ABS ACTA BIOLOGICA SLOVENICA
LJUBLJANA 2021 Vol. 64, Št. 1: 41–55

UV radiation and temperature effects on functional traits in *Helianthemum nummularium* subsp. *grandiflorum* at the alpine and montane site in the Slovenian Alps

Učinki UV sevanja in temperature na funkcionalne značilnosti *Helianthemum nummularium* subsp. *grandiflorum* na alpinskem in montanskem rastišču v Julijskih Alpah

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Abstract: Alpine plants have evolved strategies to survive harsh conditions, which include high UV and visible radiation, extreme temperatures, dryness and lack of nutrients. Survival strategies include biochemical, physiological and morphological responses, which are scarcely studied because of the time-demanding and complex experimental conditions. We researched functional traits in the alpine plant common rockrose *Helianthemum nummularium* subsp. *grandiflorum* growing under ambient UV-B and reduced UV-B radiation at different altitudes (1600 and 2000 m a.s.l.) of mount Vogel in the Slovenian Alps. Leaf anatomy, pigments and optical properties were investigated at the beginning and at the end of the growing season. Plants showed high constitutive UV-absorbing compounds content (UV-AC) throughout the season. Most leaf thickness parameters were not altered according to UV and altitude conditions. Leaves did not transmit any UV spectrum, in agreement with high UV-AC. High photosynthetic spectrum transmittance at alpine altitudes was due to complex biochemical and anatomical responses to these conditions, rather than to UV radiation. Unchanged chlorophyll content of *H. nummularium* could be related to shrub life form, where leaves shade out high UV and PAR irradiance as well as contribute to lower leaf temperature. This study shows the complexity of alpine plant response, where specific characteristics of plant species should not be overlooked.

Keywords: *Helianthemum nummularium*, leaf optical properties, pigments, UV radiation

Izvleček: Alpinske in gorske rastline so razvile strategije za preživetje v skrajnih razmerah, ki vključujejo visoko jakost UV in vidnega sevanja, skrajne temperature, sušo in pomanjkanje hranil. Strategije preživetja alpinskih rastlin vključujejo biokemične, fiziološke in morfološke odzive, ki jih raziskovalci zaradi zahtevne izvedbe redko proučujejo. V naši raziskavi smo proučevali funkcionalne značilnosti alpske rastline velecvetni popon *Helianthemum nummularium* subsp. *grandiflorum*, in sicer pod naravnim in zmanjšanim sevanjem UV-B na dveh različnih nadmorskih višinah (1600 in 2000 m n.m.) gore Zadnji Vogel v Julijskih Alpah.

Anatomijo listov, pigmente in optične lastnosti smo raziskovali na začetku in na koncu rastne sezone. Rastline so ob obeh merjenjih vsebovale veliko UV-absorbirajočih snovi (UV-AC). Večina parametrov, s katerimi smo ocenjevali debeline listnih tkiv, se ni odzivala na različne sevalne in temperaturne razmere. Listi niso prepuščali UV spektra sevanja, kar se je ujemalo z veliko vsebnostjo UV-AC. Velika prepustnost fotosinteznega spektra sevanja na alpinski nadmorski višini je bila povezana z biokemičnimi in anatomskimi značilnostmi rastline in ne z jakostjo UV sevanja. Nespremenjena vsebnost klorofila pri *H. nummularium* bi lahko bila povezana z grmičasto rastno obliko rastline, kjer zgornji listi zasenčijo spodnje liste ter tako prispevajo k manjšim prejetim odmerkom UV in fotosintezno aktivnega sevanja ter k nižji temperaturi listov. Študija kaže na kompleksen odziv alpinskih rastlin na UV sevanje in temperaturne razmere v gorah, pri čemer je pomembna tudi rastna oblika rastline.

Ključne besede: *Helianthemum nummularium,* optične značilnosti listov, pigmenti, UV sevanje

Introduction

Alpine plants come in a variety of life forms and have evolved various strategies to allow their survival under harsh mountain conditions (Körner 2003). Alpine plants adapt and acclimate to high ultraviolet (UV) and photosynthetically active radiation (PAR), low temperatures and frost, rapid temperature changes, soil dryness, lack of nutrients, low air humidity, strong winds and short growing season (Nicotra et al. 2010). Alpine environmental conditions can be stressful for plant life, where topography, exposure, diseases and human activities can also contribute to the stressful environment (Turunen and Latola 2005).

Ultraviolet-B radiation has been reported to cause changes in leaf morphology that lead to smaller and thicker leaves, with increased thickness of the epidermis or cuticle. These appear to be adaptive responses to stressful alpine environments (Jansen 2002; Robson et al. 2015). High UV-B radiation can negatively affect growth and productivity (Wargent et al. 2009; Gruber et al. 2010), carbon assimilation (Guidi et al. 2011; Hideg et al. 2013) and stomatal function (Nogues et al. 1999). Photosynthetic rates can be sustained under ambient doses of UV-B radiation through enhanced photoprotection, which depends on the induced synthesis of UV-B–absorbing compounds (UV-B–AC) and activation of the plant antioxidant defence system (Wargent et al. 2011; Kataria et al. 2014) or induction of photo-repair, which lead to the recovery of photochemical efficiency (Xu and Gao 2010).

