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Site-dependent differences in transmittance and UV-B-absorbing capacity of isolated leaf epidermes and mesophyll in *Urginea maritima* (L.) Baker[1]

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Abstract

The spectral transmittance of isolated 'intact' upper and lower epidermes as well as the extractable UV-B-absorbing capacity of epidermes and mesophyll were studied in the leaves of exposed and deeply shaded, field-grown plants of *Urginea maritima* (L.) Baker. Epidermal transmittance in the visible part of the spectrum was high (>80%) in all cases. Transmittance in the UV-B (280–320 nm) was comparatively high (*c.* 14%) in both the upper and lower epidermes of shaded plants, but more than an order of magnitude lower in exposed plants, with the lowest values observed on the upper leaf epidermis. UV-B transmittance was negatively correlated with the methanol extractable UV-B-absorbing capacity of the epidermes, but was independent of epidermal thickness. The UV-B-absorbing capacity of the mesophyll, when expressed on an area basis, was not affected by exposure. However, it was significantly higher in shaded plants, when expressed on a dry mass basis. The results indicate that although the concentrations of the UV-B-absorbing components of the whole leaf or its epidermis fluctuate according to the site-dependent radiation stress, the opposite is evident for the mesophyll. Therefore, high irradiance in *U. maritima*, apart from inducing an increase in UV-B-absorbing compounds on a whole leaf basis, also caused a change in the distribution of these compounds between epidermis and mesophyll.

Key words: *Urginea maritima*, exposure, UV-B-absorbing compounds, epidermal transmittance.

Introduction

A variety of roles have been ascribed to phenolics which abound in terrestrial plant tissues (Rice, 1979; Larson, 1988; Matern and Kneusel, 1988; Bernays *et al.*, 1989; Dakora, 1995). Among them particular attention has been given to their possible function as selective filters against ultraviolet-B (UV-B) radiation damage (Caldwell *et al.*, 1983). Phenolics such as flavonoids absorb strongly in the UV, but not in the visible region of the spectrum, they have a mainly superficial location on the cuticle (Wollenweber and Dietz, 1981), trichomes (Karabourniotis *et al.*, 1992) or epidermis (Robberecht and Caldwell, 1978) and their biosynthesis is accelerated by UV-B radiation (Beggs and Wellman, 1994). Accordingly, the flavonoid content of a leaf may be critical for its UV-B radiation resistance and this is becoming significantly important in view of the already observed increase of UV-B radiation due to the anthropogenic stratospheric ozone depletion (Zerefos *et al.*, 1995). Therefore, the measurement of UV-B-absorbing capacity of leaves has become a usual routine in almost all UV-B radiation studies.

If the UV-B protective hypothesis for phenolic compounds is valid, one may predict that their levels in a plant organ should fluctuate according to the naturally imposed UV-B radiation stress. Indeed, it has been repeatedly shown that light availability in the field and the whole leaf phenolic content are positively correlated (Mole *et al.*, 1988; Les and Sheridan, 1990; Lovelock *et al.*, 1992; Shure and Wilson, 1993; Stephanou and Manetas, 1997*a*). However, whole leaf estimations of phenolics may be misleading. For example, a phenolic which functions as a feeding deterrant or antifungal

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(Bernays *et al.*, 1989) should be present in all leaves and in all parts of a leaf. A phenolic UV-B radiation filter, however, would be most effective and less costly if its location was on those surfaces and leaves that are exposed to solar radiation. Therefore, the light-induced differences in UV-B-absorbing capacity and phenolics based on a whole leaf basis may underestimate the differences located on the most superficial leaf positions. Ideally, one should be able to distinguish between the UV-B-absorbing capacity of the upper (usually exposed) epidermis versus that of mesophyll and the lower (usually shaded) epidermis.

However, the removal of intact epidermis is not an easy task. Stomatal physiologists were very active in locating plants from which the epidermis could be peeled off in sufficient quantities, with a minimum of mesophyll contamination and with enough stiffness for easy subsequent handling. As pointed out by Weyers and Meidner (1990), this has been accomplished in very few species, while the cases where both the upper and the lower epidermes can be removed from the same plant are scarce. Therefore, the epidermal optical properties have been studied in a limited number of species and only from the surface that could be peeled off easily (Gausman *et al.*, 1975; Robberecht and Caldwell, 1980; Tevini *et al.*, 1991). To the best of our knowledge, only Day *et al.* (1996) were able to distinguish between the UV-B-absorbing capacity of upper and lower epidermes of fully exposed leaves of garden pea (*Pisum sativum*, mutant Argenteum).

