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Contrasting strategies for UV-B screening in sub-Arctic dwarf shrubs

S. I. SEMERDJIEVA[1], E. SHEFFIELD[1], G. K. PHOENIX[2], D. GWYNN-JONES[3], T. V. CALLAGHAN[2,4] & G. N. JOHNSON[1]

[1]*School of Biological Sciences, University of Manchester, 3.614 Stopford Building, Oxford Road, Manchester, M13 9PT, UK,* [2]*Department of Animal and Plant Sciences, University of Sheffield, Western Bank, Sheffield, S10 2TN, UK,* [3]*Institute of Biological Sciences, Edward Llwyd Building, University of Wales, Aberystwyth, Ceredigion, SY23 3DA, UK and* [4]*Abisko Scientific Research Station, S 981 07 Abisko, Sweden*

ABSTRACT

The content and distribution of UV-absorbing phenolic compounds was investigated in leaves of three species of *Vaccinium* co-existing at a site in north Sweden. *Vaccinium myrtillus* L., *Vaccinium vitis-idaea* L., and *Vaccinium uliginosum* L. exhibit markedly different strategies, in terms of localization and content of leaf phenolics and in their responses to UV-B enhancement. Plants were exposed to either ambient radiation or to enhancement of UV-B corresponding to 15% (clear sky) depletion of stratospheric ozone for approximately 10 years prior to commencement of this study. *Vaccinium myrtillus* contained the highest concentration of methanol-extractable UV-B-absorbing compounds, which was elevated in plants exposed to enhanced UV-B. Fluorescence and confocal laser scanning microscopy showed that these compounds were distributed throughout the leaf, and were particularly concentrated in chlorophyll-containing cells. In *V. vitis-idaea*, most phenolic compounds were cell wall-bound and concentrated in the walls of the epidermis; this pool increased in response to UV-B enhancement. It is suggested that these two plants represent extreme forms of two divergent strategies for UV-B screening, the different responses possibly being related to leaf longevity in the two species. The response of *V. uliginosum* was intermediate between the other two, with high concentrations of cell wall-bound phenolics in the epidermis but with this pool decreasing, and the methanol-soluble pool tending to increase, after exposure to enhanced UV-B. One explanation for this response is that this plant is deciduous, like *V. myrtillus*, but has leaves that are structurally similar to those of *V. vitis-idaea*.

Key-words: *Vaccinium myrtillus*; *Vaccinium uliginosum*; *Vaccinium vitis-idaea*; enhanced UV-B; fluorescence microscopy; phenolics.

Correspondence: Giles N. Johnson. Fax: +44 161275 3938; e-mail: giles.johnson@man.ac.uk

INTRODUCTION

Over the last two decades, the effects of increased UV-B on vegetation have been the subject of intense research and the molecular mechanisms involved are now well characterized (Stapelton 1992). However, the mechanisms protecting against such damage are less clear. These include reflective leaf surface structures (e.g. Semerdjieva *et al*. 2003) and selectively absorbing pigments such as phenolic compounds including flavonoids and hydroxycinnamic acid esters, mechanisms to counteract DNA damage and scavengers of free radicals formed under enhanced UV-B (Rozema *et al*. 1997). The ability to screen the leaf from UV-B is regarded as being of particular importance. UV-absorbing compounds and specific leaf anatomical features are considered to be key determinants of leaf screening efficiency (Bornman 1999).

It is generally accepted that a gradient exists in UV screening ability, from herbaceous plants (being least efficient) towards woody and perennial plants, with the conifers being most efficient (Meijkamp *et al*. 1999). Day, Vogelmann & De Lucia (1992) and Day (1993) studied a diverse group of plants and observed that UV-B radiation hardly penetrated conifer needles, whereas it reached the spongy mesophyll in some herbaceous deciduous plants. Penetration of UV-B into the leaves of woody dicotyledonous plants was found to be intermediate between that of coniferous and herbaceous leaves. Epidermal thickness and the concentration of UV-absorbing compounds were the strongest predictors of epidermal transmittance and depth of penetration of UV-B radiation. The interspecific variations found, however, suggest that other factors are also important in determining UV-B screening efficiency. One such factor likely to play an important role in determining UV-B screening ability is the location of UV-absorbing compounds in the leaf (Day, Martic & Vogelmann 1993). The high screening efficiency of the conifers, for example, has been attributed to the large amounts of cell wall-bound phenolic compounds in their leaves (Strack *et al*. 1988). Little is known, however, about the distribution of cell wall-bound phenolics in deciduous plants, although the predominant location of phenolics in such plants is thought to be

inside the cells of the epidermis and in the photosynthetic tissues (see Meijkamp *et al*. 1999 and references therein). Day *et al*. (1993) observed that a large proportion of UV-B radiation penetrated the anticlinal cell walls of the epidermis in herbaceous plants. Hence, the UV-B absorption by cell wall-bound phenolics in these might be less important than in the evergreens. Thus, the differences in the screening efficiency between herbaceous and evergreen plants could be due to a combination of factors involving quantity/quality and localization of UV-absorbing compounds in their leaves, as well as leaf longevity.

