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Enhanced UV-B radiation under field conditions increases anthocyanin and reduces the risk of photoinhibition but does not affect growth in the carnivorous plant *Pinguicula vulgaris*

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SUMMARY

The effects of enhanced UV-B radiation were investigated in the carnivorous plant *Pinguicula vulgaris* in a field experiment performed in Abisko, North Sweden (68° 21′ N, 18° 49′ E, 380 m above sea level). Potted plants were exposed to either ambient or ambient plus supplemental UV-B radiation, simulating a 15% ozone depletion. No effect was observed on either the epicuticular (external) or cellular (internal) UV absorbing capacity of the leaves. However, the anthocyanin content was more than doubled by supplemental UV-B radiation. In laboratory experiments, the anthocyanin rich, UV-B treated leaves were less susceptible to a low temperature/high light photoinhibitory treatment, as judged by *in vivo* chlorophyll fluorescence measurements. Yet, this potential benefit did not considerably affect the growth of the plant in the field (leaf area and dry mass, reproductive dry mass, flowering frequency, senescence rates, dry mass of winter buds). However, there was a marginally significant increase in root dry mass and in the root to shoot ratio, which may underlie the significant increase in the nitrogen content of the leaves. We suggest that *P. vulgaris* is resistant against UV-B radiation damage and that the possible negative effects of additional UV-B radiation on the growth of these plants may have been effectively counterbalanced by the lower risk of photoinhibition, due to the concomitant increase in anthocyanins.

Key words: anthocyanins, carnivorous plant, photoinhibition, *Pinguicula vulgaris*, UV-B radiation.

INTRODUCTION

The ongoing stratospheric ozone depletion (Müller *et al.*, 1997; Rex *et al.*, 1997) and the concomitant increase in the UV-B radiation reaching the surface of the Earth has prompted much research on the possible effects that this surplus radiation may have on plants. The main outcome from both indoor and field studies is that the effects of UV-B radiation on growth and physiology are species or even variety-specific. Thus, we can distinguish between sensitive and resistant plants (see Björn *et al.*, 1997; Rozema *et al.*, 1997 and the literature therein). Although the mechanistic basis for this distinction is not understood, attempts have been made to correlate tolerance with the levels of UV-B absorbing compounds, mostly phenolics. Evidence for such a role of phenolics are their high absorption coefficients in the UV region of the spectrum, their mostly superficial location in the epidermis (Caldwell *et al.*, 1983) and its appendages (Wollenweber, 1985; Karabourniotis *et al.*, 1992) and their induction by UV-B radiation (Beggs & Wellman, 1994). In addition, mutants of *Arabidopsis* lacking phenylpropanoid metabolism are much more vulnerable to UV-B radiation damage (Li *et al.*, 1993). Since phenolics are carbon-based

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secondary compounds, their concentrations should also depend on the competition for the allocation of photosynthetically fixed carbon to growth or defence (Herms & Mattson, 1992). Therefore, a variety of environmental factors which affect growth and/or photosynthesis, may indirectly affect phenolic levels as well. Thus water, nutrient and light availability have been reported as important determinants of a plant's phenolic levels (Waterman & Mole, 1994). Apart from their role in protection against UV-B radiation, phenolics are also considered as potent anti-herbivores (Dakora, 1995).

Carnivorous plants are exceptional in their nutrition physiology. Carbon can be autotrophically fixed through photosynthesis, yet their photosynthetic rates are extremely low compared with other growth forms (Mendez & Karlsson, 1999). Although nitrogen and phosphorus can be obtained from the usually under-developed root system, prey capture covers a considerable part of nitrogen and phosphorus requirements (Adamec, 1997) leading to increases in growth and/or reproduction (Karlsson & Pate, 1992). Concerning the possible effects of enhanced UV-B radiation, one may speculate that carnivorous species are particularly attractive as test plants for various reasons. Apart from the direct effects on the plant *per se*, UV-B radiation may affect prey behaviour and, accordingly, the plant's nutrient levels. Altered nutrient levels may affect phenolic production i.e. the UV-B absorbing capacity of the plant and, therefore, its tolerance to UV-B radiation. We have therefore studied the effects of enhanced UV-B radiation on *Pinguicula vulgaris*. Our test plant has two additional advantages. It is widespread in the subarctic environment, where considerable ozone depletion is occurring (Müller *et al*., 1997) and occupies open disturbed habitats (Hulten, 1960), being exposed to natural UV-B radiation. To the best of our knowledge this is the first UV-B radiation study with a carnivorous plant.

