites (Larcher and Wagner 1976), *R. glacialis* is found only on wet snowbed habitats or in sites continuously irrigated from melting snow, where the ground remains cool even under a strong irradiation. The assumption about the distribution of certain alpine and nordic species being possibly restricted by maximum summer temperature isotherms has already been conveyed by Dahl (1951).

The aim of this study, conducted during the summer seasons 1976-1978 and 1982, was a detailed analysis of progressing disturbances in ultrastructure and cellular functions arisen from a heat stress, and leading to the breakdown of metabolism and lethal damage. In our previous investigations, we already noticed that even at temperatures not yet producing an acute lethal damage to the leaves of heat-sensitive alpine plants, some chronic injuries had in fact been incurred, causing the leaves to die gradually within a few weeks. We believe that in view of the increasing attention paid to environmental changes at high latitudes and altitudes (Callaghan *et al.* 1992, Körner 1995) our earlier results concerning the heat sensitivity of *R. glacialis* as a bioindicator of climate warming may be of topical interest.

Materials and methods

Plants: Plants of *Ranunculus glacialis* L. in the flowering state were dug out of moraines in the Ötztal glacier region (2400-2550 m a.s.l.) between mid-July and the beginning of August. Individual plants were placed in pots filled with soil taken from the collection site, and immediately brought to the laboratory in Innsbruck. During the trials the plants were cultivated in growth rooms under 16 h photoperiod, irradiance (PPFD) of 550 μmol(photon) m^{-2} s^{-1} provided by *HQIL Osram Power Star 400 W71* lamps, day/night-temperatures 5/15 °C, and continuous irrigation (water temperature of 5-10 °C).

Experimental procedure: Two types of measurements were employed: (*1*) *during* a heat treatment to determine the inactivation of photosynthesis, and (*2*) *after* a heat exposure, at room temperatures (20-22 °C) after various time intervals (immediately, hours or longer periods), in order to estimate recovery.

For heat treatments, the shoots of potted plants (except for tests of *in vivo* Chl fluorescence) were covered with watertight plastic bags, and immersed in a water

bath (*Thermomix*; *Braun*, Melsungen, Germany; accuracy ± 0.1 °C). The heat exposure took place in the dark at temperature steps of 1-2 °C between 38 and 48 °C for half an hour. To determine the heat limits of apparent CO_2 uptake (HLC), single leaves were exposed to heat in glass tubes.

Measurement of CO_2 exchange rates in shoots: The rates of net photosynthesis (P_N) and dark respiration (R_D) were measured by infrared gas analysis (*URAS II, Hartmann & Braun*, Frankfurt, Germany) in an open system. Entire plants were fitted into a thermo-controlled sample chamber. The root zone was completely sealed off from the air in the ventilated system, thus ensuring that only the CO_2 exchange of the shoots was measured. For one experimental set, four plants were placed in one chamber, so that each point on the plots represented the average for at least 16 leaves. Experimental constants were: PPFD of 345 µmol(photon) m^{-2} s^{-1} (*HQI L Osram Power Star 400 W71*); leaf temperatures of 20 ± 1°C for monitoring after-effects in preheated plants; relative humidity 70 ± 5 %. CO_2 of the incoming air was 335 ± 20 cm^3 m^{-3}, at a flow rate of 13.9 cm^3 s^{-1}, concentration in the chamber air during gas exchange measurements was about 310 cm^3 m^{-3}.

Determination of the heat limit for CO_2 uptake: CO_2 exchange of single leaves was estimated by the semiquantitative colorimetric pCO_2 indicator method introduced by Ålvik (1939), and modified as described by Larcher and Wagner (1976). Before starting the experiment, 10 cm^3 of indicator solution was placed into open glass tubes of 50 cm^3 volume, and equilibrated with the ambient air pCO_2 (34 Pa) for one hour. The indicator solution (according to Čatský and Šesták 1966) consisted of 0.001 M $NaHCO_3$, 0.099 M KCl, 10 g m^{-3} cresol red, and 20 g m^{-3} bromothymol blue. For each run, at least ten leaves were used, one in each tube. The tubes were then closed with rubber stoppers, and exposed to PPFD of 200 µmol(photon) m^{-2} s^{-1} for 30 min.

