Physiological Adaptation Before and After Snow Melt in Green Overwintering Leaves of Some Alpine Plants

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S u m m a r y

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The physiological adaptation of *Homogyne alpina*, *Loiseleuria procumbens* and *Rhododendron ferrugineum* plants that were collected at altitudes between 2000 m and 2200 m in the Central European Alps was studied in field samples as it occurs before and after snow melt. These plants retain green leaves over the winter, well protected by snow, but showing some photosynthetic functions. During snow melt in May and June the leaves are suddenly exposed to markedly increased irradiation intensities of PAR and UV-B and to a significant increase in leaf temperature (up to 40 K). This sudden change in microclimate had no detrimental influence on the investigated photosynthetic parameters. Measurements of plastid pigments, fluorescence parameters and of antioxidant and flavonoid compounds revealed a species - specific response. The two woody dwarf shrubs *Loiseleuria* and *Rhododendron* behaved more similar as compared to *Homogyne*.

I n t r o d u c t i o n

In the High Alps many perennial plants are winter green or produce their leaves before snow cover melts (KÖRNER 1999). By this time is saved from leaf growth and development during the short vegetation period. During winter snow protects photosynthesis of winter green plants from the stressful combination of low temperature extremes and high irradiation, including UV irradiation. Under

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snow, depending on the thickness and consistency of snow (SAKAI & LARCHER 1987), plants are usually exposed to very dim light or darkness and moderate, constant temperatures around 0° C. During snow melt winter green leaves get suddenly expelled from snow after 6 up to 9 months exposure to the environmental conditions under snow coverage. This is a dramatic change of the micro - environmental condition within a few days. Knowledge about photosynthesis of winter green alpine plants during snow coverage in winter is still scare (NEUNER & al. 1999, HACKER & NEUNER 2005) and hardly an investigation has focused on the effects of the changing environmental conditions on photosynthesis during snow melt (SIEGWOLF & CERNUSCA 1984, NEUNER & al. 1999).

The plants studied here belong to two different families, one is a herb (*Homogyne alpina, Asteraceae*), while the other two (*Rhododendron ferrugineum, Loiseleuria procumbens*) are dwarf shrubs, belonging to the *Ericaceae*. The latter differ in their growth form: while *Rhododendron* may reach heights of over 60 cm (CERNUSCA 1976) and needs much snow fall for protection in winter, *Loiseleuria* mats only reach 3 cm in height, but its possibility to grow also at wind exposed ridges indicates that *Loiseleuria* belongs to the most climate resistant alpine plants.

Alpine plants have adapted to a wide range of temperatures: high irradiation can lead to high temperatures in the plant body, like 40-45°C in *Loiseleuria* (LARCHER 1977, LARCHER &WAGNER 2004), but *Loiseleuria* and *Rhododendron* tolerate even higher temperatures when tested for the most sensitive photosystem 2 (BRAUN & al. 2002). In winter, *Loiseleuria* can tolerate down to -40°C (LARCHER 1977). Changes in frost resistance, related to developmental stages, is reported by TASCHLER & al. 2004 for *Rhododendron* from Mt. Patscherkofel. For *Homogyne*, temperature resistance records are missing. The short vegetation period for alpine plants may be used more efficient, if winter green plants are able to start photosynthesis even under a thin snow cover (BERGWEILER 1987, WILDI & LÜTZ 1996). The often strong increase in irradiation during emergence from the snow is not always accompanied with favourable temperatures, as plants remain cooled by melting water. This situation can be dangerous for not adapted tissues, as was found in green alpine *Eriophorum* plants after a sudden cold period in summer (LÜTZ 1996).

In this study we compare photosynthetic performance during snow coverage and after snow melt in two typical chionophytes, i.e. *Rhododendron* and *Homogyne*, with that of *Loiseleuria*, that may not be protected by snow throughout the whole winter period. As we aimed to measure some key parameters for developmental or physiological adaptations we investigated primary photochemistry (efficiency of photosystem II), changes in chloroplast pigments, ascorbic acid and α-tocopherol, and total flavonoids to get some information on UV resistance. Photosynthetic stress parameters indicate that the leaves cope with environmental conditions quite well and hence meet the requirements in the field.

Material and Methods

Leaves from the following plants were used in this study: *Homogyne alpina* (*Asteraceae*) (**H**) needs humid soil and frost protection by snow. It grows in alpine forests or open meadows. The

leaves are herbaceous. *Loiseleuria procumbens* (*Ericaceae*) (**L**) is one of the most frost resistant alpine plants (SAKAI & LARCHER 1987). Branches with the small leaves often creep over rocks and open soil. The leaves have an anatomy to avoid water loss over a long time and they contain large amounts of intracellular stored fat. *Rhododendron ferrugineum* (*Ericaceae*) (**R**) is a mostly erected shrub which needs snow cover during winter to avoid desiccation damage.

All plants develop winter green leaves and grow in a mixed alpine shrub/meadow community a few meters from each other. The sampling site was located in 2200 m altitude on Mt. Patscherkofel, near Innsbruck, Austria. A recent description of the climate of Mt. Patscherkofel and a detailed study on *Rhododendron* species is given by LARCHER & WAGNER 2004.