MATERIALS AND METHODS

Plant material, study site and growth conditions

Pinguicula vulgaris L. (Lentibulariaceae) is a perennial, herbaceous, carnivorous plant with sticky leaves arranged in a rosette on the ground. Leaves and roots are annual and the plant overwinters as a winter bud, from which new leaves and roots sprout in the beginning of the growing season.

The present study was carried out during summer, 1998, at the Abisko Scientific Research station, northern Sweden (68° 21′ N, 18° 49′ E, 380 m above sea level), where *P. vulgaris* abounds in a range of habitats from poor mires to rich mineral soils. During early June, 180 apparently similar plants, which were just starting to grow and having three small leaves, were carefully excavated from their natural habitat (a roadside with sparse plant cover) and put in 0.5 l plastic pots filled with peat. The pots were arranged in eight plastic trays (22–24 pots per tray) and the trays distributed into eight field experimental plots (four UV-B and four controls). The plants were watered 4–5 times per week in order to keep a permanent layer of water in the trays.

Enhanced UV-B radiation was supplied from metal frames ($2.5 \times 1.3 \times 1.5$ m high) each with six fluorescent lamps (Q-Panel UV-B 313, Cleveland OH, USA) as previously described (Johanson *et al*., 1995a). In particular, the middle 70 cm of the two central lamps in each frame was covered with aluminum foil to give an even radiation distribution at plant height. Lamps were pre-burnt for 100 h before field use to achieve a stable output. Control frames had an identical design but the window glass excluded all UV-B emission from the lamps. In UV-B frames, a UV-transmitting Plexiglas (Rohm GmbH, Darmstadt, Germany) holding a cellulose diacetate filter (0.13 mm, Courtaulds, Derby, UK) excluded UV-C but permitted UV-B radiation to reach the plants. Cellulose diacetate filters were presolarized before use and changed after 50 h of irradiation. All plots received natural UV-B radiation and shading by the frames was minimal. Separate timers were used to control three UV-B lamps per frame ensuring a stepwise 'square wave' increase and decrease of additional UV-B radiation, centered at solar noon. The daily exposure time was changed every second week to follow the seasonal change in natural UV-B radiation over Abisko. Spectral irradiance at plant height was measured with an Optronic 742 spectroradiometer (Orlando, FL, USA) interfaced with a Hewlett Packard 85 computer. The absolute spectral irradiance was weighted with the generalized plant action spectrum normalized at 300 nm (Caldwell, 1971) and used in conjunction with the computer program of Björn & Murphy (1985) in order to calculate the duration that the lamps should be on each day. Calculations were based on a 15% ozone depletion scenario over Abisko under clear sky. Apparently, the anticipated ozone depletion should be higher when cloud cover is taken into account (Johanson *et al*., 1995b). Meteorological data were provided by the Abisco weather station.

Measurements

Leaf area was measured with a Delta-T (Cambridge,UK) digital image analysis system. For biomass and nutrient allocation, plants were divided into leaves, roots and reproductive structures. Dry mass to the nearest 0.1 mg was obtained after oven-drying at 70°C for a week. For nitrogen and phosphorus contents, the plant material was digested with sulphuric acid and analysed in an automatic

flow analysis system (FIA-Star 5012 Analyzer, Tecator, Hoganas, Sweden).