***In vivo* chlorophyll (Chl) fluorescence**: Photosynthetic function was assessed by the rise in basic Chl fluorescence during progressive heating (F-T-curves; Schreiber and Berry 1977) and by detection of light-induced transients. Progressive heating rates of 0.017 °C s^{-1} (1 °C min^{-1}) for the F-T-experiments were achieved using a Peltier system (*Midland Ross Co.*, Cambridge, U.S.A.). The sample temperature was continuously recorded by means of a Cu/CuNi-thermocouple pressed onto the upper leaf surface and connected to a digital voltmeter (accuracy ±0.1 °C). Fluorescence parameters were expressed relative to the reference value at 20 °C. At least five leaves were used per trial.

In vivo Chl fluorescence was recorded by a portable fluorometer (*Plant Productivity Fluorometer*; *Brancker*, Ottawa, Canada) connected to an oscilloscope (*Tektronix TM64*, Beaverton, U.S.A.). For basic fluorescence, red flashes of 1 ms in the range of 630 to 720 nm were applied at an irradiance of 10 µmol m^{-2} s^{-1}, therefore, the fluorescence intensity indicated all photosystem 2 (PS2) reaction centres being open. Fluorescence transients from leaves of preheated plants were monitored at room temperature after predarkening for 30 min prior to the experiments, and triggered with 30 µmol(photon) m^{-2} s^{-1} for 40 s.

Electron microscopy: Ultrastructural investigations were performed on leaves taken from controls, and on those taken immediately after the heat treatments by 42, 44, and 46 °C, and after different times of recovery as follows: preheated at 42 °C after 1 and 6 d recovery; preheated at 44 °C after 7 and 15 d recovery; preheated at 46 °C after 8 and 16 d recovery. Small leaf pieces were subjected to a buffered glutaraldehyde fixation, followed by osmic acid, as described in Lütz and Moser (1977). After dehydration, the samples were embedded in epoxy resin, according to Spurr (1969). Ultrathin sections were post-stained with lead citrate, and studied in a *Siemens Elmiscope 101* at 80 kV. A minimum of five different leaf pieces per treatment was sectioned, thus up to 40 cells were observed.

Assay of lethal injuries: Thermal limits of acute damage were determined by exposing potted plants to heat for 30 min. Turgor loss and the extent of necrotic patches on the leaves were estimated after full development of the symptoms. Threshold values for injury, obtained by visual rating (see Larcher 1990), were the incipient killing temperature, LT_i, at which injuries first appeared, the temperatures at 50 % lethality, LT_{50}, and at complete lethality.

Results

Effects on photosynthetic function during heat stress: When the optimum range for P_N of *R. glacialis* (15-25 °C) is exceeded, the photosynthetic activity drops to below half its capacity at 35 °C (Pisek *et al.* 1969, Moser *et al.* 1977, Körner and Diemer 1987). The heat limit for P_N is 39 °C for alpine (Pisek *et al.* 1968, Larcher and Wagner

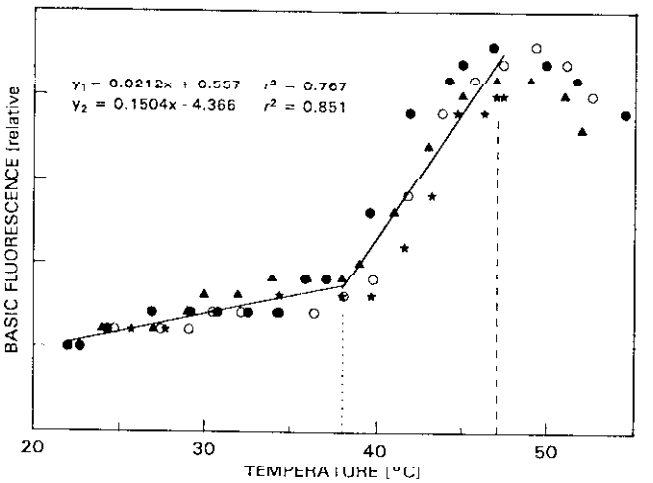

Fig. 1. Basic fluorescence (F_0) during progressive heating of leaves of *R. glacialis* at the indicated temperature. Different symbols (●, ○, ▲, ★) represent separate experimental runs. *Dotted line*: mean temperature of initial increase of the F-T curve (38.2 °C). *Dashed line*: mean temperature of onset of the peak (47.1 °C).