Although differences have been observed in UV-B screening efficiency in closely related species, no studies have adequately addressed the underlying morphological and biochemical basis for such variation (Gwynn-Jones *et al*. 1999; Semerdjieva *et al*. 2003). In 1991, a field experiment was established at Abisko, north Sweden to investigate the effects of enhanced UV-B radiation on a sub-Arctic heath ecosystem. The site is an open mountain birch forest with an under-storey dominated by four plant species, including three members of the genus *Vaccinium*: *V. myrtillus* L., *V. uliginosum* L. and *V. vitis-idaea* L. This presented an excellent opportunity to study the components of the UV-B screen of three closely related species which, although adapted to similar environments and co-existing in a single ecosystem, differ both in functional type, namely deciduous/evergreen and in morphological characteristics.

Since UV-B exposure is affected by the position of plants in natural canopies (Barnes, Flint & Caldwell 1990), and phenolic biosynthesis can be affected by herbivory (Bryant, Chapin & Klein 1983) and nutrient availability (Fajer, Bowers & Bazzaz 1992), studies of UV-B screening potential of plants taken from natural ecosystems are far more meaningful than investigations of species grown out of context. Early results from the Abisko experiments showed differences in the intrinsic amounts of UV-absorbing compounds in the dwarf shrubs and in their responses to UV-B enhancement (Johanson 1997; Phoenix *et al*. 2003). We therefore investigated the distribution of UV-absorbing compounds in the three *Vaccinium* species growing on the same experimental site. Our aim was to determine whether the UV-B screening strategy adopted was the same in all three species. We show that closely related species, occupying similar niches, vary markedly in the strategies they use to screen UV-B and in their responses to UV-B enhancement when growing together under natural conditions. These differences are interpreted in terms of the different characteristics of their leaf morphology.

MATERIALS AND METHODS

Experimental design

The UV-B enhancement experiment used in this study was established at Abisko, Swedish Lapland (68°35′ N, 18°82′ W, 360 m a.s.l) in 1991, with the treatment being applied each year since then. It consists of eight plots (1.8 m × 0.6 m each) built over the heath. In four treatment plots, enhanced UV-B simulating 15% ozone depletion (under clear skies) is provided by six fluorescent lamps; in the other four control plots, lamp irradiation is filtered through window glass. The UV-B treatment lamps have UV-transmitting Plexiglass (Rohm GmbH, Darmstadt, Germany) holding a cellulose diacetate filter (0.13 mm, Courtaulds, Derby, UK) to exclude UV-C radiation (< 280 nm). Enhanced UV-B radiation is supplied daily, beginning in early May, and is centred around noon. The lamps are controlled by timers that switch on three lamps at a time to give square wave UV-B enhancement. The daily exposure time is changed every second week to adjust for the seasonal variation in natural UV-B radiation, which is continuously monitored on site. Maximum biologically effective (BE) doses were calculated to be 5.8 kJ m^{-2} d^{-1} for the UV-B enhanced plots and 4.6 kJ m^{-2} d^{-1} for the control plots, according to Caldwell's generalized plant action spectrum. For a detailed description of the experimental design see Johanson *et al*. (1995a).

The vegetation in the plots corresponds to the *Empetrum-Vaccinium myrtillus* type (Sonesson & Lundberg 1974). The major dwarf shrubs are *Empetrum hermaphroditum* Hagerup., *Vaccinium myrtillus* L., *Vaccinium uliginosum* L. and *Vaccinium vitis-idaea* L. (see Johanson *et al*. 1995b for a description of the site vegetation, soil and climate.) Of the three *Vaccinium* species, deciduous *V. uliginosum* has the narrowest niche (Karlsson 1985a). It is restricted in its geographical distribution to high altitudes and latitudes, due to sensitivity to high summer temperatures (> 21 °C) (Jacquemart 1996). It is more heliphylous than the other species (Karlsson 1985a), dominates in nutrient rich areas (Karlsson 1985b) and is resistant to wind and exposure (Jacquemart 1996). The distribution of *V. myrtillus* is determined by winter snow cover (Welch *et al*. 1994) as it is sensitive to frost (Ögren 1996). It occurs in moist and humus-rich soils and, although more successful in open woodlands, is a facultative shade species (Ritchie 1956). The evergreen *V. vitis-idaea* is another boreal species but its distribution is more Arctic–Alpine than that of *V. myrtillus* (Grime, Hodgson & Hunt 1990). *Vaccinium vitis-idaea* fixes a significant proportion of carbon during snowmelt and in autumn when deciduous species are leafless (Karlsson 1989) and is more drought-tolerant than *V. myrtillus* (Grime *et al*. (1990).

