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Variability of Heat Tolerance in Alpine Plant Species Measured at Different Altitudes

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Abstract

We monitored variability of heat tolerance, osmotic water potential, and microclimate for seven alpine plant species at field sites and in response to a controlled in situ heat treatment (+3 K) using infrared lamps. Mean and maximum heat tolerance differed significantly between species and were clearly related to growth form and moisture conditions in the preferred microhabitat. Diurnal heat tolerance changes greater than ±1.5 K occurred on 18% of summer days at a velocity of 0.4–2.2 K h^{-1}. The diurnal heat tolerance amplitude ranged from 4.8 to 9.5 K, exceeding even the seasonal amplitudes (5–8 K) previously reported for other higher plant species. Heat tolerance increased under warmer microsite conditions and in warmer years. Long-term artificial heating by +3 K led to a significant ($P > 0.01$) increase in heat tolerance by +0.6 K. Under drought stress, heat tolerance was reduced to minimum values, with the exception of xerophytic species. The plant species investigated appeared to be very well adapted to the temperature conditions of their microhabitat, with the exception of the cushion plant *Minuartia recurva*, which recurrently experienced heat damage during the investigation.

Introduction

Common features of alpine macroclimate are low atmospheric temperatures and air pressures, causing low-vapor pressure deficits, and high maximum solar irradiation rich in shorter wavelengths (Körner, 1999). Despite reduced atmospheric temperatures, heat damage to alpine plants is a real threat (Larcher and Wagner, 1976; Gauslaa, 1984; Körner and Larcher, 1988; Körner, 1999; Neuner et al., 1999) because prostrate plant growth causes a decoupling of leaf temperature from ambient air temperature (see Körner and Larcher, 1988). Although heating above ambient temperature can be advantageous for carbon uptake, growth, and reproductive processes under certain conditions, a heat-trapping stature may increase the danger of heat damage even in a cold climate. Heat damage to alpine plants can occur under a clear sky with calm winds and potentially dry conditions (Larcher and Wagner, 1976; Gauslaa, 1984; Körner, 1999; Neuner et al., 1999).

The intraspecific variability of heat tolerance thresholds appears to be rather narrow in arctic and alpine plant species (between 0.1 and 5.0 K in response to artificial heat hardening; Gauslaa, 1984). For alpine *Silene acaulis* cushions, the range of heat tolerance thresholds under summer field conditions was much greater (9 K, 45.5–54.5°C; Neuner et al., 2000). Except for *S. acaulis*, little is known about the variability of heat tolerance thresholds in alpine plant species in the field. Our first objective was to determine the intraspecific variability of heat tolerance in seven typical alpine plant species during daily time courses and throughout the summer growing period.

Heat hardening of the leaf tissue can occur within hours or even minutes when critical high-temperature thresholds are surpassed (Alexandrov, 1977). In *S. acaulis* cushions, heat tolerance started to increase at temperatures above 30°C in response to a controlled in situ heat treatment with infrared lamps (Neuner et al., 2000). In some plant species, water deficiency can also increase heat tolerance (Alexandrov, 1977). Little is known about the driving forces of heat hardening in nature, as most investigations have been carried out under controlled environmental conditions.

Heat hardening of the most heat sensitive subprocess of photosynthesis, i.e., photosystem II (PS II), has been studied more extensively. Thermostability of PS II increased when leaves were exposed to high leaf temperatures (Schreiber and Berry, 1977; Weis and Berry, 1988; Huxman et al., 1998; Larcher et al., 1990) and, in some species, in response to drought stress (Seemann et al., 1986; Valladares and Pearcy, 1997; Hamerlynck et al., 2000). Thermostability of PS II can also be affected by irradiation intensity (Schreiber and Berry, 1977; Weis, 1982), irradiation quality (Havaux and Strasser, 1992), salinity (Larcher et al., 1990), and elevated CO_2 (Huxman et al., 1998; Taub et al., 2000).

Our second objective was to determine the driving forces of alterations in heat tolerance under alpine field conditions. We monitored leaf temperatures, irradiation intensity, and water relations in addition to heat tolerance. Each species was investigated where it grew naturally but at sites with widely contrasting environmental conditions. Three field sites at different altitudes (1950, 2150, and 2600 m a.s.l.) were chosen. Within this altitudinal range, major differences in climatic conditions could be expected.

We further aimed to investigate the effect of elevated leaf temperatures alone on heat tolerance. To simulate the effect of an assumed global temperature increase of +1.5–4.5 K within the next 50 yr (Manabe, 1998), *Loiseleuria procumbens* canopies were heated with infrared lamps, increasing leaf temperatures by +3 K throughout the whole growing period.

Materials and Methods

PLANT SPECIES AND INVESTIGATION SITES

We investigated plant species with typical alpine growth forms and heights, i.e., herbaceous and rosette plants, cushions, dwarf shrubs, and shrubs (Table 1). The selected species grow in habitats ranging from permanently wet places to wind-exposed or particularly dry sites. As plant water relations due to both transpiration and plant stature (Körner and Cochrane, 1983; Wilson et al., 1987) are decisive in determining plant temperature, the selected species were expected to have different leaf temperature maxima and hence to show differences in their intrinsic and dynamic heat tolerance.

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TABLE 1

Growth form and height, preferred microhabitat, and altitudinal range for the alpine plant species that were selected to investigate the variability of heat tolerance under alpine field conditions

Plant species	Growth form	Growth height (cm)[a]	Preferred microhabitat[a]	Altitudinal range (m a.s.l.)[b]
Loiseleuria procumbens	dwarf shrub	3–15	wind exposed	1800–2800 m
Minuartia recurva	cushion	5–15	wind exposed	2100–2900 m
Ranunculus glacialis	herbaceous	10–15	wet	2300–4000 m
Rhododendron ferrugineum	shrub	30–100	sheltered in gullies	1000–2500 m
Silene acaulis	cushion	1–3	dry	2300–3300 m
Saxifraga paniculata	rosette	5–15 (30)	dry	600–3200 m
Soldanella pusilla	herbaceous	2–10	wet, snowbed	1800–2800 m

[a] From Adler et al. (1994).

[b] From Landolt (1992).

The diurnal and seasonal dynamics of heat tolerance were monitored at three sites in the Central European Alps on Mount Patscherkofel (2248 m a.s.l.) and on the adjacent Mount Glungezer (2678 m a.s.l.) near Innsbruck. The selected species occurred naturally at these three altitudinally different sites. Above the timberline is a subalpine belt with dwarf shrubs. Farther up, alpine grass mats, rockfield and gravel vegetation, and snowbed communities are prevalent. Site 1 was located within the upper subalpine belt near the timberline on Mount Patscherkofel (1950 m a.s.l.; 47°12′39″N, 11°27′03″E). Site 2 was situated near the summit of Mount Patscherkofel (2150 m a.s.l.; 47°12′27″N, 11°27′17″E) located within the lower alpine vegetation belt. Site 3 was the peak area of Mount Glungezer (2600 m a.s.l.; 47°12′16″N, 11°3116″E), where most species found at the highest sites for higher plant growth in the nival belt of the Austrian Alps are present (Bahn and Körner, 1987). All species listed in Table 1 were found at Site 3. However, due to the small population sizes, *Rhododendron ferrugineum* and *L. procumbens* were not investigated at Site 3. Not all of the selected species grow at the lower sites. *Ranunculus glacialis* is absent on Mount Patscherkofel, and *M. recurva* can only be found at its summit.