Epicuticular (external) UV-B absorbing compounds were obtained by immersing the detached leaves for 3 min in chloroform according to Vogt *et al*. (1991). Preliminary trials showed that 3 min was sufficient to obtain all external UV-B absorbing capacity without extracting internal compounds (data not shown). It was also shown in separate trials that leakage from the cut petiole and the captured insects did not contribute to the extracted absorbance both in the UV and visible region of the spectrum (data not shown). The chloroform was allowed to evaporate over night at 40°C and the residue was redissolved in pure methanol. For total (both external and internal) phenolics, the leaves were put in boiling methanol/water/HCl (80:19:1 v/v/v) for 5 min according to Day *et al*. (1994). UV-B absorbing capacity was assessed spectrophotometrically after appropriate dilution of the cleared extracts. The relative amounts of anthocyanins were estimated from the absorbance of the same extracts in the visible part of the spectrum (peak absorbance at 531.6 nm) after applying a correction factor for the contribution of chlorophyllous pigments at this wavelength (Lindoo & Caldwell, 1978). In all cases a Shimadzu UV-160A recording spectrophotometer was used.

In vivo chl fluorescence was recorded with a PAM 2000 portable fluorometer (Walz, Effeltrich, Germany) connected to a Poquet PC (Poquet Computer Corp., Santa Clara, CA, USA) equipped with DA-2000 software (Walz). The leaves were predarkened for 25 min after which the ratio of variable (Fv) to maximum (Fm) fluorescence was measured. Fv/Fm is a measure of the potential photochemical efficiency of photosystem II (PSII) (Butler & Kitajima, 1975) and it can be linearly correlated to the photon yield of photosynthetic oxygen evolution (Adams *et al*., 1990).

Photoinhibitory treatment

The leaves were detached from the plant during early morning and Fv/Fm was measured after a dark period of 25 min at room temperatures. They were subsequently put into temperature regulated cuvettes on top of moistened filter paper. The cylindrical cuvettes were made of brass (volume 200 cm^3), their top was covered with bayonet-mount perspex lids fitted with O-rings and all metal surfaces were plated with nickel. Six cuvettes (with six leaves per cuvette) were mounted together on a heat exchanger heated by resistance rods and cooled with Peltier elements. During the experiment, the leaves were rotated between the cuvettes every hour. Outdoor ambient air (350–360 ppm CO_2) was circulated in the cuvettes at a flow rate of 12 l h^{-1} . The air inside the cuvettes was mixed with a fan and the temperature was monitored with thermocouples placed immediately under the leaves. The temperature during the experiment was maintained at 4.8 $\pm$1.0°C. Photosynthetically active radiation (Li Cor, Li-190S quantum sensor) at leaf level was either 1000 (high light) or 4.5 (low light) μmol $m^{-2}s^{-1}$ (Kodak Wratten ND grey filters). Light was provided with an Osram HQI-TS 400W metal halogen lamp. At various time intervals the leaves were removed from the cuvettes, maintained in the dark at room temperature for 25 min and their Fv/Fm was measured. Thus, the photoinhibitory treatment consisted of leaves at high light/low temperature, while the leaves at low light/low temperature were used as controls.

Sampling and statistics

At the specified dates, the indicated number of plants was randomly selected from each plot for analysis. For UV-B absorbing capacity, anthocyanins, nitrogen and phosphorus, the leaves from each individual plant were pooled. Chlorophyll fluorescence was measured on three leaves per plant and the mean value was considered representative for that plant.

Differences between treatments were analysed using nested ANOVA type II (Sokal & Rohlf, 1981). This statistical procedure was chosen to minimize the potential influence of differences between trays within treatments, since trays were not rotated between plots during the study. Normality (Kolmogorov-Smirnov test) and homoscedasticity (Bartlett test) of variables studied were tested before analyses. When required, logarithmic transformations were applied to achieve homoscedasticity. When homoscedasticity could not be achieved by means of logarithmic or square-root transformations, analyses were performed using ranks (Potvin & Roff, 1993). All statistical analyses were performed using SPSS 4.0, using Type III square sums because of unbalanced sample sizes in some of the analyses.