In a preliminary survey with Mediterranean native plants it was found that the rosette plant *Urginea maritima* (Liliaceae) easily yields both upper and lower leaf epidermal strips of several cm². The plant occupies both exposed and deeply shaded habitats and its large petiole-less, unbendable leaves have a permanent position in respect to solar radiation. Therefore, one may distinguish leaf surfaces with more or less defined irradiation history and measure their UV-B-absorbing capacity and optical properties.

Materials and methods

Plant material, sampling and statistics

Fully exposed to solar radiation and deeply shaded (under a canopy of evergreen sclerophylls) individuals of *Urginea maritima* L. (Liliaceae), growing wild in the vicinity of the Patras University campus, were used. In all cases, plants were chosen that had similar diameters (rosette plants). Leaves of the same age (5th leaf from the rosette basis) and of similar size, with a 40–45° inclination from the horizontal were used. The leaves were cut, put into air-tight plastic bags and immediately taken to the laboratory for experimentation. Experiments were performed during the spring of 1997 and 1998 and leaves were sampled at *c.* 10.00 h during clear days. On each sampling occasion, PAR received by the leaf surfaces were measured with a SF-80 Decagon Sunfleck Ceptometer. Corresponding values for PAR were 1800 ± 150, 150 ± 50, 35 ± 10, and 10 ± 5 µmol m^{-2} s^{-1} for exposed (upper and lower

surfaces) and shaded (upper and lower surfaces), respectively. *In situ* observations revealed that the distal half of the *c.* 50 cm long leaves in the exposed plants was not normally shaded by the younger, superimposed leaves. Accordingly, epidermal and mesophyll samples were taken at a distance of 10–20 cm from the tip. Preliminary trials showed that UV-B transmittance did not vary within these limits. A total of 32 leaves was used (16 exposed, 16 shaded) taken from eight individual plants. Site-dependent differences in the various measured parameters were subjected to one-way ANOVA by using the Origin 3.5 'Microcal' statistical package. Correlation analysis (Pearson's correlation test) and regression analysis were performed by SPSS statistical package.

Removal of epidermes

The method of Weyers and Travis (1981) was used with slight modification. The leaf was cut along the mid-vein, one half was used for transmittance measurements and the other half for estimation of UV-B-absorbing compounds. For removal of upper epidermis, the leaf was gently incised with a razor from the lower side, taking care not to cut the upper epidermis. The cut was parallel to the mid-vein. The leaf was subsequently inverted and peeling off the upper epidermis was achieved by lifting with forceps the cut lamina in the direction of the mid-vein. The procedure for isolating the lower epidermis was just the opposite. Epidermal pieces of several cm² were thus obtained. Examination under the microscope revealed that the epidermes were intact and completely devoid of mesophyll cells. Isolated epidermes were used immediately.

Measurements

The spectral transmittance of epidermes was measured with an Optronic (Orlando, FL) system, composed of an OL 752–10U (200 W tungsten coiled-coil filament) light source stabilized through an OL 65 precision current source, a Taylor type integrating sphere (1S 1000) and an OL 752 spectroradiometer. The diameter of the entrance port of the integrating sphere was adjusted in order to accommodate an epidermal strip of 2.3 cm² and with the minimum spatial distortion. The configuration of the system was such that the light beam was perpendicular to the epidermal strips. Spectral transmittance was measured every 2 nm and the mean across each waveband is given. The values were corrected with reference to the spectral characteristics of the lamp.

Epidermal strips similar to those used for transmittance measurements (same area and position on the leaf) were immersed in a mixture of methanol:H$_2$O:HCl (90:1:1, by vol.) and boiled for 10 min (Day *et al.*, 1994). UV-B-absorbing compounds were assessed from UV spectra of methanolic extracts. The same procedure was used with leaf discs without epidermes for the estimation of UV-B-absorbing compounds of the mesophyll. In all cases a Shimadzu UV-160 A recording spectrophotometer was used.

Epidermal thickness was assessed microscopically from hand-cut transverse sections of fresh leaves viewed immediately under a light microscope. In order to avoid variability due to possible differential epidermal thickness along the leaf, the sections were taken from leaf regions similar to those used for spectral transmittance. On each section of either exposed or shaded leaves (16 independent measurements for each case), upper and lower epidermal thickness was measured at seven different points.

Leaf thickness was measured with a friction-stop caliper (Mitutoyo, Japan). Values obtained with the caliper were almost identical with some randomly performed measurements

Table 1. *UV-B-absorbing compounds (A_{300} cm^{-2}) and transmittance (T%) of upper and lower epidermes as well as of mesophyll tissue of* U. maritima, *taken from fully exposed and deeply shaded plants*

Values are means ± SD from 16 independent measurements. Different letters in each row indicate statistically significant differences at $P < 0.05$. A_{300} cm^{-2} denotes the 1 cm light path absorbance at 300 nm of a 1 cm^3 methanolic extract taken quantitatively from 1 cm^2 leaf area.