1976) and between 36-42 °C for arctic proveniences (Semikhatova *et al.* 1992). During three summers we found the HLC of 38 ± 0.8 °C using the colorimetric pCO_2 indicator method.

Alterations of thermotropic properties of the thylakoid membranes were revealed by sharp deviations in the rise of basic fluorescence during a gradual heating (Schreiber and Bilger 1987, Terzaghi *et al.* 1990). Break in the F-T curve at 38.2 ± 1.2 °C indicated the beginning of heat-inactivation of photosynthesis in *R. glacialis* (Fig. 1). Smillie and Nott (1979) obtained similar values (38-40 °C) for herbaceous alpine plants near snow banks below the summit of Mt. Kosciusko (Australia). At the onset of the F-T curve peak (mean value: 47.1 ± 0.3 °C) the thylakoid membranes were irreparably damaged.

After-effect of heat treatment on the P_N and R_D: Previous high temperature stress may cause a long-lasting strain of vital processes after the return to a normal temperature. Continuous measurements using IRGA allow the quantification of the degree and duration of decline or recovery of the P_N (see Fig. 2), whereas very small departures from the CO_2 compensation concentration of a large number of samples can be estimated conveniently by the pCO_2 indicator method (see Table 1).

Table 1. Final result after heat pretreatment at indicated temperatures [°C] for 30 min on CO_2 exchange [% per set, $n \geq 10$] of leaves of *Ranunculus glacialis* assayed with the colorimetric indicator method at 20 °C.

CO_2 exchange	40	41	42	43	44	45	46
CO_2 uptake	100	100	75	40	26	-	-
CO_2 compensation			17	20	13	-	-
CO_2 release	-	-	8	60	61	100	100

Immediately after a heat treatment at temperatures above HLC, after transfer to room temperature, the P_N was still inhibited. However, after heating for 30 min in the dark at 40 °C, a positive P_N was observed within several hours. After preheating at 42 and 44 °C, recovery of the photosynthetic function was delayed and incomplete. The CO_2 exchange balance of the entire plants frequently did not approach positive values, since the enhanced respiratory CO_2 efflux (see below) of all plant parts, including non-photosynthetic tissues, exceeded the small foliar photosynthetic activity. In order to determine the degree of recovery of photosynthetic function a *recovery index*, RI, was calculated: $RI = (CE_L\text{-}CE_D)_h/(CE_L\text{-}CE_D)_c$, where $(CE_L\text{-}CE_D)_h$ is the difference between the rates of CO_2 exchange in the light (CE_L) and in the dark (CE_D) for the preheated samples, and $(CE_L\text{-}CE_D)_c$ is the difference for the controls. The variability of heat sensitivity of the individual leaves was greatest in the temperature range of 42-44 °C (Fig. 2, Table 1), which indicated a particularly labile phase in the stress syndrome. The same variability was also detectable in the *in vivo* Chl fluorescence. Whereas after heating up to 41 °C typical fluorescence induction transients were still detected in all tested leaves, after preheating to 42 °C only 2/3 of

the leaves had recovered, and after 44 °C only about 1/10 (not shown). Above 45 °C, neither fluorescence nor CO_2 exchange assays revealed any resumption of the photosynthetic activity. Such persistent depression of photosynthesis may be the result of destructing the organization and functions of chloroplasts, in particular of the water-splitting system and the transport chain between the two photosystems (Santarius 1975, Berry and Raison 1981, Weis and Berry 1988).