RESULTS

Table 1 shows that supplemental UV-B radiation had no effect on leaf area and on the total above-ground biomass measured during the mid-growing period, nor on the dry mass of the over-wintering buds, measured after the senescence of plants. Flowering frequency was also unaffected. Furthermore, the allocation of the above-ground dry mass to assimilative (leaves) and reproductive functions was similar in the control and UV-B treated plants. However, there was a trend towards increased root dry mass, and for a decrease in the shoot/root ratio under supplemental UV-B radiation.

Table 2 shows a small (16.1%) positive UV-B radiation effect on leaf nitrogen and a corresponding

Table 1. *Mean ± SD (sample size) for several vegetative and reproductive variables of* Pinguicula vulgaris *grown under ambient or ambient + supplemental UV-B radiation*

Variable	Control	UV-B	% change	Trays (within treatment)	Treatments
Leaf area (cm^2)	5.9 ± 1.6 (32)	5.2 ± 1.8 (32)	−11.3	$F_{6.56} = 2.44$ $P = 0.036$	$F_{1.6} = 0.29$ $P = 0.611$
Leaf DM (mg)	15.3 ± 4.5 (16)	14.3 ± 4.8 (16)	−6.5	$F_{6.24} = 1.85$ $P = 0.132$	$F_{1.6} = 0.20$ $P = 0.669$
Root DM (mg)	8.0 ± 4.6 (15)	12.3 ± 5.6 (16)	53.8	$F_{6.23} = 0.94$ $P = 0.486$	$F_{1.6} = 4.93$ $P = 0.068$
Shoot : root ratio	2.4 ± 1.2 (15)	1.3 ± 0.9 (16)	−45.8	$F_{6.23} = 2.15$ $P = 0.086$	$F_{1.6} = 3.64$ $P = 0.105$
Reproductive DM (mg)	9.6 ± 1.3 (13)	8.8 ± 2.0 (10)	−8.3	$F_{6.15} = 1.12$ $P = 0.339$	$F_{1.6} = 0.98$ $P = 0.361$
Total DM (mg)	31.2 ± 7.7 (15)	32.2 ± 8.7 (16)	3.2	$F_{6.23} = 0.57$ $P = 0.747$	$F_{1.6} = 0.12$ $P = 0.742$
Flower frequency	76.7% (90)	80% (90)	4.3	x^2 test: $X_1^2 = 0.131$ $P = 0.718$	
Winter bud DM (mg)	9.7 ± 4.6 (33)	10.8 ± 6.1 (33)	11.3	$F_{6.58} = 0.91$ $P = 0.496$	$F_{1.6} = 0.12$ $P = 0.413$

Results of nested ANOVA for comparison between treatments are given in the last two columns. Flowering frequency refers to the whole growing period. Winter buds were measured after plant senescence (mid-September). All other variables were measured late July/early August, 1998.

Table 2. *Mean ± SD (sample size) for N and P (mg g^{-1}) of various plant parts*

Variable	Control	UV-B	% change	Trays (within treatment)	Treatments
Leaf N	14.3 ± 2.3 (32)	16.6 ± 2.9 (32)	16.1	$F^{6.56} = 1.05$ $P = 0.401$	$F_{1.6} = 4.19$ $P = 0.013$
Reproductive N	16.7 ± 2.2 (25)	18.1 ± 2.2 (21)	8.4	$F_{6.38} = 1.35$ $P = 0.259$	$F_{1.6} = 4.19$ $P = 0.087$
Winter bud N	22.4 ± 3.9 (33)	20.6 ± 5.3 (32)	−8.0	$F_{6.57} = 1.13$ $P = 0.359$	$F_{1.6} = 1.69$ $P = 0.242$
Leaf P	1.2 ± 0.2 (31)	1.2 ± 0.4 (32)	0.0	$F_{6.56} = 1.20$ $P = 0.321$[1]	$F_{1.6} = 0.44$ $P = 0.534$[1]
Reproductive P	1.9 ± 0.4 (25)	1.6 ± 0.3 (21)	−15.8	$F_{6.38} = 1.28$ $P = 0.301$[2]	$F_{1.6} = 4.74$ $P = 0.072$[2]
Winter bud P	1.9 ± 0.6 (33)	2.5 ± 1.2 (32)	31.6	$F_{6.57} = 1.01$ $P = 0.430$[1]	$F_{1.6} = 3.81$ $P = 0.099$[1]