Damaging UV-B effects at high altitudes and low latitudes are an exception rather than norm (Searles et al. 2001). The responses of high-altitude plants to UV-B radiation are often less pronounced compared to those of low-altitude plants, which suggests that alpine plants are acclimated and well-adapted to greater intensities of UV-B radiation. Indeed, these plants might have simultaneous co-tolerance to several stress factors, as acclimation or adaptation to harsh climates can also increase tolerance to UV-B radiation, and *vice versa.* Such interactions might aggravate or mitigate plant responses. Plant can increase their tolerance to UV-B, low temperatures or drought through increased acclimation to a second stressor (Chalker-Scott and Scott 2004; Trošt Sedej and Gaberščik 2008; Terfa et al. 2014).

The present study investigated adaptations and acclimatisation to increased UV-B radiation at two different alpine altitudes in the alpine plant common rockrose *Helianthemum nummularium* subsp. *grandiflorum* (Scop.) Schinz & Thell.). Common rockrose is widespread from upper montane (1200-1600 m a.s.l.) to alpine (1600-2000 m a.s.l.) altitudes in Central and Southern European mountains. This indicates its successful adaptation and acclimation to the alpine environment. Current research of *Helianthemum* has been limited to phylogenetics (Volkova et al. 2016).

The objective of the present study was to investigate *H. nummularium* responses to different UV radiation doses at different altitudes in its natural habitat. We aimed to study the interactive effects of diverse environmental conditions on the responses of *H. nummularium* in its natural habitat.

Materials and methods

Plant species

Helianthemum grandiflorum subsp. *grandiflorum* is an alpine herbaceous perennial that sometimes has a woody base. It grows 10 to 30 cm high, and flowers from June to September. *H. nummularium* is commonly distributed over warm, dry, rocky limestone meadows from montane to alpine altitudes throughout the Alps and other high mountains of Central Europe (Martinčič et al. 2010).

Experimental site

The experimental site was located at Mount Zadnji Vogel in the Julian Alps, Slovenia, and comprised a montane and an alpine altitude plot (1600 m a.s.l.: 46°19'04.4"N, 13°48'33.9"E; 2000 m a.s.l.: 46°19'46.7"N, 13°48'07.3"E) (Fig. 1). Both plots were above the timberline, on southerly exposed slopes. Slope steepness was 70% to 100%. The vegetation consisted of meadows on calcareous ground that lacked grazing and tourism. Their mean annual precipitation from 1981-2010 was 2600 to 3200 mm (Slovenian Environment Agency).

Two different UV exposures were provided to the plants using two types of filters: a Quinn XT UV filter (UV-), which absorbed UV-B and UV-A; and a Quinn cast UVT filter (UV), which was transparent to UV-B and UV-A (Quinn-Plastics, UK). Five UV and five UV- filters (each 20 × 20 cm) were positioned 15 cm above a group of three to six plants at each altitude. Similar soil water contents near and under the filters were maintained due to slope steepness, heavy rainfall and small size of the filters.

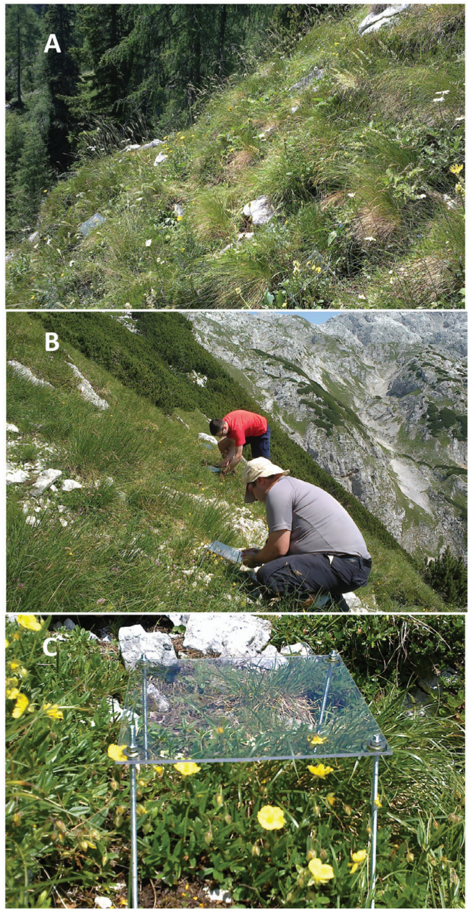

Figure 1: Experimental plot at Zadnji Vogel. **A** - at 1600 m a.s.l. **B** - at 2000 m a.s.l. **C** - *Helianthemum nummularium* subsp. *grandiflorum.*

Slika 1: Poskusna ploskev na Zadnjem Voglu. **A** - na 1600 m n.m. **B** - na 2000 m n.m. **C** - *Helianthemum nummularium* subsp. *grandiflorum.*

UV-B radiation was monitored over four days with a clear sky for all four different treatments (two altitudes × two UV exposures), using a radiometer with a UV-B radiometric sensor (RM-22; Opsytec Dr. Gröbel, Germany). The data collected were used to determine the proportion of the UV-B radiation that reached the plants under the different UV filters. The standard daily UV-B doses (UV) and the reduced daily UV-B doses (UV-) were calculated over three months (July, August, September) and for the four treatments using a model (Björn and Murphy 1993) that included Caldwell's generalised plant action spectra (Caldwell et al. 1986) for daily clear-sky measures (Tab. 1).