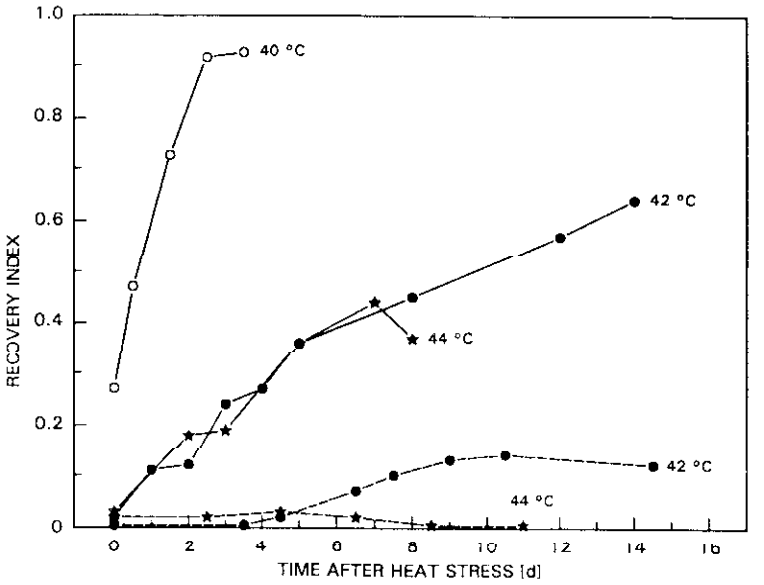

Fig. 2. Representative responses of CO_2 exchange at PPFD of 345 µmol(photon) m^{-2} s^{-1} and 20 °C after preheating entire plants of *R. glacialis* for 30 min in the dark at (○) 40 °C, (●) 42 °C, and (★) 44 °C. Recovery index is the ratio of gas exchange between preheated samples and control prior to heat treatment (for explanation see text). Mean P_N of non-stressed plants: 12.9±4.2 µmol m^{-2} s^{-1}; SLA: 16.6 m^2 kg^{-1}(dry mass). Each point represents the mean of 4 plants in one measurement chamber. Note large variation (dashed *versus* solid lines) between values of samples preheated at 42 and 44 °C.

Abnormal increase of R_D during and after heat stress is a valuable criterion for indicating stress in susceptible plant species (Lange 1965, Larcher and Bodner 1987). The critical temperature for mitochondrial respiration during heat exposure, defined by Semikhatova *et al.* (1976) as the highest rate before a drop due to disturbance to respiratory processes, was 38 °C for *R. glacialis* from the Wrangel Island (71° N). After preheating potted plants at 40-42 °C, the activity of R_D (at 20 °C) went up for one or two days, and became normalized not earlier than 4-7 d after the treatment (Fig. 3). Following heating at 44-46 °C, the R_D was strongly enhanced for 5 to 10 d, which recalled a climacteric rise. This respiratory overshoot indicated pathological catabolism. Also after an exposure to 48 °C the R_D remained high, until complete collapse within 1-2 d, due to a tissue decay. During exposure to 50 °C large areas of the leaves were destroyed, so that only a few portions of sublethal tissue released any CO_2 at all.

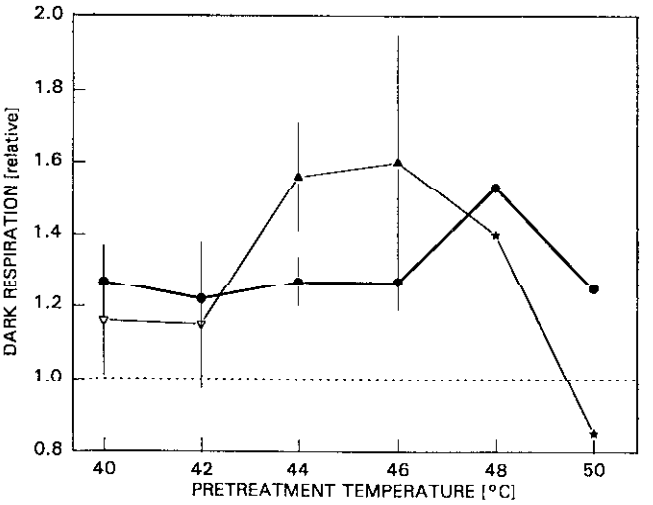

Fig. 3. Relative dark respiration (ratio between preheated and non-stressed plants) of *R. glacialis* at 20 °C after heat exposure at the given temperatures for 30 min: ● after effect within one day; ▽ recovered after few days; ▲ overshoot during progressive irreversible perturbation after 1-2 weeks; ★ acute breakdown of the metabolism.

Limits for lethal injuries: Visible signs of heat stress became apparent at 42-43 °C as turgor loss and infiltrated patches on the leaves, which were reversible within one day. The first necrotic injuries (LT_i) were recognized on the lamina and petioles after a heating to 44 °C, that led to yellowing of entire leaves after one or two weeks. An acute heat damage of LT_{50} was observed in plants exposed to 45-46 °C, and a complete lethality of the whole plants at 48 °C and higher temperatures. Values of LT_{50} at 46 °C were reported by Pisek *et al.* (1968) and at 47 °C by Gauslaa (1984).