MICROCLIMATE

Standard meteorological data for the two sites on Mount Patscherkofel are available. However, knowledge of the actual microclimate is a prerequisite for the interpretation of stress physiological field data. At all three sites, micrometeorological measurements were conducted continuously within each species canopy throughout the investigation period (1998–1999).

Leaf temperatures of all species were determined with thermocouples (Type T; solder junction diameter 0.3 mm), which were mounted to the leaves with a special adhesive (Transpore, BM-Austria GmbH, Vienna, Austria). Soil temperatures were measured with thermistors (107, Campbell Scientific, Logan, Utah, USA). Irradiation was recorded with quantum (QS, Delta-D, UK) and pyranometer sensors (SP1110, Skye Instruments, UK). All sensor types were connected to data loggers (CR10X, Campbell Scientific, Logan, Utah, USA) collecting data every 10 s from each sensor and recording mean values at 30-min intervals.

HEAT TOLERANCE

Heat tolerance was determined with solar-powered portable field heat tolerance test equipment described in detail by Buchner and Neuner (2001). The heat tolerance test equipment consists of 6 exposition chambers, each of which runs a different preset target temperature for an exposure time of 30 min. Temperatures were chosen in such a way that the lowest should cause no damage and the highest should produce 100% damage.

Heat tolerance measurements were conducted exclusively on fully expanded leaves (5 leaves per species and target temperature). Leaves were fixed to heat-stable transparencies (100 × 130 mm) with an adhesive. Transparencies were then enclosed in the exposition chambers. After the heat treatment and a species-specific latent period, the leaves still mounted on the transparencies were digitized with a scanner for later quantification of heat damage by digital image analysis (Neuner and Buchner, 1999). *Minuartia recurva* and *S. acaulis* did not show damage-induced discoloration of the leaves; therefore, heat damage of these species was assessed by the conductivity test according to the procedure of Neuner and Buchner (1999). The percentage of heat damage for each sample was then plotted against heat-treatment temperature, and a classic logistic function was fitted to the data (P-Fit, Biosoft, Durham, North Carolina, USA). LT_{50} values, i.e., the temperature at 50% heat damage, can then be read directly from the curve-fitting protocol.

To cover a wide range of diurnally and seasonally occurring natural environmental conditions, actual heat tolerance was determined throughout two successive growing periods (1998, 1999) at the three investigation sites, at a minimum of weekly intervals. At each of the three sites daily time courses for heat tolerance of leaves were determined in parallel. The first measurement was conducted before dawn and the second around midday (12:00–14:00 mean European time). Additional measurements were performed during the afternoon and in the evening. ΔLT_{50} is the difference in heat tolerance between predawn and midday. The velocity of heat tolerance changes during the morning was calculated for the same time period.

WATER POTENTIAL

Samples for determination of leaf water potential were taken at the same time as heat tolerance measurements. Actual osmotic water potential ($\Psi_{0(\mathrm{act})}$) was determined on leaves that immediately after excision had been wrapped in aluminium foil and freeze-killed in liquid nitrogen (Kikuta and Richter, 1992). To determine the osmotic water potential at full saturation ($\Psi_{0(\mathrm{sat})}$), the petiole of each leaf was dipped in water and the laminas were exposed to humid air inside closed plastic boxes until they were fully saturated with water. These leaves were also freeze-killed in liquid nitrogen, as described earlier. Water potential measurements were conducted in calibrated C-52 Sample Chambers (Wescor, Logan, USA) connected to a HR-33T Dew Point Microvoltmeter (Wescor, Logan, Utah, USA) which was operated in the hygrometric mode.

IN SITU LONG-TERM HEAT TREATMENT

During the 1999 growing period, two *L. procumbens* canopies were heated by +3 K and compared to an untreated canopy (Fig. 1) to study the effect of a leaf temperature increase under otherwise unchanged environmental conditions. All investigated *L. procumbens* canopies had the same orientation and exposure toward wind and irradiation. Heating was provided by infrared lamps (Type 13713Z/98, 1000 W, Phillips, Vienna, Austria), using the method of Nijs et al. (1996). 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 70 cm above the canopy surface and were mounted to the north of the investigated plants to prevent shading. Canopy leaf temperatures were recorded with 5 thermocouples (Type T, 0.2 mm copper-constantan thermocouples) every 0.125 s on a Multiplexer (AM25T, Campbell Scientific, Logan, Utah, USA) connected to a data

TABLE 2

Mean, minimum, and maximum heat tolerance (LT_{50}[°C] of leaves of different alpine plant species measured during the growing periods of 1998 and 1999 at three investigation sites (site 1: 1950 m a.s.l., site 2: 2150 m a.s.l., and site 3: 2600 m a.s.l.). Species are listed in order of decreasing mean heat tolerance. Heat tolerance is compared to LT_{50} values obtained by other authors. The maximum summer range for heat tolerance, the maximum diurnal range for heat tolerance, the maximum change in heat tolerance from predawn until midday (ΔLT_{50}; [K]), and the maximum velocity of changes (K/h) are also shown. Significant differences between mean values of LT_{50} for different species are indicated by different letters (Bonferroni, $P > 0.05$*)*

		LT_{50}					ΔLT_{50}			
Species	n	Mean	SD	Min	Max	Other authors	Summer	Diurnal	Predawn–midday	Maximum velocity
Saxifraga paniculata	811	52.6^a	1.8	47.2	57.5	52.0/54.0^1 45.5/46.5^2	10.3	7.4	4.6–4.2	2.2–1.5
Minuartia recurva	425	50.8^b	2.0	44.3	55.5		11.2	9.5	4.7–7.2	0.9–1.8
Loiseleuria procumbens	871	50.6^b	2.0	44.8	56.5	51.8^4 49.9^3 49.0^5	11.7	9.1	8.7–4.6	1.5–1.3
Silene acaulis	454	50.3^c	1.9	45.5	54.9	51.0/55.0^6 51.0^7 49.0/51.4^4 46.6^3	9.4	7.2	5.8–7.2	1.4–1.0
Soldanella pusilla	802	49.9^d	1.6	44.4	54.6	46.0^5	10.2	7.0	4.4–5.7	1.3–1.2
Rhododendron ferrugineum	692	48.4^e	1.8	43.8	53	43.5/46.5^8	9.2	5.3	4.1–4.6	1.4–1.3
Ranunculus glacialis	129	47.8^f	1.2	45.3	51.3	47.1/49.3^4 47.0^3 46.5^9 45.5^{10}	6.0	4.8	3.3–3.1	0.4–0.7

^{1} Biebl and Maier (1969), ^{2} Sapper (1935), ^{3} Kjelvik (1976), ^{4} Gauslaa (1984), ^{5} Larcher and Wagner (1976), ^{6} Kainmüller (1974), ^{7} Biebl (1968), ^{8} Schwarz (1970), ^{9} Pisek et al. (1968), ^{10} Larcher et al. (1997).

logger (CR10X, Campbell Scientific, Logan, Utah, USA). The mean leaf temperature of each heated canopy was then compared with the mean leaf temperature of the reference canopy. When the mean leaf temperature of the heated canopies dropped below the target temperature, the lamps were activated; in the opposite case they were turned off.

