



Morphological and biochemical variations in St. John's wort, *Hypericum perforatum* L., growing over altitudinal and UV-B radiation gradients

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Abbreviations:

DM – dry mass
UV AC – UV absorbing compounds

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Abstract

Background and Purpose: The climate of the Alpine region is marked by a higher proportion of UV radiation which, combined with other stresses, plays an important role in determining the differences between highland and lowland plant populations. The study was aimed at identifying the plant habitus as well as selected morphological and biochemical properties that enable optimised growth under given conditions, and at determining the potential of plants to cope with UV-B radiation stress.

Material and Method: *St. John's wort*, *Hypericum perforatum* L., thrives in a continuum of habitats from lowlands to highlands. Chlorophylls *a* and *b* and UV absorbing compounds (AC) were determined. The height of the plants, internodes and shoots, and the angle of the shoots were measured. Thicknesses of epidermis, palisade and spongy tissues were examined and evaluated by the computer program AnalySIS 3.0 (Soft Imaging System, Münster, Germany). The number of seeds was counted and their weight determined at the end of the growing season.

Results and Conclusions: The content of total UV absorbing compounds (AC) in leaves increased with altitude. The average content of total UV AC in plants growing at 1300 m a.s.l. was about 25% higher than at 400 m a.s.l... The content of chlorophyll showed the opposite trend. At high altitudes plants were shorter; due to shorter and reduced numbers of internodes. Reproductive success was much lower in plants growing at high altitudes; they developed fewer flowers (on average 147 at 400 m a.s.l. and 50 at 1700 m a.s.l.) and seeds per plant than those growing in lowlands. The present study indicates that *St. John's wort* growing at high altitudes has developed an effective strategy to mitigate the effects of the severe alpine climate.

INTRODUCTION

The climate of the Alpine region is very complex, due to interactions between the mountains and the general circulation of the atmosphere (1). The altitudinal gradient is associated with decreased air temperature and pressure, increased precipitation, and changes in wind exposure, soil fertility and duration of snow cover (2). Lower temperatures and longer persistence of snow cover at higher altitudes result in a shorter vegetation period. Marked differences are also observed in the quality of the incident radiation. The intensity of UV-B radiation in-

creases steeply at higher altitudes, even though the daily total solar radiation is usually nearly the same along the gradient (3). The increase in UV-B radiation (290–320 nm) has been reported to range from between 6 and 8% (3) to 20% (4) per 1,000 m of elevation.

The higher proportion of UV radiation, combined with other stresses in the mountains, plays an important role in determining the differences between highland and lowland plant populations (5). The environmental constraints result in changes, not only in plant morphology and anatomy, but also in their habitus, physiology and productivity (2). Many adaptations found in mountain plants are similar to those shown to result solely from increased UV-B radiation. These include photomorphogenetic changes, disturbances to physiological processes, modification of plant enzyme content, biomass reduction, disturbances in pollen germination and reduced reproductive success (6–9). An increased content of UV-B screening compounds, in response to UV-B radiation, has been widely reported (9–14).

Sensitivity and adaptation to UV-B radiation are highly species specific but also depend on the environment (10, 15–18). Species and populations originating from naturally high UV-B radiation locations, i.e. from high elevations or low latitudes, have been shown to be less sensitive to UV-B radiation than those from low intensity UV-B radiation locations (15, 19–23). Species adapt their epidermal UV-B attenuation to match solar UV-B dose along latitudinal and altitudinal gradients, providing one of the bases for tolerance to UV-B radiation in plants colonizing high altitude regions (24). Körner (25) claimed that, due to the high level of adaptation of plants to solar radiation, UV-B radiation does not present an important constraint for growth and development of plants in alpine regions. This holds true for present conditions, but given the presumed increase of ambient UV-B radiation in combination with global warming (26), the level of changes might exceed the tolerance of high altitude plant populations. In those regions snow-cover determines the period over which plants intercept solar radiation. The potential prolongation of the growth period due to global warming might also result in increased cumulative UV-B doses received by plants.