Table 1. Total and biologically active UV-B doses calculated for daily clear-sky (UV) and reduced daily clear-sky (UV-) UV-B according to month and altitude (Björn and Murphy 1993).
Tabela 1. Celokupni in biološko aktivni odmerki UV-B, izračunani za sončen dan (UV) in sončen dan pri zmanjšani jakosti (UV-) UV-B glede na mesec in nadmorsko višino (Björn in Murphy 1993).

		UV-B dose (kJ m^{-2} day^{-1})					
		July		August		September	
Filter	UV-B	1600 m	2000 m	1600 m	2000 m	1600 m	2000 m
UV	Total	55.91	57.58	47.75	49.27	34.52	35.73
	Biologically active	7.13	7.34	5.86	6.05	3.9	4.04
UV-	Total	16.77	17.28	14.32	14.78	10.36	10.38
	Biologically active	2.14	2.2	1.76	1.82	1.17	1.18

For both research plots, temperatures were monitored 10 cm underground once per hour during the experimental period, using temperature data loggers (HOBO TidbiTv2; Onset Computer Corporation, USA) (Fig. 2).

Measurements

Plants were analysed in July and September. The UV filters were placed above the plants at the end of June. The first sampling and measurements were conducted three weeks after the UV filters were placed. Measurements were performed on 10 randomly selected fully developed upper leaves per treatment at two altitudes. All the analysed leaves developed under the filters.

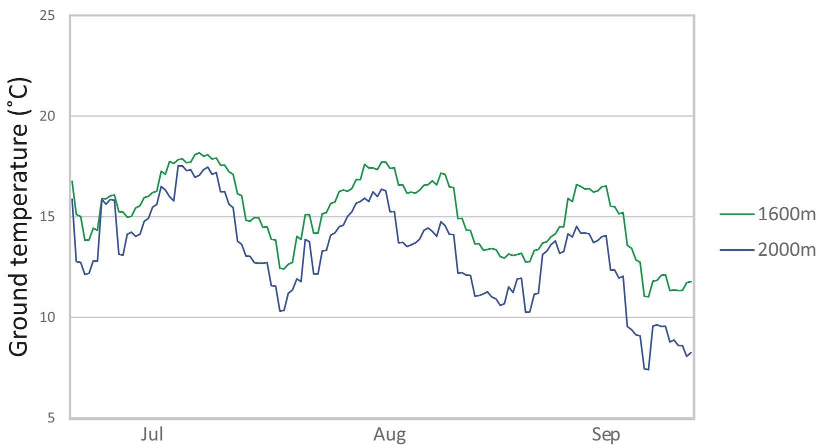

Figure 2. Mean daily ground temperatures at 1600 m and 2000 m a.s.l. at Zadnji Vogel, monitored once per hour in July, August and September, using temperature data loggers (HOBO TidbiTv2).
Slika 2. Srednje dnevne temperature tal na 1600 m in 2000 m n.m. na Zadnjem Voglu, merjene vsako uro julija, avgusta in septembra s pomočjo temperaturnih senzorjev (HOBO TidbiTv2).

Anatomical analysis

For the anatomical studies, 10 fully developed leaves were sampled from 10 plants per treatment at each altitude. Anatomical observations of the leaf cross-sections, which included palisade and spongy parenchyma thickness, and epidermis and cuticle thickness measurements, were performed under an optical binocular microscope (CX41; Olympus, Japan), with the measurements recorded and photographs taken with a digital camera (XC30; Olympus, Japan), using CellSens software (Olympus, Japan). The nail polish peel method was used to measure the stomatal features of the upper and lower leaf surfaces, under the same optical binocular microscope.

Pigment analyses

Photosynthetic pigments

Fully developed leaves from 10 plants per treatment at each altitude were collected at noon and were kept on moist paper in a refrigerated box. Fresh mass (FM) of the leaf tissue (10 mg) was extracted in 80% (v/v) acetone in buffered distilled water (pH 7.8). Absorbances were measured using a UV/VIS spectrophotometer (Lambda 25; Perkin Elmer, USA). Chlorophyll a (Chl a) and chlorophyll b (Chl b) contents were calculated from the absorbances measured at 663.6 nm and 646.6 nm (Porra et al. 1989). Pigment contents were expressed per leaf dry weight (mg g^{-1}).

UV-B- and UV-A-absorbing compounds

The leaf tissue that was collected as described above (10 mg FM) from 10 fully developed leaves from 10 plants per treatment at each altitude were extracted in 5 mL of acidified MeOH (MeOH/H_2O/HCl (37%), 79:20:1, v/v) (Caldwell 1968). Absorbance was measured over the spectral ranges for UV-B (280-315 nm) and UV-A (316-400 nm) using a UV/VIS spectrometer (Lambda 25; Perkin Elmer, USA), and were calculated per dry weight (g^{-1}) and integrated to estimate the total content of UV-B- (UV-B-AC) and UV-A-absorbing compounds (UV-A-AC) (a.u. g^{-1}).