Samples were taken between May and July, 1997 (May 26th; June 4th; June 16th; July 10th) and either measured at the growing site (chlorophyll fluorescence) or put into liquid N_2 after fresh weight determination. Area assays could be performed via scanning of the leaves after pigment extraction.

Developmental stages of plants for this study: Plants were followed over a period of about 8 weeks (spring to summer). **H, L, R 2**: plants at the first sampling date: under up to 10 cm snow (L2 could not be harvested at May 26th). **H, L, R 3**: plant distance to snow at the first sampling date: 0.1 to 1 m. **H, L, R 4**: plant distance to snow at the first sampling date: 2-10 m. These three stages represent different acclimation stages at the beginning of the observations.

Leaf area and leaf fresh weight were determined for possible drastic changes which may influence the calculation of measured parameters. The ratio of weight to area (mg/cm^2) did not change significantly, as the example from June 4th, 1997 shows: *Homogyne*: H2: 39.3; H3: 39.2; H4: 39.0. *Loiseleuria*: L2: 56.4; L3: 55.4; L4: 47.9. The higher values of L2.3 can be explained by the adsorbed humidity from melting snow water. *Rhododendron*: R2: 33.0; R3: 30.9; R4: 32.6.

Pigments, tocopherol and ascorbic acid were analyzed by means of HPLC (WILDI & LÜTZ 1996, LÜTZ 1996). For semi-quantitative flavonoid determinations leaves were extracted with pure methanol according to JUNGBLUTH & al. 1995, the solution diluted to 75% methanol, clarified by centrifugation, and the spectra were recorded from 270 to 420nm. The calculation for relative absorption units per unit area was made using the relative peak height at the individual absorption peak (see figures) between 320 and 340 nm.

Chlorophyll fluorescence (PEA-equipment, Hansatech, England) was measured in the field as Kautsky effect and data are given as ratio Fv/Fm. From the induction curves the data were taken to address the biophysical parameters of primary photochemistry according to the formula given by STRASSER & al. 1995 and SRIVASTAVA & al. 1999.

UV-measurements in the field were performed with a Solar Light UV-B quantum sensor (Philadelphia, USA, units: MED/h). This sensor is cosinus corrected and allows temperature compensation of sensitivity.

Leaf temperatures were measured with type T 0,2 mm copper constatan, fine wire thermocouples, and recorded on a data logger (CR10X Micrologger, Campbell Scientific, Logan, USA). Temperature data were sampled every 30 seconds and averages were calculated and stored every 30 minutes.

Statistics: All mean values are based on the average of 5 individual assays, and standard error is given.

Results

Leaf temperature records on *Loiseleuria* during May 1997 give an impression of the dramatic changes in microclimate during snow melt when leaves are suddenly expelled from snow after 6 to 9 months in darkness or dim light and exposed to more or less 0° C (Fig. 1).

The pool sizes of most chloroplast pigments extracted from these winter green leaves did not change greatly, as the ratio of total chlorophylls to total caro-

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tenoids shows (Table 1). For *Homogyne*, there is practically no change despite that some samples (H2, first two sampling dates, H3 first sampling date) were still covered by snow or just emerged. H4, at all dates free from snow, accumulated somewhat more carotenoids, resulting in a lower, but non-significant ratio. The absolute amounts of chlorophylls or of carotenoids increased in H2 from May 26th to June 16th and is found reduced at July 10th only slightly higher than at the beginning (for chlorophylls: 0.43 - 0.57 - 0.7 - 0.48 g/m²). For leaves from *Rhododendron*, both pigment classes developed a similar kinetic as *Homogyne*, but the absolute amounts are lower (for chlorophylls: range 0.3 - 0.46 g/m²). Also, chl/car ratios were again highest at the first sampling date when snow still covered leaves up to 10 cm. From the beginning of June until July a non significant increase in carotenoids lowered this ratio. A slightly different pigment accumulation was measured in *Loiseleuria*: In sample L4, free of snow for the whole observation period, the chl/car ratio was lowered from 4.16 to 3.76, probably by an intense production of ß-carotene (see below). A highly accelerated chlorophyll synthesis in comparison to carotenoid accumulation was found when *Loiseleuria* leaves emerged from snow and warmed up (**L2**, 3.40 to 4.36 in about 12 days). Until July 10th, this ratio remained significantly higher than the L4 (always snow-free) sample.
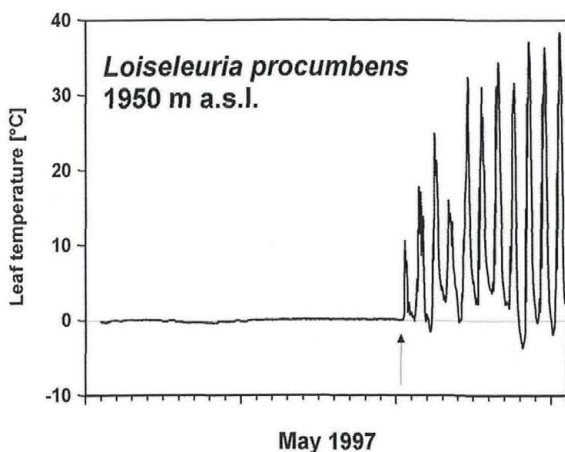

Fig. 1. Leaf temperature record of *Loiseleuria procumbens* throughout May 1997 at Mt. Patscherkofel. Arrow: first emergence of leaves from snow. The diurnal cycles show that at the end of May occasionally night frosts occurred.