Hypericum perforatum (L.) (St. John's wort) thrives in a continuum of habitats from lowlands to highlands. The study was aimed at identifying the characteristics, i.e. habitus and selected morphological and biochemical properties, that enable optimised growth under given conditions, and at determining the potential of plants to cope with UV-B radiation stress.

MATERIAL AND METHODS

Site description

Plants were collected for analysis from June to August 2001 from locations at different altitudes in the Slovenian Alps. The chosen locations were Pšata near Cerklje (14.5103°E, 46.2606°N) at 400 m, Škrjančevo

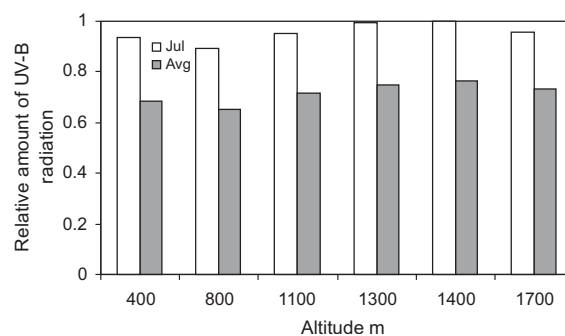


Figure 1. Relative amount of UV-B radiation in the locations over an elevation gradient in July and August.

(14.5160°E, 46.2741°N) at 800 m, Ambrož under Kravec (14.5337°E, 46.2757°N) at 1100 m, half way between Ambož and Alp Jezerca (14.5277°E, 46.2823°N) at 1300 m, Alp Jezerca (14.5349°E, 46.2857°N) at 1400 m, and Kravec (14.5358°E, 46.2846°N) at 1700 m. The average temperatures in 2001 at Brnik near Cerklje (14.4882°E, 46.2461°N) at 384 m were 16.6 °C in June, 19.5 °C in July and 20.2 °C in August, and on Kravec (14.5395°E, 46.3011°N) at 1740 m, 8.5°C in June, 11.8°C in July and 13.6°C in August (27).

The relative solar UV-B radiation at the localities studied (Figure 1) was estimated on clear days at noon using a UV-B sensor with integrator (Delta T Device).

All analyses were made on first, fully developed leaves and on flowers.

Biochemical analyses

Chlorophylls *a* and *b* (Chl *a*, *b*) were estimated after Jeffrey & Humphrey (28). A weighed leaf was homogenised in 8 cm³ of 90% (v/v) acetone and centrifuged (19 000 ×g, 3 min, 4 °C) in a top refrigerated ultracentrifuge (2K15, Sigma, Osterode, Germany). Absorbance was measured with a UV/VIS Spectrometer System Lambda 12 (Perkin-Elmer, Norwalk, CT, USA). The amount of Chl per sample dry mass (DM) was calculated.

UV absorbing compounds (AC) were extracted from freshly homogenised plant material with methanol : distilled water : HCl (37% (v/v)) = 79 : 20 : 1 (v/v/v). After 20 minutes incubation the samples were centrifuged in a top refrigerated centrifuge (1600xg, 10 °C, 10 min). The supernatants of the samples were scanned in the range from 280 to 320 nm (UV-B) and from 320–400 nm (UV-A) at intervals of 1 nm. The absorbance values were integrated and expressed per dry mass (DM) of the sample, according to Mirecki & Teramura (29).

Morphological analyses

The height of the plants, internodes and shoots, and the angle of the shoots were measured. Cross sections of the leaves, thickness of epidermis, palisade and spongy tissues were all prepared, using a microscope, and evaluated by the computer program *AnalysIS 3.0* (*Soft Im-*