	Exposed		Shaded	
	Upper	Lower	Upper	Lower
A_{300} cm^{-2}, epidermal	3.93 ± 1.54 a	3.02 ± 1.09 b	0.93 ± 0.41 c	0.70 ± 0.32 c
A_{300} cm^{-2}, mesophyll	3.13 ± 1.13 a		3.33 ± 0.69 a	
T, 280–320 nm	1.2 ± 0.7 a	2.5 ± 1.4 b	13.8 ± 7.1 c	14.3 ± 3.0 c
T, 320–400 nm	6.4 ± 2.7 a	11.4 ± 4.3 b	33.0 ± 9.9 c	40.0 ± 3.3 c
T, 400–700 nm	81.3 ± 3.3 a	82.5 ± 3.2 a	84.0 ± 2.5 a	85.4 ± 2.9 a

of leaf thickness in transverse sections with the microscope. Mesophyll thickness was calculated as the difference between leaf thickness and the sum of upper and lower epidermal thicknesses. For specific mass (mg DW cm^{-2}) determination, discs of known surface area were dried to constant mass at 80 °C.

Results

Table 1 shows that the methanol extractable UV-B-absorbing capacity of isolated epidermes is considerably higher in exposed compared to shaded plants. In addition, in an exposed leaf, the higher values are obtained from the fully exposed, upper epidermis, while in a shaded leaf both epidermes show the same A_{300} cm^{-2}. The *in vivo* spectral transmittance of intact epidermes in the UV-B region of the spectrum (Table 1) are compatible with the above results, showing that the more exposed the leaf surface, the less UV-radiation is transmitted to the mesophyll. On the contrary, transmittance in the photosynthetically active region (400–700 nm) is always high and independent of radiation conditions. Corresponding values of A_{300} cm^{-2} of the mesophyll were also independent of light or shade. The results also show that on an area basis, epidermes of exposed leaves contain 69% of the total UV-B-absorbing capacity, while the corresponding value in deep shade is only 33%.

Although the observed differences in epidermal transmittance can be attributed to their UV-B-absorbing compounds, a contribution from epidermal thickness cannot be excluded. However, the thickness of both upper and lower epidermis in shaded plants as well as the lower epidermis of exposed plants were the same (Table 2). Only the upper epidermis of exposed plants was slightly (c. 16%) thicker. In addition, statistical analysis (Pearson's correlation test) between epidermal thickness and UV-B transmittance provided an insignificant correlation ($P = 0.244$). However, epidermal transmittance in the 280–320 nm region and A_{300} cm^{-2} were negatively correlated ($P < 0.001$) as shown in Fig. 1, indicating that the UV-B-absorbing compounds may be the main determinants of UV-B radiation attenuation by the epidermis.

Table 2. *Thickness (μm) and specific mass (mg cm^{-2}) of upper and lower epidermes as well as of mesophyll tissue of* U. maritima *grown in exposed or shaded sites*

Values are means ± SD from 16 independent measurements. Different letters in each row indicate statistically significant differences at $P < 0.05$.

	Exposed		Shaded	
	Upper	Lower	Upper	Lower
Epidermal thickness	56 ± 3 a	48 ± 5 b	49 ± 4 b	47 ± 3 b
Mesophyll thickness	819 ± 72 a		665 ± 69 b	
Specific mass,				
Epidermal	1.17 ± 0.07 a	1.14 ± 0.11 a	0.82 ± 0.16 b	0.72 ± 0.09 b
Mesophyll	8.09 ± 1.30 a		5.50 ± 0.75 b	

Table 3. *UV-B-absorbing compounds (A_{300} mg^{-1}) expressed on a dry mass basis*

Values are means ± SD from 16 independent measurements. Different letters in each row indicate statistically significant differences at $P < 0.05$. A_{300} mg^{-1} denotes the 1 cm light path absorbance at 300 nm of a 1 cm^3 methanolic extract of 1 mg leaf dry weight.