Despite the partially very different light- and temperature exposures of the plants, the chl a/b ratio showed no remarkable changes, but distributed between 2.5 to 3.0 for all sampling dates and variants (data not shown).

Remarkable changes were found for ß-carotene, as this pigment increased higher than other secondary pigments like the mostly stable lutein: if a comparison is made between first (May 26th) and last (July 10th) samplings, *Homogyne* could increase ß-carotene from about 9 to about 22 mg/m^2, *Rhododendron* from about 5.3 to 13 mg/m^2, and *Loiseleuria* had the strongest increase by about 2.8 to 12.4 mg/m^2.

Table 1. Ratio of total chlorophylls to total carotenoids for different developmental stages and sampling times. Mean values and standard error of 5 measurements.

	26. 5.		4. 6.		16. 6.		10. 7.	
H2	5.70	0.12	4.77	0.13	4.86	0.08	4.65	0.21
H3	5.40	0.23	4.52	0.22	4.68	0.11	4.55	0.25
H4	5.55	0.16	4.73	0.27	4.57	0.22	4.19	0.09
R2	4.33	0.14	3.64	0.09	3.94	0.11	4.28	0.13
R3	4.54	0.16	3.82	0.18	4.29	0.13	4.14	0.14
R4	4.30	0.21	3.39	0.22	4.01	0.08	3.74	0.18
L2			3.40	0.17	4.36	0.20	4.21	0.14
L3	3.72	0.13	3.43	0.05	3.25	0.06	4.08	0.17
L4	4.16	0.16	4.09	0.11	3.84	0.31	3.76	0.12

The range of absolute amounts of xanthophyll (x) cycle pigments was similar in all plants over the observation period (0.02 to 0.04 g/m^2) (Fig. 2). This pigment class showed the strongest adaptations to the changing light and temperature conditions during emerging from snow and increased leaf temperatures by longer distances from melting snow fields. In H2 and R2 the x-cycle pigments were about doubled from May to mid-June (from snow covered leaves to full light exposed leaves), with an intermediate reaction in H3 and R3, and finally reduced to a value still higher than in the first sample. In *Loiseleuria*, the L2 and L3 variants developed a similar characteristic compared to H2 and R2, but started on a much higher level than L4. Increasing light intensity and temperature over this springtime did not affect *Loiseleuria* x-cycle pigments as strong as in both other plants; the stage L4 (fully sun exposed leaves over the entire period) showed no significant changes. Nevertheless, at July 10th, the three variants in all plants levelled off at similar values. This might represent the pigment accumulation for the main summer period after the spring period has finished.

The pigment data show that the plants have a fully developed, well protected photosynthetic apparatus already in spring under snow, which becomes modulated according to the climate impact until summer conditions prevail.

α-Tocopherol and ascorbic acid represent the main antioxidant systems for the lipophilic and hydrophilic compartments, respectively, in plastids. The average

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content of α-tocopherol is highest in *Loiseleuria* (Fig. 3) and lowest in *Homogyne* leaves. *Loiseleuria* needs a lipophilic antioxidant to protect the accumulated leaf lipids. The tocopherol formation in *Homogyne* seems to be partially dependent from temperature, as the H2 samples were not able to produce higher amounts when leaves were under snow or near snow beds cooled by melting water. From mid of June H2, H3, H4 showed similar amounts which decreased strongly until July 10th. All stages studied in *Loiseleuria* followed a similar kinetic, which peaked mid-June when all samples were no longer affected by snow or melting water.