[1]Rank transformed for analysis.
[2]Log transformed for analysis.
Statistics and other details as in Table 1

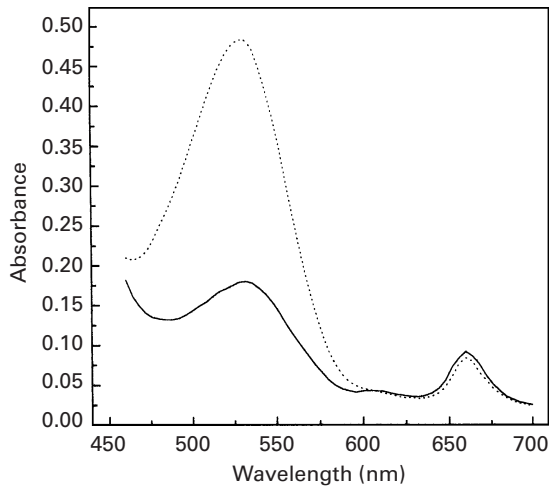

Fig. 1. Absorbtion spectra (450–700 nm) of acidified methanol extracts of *Pinguicula vulgaris* leaves grown under ambient or ambient plus supplemental UV-B radiation. Data from a single, characteristic replication out of 16 (see Table 3). UV-B, dotted line; control, solid line. Absorbances in the 250–450 nm range are omitted, as no UV-B radiation effect was found. The peak at 660 nm apparently corresponds to pheophytins.

trend for reproductive nitrogen. These changes were abolished in the over-wintering bud. Concerning phosphorus, the negative trend observed in the reproductive plants was reversed in the over-wintering structures.

Absorption spectra in the 250–700 nm spectral range of the epicuticular material in pure methanol had a single peak at *c.* 277 nm. This was also the main peak for the acidified methanol extracts of the whole leaf, where two additional maxima at *c.* 332 nm and 532 nm were also observed (data not shown). The latter maximum apparently belongs to anthocyanins. UV-B radiation had no effect on the position of the peaks. However, the absorbance corresponding to anthocyanins was more than doubled in the UV-B treated plants (Fig. 1, Table 3) while the absorbance in the UV spectral band was not changed (Table 3). In fact, plants under UV-B supplementation were more red and microscopic examination of leaf cross sections revealed that the red pigment was confined to the epidermal cells, mainly of the upper leaf surface.

Since anthocyanins absorb in the visible spectrum, we argued that their higher levels in the UV-B treated plants may attenuate photosynthetically active radiation reaching the mesophyll and, in this way, alleviate the risk of photoinhibition. Fig. 2 shows the results of a laboratory experiment designed to test this possibility. Fv/Fm values from leaves detached from the plant during early morning were comparatively low (*c.* 0.62–0.64) with no differences between control and UV-B treated plants. Low temperature at low light had no influence on Fv/Fm in both groups of plants.

Table 3. *Mean ± SD absorbances at the indicated wavelengths of extracts (see the Materials and Methods section) of* Pinguicula vulgaris *leaves grown under ambient or ambient + supplemental UV-B radiation*

Wavelength (nm)	Control	UV-B	% change	Trays (within treatment)		Treatments	
277.4 (external)	3.20 ± 0.54	3.36 ± 0.66	5.0	$F_{6,24} = 2.07$	$P = 0.095$	$F_{1,6} = 0.35$	$P = 0.576$
276.4	26.61 ± 2.04	27.70 ± 3.07	4.1	$F_{6,24} = 1.91$	$P = 0.121$	$F_{1,6} = 0.87$	$P = 0.387$
331.6	17.86 ± 1.52	19.32 ± 2.12	8.2	$F_{6,24} = 1.33$	$P = 0.284$	$F_{1,6} = 3.98$	$P = 0.093$
531.6	0.20 ± 0.05	0.44 ± 0.19	120.0	$F_{6,24} = 1.40$	$P = 0.254$[1]	$F_{1,6} = 18.14$	$P = 0.005$[1]