STATISTICAL DATA ANALYSIS

More than 4000 single measurements of heat tolerance and simultaneous recordings of microclimate parameters represent a substantial database for the analysis of short- and long-term changes of heat tolerance and its possible driving forces. For descriptive statistical data analysis and comparisons of mean values, we used SPSS software (SPSS Version 9.0, SPSS Inc., Chicago, Illinois, USA). After the data had passed the test for normality (Kolmogorov-Smirnov test), the significance of the difference between mean values (n = 5) for heat tolerance from different times during the day and between different plant species, years, or investigation sites was tested by analysis of variance (ANOVA) and the Bonferroni test. When only two means were compared, the Student's t-test was used.

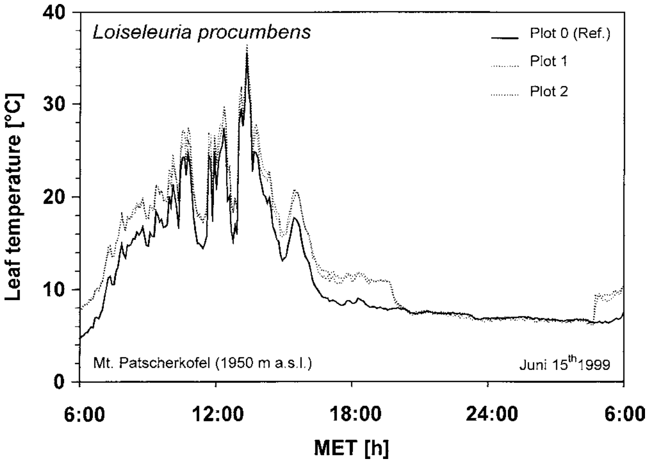

FIGURE 1. Typical daily time courses (15 June 1999) for mean leaf temperature measured in two L. procumbens *canopies (Plots 1 and 2) that were heated with infrared lamps to increase temperatures by +3 K, compared to an untreated reference canopy (Plot 0) at site 1 on Mt. Patscherkofel (1950 m a.s.l.).*

Results

DYNAMIC OF HEAT TOLERANCE

Heat tolerance differed significantly between species (Table 2). *Saxifraga paniculata* had the highest mean heat tolerance of 52.6°C

TABLE 3

Mean, minimum, and maximum leaf temperatures (°C, half-hourly means) for the investigated plant species measured during the growing periods of 1998 and 1999 at three investigation sites (site 1: 1950 m a.s.l., site 2: 2150 m a.s.l., and site 3: 2600 m a.s.l.). The maximum summer range for leaf temperatures (K) and the maximum diurnal range for leaf temperatures (K) are also shown

	Leaf temperature				Maximum leaf temperature range	
Species	Mean	SD	Min	Max	Summer	Diurnal
Saxifraga paniculata	10.6	7.0	−3.8	47.7	51.5	41.9
Minuartia recurva	9.7	8.0	−3.6	57.3	60.9	55.9
Loiseleuria procumbens	11.5	7.6	−5.6	43.7	49.3	42.8
Silene acaulis	9.6	7.1	−3.7	40.1	43.8	40.1
Soldanella pusilla	9.2	5.5	−4.2	43.4	47.6	34.5
Rhododendron ferrugineum	10.7	6.0	−3.7	36.0	39.7	35.0
Ranunculus glacialis	7.9	5.5	−3.6	34.8	38.4	37.2