Irrespective of snow cover or fully exposed and at higher average leaf temperatures, the leaves of *Rhododendron* did only slightly change α-tocopherol contents.
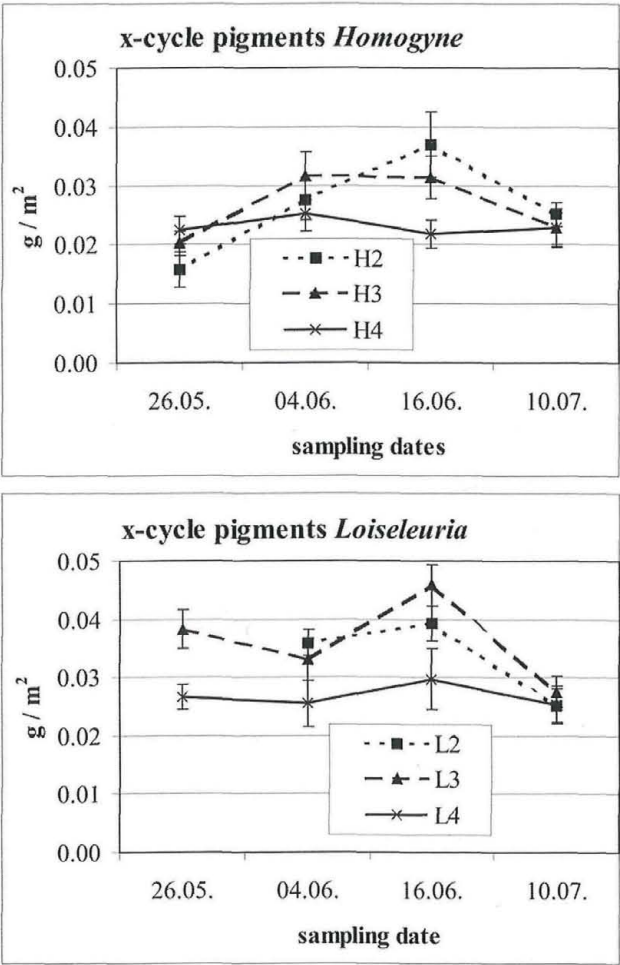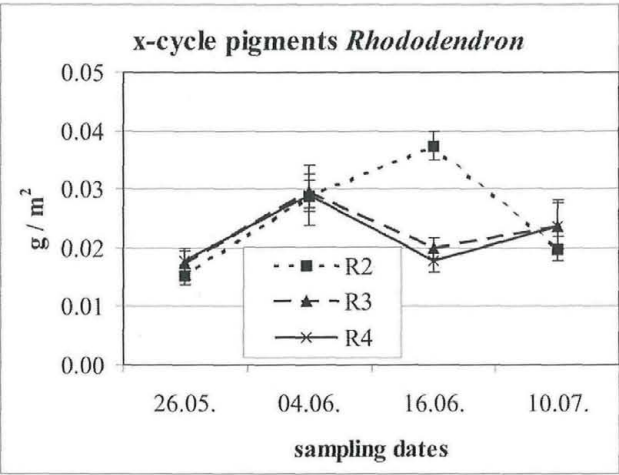
Fig. 2. Development of xanthophyll (x-) cycle pigments from leaves of the individual plants at different developmental stages.

For ascorbic acid, again *Homogyne* accumulated less antioxidant (Fig. 4), which changed obviously according to light exposure, as the snow covered leaves of H2 and H3 indicate for May 26th. After June 4th, a continuous decrease is found to an amount comparable to the snow covered leaves. A non-consistent change over time and developmental stage shows *Loiseleuria*: while light or temperature effects are hardly visible for L2 and L3, the L4 samples changed drastically. Only the leaves from *Rhododendron* contained increasing amounts of ascorbic acid with increasing exposure of leaves to light and prevailing temperatures.