$n = 16$ in all cases. Measurements were performed at late July 1998. Statistics as in Table 1. 'External' denotes epicuticular extract. All other cases refer to a total leaf extract. Absorbances were normalized at 1 cm light path and 1 cm^3 of extract obtained from 1 cm^2 of leaf surface.
[1]Rank transformed for analysis.

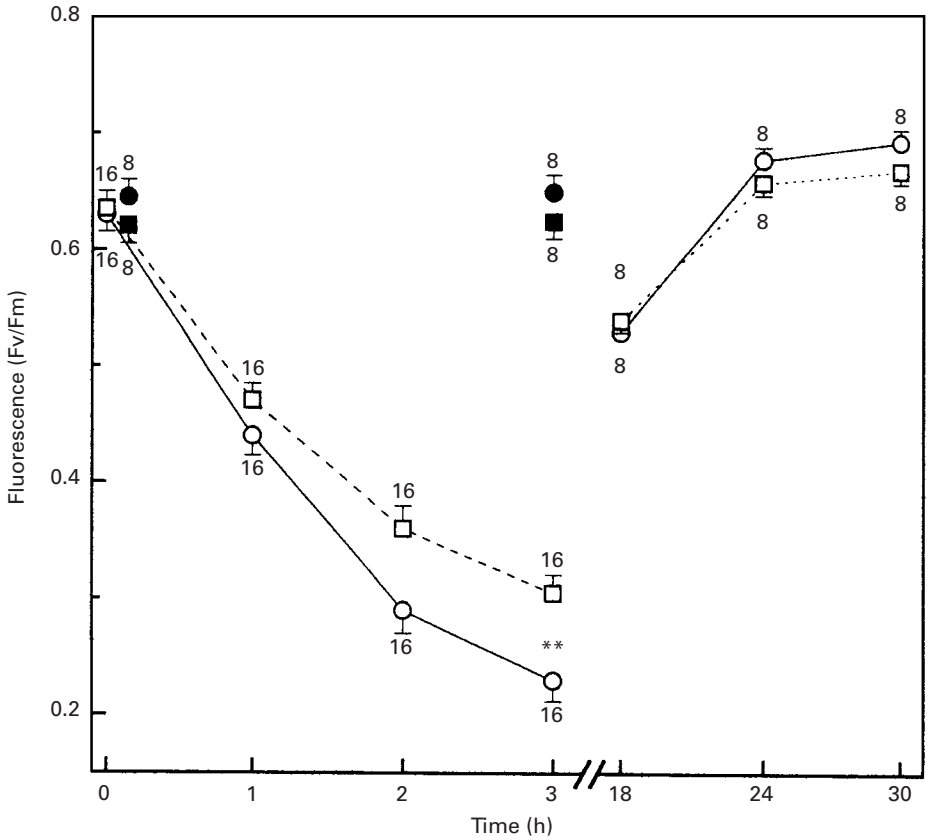

Fig. 2. Fv/Fm of *Pinguicula vulgaris* leaves vs time at low temperature (4.8 ± 1.0 °C) and high (1000 μmol $m^{-2} \cdot s^{-1}$, open symbols) or low (4.5 μmol $m^{-2} \cdot s^{-1}$, closed symbols) light in early August 1998. Squares, UV-B treated plants; circles, control plants. The photoinhibitory treatment lasted for 3h, after which Fv/Fm was allowed to recover in the dark at room temperatures. Values are means ± SD from 16 or 8 plants as shown in the figure and described in the Materials and Methods section. For clarity, symbols at time 0 for low light have been slightly moved to the right. The asterisks (* or **) denote significant differences between control and UV-B treatments at $P < 0.05$ or $P < 0.001$, respectively.