Ultrastructural disorders due to high temperatures: The ultrastructure of leaf cells of control samples was typical for mesophyll cells (Fig. 4*a*); it was comparable to the ultrastructure of *R. glacialis* leaf cells taken directly from different field sites (Lütz and Moser 1977). This picture changed when the leaves were exposed to the 42 °C treatment: samples taken instantly after the heat exposure (Fig. 4*b*) contained mostly swollen chloroplasts and occasionally damaged tonoplasts together with a high vesiculation in the cytoplasm. Mitochondria or the thylakoid system did not change in appearance, but the plastids often contained clusters of electron dense material, probably heat shock granules (HSG, see further). After one day of recovery, plastid swelling was reduced, and increased numbers of polyribosomes as an indicator of enhanced protein synthesis occurred in the cytoplasm. On the 6th day of recovery most leaf cells showed a normal ultrastructure including disappearance of HSG.

Heat treatment at 44 °C resulted in tonoplast damage and in strong swelling of plastids and mitochondria (Fig. 4*c*). In both organelles HSG were clearly visible. They occurred also in mitochondria and in microbodies (not shown). From 7 till 15 d of recovery (Fig. 4*d*) swelling of mitochondria was almost completely reduced, but

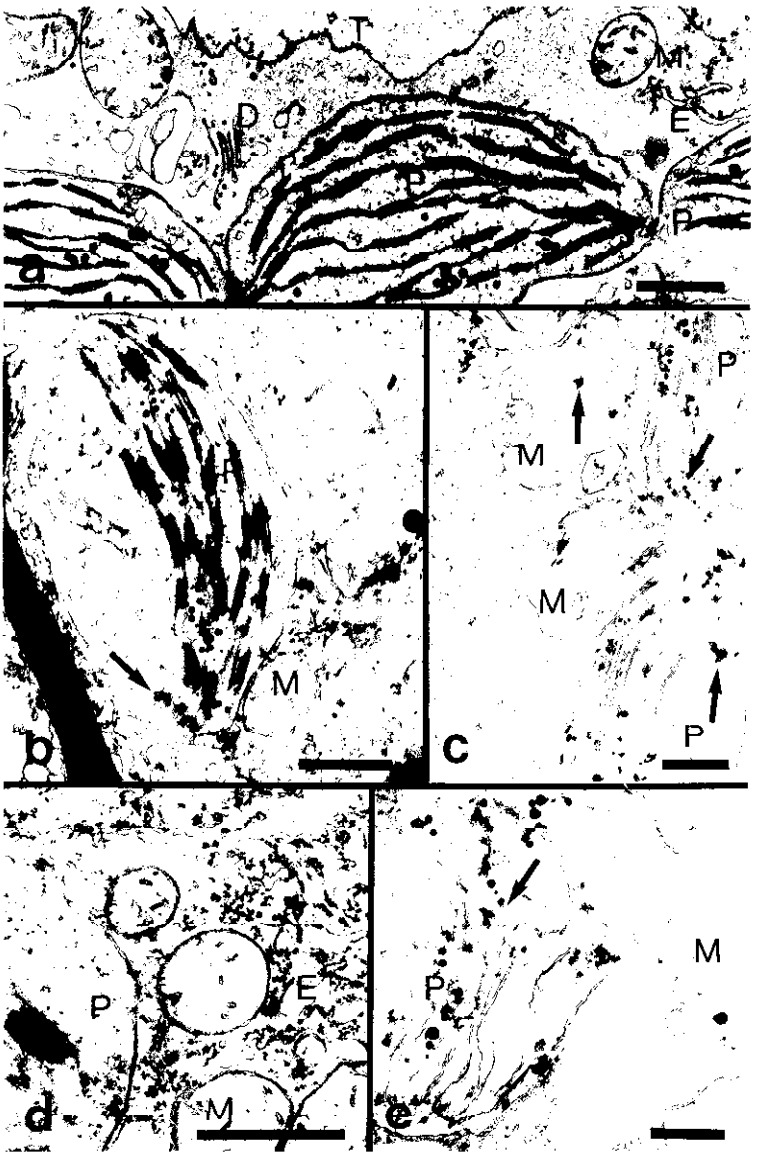

Fig. 4. Electron micrographs of typical structures in the protoplasma of leaf cells of *R. glacialis*. *a*: Control with plastids (P), mitochondria (M), dictyosomes (D), endoplasmatic reticulum (E), and tonoplast (T). *b*: heated at 42 °C, immediately after treatment; plastids appear strongly swollen, containing occasionally heat shock granules (*arrows*). *c*: 44 °C, immediately after treatment: plastids and mitochondria show strong swelling, and contain many heat shock granules. *d*: preheated at 44 °C, after 15 d recovery: while plastids remain swollen, mitochondria appear normal; rough endoplasmatic reticulum and polyribosomes indicate enhanced protein biosynthesis. *e*: heated at 46 °C, immediately after treatment: very similar to *c*; in addition, the thylakoid membrane system is dilated. Numerous heat shock granules developed. Bars: 1 μm.

not that of chloroplasts. The HSG persisted only in plastids and microbodies. Some vesiculations in the endoplasmatic reticulum still indicated the previous heat stress, while ribosomes and dictyosomes (not shown) were frequently distributed in the cytoplasm, again indicating repair activities.