Measurement of Fv/Fm serves as a widely used stress parameter for PS II primary activity with the benefit to obtain non-destructive and fast many records in the field. Photosystem II efficiency (Table 2) differed markedly between the species at the first sampling dates. In *Homogyne*, PS II activity of plants under snow was relatively well developed nearly as in the stages far from the snow bank. *Rhododendron* leaves had low efficiencies, which only slowly increased, in all stages for the two first sampling dates, even in the fully exposed stage R4. The leaves of *Loiseleuria* in the stages L2 and L3 needed similar time for development of PS II activity as *Rhododendron* did, starting from the lowest values of all plants.
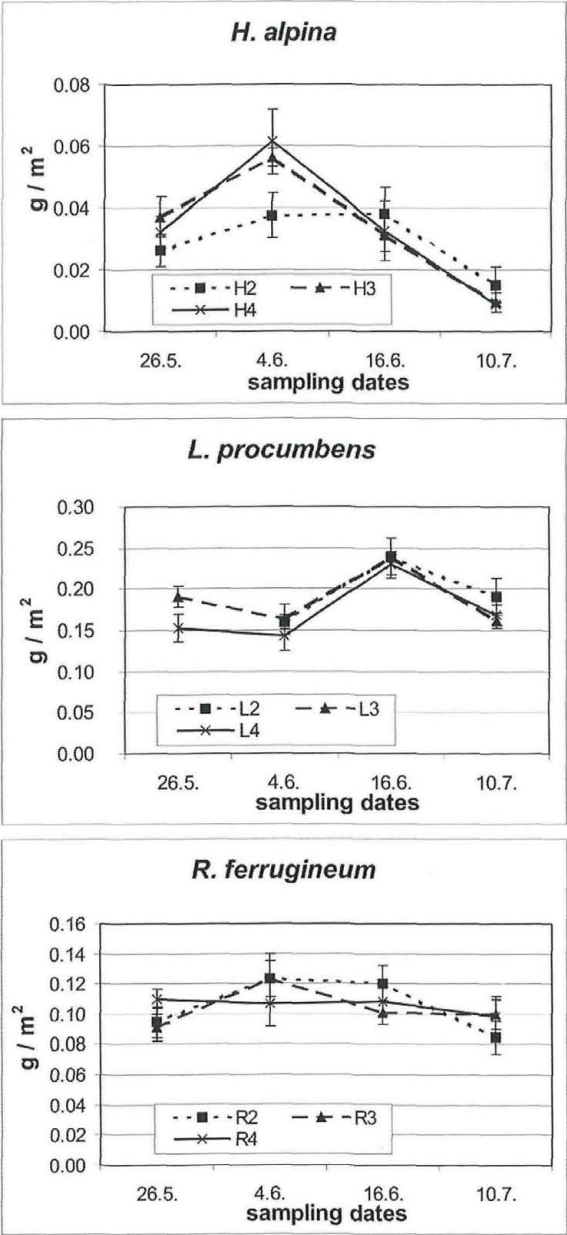
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Fig. 3. Development of α-tocopherol contents from leaves of the individual plants at different developmental stages.
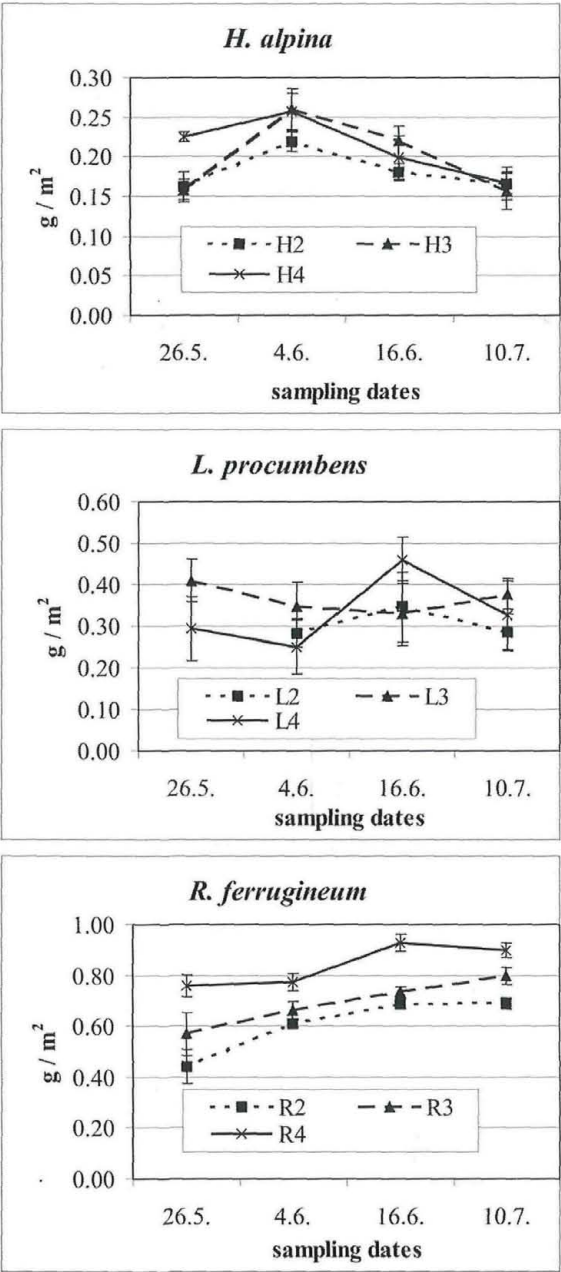
(147)

Fig. 4. Development of amounts of total ascorbic acid from leaves of the individual plants at different developmental stages.

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In L4, which was snow free for days already at the first sampling date, Fv/Fm is highest with some reduction during July 10th probably because of drought or photo-inhibition. The data show that every plant has a different kinetic in PS II development of the green leaves from early spring to early summer, with *Homogyne* as most efficient plant in all stages.

Table 2. Photosystem II activity expressed as Fv/Fm values for different developmental stages and sampling times. Mean values and standard error of 5 measurements.

	26. 5.		4. 6.		16. 6.		10. 7.	
H2	0.75	0.02	0.76	0.01	0.83	0.01	0.78	0.02
H3	0.79	0.03	0.80	0.01	0.82	0.00	0.80	0.01
H4	0.78	0.01	0.81	0.01	0.81	0.01	0.78	0.01
R2	0.41	0.10	0.45	0.09	0.74	0.02	0.69	0.02
R3	0.47	0.03	0.52	0.03	0.75	0.01	0.74	0.02
R4	0.49	0.04	0.53	0.05	0.81	0.01	0.75	0.02
L2	0.37	0.05	0.55	0.06	0.74	0.03	0.63	0.07
L3	0.38	0.03	0.58	0.05	0.74	0.03	0.75	0.01
L4	0.76	0.02	0.65	0.05	0.73	0.05	0.64	0.06

The chlorophyll fluorescence induction curves contain information about first biophysical events after photon absorption in PS II. STRASSER & al. 1996 developed data evaluation via spider plots (Fig. 5). These plots demonstrate the time dependent development of fluorescence parameters. The data are shown for stage H2, R2, L2 when plants were still covered by a few cm of snow. Reference date is July 10th. Surprisingly, the curve parameters of *Loiseleuria* and *Rhododendron* changed very similar during the measurement period. The absorption per reaction