However, at low temperature and high light, there was a progressive reduction in photosystem II photochemical efficiency, which was less pronounced in the anthocyanin-rich UV-B treated plants. In subsequent darkness, recovery of Fv/Fm values was slow but complete within 24 h. The photon fluence rates and temperatures used in this laboratory photoinhibition experiment are realistic for Abisco, especially towards the end of the growing season. Fig. 3 shows that in a clear day at late August, photon fluence rates around noon (10–14 h) exceed 800 μmol m^{-2} s^{-1}, while air temperature varies between 5 and 9°C. Since the differences in the risk of photoinhibition could influence the longevity of the leaves and plants, we followed the senescence rates during the late summer period with decreasing temperatures (from 15 August and up to 19 September, when the first frost killed all leaves). Senescence rates did not differ between treatments either considering plant (G test: G5 = 0.321, P = 0.997) or leaf (G5 = 0.778, P = 0.978) senescence (data not shown).

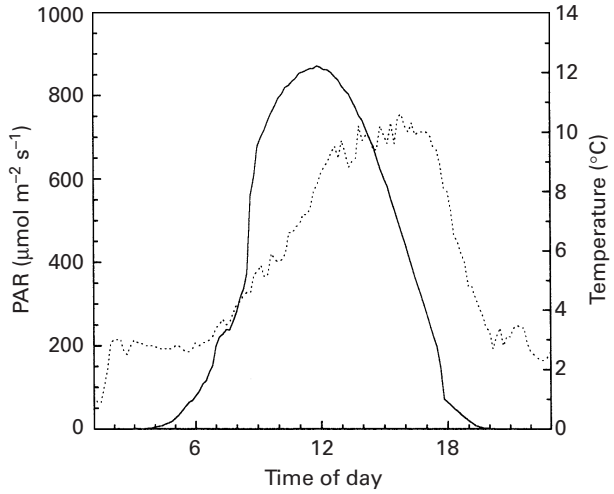

Fig. 3. Photon fluence rate (continuous line) and air temperature (broken line) vs time in the vicinity of the experimental plots on a clear day (August 30, 1998).

DISCUSSION

It is evident that *P. vulgaris* is resistant to UV-B radiation, at least to the mild doses used in this investigation. This may be due to the high UV-B absorbing capacity of its leaves. *Pinguicula vulgaris* is known to contain up to 0.08% (f. wt) benzoic acid (Banquis & Miriamanoff, 1970). This compound has peak absorbance at 225 nm and a secondary peak at 275 nm (Sodium salt, Sigma, in methanol: water : HCl 80 : 19 : 1 v/v/v) which may well account for the 276 nm peak observed in the present study. Supplemental UV-B radiation had no effect on the absorbance at 276 nm (apparently benzoic acid) nor at 332 nm (apparently flavonoids). Yet, a considerable increase was observed at 532 nm (Table 3, Fig. 1), indicating a differential acceleration in some terminal reaction(s) of anthocyanin formation. In laboratory experiments, simple phenolics, flavonoids and anthocyanins could be induced by UV-B radiation, although the corresponding photoreceptors might be different (Lindoo & Caldwell, 1978; Hashimoto *et al.*, 1991; Brandt *et al.*, 1995). Under field conditions however, with balanced visible/UV-A/UV-B radiation and mild UV-B supplementation, the anthocyanin content remained unaffected (Dillenburg *et al.*, 1995). To the best of our knowledge, our investigation is the first to report a considerable UV-B induced increase in anthocyanins under field conditions.