Leaf optical properties

Fully developed leaves from 10 plants per treatment at each altitude were collected at noon and were kept on moist paper in a refrigerated box. The leaf transmittance and reflectance spectra were measured in the laboratory on fresh leaves, using the Jaz Modular Optical Sensing Suite (Ocean Optics, USA) with a measurement sphere (ISP-30-6-R; Ocean Optics, USA), applying UV/VIS/near infrared light from a deuterium and halogen light source (DH-2000; Ocean Optics, USA). The leaf reflectance spectra were measured for the upper leaf surface. The spectrometer was calibrated to 100% reflectance using a white reference panel with >99% diffuse reflectance (Spectralon; Labsphere, North Sutton, NH, USA). The leaf transmittance spectra were measured for the lower leaf surface by illumination of the upper surface. The spectrometer was calibrated to 100% transmittance with a light beam that passed directly into the interior of the integrating sphere. The transmittance and reflectance measurements were processed using SpectraSuite software (Ocean Optics, USA).

Data analysis

The plant responses and characteristic data were analysed using the SPSS Statistics 22.0 software (IBM, USA). Statistical tests were performed on 10 samples. The normal distributions of the data were evaluated using Shapiro-Wilk tests. Homogeneity of variance was analysed using Levene's tests. One-way ANOVA with multiple comparison tests and Tukey's *post-hoc* tests or Kruskal-Wallis tests and Bonferroni *post-hoc* tests were used to compare differences between the four treatments at each experimental site. Factorial ANOVA was performed to investigate the effects of UV radiation and temperature, and their interaction on the measured parameters. Redundancy analysis (RDA, CANOCO 5) was used to determine whether variations in the measured variables were related to the differences in temperature and UV radiation between experimental plots.

Results

Leaf anatomical characteristics

Most of the *H. nummularium* leaf thickness parameters were significantly higher at the montane altitude compared to the alpine altitude in July, which might be due to the delayed phenological events at the alpine altitude. The upper and lower cuticle thicknesses significantly increased under reduced UV radiation (UV-) at the montane and alpine altitudes in September, while spongy mesophyll thickness decreased. Total leaf thickness and specific leaf area did not change according to the different UV radiation and altitude conditions in September. The measured stomatal characteristics showed no changes at all (Tab. 2).

Table 2: Leaf anatomical characteristics for *Helianthemum nummularium* according to month and altitude for near-ambient (UV) and reduced (UV-) UV radiation.
Tabela 2: Anatomske značilnosti listov pri *Helianthemum nummularium* julija in septembra v montanskem in alpinskem pasu pri naravnem (UV) in zmanjšanem (UV-) UV sevanju.

Measure	Detail	July		September						
		1600 m	2000 m	1600 m		2000 m		Factorial ANOVA		
		UV	UV-	UV	UV-	UV	UV-	T	UV	T*UV
Stomatal density (mm^{-2})	Upper	87.7 ±5.1^{a}	90.3 ±3.4^{a}	96.6 ±4.8^{a}	92.5 ±7.7^{a}	95.5 ±3.6^{a}	92.7 ±2.0^{a}	ns	ns	ns
	Lower	157.8 ±5.3^{a}	154.5 ±3.9^{a}	164.6 ±12.5^{a}	161.1 ±9.3^{a}	160.2 ±3.9^{a}	152.1 ±3.5^{a}	ns	ns	ns
Stomatal length (µm)	Upper	32.9 ±0.5^{a}	32.3 ±0.5^{a}	34.7 ±0.4^{a}	34.8 ±0.8^{a}	33.9 ±0.3^{a}	34.2 ±0.5^{a}	ns	ns	ns
	Lower	33.7 ±0.2^{a}	34.7 ±0.5^{a}	34.1 ±0.4^{a}	34.3 ±0.2^{a}	33.7 ±0.3^{a}	34.5 ±0.7^{a}	ns	ns	ns
Cuticle thickness (µm)	Upper	3.2 ±0.1^{a}	2.2 ±0.1^{b}	2.9 ±0.1^{a}	3.6 ±0.2^{b}	3.5 ±0.2^{b}	4.0 ±0.2^{c}	ns	*	*
	Lower	2.2 ±0.2^{a}	1.5 ±0.1^{b}	2.5 ±0.2^{a}	2.9 ±0.2^{a}	3.0 ±0.2^{b}	3.1 ±0.2^{b}	ns	**	*
Epidermis thickness (µm)	Upper	22.5 ±0.9^{a}	21.7 ±1.0^{a}	22.8 ±0.8^{a}	25.2 ±0.8^{a}	24.3 ±0.6^{a}	22.6 ±0.7^{a}	ns	ns	ns
	Lower	17.9 ±0.9^{a}	18.0 ±0.7^{a}	19.9 ±1.0^{a}	23.6 ±1.0^{a}	22.4 ±0.7^{a}	19.2 ±0.7^{a}	ns	ns	ns
Leaf thickness (µm)	-	302.8 ±6.3^{a}	259.9 ±7.9^{b}	320.6 ±12.5^{a}	316.2 ±9.3^{a}	309.2 ±3.9^{a}	288.5 ±6.5^{a}	ns	ns	ns
Mesophyll thickness (µm)	Palisade	133.0 ±3.7^{a}	112.4 ±3.9^{b}	140.7 ±7.7^{a}	140.2 ±5.3^{a}	127.8 ±2.0^{ab}	124.6 ±4.0^{b}	**	ns	*
	Spongy	132.7 ±3.9^{a}	103.5 ±4.4^{b}	131.6 ±5.1^{a}	120.4 ±4.9^{a}	128.0 ±3.0^{ab}	114.4 ±3.8^{b}	ns	*	ns
Specific leaf area (cm^2 g^{-1})	-	0.2 ±0.0^{a}	0.2 ±0.1^{a}	0.1 ±0.0^{a}	0.1 ±0.0^{a}	0.1 ±0.0^{a}	0.1 ±0.0^{a}	ns	ns	ns