Fig. 5. Spider plot presentation of key parameters derived from the fluorescence induction curves obtained by PEA - measurements. Parameters are as follows (SRIVASTAVA & al. 1999): **ABS/RC**: absorption flux of photons per reaction centre (PS II); expression of antenna size; **TR$_0$/RC**: flux of excitation energy trapped per reaction centre (PS II) at time 0; **ET$_0$/RC**: electron transport behind Q$_A^-$ per reaction centre (PS II) at time 0; **RC/CS**: reaction centres per cross section; density of photosynthetic units per leaf area; **ABS/CS**: absorption flux of photons per cross section; **TR$_0$/CS**: flux of excitation energy trapped per cross section at time 0; **ET$_0$/CS**: electron transport behind Q$_A^-$ per cross section at time 0; **Phi(Po)**, or **TR$_0$/ABS**: probability that an absorbed photon is trapped by a reaction centre at time 0; maximal yield of photochemistry; F$_V$/F$_M$. All data are from stage 2 (H2, L2, R2) of the plants studied. The curves represent: _____ July 10th (reference line); _ _ _ June 16th; _ . _ . _ June 4th, ... May 27th.

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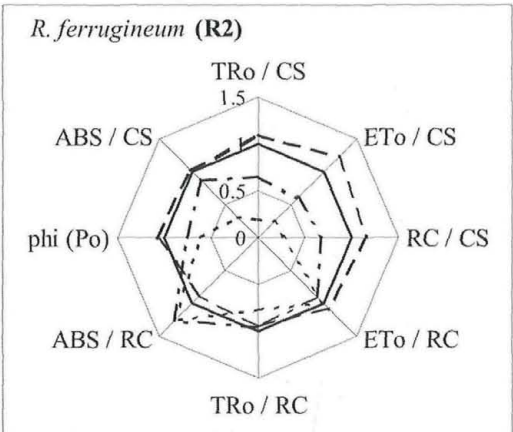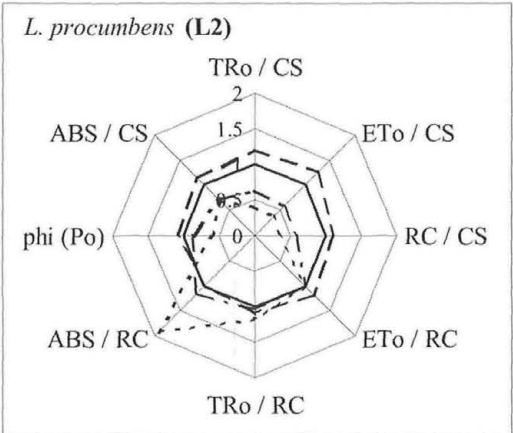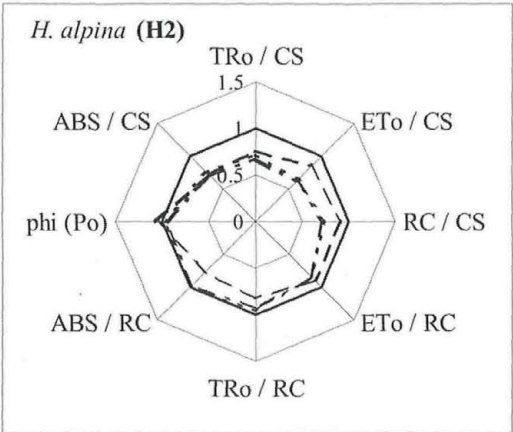
center (ABS/RC) value in May amounts to nearly 200% of the reference in July, while e.g. electron transport per reaction center (ETo/RC) appear similar at any time. TRo/CS, ETo/CS and RC/CS were highest at June 16th, showing that PS II development might be finished. Despite the observation that Fv/Fm did not change greatly in *Homogyne*, the higher resolution by spider plot analysis demonstrates that individual processes of energy transformation in PS II developed continuously for all parameters until July 10th - a different mechanism compared to the other samples. Already at a high level appears the parameter phi(po), which accounts for maximum quanta efficiency for reduction of Q_A.

With increasing altitude alpine ecosystems are exposed to enhanced UV irradiation (BLUMTHALER & al. 1994), but snow fall, even in summer, can shield effectively against short wave irradiation. The following data give an example for the attenuation of visible and of UV-light by snow during alpine spring, measured during May 25th at 12:30 CET at 2200m altitude, the snow was lightly covered with dust. The sensor unit is calibrated for the biological weighing function MED/h. Clear sky, sunny: 4 MED/h (approx. 2300 μMol photons/m^2 sec PAR). Snow depth 2cm: 0.4 MED/h; 6 cm: 0.02 MED/h; 10 cm: $1*10^{-3}$ MED/h; 20 cm: $1*10^{-6}$ MED/h.

A great part of UV shielding comes from soluble epidermal flavonoids. Their methanolic extracts have a broad peak in the UV-A/B region and show plant individual maxima: *Loiseleuria* has a main absorbance at 285 nm with an additional peak near 328 nm, while *Homogyne* absorbs maximal at 331 nm with a shoulder at 290 nm. *Rhododendron* extracts have a main absorbance at 287 nm and another peak at 334 nm. The absorption near 330 nm can be used for semi-quantitative comparisons of changes in total extractable flavonoids. Fig. 6 shows the changes in methanol soluble compounds, extracted from the leaves for different developmental stages and over time. *Homogyne* increased protective compounds from the stage under snow (H2, H3 May 26th) up to twofold with time, while the already sun exposed sample, starting at a high level, accumulated much less. The UV protection in *Rhododendron* seems to be relatively high in all stages and is only slightly reduced (acclimated) with time. Similarly, *Loiseleuria* leaves appeared always well equipped with soluble flavonoids, if compared e.g. to *Homogyne*, and is able to increase these amounts further, mainly in the longer sun exposed stages.