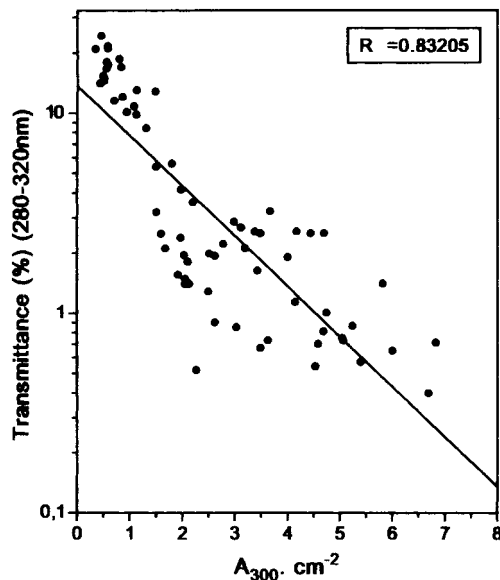|	Exposed		Shaded	
	Upper	Lower	Upper	Lower
Epidermis	3.33 ± 0.35 a	2.63 ± 0.25 b	1.09 ± 0.56 c	0.97 ± 0.45 c
Mesophyll	0.38 ± 0.14 a		0.60 ± 0.12 b	

Fig. 1. Correlation of the % epidermal transmittance (logscale) in the UV-B region (280–320 nm) and the epidermal UV-B-absorbing capacity (A_{300} cm^{-2}). Data include all measurements (upper and lower surface from both exposed and shaded leaves). A_{300} cm^{-2} denotes the 1 cm light path absorbance at 300 nm of a 1 cm^3 methanolic extract taken quantitavely from 1 cm^2 leaf area. Correlation was significant at $P < 0.001$. (Pearson's correlation test).

The expression of A_{300} on an area basis is useful in order to assess the UV-B-absorbing capacity of the tissues of a leaf. However, information on the differential allocation of biomass to UV-B-absorbing compounds in the various tissues (epidermis, mesophyll) can be given by expressing the A_{300} on a dry mass basis (Table 3). In this case, the changes in A_{300} mg^{-1} DW in the epidermes follow the same pattern as the A_{300} cm^{-2} given in Table 1. The picture, however, is different for the mesophyll. Since the investment of mesophyll biomass cm^{-2} of leaf surface is considerably higher in exposed leaves, (see Table 2), the A_{300} mg^{-1} DW is much lower (Table 3). The same table also shows that on a dry mass basis, epidermes contain 94% and 77% of the total UV-B-absorbing capacity of exposed and deeply shaded leaves, respectively.

Discussion

It is evident from the results of this investigation that epidermes taken from leaves of the same plant but with different irradiance histories, show considerable differences in UV-B transmittance and the corresponding UV-B-absorbing compounds. These results are in accordance to those of Robberecht and Caldwell (1980) who found the epidermal transmittance in a variety of plant species to be generally lower at sites of high UV-B radiation doses. However, these investigations examined only the upper epidermes of fully exposed plants.

These results do not permit an assessment of the relative

influence of the various spectral regions of solar radiation, as both UV and visible irradiation varied. Day *et al.* (1996), working with garden pea at ambient visible and ambient or ambient plus supplemental UV-B radiation, found no effect on UV-B-absorbing capacity of both upper and lower epidermes as well as of mesophyll. Similarly, Stephanou and Manetas (1997*a*) found no effect of supplemental UV-B radiation on both epicuticular (external) and cellular (internal) UV-B-absorbing compounds in *Cistus creticus*, although these compounds varied considerably between sunny and shadow sites. Yet, it seems that the response of superficial (epicuticular or epidermal) UV-B-absorbing compounds to supplemental UV-B radiation is species-specific, since epicuticular (but not cellular) compounds did increase by supplemental UV-B radiation in *Dittrichia viscosa* (Stephanou and Manetas, 1997*b*). Therefore, it is possible to predict that in some cases the effects of the expected increase in solar UV-B radiation on these compounds may be masked by the much larger site-dependent changes.

Differential responses of UV-B-absorbing compounds in various leaf tissues in respect to irradiance levels have also been observed by Liakoura *et al.* (1997). Thus, trichome (but not mesophyll) compounds were considerably higher in exposed compared to shaded leaves in *Verbascum speciosum* and *Quercus ilex*. These authors expressed their results on a leaf area basis. If it is taken into account that exposed leaves usually have a higher leaf specific mass, it may be concluded that in *V. speciosum* and *Q. ilex* (Liakoura *et al.*, 1997) as well as in *U. maritima* (see results of this investigation), solar radiation causes antiparallel changes in leaf UV-B-absorbing compounds, i.e. an increase in their concentration at the surface, but a corresponding decrease in the interior of the leaf. Further work is needed in order to confirm the above and elucidate the reasons for this differential response. It may also be noted that in the present investigation, insoluble or wall-bound UV-B-absorbing compounds were not measured and this may have introduced a source of error in these results.

It is also suggested that the quick, easy and quantitative isolation of intact, mesophyll free epidermes, combined with the high responsiveness of the UV-B-absorbing compounds to the radiation environment, make *U. maritima* a very useful plant for a detailed study of the effects of spectral irradiance on the allocation of phenolics to various leaf tissues.

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