Data are means ± standard error (n = 10 plants). Different letters indicate significant differences between the treatments ($p \le 0.05$; one-way ANOVA); *, $p \le 0.05$; **, $p \le 0.01$; ns, $p > 0.05$, as significant responses to environmental factors temperature (T) and UV radiation, and their interaction (Factorial ANOVA).

Pigment contents

Pigment contents of *H. nummularium* showed the least changes for UV radiation and altitude for these three experimental plant species. The UV-B–AC contents in these leaves were lower at the montane than the alpine altitude in July and September (Tab. 3).

Leaf optical properties

Leaf reflectance for *H. nummularium* showed minor changes according to UV radiation and altitude, while leaf transmittance of the total photosynthetic spectrum (except for violet) was significantlly higher at the alpine than the montane altitude. Reduced UV radiation (UV-) increased the transmittance of the green and yellow spectra of all the three plant species. (Fig. 3, Tab. 4).

Table 3: Pigment contents of *Helianthemum nummularium* according to month and altitude for near-ambient (UV) and reduced (UV-) UV radiation.

Tabela 3: Vsebnost barvil v listih pri *Helianthemum nummularium* glede na mesec in nadmorsko višino pri naravnem (UV) in zmanjšanem (UV-) UV sevanju.

Measure	July		September						
	1600 m	2000 m	1600 m		2000 m		Factorial ANOVA		
	UV	UV-	UV	UV-	UV	UV-	T	UV	T*UV
Chlorophyll a (mg g^{-1} DW)	1.60 ±0.20[a]	1.93 ±0.16[a]	1.65 ±0.16[a]	1.61 ±0.15[a]	1.44 ±0.09[a]	1.40 ±0.13[a]	ns	ns	ns
Chlorophyll b (mg g^{-1} DW)	0.83 ±0.18[a]	1.36 ±0.19[a]	1.44 ±0.42[a]	1.37 ±0.26[a]	1.06 ±0.16[a]	0.99 ±0.12[a]	ns	ns	ns
UV-A–AC (a.u. g^{-1})	5.80 ±0.20[a]	6.14 ±0.70[a]	5.00 ±0.21[a]	4.46 ±0.30[a]	4.89 ±0.22[a]	4.65 ±0.43[a]	ns	ns	ns
UV-B–AC (a.u. g^{-1})	5.08 ±0.40[b]	7.30 ±0.23[a]	4.80 ±0.28[b]	6.42 ±0.68[ab]	7.02 ±0.25[a]	6.37 ±0.54[ab]	*	ns	ns

Data are means ±standard error (n = 10 plants). Different letters indicate significant differences between the treatments (one-way ANOVA); *, p ≤0.05; ns, p >0.05, as significant responses to environmental factors temperature (T) and UV radiation, and their interaction (Factorial ANOVA).