Table 2. Threshold temperatures [°C] for photosynthetic activities and structures during the gradual progress of impairment due to heat stress in leaves of *Ranunculus glacialis*.

Stress response	Transient effect on metabolism	Reversible disturbance, full recovery	Long lasting perturbation becoming irreversible	Breakdown of functions and irreparable disruption of cell structures
Heat limit for P_N	38 ± 0.8			
Discontinuities on the basic fluorescence curve	38.2 ± 1.2			47.1 ± 0.3
After-effects on photosynthesis				
recovery index		40	42-44	44-46
gas exchange balance		41	42-44	45
fluorescence transients	< 39	41	42-44	45
After-effects on dark respiration		42	44-46	48
Abnormal alterations of protoplasmatic ultrastructure				
chloroplasts		42	44	46
mitochondria		44	46	
cytoplasm, ER		44	44	46
tonoplast		44	46	> 46
Lethal injuries			44	45-46 (partial) 48 (total)

Samples taken for electron microscopy immediately after the 46 °C treatment showed an ultrastructure similar to the 44 °C samples in plastids and mitochondria. In addition, the thylakoid membranes developed dilatations, tonoplasts were often destroyed, and dictyosomes were not visible. All cells still contained numerous HSG. Even a "recovery" period of up to 16 d was not sufficient to change this general ultrastructural appearance. Because even polyribosomes were not found with the increasing recovery time, the severe heat stress had probably prevented the repair of cell metabolism and membrane structure. None of the treatments did change the average number of plastoglobules seen in the plastids.

Discussion

With increasing intensity and duration of a heat stress it was possible to recognize different degrees of impairment (Table 2). One of the earliest events due to the heat

stress was the temporary depression of photosynthesis (down to CO_2 compensation concentration at HLC) and a blockage of the photochemical reaction centres of PS2 (lower deflection on the F-T curve). Seemann *et al.* (1984) found in leaves of desert plants and Larcher *et al.* (1991) in those of evergreen woody species a close agreement between the temperature of the heat limit for P_N and the early breakpoint of basic fluorescence. Up to 41 °C the P_N could recover completely, at 42 °C the recovery index reached 0.5, and at higher temperatures the photosynthetic function was gradually extinguished with the structural damage of chloroplasts in progress. Respiration was somewhat more stable to heat than photosynthesis; only above 44 °C there was a marked R_D overshoot, indicating the onset of declining cellular integrity.