Apart from their absorption in the visible spectrum, anthocyanins absorb also in the UV region of the spectrum (270–290 nm, see Markham, 1982). Therefore, they have been empirically implicated in UV-B protection of young leaves (Lee & Lowry, 1980). More recently Burger & Edwards (1996) provided experimental evidence that the anthocyanin-rich red varieties of *Coleus* were less damaged by UV-B radiation, compared to anthocyanin-less green varieties. In addition, Stapleton & Walbot (1994) showed that the DNA of maize varieties containing anthocyanins was better protected against UV-B radiation damage. However, Woodall & Stewart (1998) questioned the above on the basis that anthocyanins do not absorb appreciably in the UV-B (290–315 nm) spectral band, unless they are acylated with aromatic organic acids (Markham, 1982). In this case, their 270–290 nm UV peak is shifted to the UV-B region. However, this shift does not necessarily result in a considerable increase in their specific absorbance in the UV-B region of the spectrum (Woodall & Stewart, 1998). In anthocyanins, the UV and visible absorption coefficients are almost the same (Woodall & Stewart, 1998). Accordingly, if we assume that anthocyanins in *P. vulgaris* are indeed acylated, their normalized absorbance at 300 nm would be as low as 0.20 and 0.44 for the control and UV-B treated plants respectively (see Table 3). Since the corresponding total normalized absorbances at this wavelength are 13.83 and 14.67 (not shown but see Table 3 for values at adjacent wavelengths), the relative contribution of anthocyanins to UV-B attenuation would be 1.4% for the controls and 3% for the UV-B treated plants. We may therefore assume that the UV-B induced increase in anthocyanins of *P. vulgaris* can not afford significant protection against UV-B radiation damage since the absorbances of other co-occurring phenolics are much higher.

Absorption of visible light by epidermal anthocyanins could reduce the photosynthetically active radiation reaching the mesophyll and, accordingly, suppress the already low (Mendez & Karlsson,1999) photosynthetic rates of this plant. However, corresponding reductions in growth or reproduction were not observed. On the other hand, anthocyanins may protect against photoinhibition by visible radiation, as suggested by Gould *et al.* (1995) and Ntefidou & Manetas (1996). Although previous attempts to verify this hypothesis were negative (Burger & Edwards, 1996; Dodd *et al.*, 1998), the results of the present investigation clearly showed that the anthocyanin-rich, UV-B treated leaves were less prone to photoinhibition imposed by high light and low temperature (Fig. 2). However, it is possible that the apparent correlation between high anthocyanin and lower photoinhibitory risk found in the present study could be coincidental, and that other processes induced by UV-B could be responsible for the increase in resistance to photoinhibitory stress. Regardless of this, the differences in the extent of photoinhibition observed in the laboratory did not result in corresponding changes in the above-ground biomass accumulation in the field, nor on dry mass of overwintering buds. In addition, the leaf and plant senescence rates measured during late season, where the slightly above zero temperatures could have enhanced the photoinhibitory risk, were the same in control and UV-B treated plants. Therefore, we

have to accept either that the increase in anthocyanins was of no adaptive significance or that the lower photoinhibitory risk counterbalanced the possible negative effects of UV-B radiation. *In situ* fluorescence measurements and photosynthetic rates of control and UV-B treated plants could help to express an opinion on the above alternatives.

Anthocyanins could also be induced by nutrient (P and N) limitation (Gershenzon, 1983). If, for some reason, the insects avoid the UV-B frames (Stephanou *et al*., 1999), this could result in less prey capture and, accordingly, lower tissue nitrogen (Aldenius *et al*., 1983) and decreased reproductive effort (Thoren & Karlsson, 1998). However, these usual symptoms of prey absence were not observed in this investigation. Thus, flowering frequency and allocation of biomass to reproductive structures was not affected by UV-B radiation, at least at the short term scale investigated here. Furthermore, the nitrogen content of the leaves was improved under UV-B supplementation. Since prey capture was not measured in this study, we can not explain the increase in nitrogen. However, this can be correlated with the increased root mass under UV-B supplementation (Table 1).

In conclusion, this first study of the UV-B radiation effects on a carnivorous plant showed that *P. vulgaris* is very well equipped to cope with the ongoing increase of UV-B radiation reaching the surface of the earth. In addition, the preferential increase in leaf anthocyanins may be beneficial to this plant under certain environmental conditions.

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