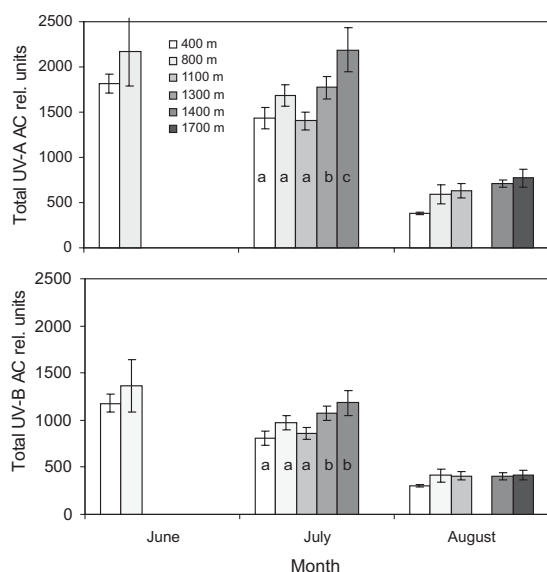


Figure 2. The amount of UV-A and UV-B absorbing compounds in leaves of *H. perforatum* growing over an elevation gradient. Letters represent variance differences (one way ANOVA and Post Hoc Tukey's test), (n=4).

aging System, Münster, Germany). The numbers of seeds were counted and their weight determined at the end of the growing season.

Statistical analyses

Measurements were made on 5 to 8 parallel samples. Variance (one way ANOVA and Post Hoc Tukey's test, SPSS for Windows 11.0.0.) of all parameters was analysed to establish the differences between different locations.

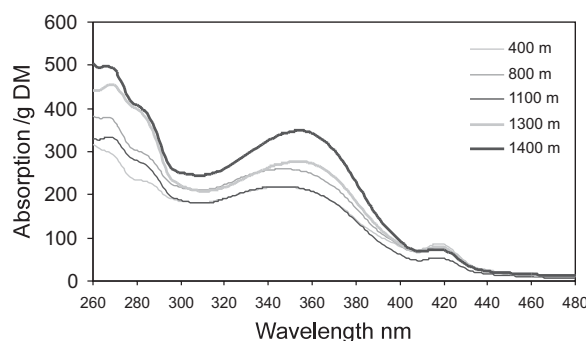


Figure 3. The absorbance spectra of methanol extracts of leaves of *H. perforatum* growing over an elevation gradient (n=4).

RESULTS

In July the production of substances filtering radiation in the UV-B and UV-A ranges in leaves (Figure 2) correlated with altitude ($r^2=0.62$ and $r^2=0.72$, respectively). The average content of total UV AC in plants growing at 1300 m a.s.l. was about 25% higher than in plants growing at 400 m a.s.l.. From Figure 2 it is evident that the production of UV-B and UV-A AC was highly dependent on seasonal changes. The timing of phenological phases depends on the length of the vegetation period, which changes with elevation. Flowering started in June at 400 and 800 m a.s.l., in July at 1100, 1300 and 1400 m a.s.l., but not until August at 1700 m a.s.l.. That is why it was not possible to compare samples from all altitudes at the same time. The spectra of methanol extracts of leaves in July showed two peaks, at 290 nm and at 360 nm (Figure 3). The increase of UV-B and UV-A AC with increased elevation was not found in the flowers (Table

TABLE 1

Chlorophyll a and b contents in leaves and the amounts of UV-A and UV-B absorbing compounds in flowers of *H. perforatum* growing over an elevation gradient. Letters represent variance differences (one way ANOVA and Post Hoc Tukey's test), (n=4).

	Month	Altitude					
		400	800	1100	1300	1400	1700
Chl a	June	3.70a	3.65a				
	July	4.94a	4.02b	2.83c	3.69b	3.16c	
	August	2.74a	2.77a	2.79a		2.76a	2.29b
Chl b	June	2.43a	2.39a				
	July	3.28a	2.62b	1.78c	2.37b	2.03c	
	August	1.66a	1.65a	1.66a		1.65a	1.35b
UV-A AC	June	1576a	1351a				
	July	1001a	1106a	968a	962a	1043a	
	August	372a	343a	420b		493c	451b
UV-B AC	June	1174a	1062a				
	July	567a	715b	684a	752b	752b	
	August	309a	310a	345a		418b	352a