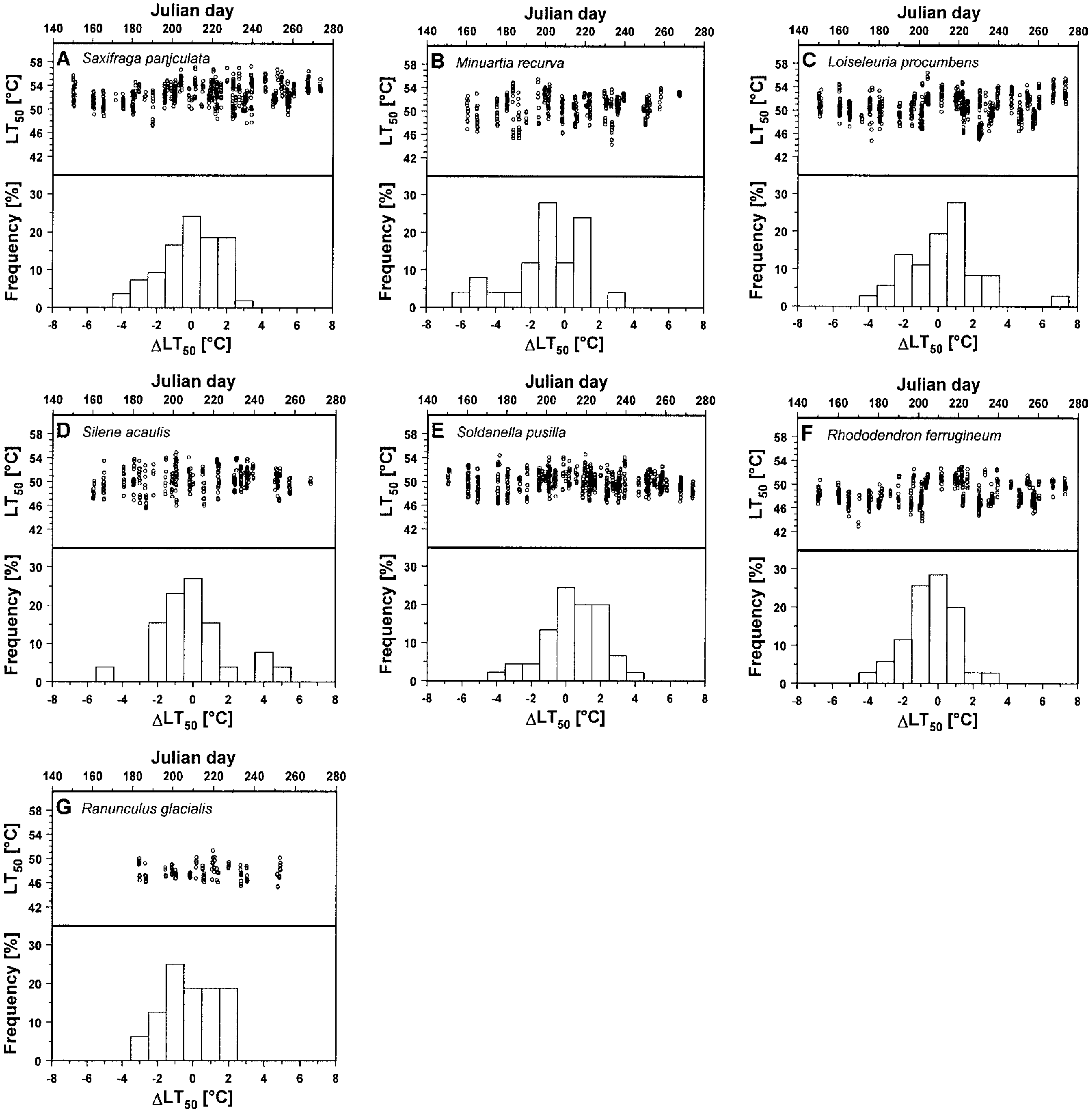

FIGURE 2. Upper graphs: Heat tolerance of different alpine plant species, (A) S. paniculata, *(B)* M. recurva, *(C)* L. procumbens, *(D)* S. acaulis, *(E)* S. pusilla, *(F)* R. ferrugineum, *and (G)* R. glacialis *measured during the growing period of both investigation years (1998, 1999) and at three different sites: Mt. Patscherkofel (1950 m a.s.l and 2150 m a.s.l.) and Mt. Glungezer (2600 m a.s.l.). For sample size, see Table 2. Lower graphs: Open bars indicate the frequency of heat hardening and dehardening processes from predawn until midday (ΔLT_{50}). The ΔLT_{50} values were grouped in 1 K temperature classes.*

(LT_{50}), followed by the cushion plant *M. recurva* (50.8°C) and the dwarf shrub *L. procumbens* (50.6°C). Significantly lower values were measured for *S. acaulis* (50.3°C), *S. pusilla* (49.9°C), *R. ferrugineum* (48.7°C), and finally *R. glacialis* (47.8°C). The maximum heat tolerance for the investigated species was found in leaves of *S. paniculata* (57.5°C) followed by *L. procumbens* (56.5°C). Heat tolerance for all investigated species varied markedly during the summer growing period, with a total range of 9.2 to 11.7 K in all species except *R. glacialis* (6.0 K).

The diurnal variability of heat tolerance was only slightly lower than the seasonal variability during the growing period, with the exception of *R. ferrugineum*, where the diurnal heat-hardening capacity was half of the seasonal capacity. The maximum diurnal variability in leaf temperature was also nearly as great as the leaf temperature range over the whole investigation period (Table 3).

The investigated species did not show a pronounced seasonal change in heat tolerance (Fig. 2) but did show frequent diurnal heat tolerance adjustments. From predawn until midday, heat hardening ($\Delta LT_{50} > 1$ K) took place in 26–47% of summer days studied, and heat dehardening ($\Delta LT_{50} < -1$ K) occurred during 27–60% of summer days. Heat hardening and dehardening were frequently occurring

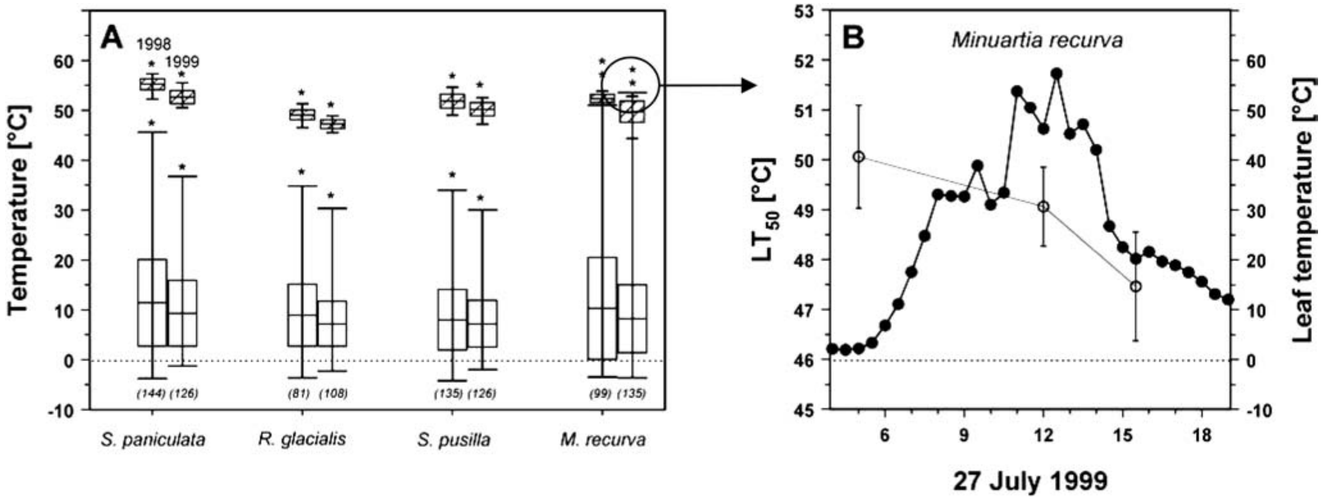

FIGURE 3. (A) Mean heat tolerance of leaves (LT_{50}) of four alpine plant species (hatched box: mean value ±SD; minimum and maximum indicated by a vertical line) on Mt. Glungezer (2600 m a.s.l.) compared to mean leaf temperatures of the respective species (open box: mean value ±SD; minimum and maximum indicated by a vertical line) measured during two successive summer periods (1998 and 1999). The difference between mean values of heat tolerance and leaf temperature was tested by Students t-test and was (*) significant ($P > 0.05$*) in all cases. (Numbers in italics = number of samples.) (B) Daily time course of heat tolerance (LT_{50}) (open circle: mean value ±SD) and leaf temperature (solid circle) of* M. recurva *at Mt. Glungezer (2600 m a.s.l.) on a clear and hot summer day (27 July 1999).*

physiological processes under natural alpine field conditions. Under the experimental conditions, the maximum mean velocity of changes in heat tolerance was 2.2 K h^{-1} in the leaves of *S. paniculata* (see Table 2). Most of the other species hardened at slower rates (0.9–1.4 K h^{-1}). Heat hardening of *R. glacialis* leaves occurred at the distinctly lower velocity of 0.4 K h^{-1}. Similar observations were made with respect to heat dehardening: Heat dehardening was slower in leaves of *R. glacialis* (− 0.7 K h^{-1}) than all of the other species (−1.0 and −1.8 K h^{-1}). Natural heat damage to leaves was observed for *M. recurva*. Leaf temperature maxima of *M. recurva* exceeded the minimum, and in 1999 even the maximum heat tolerance of the species at Site 3. It is thus not surprising that heat damage to *M. recurva* cushions recurred (e.g., at 27