Observations of the leaf cells ultrastructure supported the results of physiological measurements, as plastids were gradually damaged with increasing heat stress, and only the 42 °C treatment allowed for a slow recovery of normal ultrastructure. A visible damage to mitochondria required higher temperatures in comparison with chloroplasts. Regeneration of their membranes and functions following the 44 °C treatment needed at least 15 d of recovery. The strong, non-reversible damage to most cytoplasmatic components explained the cessation of metabolic functions. The fluorescence assays responded earlier to heat stress in comparison with visible ultrastructural changes in thylakoids. This is consistent with observations of Smillie (1979), who described injuries on thylakoid membranes above 44 °C in heat-stressed leaves of *Passiflora*, occurring first in PS2, while PS1 was inactivated above 48 °C. Also in both heat-tolerant and heat-resistant Indian rice cultivars PS1 was much more resistant to temperatures over 40 °C than PS2 (Bose and Ghosh 1995). Bauer (1978) and Bauer and Senser (1979) observed a similar swelling of plastids and thylakoids of *Hedera helix* leaves exposed to 48 °C for 30 min. In this species the Hill reaction was very heat-sensitive, even at 42 °C, whereas the ribulose-1,5-bisphosphate carboxylase/oxygenase activity had not been significantly reduced up to 50 °C. A freeze-etch study of *Nerium oleander* showed a clear relation between temperature increase above 41 °C, and a concomitant loss of connection between particles of the PS2 core and the light-harvesting complex, resulting in a loss of photosynthetic activity (Armond *et al.* 1980).

According to the dynamic stress concept of Selye and Stocker (see Larcher 1987, Lichtenthaler 1996) and the dualism of responses to environmental constraints (Alexandrov 1977), the stages of progressive strain include structural and functional destabilization (primary response phase), restabilization (restitution phase), recovery (repair) or decay (chronic exhaustion). During and after a heat stress in leaves of *R. glacialis* the following temperature ranges could be regarded as delimiting the successive events: 38-39 °C for a temporary deviation from normality, 40-42 °C for a reversible response phase, 42-44 °C for a chronic decline, and above 45 °C for a breakdown and lethal damage. Already at 42 °C some cells showed injured tonoplasts which might be responsible for observed turgor losses, and at exposures to a higher temperature, with an increased tonoplast destruction, for necroses in the leaves. This is different from observations of the heat stability in *Valerianella* tonoplasts, which are resistant up to 50 °C, while photosynthesis is affected already over 40 °C (Weigel 1983).

Under a moderate heat stress, the cells develop heat shock proteins, which assist in and protect for an undisturbed assembly, folding, and translocation of other proteins (Nover *et al.* 1989). Heat shock proteins can aggregate to heat shock granules (HSG) as ubiquitous structures at high temperatures, not only in vascular plants, but also in green algae (Meindl 1990). Such structures develop in the cytoplasm, plastids, mitochondria, and occasionally in microbodies of leaves under the heat stress. The granules found in *R. glacialis* were similar to those described by Fransolet *et al.* (1979) and by Neumann *et al.* (1989) for agricultural plants. This strongly supports the presence of heat shock proteins also in *R. glacialis*. A further proof is their complete disappearance in cells able to restore ultrastructure and photosynthesis. This is the first time that HSG are described for an arctic-alpine psychrophytic plant.

In comparison with the findings in leaves of herbaceous plants of boreal and temperate zones listed by Bauer *et al.* (1975) and Larcher (1994), and even of many alpine and arctic vascular plants (Denko *et al.* 1981, Gauslaa 1984), *R. glacialis* can be ranked as a peculiar heat-sensitive species. A characteristic feature of the pronounced thermosensitivity is the fact that between reversible disturbances and the appearance of incipient necrosis there is a wide range of chronic degradation (similar to the decline leading to a damage in chilling-sensitive species). Furthermore, the thresholds for heat-inactivation and visual injuries obtained in this study during various summers were remarkably close together, differing only by less than ±1 °C, which suggested that the capacity of *R. glacialis* for heat acclimation was limited. By acclimation of *R. glacialis* at supraoptimal temperatures, Gauslaa (1984) achieved a relatively small rise of 2 °C in the LT_{50}: the average for 28 hardened northern species was an increase of 2.6 °C.

Under natural conditions, overheating comes from a strong irradiation which may cause photoinhibition. In experiments with *Oxyria digyna,* Engel *et al.* (1986) found a progressive decline in photosynthesis as a result of strong irradiation coupled with high temperature. However, many alpine plants, including *R. glacialis*, contain considerable amounts of antioxidants that increase with altitude (Lütz 1996, Wildi and Lütz 1996), that are able to avoid photoinhibition even under a high irradiation. Thus, an increase in the leaf temperature above a certain threshold may damage alpine plants rather than a strong irradiation *per se*.

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