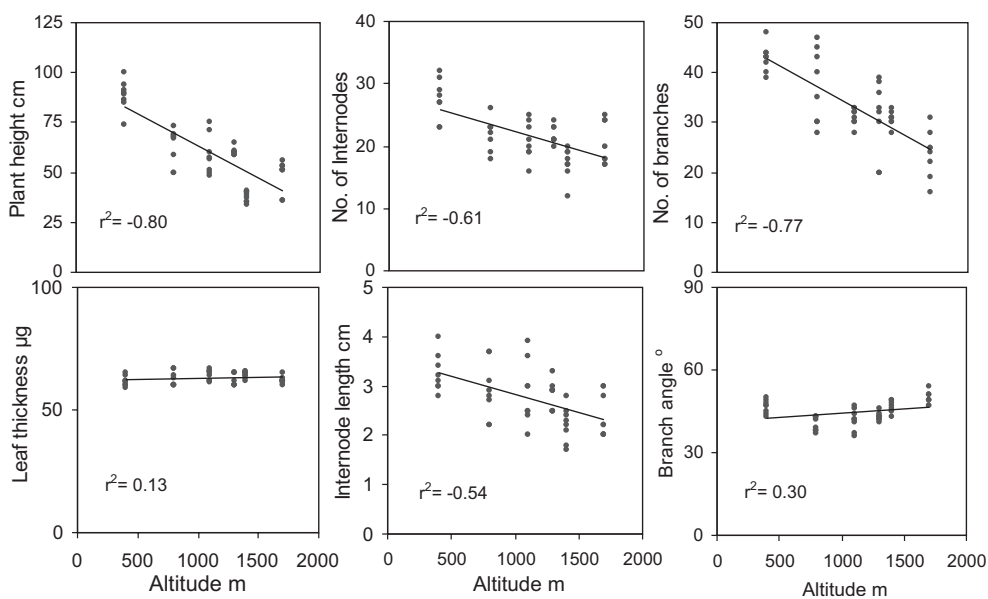


Figure 4. Plant height, number and height of internodes, number of branches, branch angle and leaf thickness in *H. perforatum* growing over an elevation gradient ($n=8$).

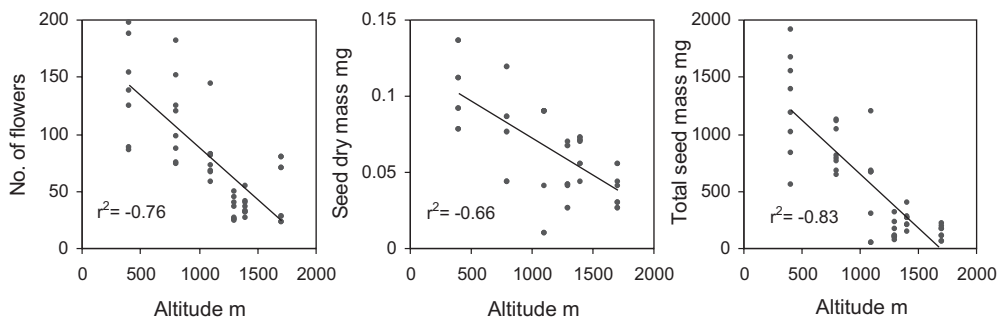


Figure 5. Number of flowers per plant and seed dry mass in *H. perforatum* growing over an elevation gradient ($n=8$).

1), the correlation coefficients in July being 0.38 and 0.14, respectively. The synthesis of photosynthetic pigments Chl *a* and Chl *b* in July (per DM) was also dependent on elevation ($r^2=0.72$ and $r^2=0.73$, respectively) but there were also marked differences during the vegetation period. The decrease in July was not the consequence of the changes in the leaf thickness, since it did not differ between locations (Figure 4).

The analysis of *H. perforatum* growth was made at the end of the growth period, at the time of fruiting, and showed the traits typical of alpine plants. A trend of lower growth with increased elevation was observed (Figure 4). The average height of plants was 89 cm at 400 m a.s.l., 38 cm at 1400 m a.s.l. and 48 cm at 1700 m a.s.l.. This was the consequence of both shorter and reduced numbers of internodes. The height of plants was also slightly related to plant branching ($r^2= 0.54$). High altitude plants developed fewer branches (Figure 4) while the differences in the branch angle were negligible.