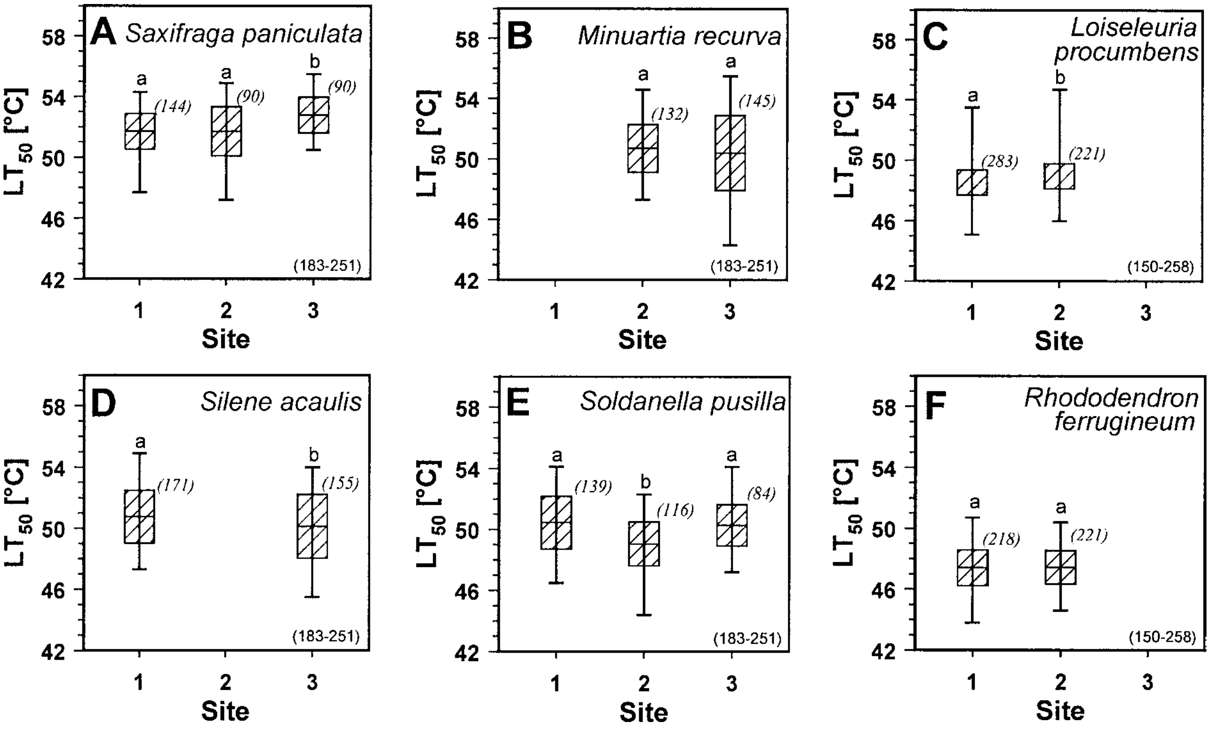

FIGURE 4. Mean heat tolerance of leaves (LT_{50}; mean value ±SD; minimum and maximum indicated by a vertical line) of selected alpine plant species (A) S. paniculata, *(B)* M. recurva, *(C)* L. procumbens, *(D)* S. acaulis, *(E)* S. pusilla, *and (F)* R. ferrugineum *measured during summer 1999 at three different investigation sites (site 1: Mt. Patscherkofel [1950 m a.s.l.], site 2: Mt. Patscherkofel [2150 m a.s.l.], site 3: Mt. Glungezer [2600 m a.s.l.]). Significant differences between mean values of heat tolerance at each site were tested for using Students t-test and with ANOVA and the Bonferroni test at* $P > 0.05$*. Significant differences are indicated by different letters. The numbers given in brackets are julian days and indicate the measurement period for each species. (Numbers in brackets in italics = number of samples.)*

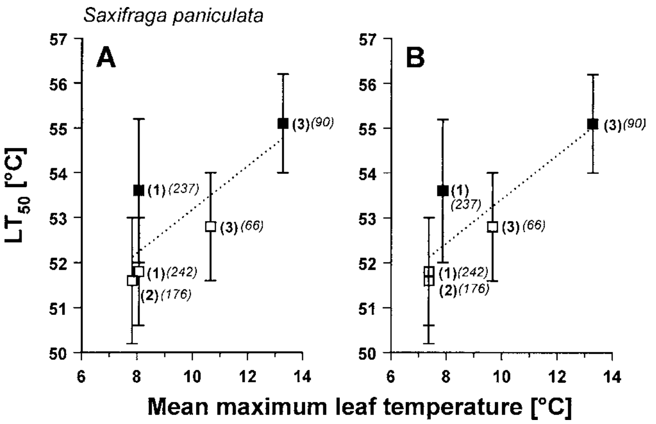

FIGURE 5. *Effects of mean maximum leaf temperature (arithmetical means of half-hourly maxima) from (A) 1 h and (B) 3 h before sampling of* S. paniculata *leaves to measure mean heat tolerance. Data from two years (* ■ 1998, □ 1999) and three sites (numbers in brackets). (Numbers in brackets in italics = number of samples.)

July 1999) at this site during the investigation period (Fig. 3). All other species showed sufficiently high safety margins of several K between the highest leaf temperature and the minimum heat tolerance.

EFFECT OF LEAF TEMPERATURE ON HEAT TOLERANCE

Under natural alpine conditions, absorption of irradiation by leaves causes an increase in leaf temperature. Aside from exposure to wind, leaf temperature is mainly controlled by irradiation, so effects of leaf temperature on heat tolerance are intertwined with potential irradiation effects.

The summer of 1998 was significantly warmer than the summer of the following year, as shown by higher mean leaf temperatures (see Fig. 3). Mean, maximum, and minimum heat tolerance was also significantly higher in 1998 than during the colder summer of 1999.

Significant differences between heat tolerances for the same species at different sites were observed, but not for all species (Fig. 4). We cannot deduce an effect of elevation on heat tolerance from our data because our sites differed largely in exposure. Heat tolerance either increased (*L. procumbens*), decreased (*S. acaulis*), or remained unchanged (*M. recurva*, *R. ferrugineum*) with changes in elevation. The higher the mean maximum leaf temperatures at a particular growing site, the higher the mean heat tolerance in leaves of *S. paniculata* (Fig. 5). The same tendency can also be found among the other species being examined (data not shown). Mean maximum temperature appears to be an important factor affecting heat tolerance. When the actual heat tolerance value is plotted against mean leaf temperature for the 3 h prior to the heat tolerance measurement, the relationship between leaf temperature and heat tolerance is less clear (Fig. 6). When mean leaf