Fig. 6. Development of total extractable flavonoids, measured at their indicated maximum in the UV-A region, in relative units per standard area from leaves of the individual plants at different developmental stages.

H. alpina 331nm

L. procumbens 328nm

R. ferrugineum 334nm

(152)
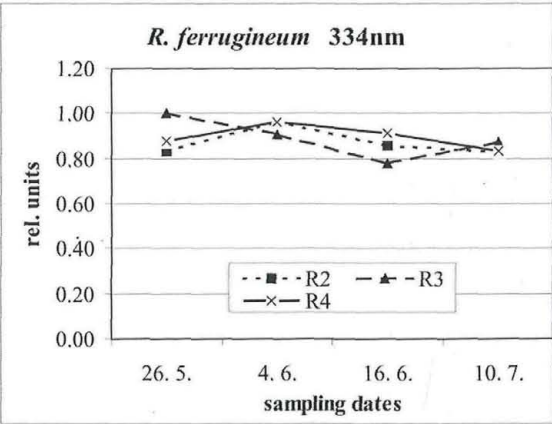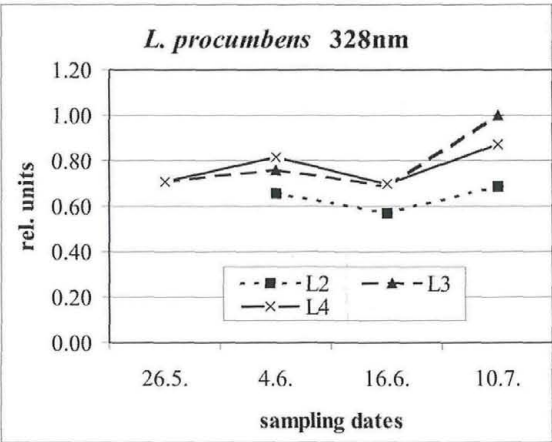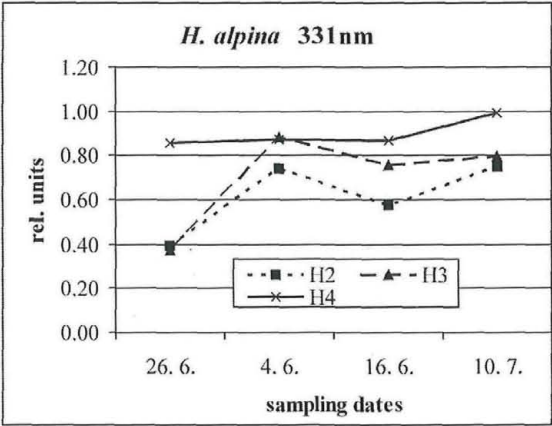
Discussion

The strongest changes in plastid pigments were observed in samples from the snow covered stage to the open condition near the snow field. Similarly, HALDIMANN 1996 described similar increases in chl contents and ß-carotene in maize plants first grown at lower temperature, if temperatures were increased. Thus not only light but in addition temperature changes affect the physiological adaptation in the winter green leaves, which still form more chlorophyll; its synthesis in the light is temperature controlled (LÜTZ 1996). From end of May the weather conditions improved. In general, also total carotenoids increased with distance from the snow field resp. developmental time. A part of this pool is composed by the x-cycle pigments, which increased similarly to all pigments during the spring period, levelling off in summer (July). Therefore they will be able to protect the photosynthetic apparatus in all stages of leaf exposure to the climate from a damaging surplus in energy (PFÜNDEL & BILGER 1994, YOUNG & FRANK 1996). The absolute amounts of x-cycle pigments was found similar in all three plants.

The strong increases for ß-carotene in *Rhododendron* and in *Loiseleuria* indicated an increase in PS II reaction centers, which is mirrored in the spider plot analysis. GRABHERR 1974 studied *Loiseleuria* leaves from March to August and also observed increasing ß-carotene contents. One possible reason for the extraordinary accumulation of ß-carotene in this small shrub could be that the leaves contain massive fat droplets, and this pigment may help to avoid oxidation of the storage fat.

Another lipophilic antioxidant is α-tocopherol (BUETTNER 1993, FRYER 1992). It is located in the plastid envelope and in the thylakoid membranes and can protect membranes by inactivation of chemical radicals like singulett oxygen, superoxide radicals etc., or by quenching mechanisms. *Homogyne* and *Rhododendron* leaves contain considerable amounts of this antioxidant already under snow, while *Loiseleuria* needs several days for an increase in α-tocopherol. This equipment of antioxidant defence seems to best developed in *Rhododendron* showing nearly no changes in all samples harvested, while in Homogyne an intense adaptation to higher amounts in the first days of emergence from snow could be observed, strongly reduced in the last sample in July. Taken both lipophilic antioxidants together, each plant did react individually, but if one compound decreased, the partner compound increased (however, not in stoichiometric relation).