Reproductive success was significantly affected by the severe environment and enhanced UV-B radiation at

higher elevations. Analysis revealed a reduced number of reproductive organs and delayed time of reproduction. The number of flowers and seeds per plant and the weight of each seed decreased with elevation (Figure 5). The average number of flowers per plant was 147 at 400 m a.s.l. and 50 at 1700 m a.s.l.. There was a negative correlation between the measured UV-B dose and the number of flowers and seeds ($r^2= -0.68$ and -0.71 , respectively) (Figure 5). The strong correlation between the number of flowers and the number of seeds ($r^2= 0.96$) showed that the length of the vegetative period for *H. perforatum* was long enough to complete the reproduction cycle, even at the highest locations.

DISCUSSION

St. John’s wort grown at different altitudes exhibited differences in plant architecture, biochemistry and reproduction. These changes resulted from the combined effects of seasonal dynamics and the altitudinal gradients of environmental factors and, presumably, UV-B radiation.

Important protection against UV-B in plants growing in high altitudes is provided by the production of UV AC, which protect vulnerable targets in the plant tissue (Figure 2). These compounds are located in epidermal cells, and constitute an effective, selective filter that absorbs UV radiation without interfering with photosynthetically active radiation (30–32). Quantitative changes with elevation of these substances in leaves of *H. perforatum* appear to be important in determining sensitivity to UV-B. Jüngenliemk & Nahrstedt (33) report that St. John's wort contains 22 different phenolic substances. The content is also significant in flowers, being the highest in buds (34). The amount of these substances produced in leaves obviously depends on UV-B radiation level, but not in blossoms, as was seen from our results (Table 1). Fillela & Penuelas (35) stated that the amount of UV-B AC was 15% higher in the leaves of *Quercus ilex* thriving at 1200 m a.s.l., than in those at 200 m a.s.l.. A positive correlation was also found between the amount of UV-B absorbing compounds and elevation for leaves of *Fagus sylvatica* (36) and the previous year's needles of *Picea abies* (37). The content of photosynthetic pigments however was higher in populations from lowlands (Table 1). The decrease of chlorophyll content with elevation might also be the consequence of increased UV-B radiation, since this effect has been shown for many UV-B treated plants (37–39).

Many effects of UV-B radiation concern morphogenetic changes (8). The plants grew less at higher elevations (Figure 4). Internodes were fewer and they were shorter. The results were similar to those of Teramura (40), on the specimens of *Glicine max*, which also grew less because of shorter internodes. Stunting of plants that thrive at high altitudes is an adaptive strategy to avoid the damaging mechanical effect of strong winds (2). Negative effects on plant growth have also been documented for UV-B stressed *Gnaphalium luteo-album* (41). The reason for stunting effects on plants is not totally clear, but the authors suggested that this was a consequence of hormone imbalance or direct DNA damage. Another possibility has been proposed by Teramura & Sullivan (16), who stated that direct damage to the photosynthetic processes by UV-B, could be a mechanism for growth reduction. Kao et al. (42) pointed out that growth does not depend on the sensitivity of the species to UV-B radiation alone, but is also a response to all other constraints, such as shorter growth period, lower temperatures, nutrient and water limitations, pests and competition. Coping with any kind of stress is energy consuming. The production of UV AC in primary producers demands an additional supply of energy, which is provided by higher respiratory potential, and results in lower growth rate (12, 14). Therefore cost-benefit relations in plants can either depress or stimulate certain processes, i.e. growth, reproduction, the production of secondary substances or establishment of other protective mechanisms.

Other important changes on St. John's wort on the elevation gradient were the reduced numbers of flowers and seeds per plant and the altered time of flowering (Figure

5). The effects are in line with findings on UV-B treated plants (43, 44, 9). The additional delay of reproduction due to enhanced UV-B could have an effect on pollinators, which appear before or after the plants flower (45).

The present study indicates that St. John's wort growing at high altitudes has developed an efficient strategy to mitigate the effects of the severe alpine climate. The production of UV absorbing substances and other traits enables plants to cope with the high level of UV-B radiation. However, the lower reproductive success at high altitudes constitutes a risk for *H. perforatum* highland populations if UV-B radiation dose were to increase or if some other events, such as air pollution and global warming, were to become more pronounced. Many characteristics of alpine species that enable their survival under present condition might limit their responses to environmental changes, as well as to biological invasions, and they are very likely to change their abundance and distribution range.

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