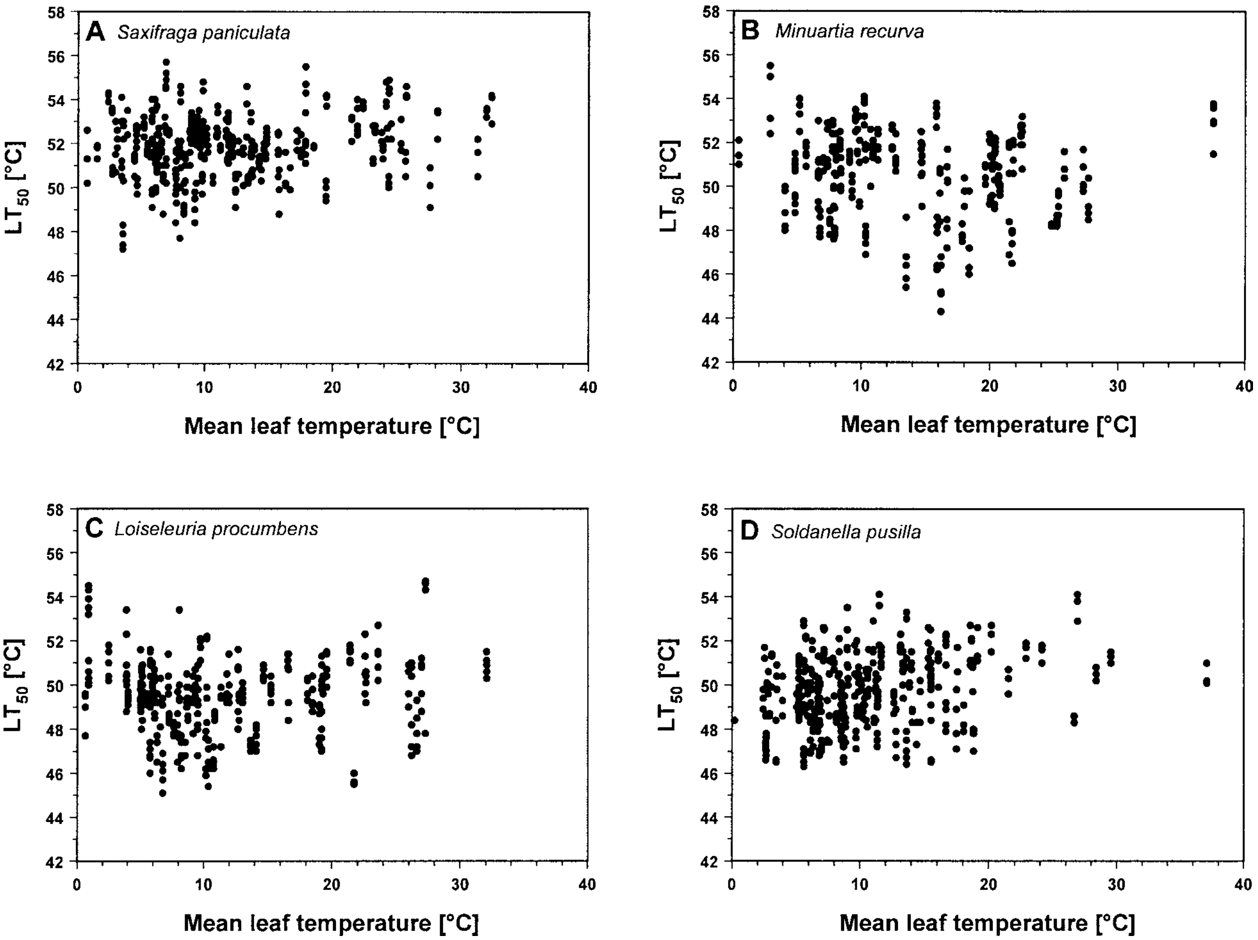

FIGURE 6. *Effect of mean leaf temperature (mean values from 3 h before sampling of leaves of (A)* S. paniculata, *(B)* M. recurva, *(C)* L. procumbens, *and (D)* S. pusilla *for heat tolerance (LT$_{50}$) at three different investigation sites (site 1: Mt. Patscherkofel [1950 m a.s.l.], site 2: Mt. Patscherkofel [2150 m a.s.l.], site 3: Mt. Glungezer [2600 m a.s.l.]). When mean leaf temperatures exceed a specific high temperature threshold, low heat tolerance values are absent. The numbers given in brackets are julian days and indicate the measurement period (1999). For sample size, see Table 2.*

temperatures exceed a specific high temperature threshold, low heat tolerance values are absent. Below the high temperature threshold, there is considerable variability in heat tolerance, suggesting that a factor other than leaf temperature induces heat tolerance adjustments.

Artificial heating of *L. procumbens* canopies by +3 K led to a significant ($P > 0.01$) increase in heat tolerance compared to an untreated canopy. Mean heat tolerance of leaves of the untreated canopy was 49.4°C, while mean heat tolerance of heated plants had increased to 50.1°C and 50.2°C, respectively (Fig. 7).

EFFECT OF WATER RELATIONS ON HEAT TOLERANCE

The difference between $\Psi_{0(act)}$ and $\Psi_{0(sat)}$ is a measure of drought stress. In unstressed leaves ($\Psi_{0(act)} = \Psi_{0(sat)}$) the greatest range of heat tolerance values was measured for most species (Fig. 8). As drought stress increased (increasing difference between $\Psi_{0(act)}$ and $\Psi_{0(sat)}$), recorded maximum heat tolerance values decreased. In contrast, *S. paniculata* showed maximum heat tolerance during drought stress at water potential differences ($\Psi_{0(act)} - \Psi_{0(sat)}$) of between −0.5 and −1.5 MPa.

Discussion

INTERSPECIFIC HEAT TOLERANCE DIFFERENCES

Heat tolerance was clearly related to the growth form and the preferred microhabitat moisture conditions of each species. Prostrate growth forms such as rosettes of *S. paniculata*, cushions, and dwarf shrubs tolerate significantly higher temperatures than herbs and tall *R. ferrugineum* shrubs. This evidently mirrors the growth form–specific differences in leaf temperature climate. In addition, species on dry sites have a higher heat tolerance than species that occupy permanently moist sites, allowing a greater transpirational cooling. Even a south-facing individual of *R. glacialis* experienced a maximum leaf temperature of only 34.8°C because it grew on moist soils that did not restrict transpiration. Therefore, the slow heat-hardening rate (+0.4 K h^{-1}) and low heat tolerance adjustments (4.8 K) seem to be sufficient for this species. *R. ferrugineum* experienced lower leaf temperature maxima (36.4°C) than the other species due to the thickness of its canopy, and was the second-most hardy species. Although *S. pusilla* is typical of moist snowbed communities, it has a high maximum heat tolerance ($LT_{50} = 54.6$°C). Immediately after snowmelt, leaves are close to the ground and not shaded by other species. Under these conditions, critically high leaf temperatures of up to 43.3°C were measured.

Heat damage was observed only on leaves of *M. recurva* but has previously been reported to occur under natural conditions for *S. acaulis* (Gauslaa, 1984; Körner, 1999) and *S. paniculata* (Neuner et al., 1999). Heat damage, although not very frequent, occurs today in alpine environments. The probability of heat damage to alpine plants could increase as temperature extremes are expected to increase with global climate change (Wagner, 1996).