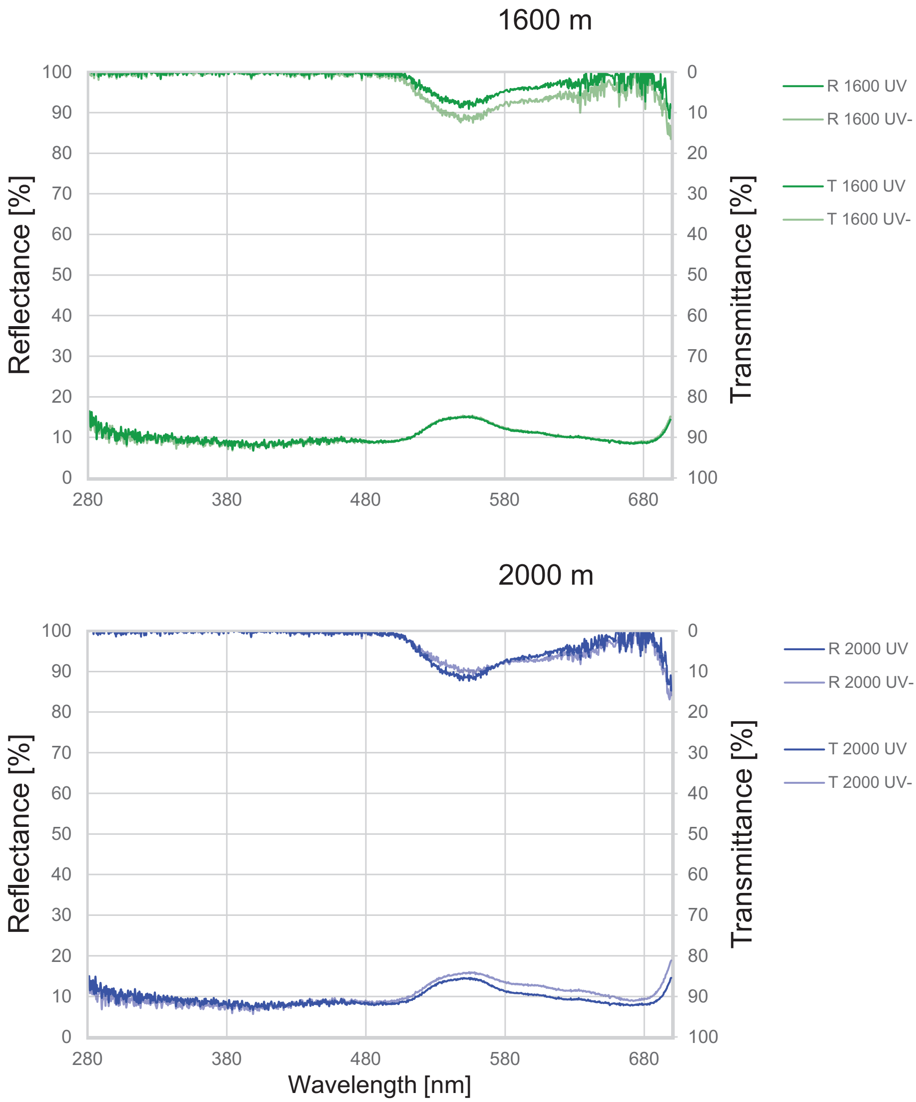

Figure 3: Leaf optical properties in September, as leaf transmittance and reflectance of *Helianthemum nummularium* at the indicated altitudes under near-ambient (UV) and reduced (UV-) UV radiation. Data are means for every 5-nm interval (n = 10 plants).

Slika 3: Optične lastnosti listov v septembru pri *Helianthemum nummularium* na označenih nadmorskih višinah pod naravnim (UV) in zmanjšanim (UV) UV sevanjem. Podatki so aritmetične sredine 5-nm intervalov (n = 10 rastlin).

Table 4: Significance of the effects of temperature and UV radiation on leaf reflectance and transmittance for *Helianthemum nummularium* over the light spectral ranges in September.
Tabela 4: Učinek temperature in UV sevanja na odbojnost in prepustnost listov *Helianthemum nummularium* v različnih spektralnih območjih v septembru.

Light spectrum	T	UV	T*UV
Reflectance			
UV-B	ns	ns	ns
UV-A	ns	ns	ns
Violet	ns	ns	ns
Blue	ns	ns	ns
Green	ns	ns	ns
Yellow	ns	ns	ns
Red	*	ns	ns
Near-infrared	*	ns	ns
Transmittance			
UV-B	ns	ns	ns
UV-A	ns	ns	ns
Violet	ns	ns	ns
Blue	*	ns	ns
Green	**	*	ns
Yellow	**	*	ns
Red	**	ns	ns
Near-infrared	*	ns	ns

*, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$; ns, $p > 0.05$, as (light colour coded) significant effects of environmental factors temperature (T) and UV radiation, and their interaction (Factorial ANOVA).

In July, the diversity among the treated plants was low; the morphological variables and UV-absorbing compounds content accounted for 66% of the total variation between the two altitudes and different UV-B radiation treatments according to RDA analyses. In September, the diversity between the treated plants was high; the morphological variables and UV-absorbing compounds content accounted for 19% of the total variation between the different UV-B radiation and altitudinal treatments according to RDA analyses. Explanatory variables accounted for 19.9% of the total variation, where T explained 14.7% and UV-B 5.1%. (Fig. 4).

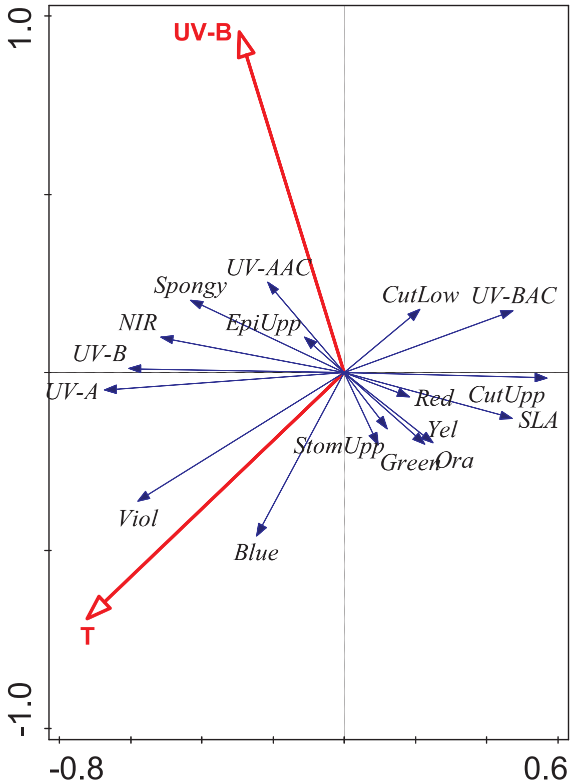

Figure 4: Redundancy analysis ordination diagram showing the strengths of the associations between the environmental factors (T, UV-B) and functional traits of *Helianthemum nummularium.*