The hydrophilic antioxidant ascorbic acid showed similar alterations as α-tocopherol. Again, *Homogyne* contains the lowest amounts per leaf area, *Rhododendron* a remarkably high content, in average double the amount of *Loiseleuria*, continuously increasing with exposure time to summer conditions. Similarly, WILDI & LÜTZ 1996 found during a study of antioxidants in several alpine plants, that there is no common strategy but individual behaviour in the accumulation of different antioxidants. Ascorbic acid formation occurs during active photosynthesis, thus green overwintering leaves may store some antioxidant under the snow or start already synthesis, if a thin snow cover allows for some photosynthetic activity - to be prepared for the snow melt with relatively fast exposure of leaves to high

energy input. STREB & al. 1997 described that ascorbate contents in plastids of *Homogyne*, *Soldanella alpina* and *Ranunculus glacialis* were much higher than in comparable low land species - a possible indication of the higher irradiation load in higher altitudes.

Photosystem II activity (as Fv/Fm) is reduced under winter conditions in overwintering leaves (BOLHAR-NORDENKAMPF & al. 1989, LÜTZ & CZAPALLA 1996, NEUNER 1996, NEUNER & al. 1999, LEHNER & LÜTZ 2003), but easily recovers under spring conditions. The two *Ericaceae* species of this study show a similar behaviour. The highest efficiencies were recorded in the samples that had emerged earliest from snow (L4, R4). In contrast, *Homogyne* did not significantly reduce Fv/Fm under cold conditions. This plant should be able to start photosynthesis with high efficiency much faster. The spider plot analyses (according to SRIVASTAVA & al. 1999) support these results: Trapping and electron transport per reaction centre did not change greatly during spring development in all plants, indicating a well developed structure, while the number of reaction centres per cross section is reduced in the first stages. This can help to avoid over-reduction of the electron transport system. *Rhododendron* and *Loiseleuria* increased the light harvesting antenna in the earlier stages, when leaves were more shaded, as for *Loiseleuria*, when some anthocyanins accumulated in the leaves. Again, *Homogyne* shows the smallest deviation from the reference curve (based on July 10th). LEHNER & LÜTZ 2003 followed the kinetics of primary photosynthesis in cembran pine and dwarf pine from late winter until May. Their spider plot study showed similar changes in key parameters of these timberline trees: antenna sizes decreased from winter to May, trapping per reaction centre changed only slightly, but electron transport (behind Q_A-) per cross section developed strongly with increasing springtime. In a different system (winter wheat and rye), which developed at 5°C, photosystem II was much more resistant against photoinhibition compared to 20°C grown plants, because a higher amount of Q_A as primary electron acceptor was kept in the oxidized stage (ÖQUIST & al. 1993, ÖQUIST & HUNER 1993).

Can alpine plants store their UV-protection from one year to the next year or is this protection changed under snow? Both *Ericaceae* have developed passive UV-avoidance mechanisms, as their upper leaf surfaces are covered by a thick wax- and cuticular layer (*Loiseleuria*, LÜTZ & GÜLZ 1985) or leaf movements and thick cuticula (*Rhododendron*, LARCHER & WAGNER 2004). These anatomical adaptations reduce UV penetration (CEN & BORNMAN 1993, ROZEMA & al. 1997). Leaves of *Homogyne* have a thinner, but often reflectant cuticular layer (own observations). These differences may explain, why the *Ericaceae* did not very much differ in soluble flavonoid concentrations between the three developmental stages, but *Homogyne* increased these compounds from May 26th to June 4th drastically and was later comparable with the kinetic found in *Loiseleuria*. In July, the absolute amounts of total extractable flavonoids per leaf area was very similar between all plants. Fluorescence and pigment data suggest, that at all stages of leaf development, and with increasing time of the year, the UV - protection by passive plus actively regulated mechanisms (flavonoids) was sufficient. In spring time, when frost resistance is of prime importance (TASCHLER & al. 2004), exposure to UV-B

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irradiation may increase cold hardiness, as was found for a garden form of *Rhododendron* (DUNNING & al. 1994). Our studies are supported by SCHNITZLER & al. 1997, that leaf exposure to UV irradiation induces synthesis of flavonoids; or by reports of BARNES & al. 1987 and RAU & HOFFMANN 1994 that several alpine plants contain already higher amounts of UV-screening substances per se.

The plants studied retain green leaves over the winter. The leaves remain photosynthetically active to be able to start with photosynthesis even under a thin snow cover as soon as there is enough irradiation available. Possible stresses immediately after snow melt, caused by sudden warming and exposure to the strong solar irradiation is tolerated by a species - specific set of antioxidants and pigments together with a fine-tuned regulation of energy transfer in photosynthesis. Therefore these winter green plants have developed a strategy of adaptation to start their energy harvest from the sun as soon as possible to cope with the short vegetation period in high altitudes.

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