Mean heat tolerances for the investigated alpine plant species correspond well with findings of earlier investigations (see Table 2). However, the range from minimum to maximum heat tolerance distinctly exceeds the range deducible from the earlier data. Only dehardened greenhouse plants of *S. paniculata* had a heat tolerance lower than we found at field sites (Sapper, 1935). Taking all data into consideration, minimum heat tolerances for all investigated species ranged from 43.8 to 45.5°C (1.7 K). The similarity between species in this respect could reflect a substantial similarity in the so-called primary thermostability of all species (Alexandrov, 1977), i.e., a minimum level of heat tolerance that occurs as long as environmental factors do not cause heat hardening.

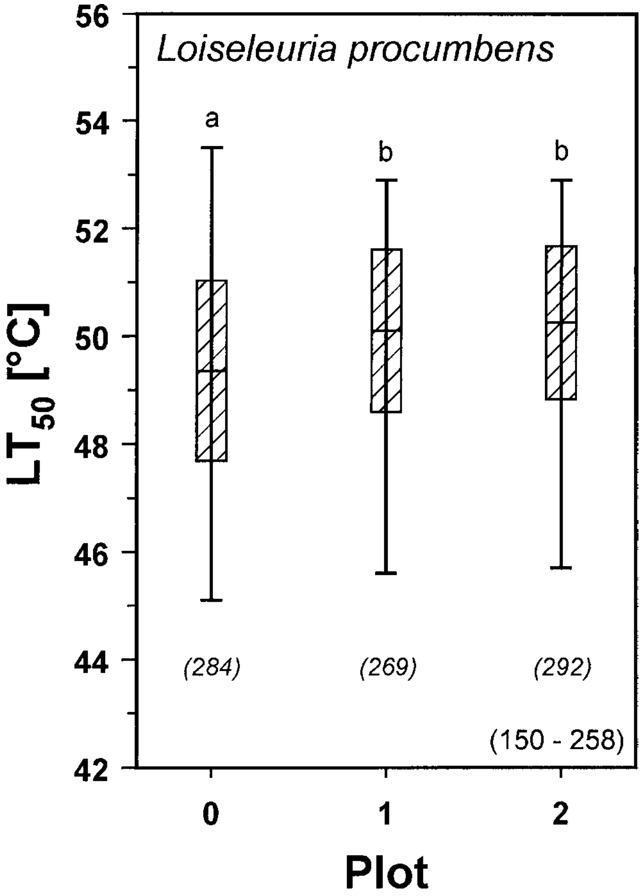

FIGURE 7. Mean heat tolerance of leaves (LT_{50}; mean value ±SD; minimum and maximum indicated by a vertical line) of L. procumbens *at Mt. Patscherkofel (1950 m a.s.l.) during the summer of 1999 from an untreated reference canopy (Plot 0) and from canopies (Plot 1, Plot 2) heated during daytime hours by +3 K relative to this reference canopy. Significant differences between mean values of heat tolerance at each plot were tested for using ANOVA and the Bonferroni test at* $P > 0.05$. *Significant differences are indicated by different letters.*

DIURNAL CHANGES IN HEAT TOLERANCE

Diurnal changes in heat tolerance (ΔLT_{50}) were frequent, indicating that heat hardening and dehardening are commonly occurring physiological processes under alpine field conditions. All species showed significant diurnal changes in heat tolerance, from a minimum change of 4.8 K (*R. glacialis*) to a maximum of 9.5 K (*M. recurva*). This range exceeds that found in earlier field investigations by several degrees (1.0–2.5 K: Alexandrov, 1977; 4 K: Larcher, 1980; 2 K: Larcher et al., 1989). The extent of diurnal heat hardening observed in our investigation even exceeds the maximum extent after artificial heat hardening of arctic and alpine plants reported earlier (0.1–5 K: Gauslaa, 1984; 4.7 K: Neuner et al., 1999). Gauslaa (1984) was able to provoke maximum heat hardening of +5 K in *Oxyria digyna* with a harsh heat treatment at sublethal temperatures for 24 h. In most of the other investigated plants the heat-hardening response was much less (+2–3 K). Experiments with *S. acaulis* showed that the extent of heat hardening depended on the heating rate employed (Neuner et al., 2000). Immediate exposure to extremely high temperatures appeared to retard heat hardening, whereas exposure to moderate temperatures above a critical high-temperature threshold caused a maximum increase in heat tolerance. These factors could explain the differences in reported results. In addition, species may respond differently to high altitudes than to high latitudes because of the difference in irradiation extremes, which potentially affect heat tolerance (Maier, 1971).