Slika 4: Diagram redundančne ordinacijske analize prikazuje moč povezav med okoljskimi dejavniki (T, UV-B) in funkcionalnimi značilnostmi *Helianthemum nummularium.*

Discussion

Unaffected Chl a and Chl b contents of a shrub life form

Chl a and Chl b contents did not change in *H. nummularium* according to UV radiation and altitude throughout the season. Previous studies have reported that UV radiation can lead to reductions, no changes, and increases in chlorophyll contents (Smith et al. 2000; Bassman et al. 2003). It has been shown that UV radiation generates free radicals and leads to degradation of chlorophylls and carotenoids (Middleton and Teramura 1993), and also to increased biosynthesis of photosynthetic pigments under adequate intensities of PAR and UV-A (Verdaguera et al. 2017). Furthermore, heterogeneous responses can reflect differences in species and/or cultivars (Teramura 1983). Decreases in chlorophyll contents under strongly reduced solar UV-B radiation have been shown for *Zea mays* and *Citrus aurantifolia* (Barsig and Malz 2000; Ibanez et al. 2008). John et al. (2001) demonstrated that exposure to UV radiation can lead to the induction of genes associated with senescence, which can result in loss of plant chlorophyll content. Trošt Sedej et al. (2020) showed a significant decrease in chlorophyll contents in an alpine plant *Saxifraga hostii* under the ambient UV radiation at alpine altitudes at the end of the growing season, which might suggest earlier senescence. The different response of *H. nummularium* and *S. hostii* could be due of different life forms, where the leaves of *S. hostii* rosettes are more exposed to irradiance than the leaves of *H. nummularium* shrub growth form.

High levels of constitutive UV-absorbing compound contents

H. nummularium showed high UV-AC contents throughout the season. In agreement with this, other studies have demonstrated that species from locations with naturally high UV radiation at either high altitude or low latitude are less sensitive to UV radiation than species from low UV radiation locations (Biswas and Jansen 2012). Flavonoids have key roles in plant UV protection due to their antioxidant and UV-screening properties (Smith et al. 2000). Flavonoid formation is not only induced by UV radiation, but is also affected by other environmental variables, such as photosynthetic irradiance, temperature and nutrient supply (Bassman et al. 2003).

In *H. nummularium*, UV-AC contents were generally not affected by the different environmental factors, which demonstrated high constitutive UV-AC contents. Constitutive levels of UV-AC under increased UV radiation (flavonoids and other phenylpropanoid derivatives) have been correlated with plant tolerance to UV radiation (Qaderi et al. 2008). High constitutive and inducible UV-AC explain the good protection of the photosystem from UV radiation penetration of the plants at the alpine altitude. Constitutive and inducible UV radiation tolerances reflect phenotypic plasticity in response to the different UV environments; lowland *Arabidopsis* ecotypes show more inducible UV radiation tolerance, whereas highland *Arabidopsis* ecotypes show constitutive UV radiation tolerance (Jansen et al. 2010). These studies suggest that for alpine plants, constitutive defence is a more advantageous strategy than inducible defence, where UV stress is constantly high.

Most leaf thickness parameters showed no response to the UV and altitude conditions

Most of the *H. nummularium* leaf thickness parameters and all of the stomatal characteristics showed no changes according to the UV radiation and altitude in September. The exceptions were for spongy mesophyll thickness, upper and lower cuticle thickness, which increased and decreased, respectively, under the near-ambient UV radiation at the montane and alpine altitudes in September. UV radiation can induce several photomorphogenic responses, which include decreased leaf area and increased leaf thickness. Such modifications represent effective mechanisms that reduce the transmittance of UV radiation to the inner leaf tissue (Czégény et al. 2016). Increases in leaf thickness were attributed to increases in the number of spongy parenchyma cells in *Brassica carinata* and *Medicago sativa* (Bornman and Vogelmann 1991). It has been reported that cuticle thickness of the upper needle surface of *Abies balsamea* decreases at high altitudes (DeLucia and Berlyn 1984). These studies indicate that plants that are less adapted to UV radiation show more changes to their leaf parameters than those that are better adapted to UV radiation. This suggests that *H. nummularium* plants are well-adapted to UV radiation and high altitude.

Leaves did not transmit any UV spectrum

The leaf reflectance of *H. nummularium* showed small changes according to UV radiation and altitude. Another study on *S. hostii* showed that alpine plants, but not montane plants, can be grouped according to their different UV radiation exposures in September, meaning that UV radiation had a distinct influence on leaf optical properties (Trošt Sedej et al. 2020).

The leaves of *H. nummularium* did not transmit any UV spectrum, which corresponded to their high constitutive and inducible UV-AC contents. Similar responses were shown for *Arnica montana,* where enhanced UV radiation was not the key factor that triggered changes in the flavonoid composition. The key factor was temperature, which decreased with altitude (Albert et al. 2009). A tropical alpine study indicated that high levels of UV radiation screening are common to native and non-native plant species, and to different growth forms in an alpine environment. The plasticity of the epidermal UV radiation transmittance is a mechanism that is used by some, but not all, species to cope with varying solar UV radiation exposure. In the non-native *Verbascum thapsus*, leaf transmittance of UV-A was shown to be variable along an alpine altitude gradient, and to be strongly correlated with UV-B radiation and altitude. However, in the native *Vaccinium reticulatum*, leaf transmittance of UV-A was consistently low and did not change with altitude (Barnes et al. 2017).