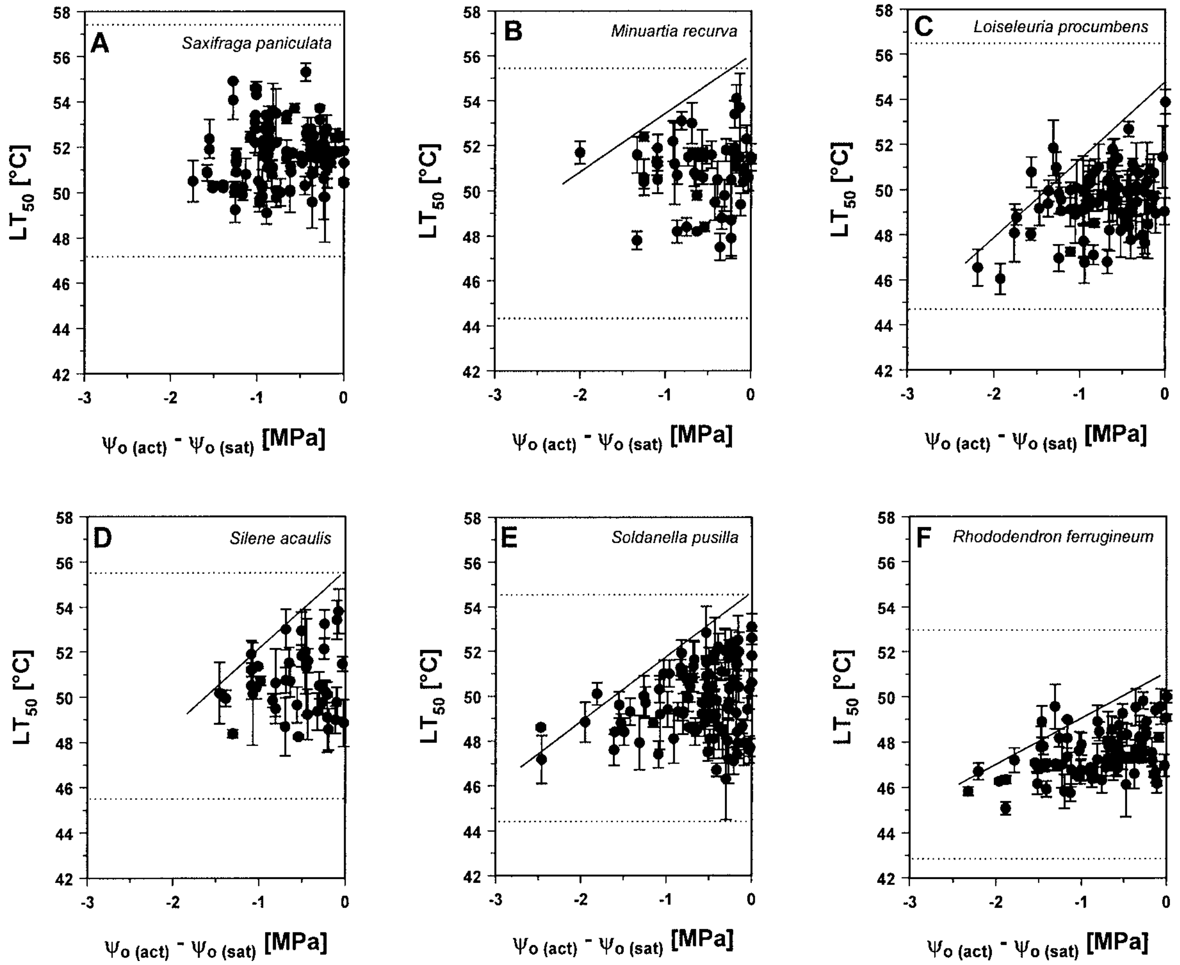

FIGURE 8. Effect of drought stress indicated by increasing differences between $\Psi_{0\ (sat)}$ and $\Psi_{0\ (act)}$ on actual heat tolerance of leaves (LT_{50}; mean value ±SD) of (A) S. paniculata, *(B)* M. recurva, *(C)* L. procumbens, *(D)* S. acaulis, *(E)* S. pusilla, *and (F)* R. ferrugineum *measured at three different investigation sites in 1999. Dotted lines indicate the maximum and minimum heat tolerance (LT_{50}) measured during both investigation periods (1998 and 1999). For sample size, see Table 2.*

The high diurnal heat tolerance variability could be a peculiar feature of alpine plants. In European plant species from low altitudes, maximum diurnal changes in heat tolerance ranged from 1 to 2.5 K (Alexandrov, 1977; Kappen, 1981; Larcher et al., 1989). These changes were not observed every summer but occurred exclusively during hot, dry summers (Lange, 1961). At high altitudes, environmental conditions such as temperature and irradiation typically change rapidly during the day. Daily temperature amplitudes were similar to total summer amplitudes. High daily leaf temperature amplitudes exceeding 30 K were frequent events (e.g., at Site 2 *L. procumbens*: 21.5%, *S. paniculata*: 23.2%). Under such environmental conditions rapid diurnal adjustments of heat tolerance are ecologically significant and can be critical in surviving high temperatures.

VARIABILITY OF HEAT TOLERANCE OVER SUMMER

The range of diurnal changes in heat tolerance was only a few degrees less than the range over the whole summer. For *R. ferrugineum*, the summer range for heat tolerance (9.2 K) exceeds the total seasonal amplitude of 7 K reported earlier (Schwarz, 1970). With the exception of *R. glacialis* (6.0 K), we observed greater variations in heat tolerance during summer (9.4–11.7 K) than the maximum seasonal amplitude (5–8 K) commonly observed in other higher plant species (see Larcher, 1973; Kappen, 1981; Körner, 1999). Heat tolerance varied between a minimum and a species-specific maximum value during summer rather than showing a trend over the summer. In contrast to our investigation, earlier studies of minimum heat tolerance found distinct species-specific seasonal trends in heat tolerance (see, e.g., Larcher, 2001).

FORCES DRIVING HEAT TOLERANCE ADJUSTMENTS

Temperature conditions have a strong impact on heat tolerance of plants. Previous studies have shown that if leaf temperature exceeds a species-specific high-temperature threshold, heat tolerance usually increases rapidly (Alexandrov, 1977; Neuner et al., 2000). Our results under natural conditions, comparing years and sites and in response to artificial heating, also suggest that leaf temperature has a large impact on heat tolerance. The year 1998 has been one of the warmest years in the Northern Hemisphere since the beginning of systematic worldwide climate registrations in 1856 (Schönwiese, 2001). That year may thus serve as a good indication of the potential response to global warming. Under the warmer conditions of 1998, all species investigated had a higher heat tolerance and showed sufficient heat hardening to overcome high temperatures. However, as seen for *M. recurva*, this mechanism works only as long as leaf temperature extremes do not exceed the maximum heat tolerance of a species.

Artificial heating of *L. procumbens* canopies (+3 K) corroborates the leaf temperature effect because it led to a significant increase in heat tolerance in heated plants. Similar effects of leaf temperature on LT_{50} are reported for *Potentilla gracilis*, a widespread herb of subalpine meadows (Loik and Harte, 1996). Nevertheless, when heat tolerance and leaf temperature were directly plotted against each other, the effect of leaf temperature on heat tolerance was less clear. At temperatures lower than those leading to heat hardening, other factors must have an effect. This corroborates the findings of Alexandrov (1977) that heat tolerance sometimes increases even though leaf temperatures are lower than the temperature threshold for heat hardening.

Thermostability of PS II can be affected by irradiation intensity (Schreiber and Berry, 1977; Weis, 1982), but there is little evidence for direct effects of irradiation intensity on overall heat tolerance of leaves (Maier, 1971). As irradiation and leaf temperature are two strongly coupled environmental factors, they can be hard to distinguish from each other in field investigations. Preliminary results of in situ heating experiments in which optical photosynthetic photon flux density (PPFD) filters were employed to produce different irradiation intensities suggest that high irradiation ($>1000\ \mu mol\ m^{-2}\ s^{-1}$) can cause heat hardening in *L. procumbens* leaves even at low leaf temperatures (Neuner and Eder, unpublished data). This observation could explain high heat tolerances at low leaf temperatures.

High heat tolerances were, in most species, absent under drought stress. In contrast, *S. paniculata* showed maximum heat tolerance values under drought stress. This capability is ecologically significant for a species that grows preferentially on dry sites where a decrease in $\Psi_{0(act)}$ occurs with high leaf temperatures and strong irradiation (Neuner et al., 1999). Drought stress is known to lead to an increase in heat tolerance at low leaf temperatures, particularly in xerophytic plants (Bannister, 1970; Falkova, 1973; Alexandrov, 1977) such as *S. paniculata* (Neuner et al., 1999). In contrast, mesophytic species often do not respond to decreasing water content with an increase in heat tolerance (Zavadskaya and Denko, 1968). Our investigation of alpine species appear to corroborate these findings.

The plant species investigated reacted sensitively to climate changes, especially long-term temperature increases, leading to increases in mean heat tolerance. Therefore, mean heat tolerance may serve as a climate indicator. Knowledge of the species-specific heat-hardening capacity under natural conditions may also allow us to predict future distribution shifts for certain plant species.

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