The leaf transmittance of the photosynthetic spectrum increased at the alpine altitude, while the transmittance of the green and yellow spectra increased under the reduced UV radiation in September. The leaf optical properties are dependent on the anatomical and biochemical characteristics of the leaf, such as the structure of the leaf, the concentration and type of flavonoids it contains, chlorophylls and other pigments, and the thickness of the epidermis, cuticle, waxes and hairs (Ziska et al. 1992), and they are correlated with plant life form (Day et al. 1992). The optical properties can be species-specific, but can also vary within a species, due to ontogenetic development of plants (Liew et al. 2008). The environmental conditions, to which a plant is exposed to, can alter leaf optical properties, including for UV radiation and PAR, temperature, and water and soil properties (Ustin and Jacquemoud 2020). Therefore, the high photosynthetic spectrum transmittance of *H. nummularium* at the alpine altitude is more due to complex biochemical and anatomical responses to the alpine environmental conditions than to UV radiation itself.

Conclusions

H. nummularium showed high UV-AC contents throughout the season. The UV-AC contents were generally not affected by the different environmental factors, which indicated high constitutive UV-AC contents. Constitutive and inducible UV radiation tolerances reflect phenotypic plasticity in response to different UV environments. Most leaf thickness parameters showed no response to the different UV radiation and altitude conditions. Plants, less adapted to UV radiation, show more changes in leaf parameters than plants, well-adapted to UV radiation, which suggests that *H. nummularium* is well-adapted to UV radiation and high altitude. The leaf reflectance of *H. nummularium* showed small changes according to UV radiation and altitude. The leaves of *H. nummularium* did not transmit any UV spectrum, which corresponded to their high constitutive and inducible UV-AC contents. Leaf transmittance of the photosynthetic spectrum increased for the alpine altitude, while the transmittance of the green and yellow spectra increased under the reduced UV radiation in September. The high photosynthetic spectrum transmittance of *H. nummularium* at the alpine altitude was due to complex biochemical and anatomical responses to the alpine environmental conditions, rather than to UV radiation. Unchanged chlorophyll content of *H. nummularium* could be related to shrub life form, where the leaves shade out the high UV and PAR irradiance as well as contribute to lower leaf temperature. The study shows complexity of alpine plant response, where specific characteristics of plant species should not be overlooked.

Povzetek

Alpinske in gorske rastline so razvile strategije za preživetje v skrajnih razmerah, ki vključujejo visoko jakost UV in vidnega sevanja, skrajne temperature, sušo in pomanjkanje hranil. Strategije preživetja alpinskih rastlin vključujejo biokemične, fiziološke in morfološke odzive, ki jih raziskovalci zaradi zahtevne izvedbe redko proučujejo. V naši raziskavi smo proučevali funkcionalne značilnosti alpske rastline velecvetni popon *Helianthemum nummularium* subsp. *grandiflorum* pod naravnim in zmanjšanim sevanjem UV-B na dveh različnih nadmorskih višinah (1600 in 2000 m n.m.) gore Zadnji Vogel v Julijskih Alpah. Anatomijo listov, pigmente in optične lastnosti smo raziskovali na začetku in na koncu rastne sezone. *H. nummularium* je ob obeh merjenjih vseboval veliko UV-absorbirajočih snovi (UV-AC) v listih. V splošnem različni okoljski dejavniki na vsebnost UV-AC niso vplivali, kar kaže na visoko vsebnost konstitutivnih UV-AC. Konstitutivne in inducibilne UV-AC odražajo fenotipsko plastičnost rastline v

odzivu na različna UV okolja. Večina parametrov, s katerimi smo ocenjevali debeline listnih tkiv, ni pokazala odziva na različno UV sevanje in temperaturne razmere. UV sevanje pri manj prilagojenih rastlinah izzove večje spremembe anatomije listov kot pri rastlinah, prilagojenih na veliko UV sevanje, kar kaže, da je *H. nummularium* dobro prilagojena alpinska rastlina. Listi niso prepuščali UV sevanja, kar ustreza njihovi visoki konstitutivni in inducibilni vsebnosti UV-AC. Prepustnost fotosinteznega spektra sevanja skozi liste se je povečala na alpinski nadmorski višini, medtem ko se je prepustnost zelenega in rumenega spektra povečala pod zmanjšanim UV sevanjem septembra. Velika prepustnost fotosinteznega spektra sevanja na alpinski nadmorski višini je bila povezana z biokemičnimi in anatomskimi značilnostmi rastline in ne z jakostjo UV sevanja. Nespremenjena vsebnost klorofila pri *H. nummularium* bi lahko bila povezana z grmičasto rastno obliko rastline, kjer zgornji listi zasenčijo spodnje liste ter tako prispevajo k manjšim prejetim odmerkom UV in fotosintezno aktivne svetlobe ter k nižji temperaturi listov. Študija kaže na kompleksen odziv alpinskih rastlin na UV sevanje in temperaturne razmere v gorah, pri čemer je pomembna tudi rastna oblika rastline.

Acknowledgements

We thank the Slovenian Research Agency for financing the research programme P1-0212. We are grateful to Dragan Abram for technical support.

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