Total methanol extracts

Leaves from the three *Vaccinium* dwarf shrubs from control and UV-B treatment plots were collected in the second half of July 2000 for assessment of their UV-B-absorbing capacity. Only the third youngest fully expanded leaf was collected, to minimize developmental effects. One hundred milligrams of leaf material from each species were frozen in liquid nitrogen and ground into a fine powder prior to methanol extraction, as described by Schnitzler *et al*. (1996). Two millilitres methanol was added to the powder and extraction was carried on for 1 h at room temperature

in the dark. The solids were removed by centrifugation at 16 000 *g* for 15 min and the clear supernatant removed. The remaining cell debris was re-extracted twice with 1 mL methanol. The supernatants from the three extractions were pooled and left to dry at room temperature. The residues were re-suspended in 1 mL methanol. These extracts were stored at 4 °C prior to subsequent spectrophotometric measurements.

Cell wall preparations for analysis of cell wall-bound alkali-extractable phenolic compounds

Cell wall debris remaining from the procedure described above was incubated for 20 min in 1 M NaCl, then in 0.5% sodium dodecyl sulphate (w/v) (20 min; twice) and then in chloroform/methanol (1 : 1, v/v) (20 min; twice). After each incubation, extracts were centrifuged at 16 000 *g* for 5 min. The pellet was washed in acetone, dried in a vacuum drier (DNA Speed Vac 120; Savant, New York, USA) and stored at −70 °C. Before each measurement, 1 mg of crude cell wall material was incubated in 0.5 mL 1 M NaOH for 16 h at room temperature in the dark, following the method of Strack *et al*. (1988). After centrifugation at 16 000 *g* for 15 min, aliquots of 200 μL were mixed with an equal volume of 1.5 M formic acid and centrifuged for 5 min at 16 000 *g*. The supernatant was used for spectrophotometric measurements.

Spectrophotometric measurements

Total methanol extracts were diluted 200 fold and their absorbance measured in the range 250–400 nm using a UV-VIS dual beam spectrophotometer (UV-2401 PC; Shimadzu, Kanagawa, Japan), using pure methanol as a blank. UV-B-absorbing capacities were quantified as the integrated absorbance in the range 280–320 nm.

The supernatant resulting from the NaOH extraction of the cell wall-bound phenolic compounds was diluted 10 fold with distilled water and its absorbance measured in the range 250–400 nm using distilled water as a blank. UV-B-absorbing capacities were compared in the same way as for the total methanol extracts. Spectra were normalized to the absorbance at 283 nm. Difference spectra were calculated by subtracting the normalized absorbance of the extracts from control plots from those from the UV-B-treated plots for each wavelength.

Localization of UV-absorbing compounds in leaf tissues

Small pieces of fresh leaves from all the species from both control and treatment plots were placed in aluminium foil wells and embedded in Tissue-Tek O.C.T. compound (Raymond A Lamb Sakura, Torrance, CA, USA). They were rapidly frozen in liquid nitrogen and stored at −70 °C. Cross sections (30–50 μm) were cut with a cryotome (Leica CM 3000; Leica, München, Germany) and placed on slides, then stored at −70 °C.

Transverse sections of frozen leaves were cut using a razor blade and placed in 10 mM phosphate buffer (pH 6) then stained with 0.5% Naturstoffreagenz A (diphenylboric acid 2-aminoethylester; Sigma, Poole, Dorset, UK) in 10 mM phosphate buffer (pH 6) with 10% sucrose (Sigma) and 2% dimethyl sulfoxide (Sigma) (Vogt *et al*. 1994). Epidermal peels were separated from small pieces of leaves and treated in the same way as the cross sections.

Epi-fluorescence microscopy was performed (Leitz Dialux 200; Leitz, München, Germany) with excitation: 450–490 nm; reflection short-pass filter: 510 nm; long-pass filter: 515 nm and recorded on Kodak Elite Chrome 400 film (Kodak, Hemel Hempstead, Herts, UK).

For confocal laser scanning microscopy (BioRad MRC 1024 MP; BioRad, Hercules, CA, USA on a Nikon TE 300 fluorescence microscope; Nikon, Tokyo, Japan) specimens were imaged first in transparent mode for reference. Subsequently, excitation at 488 nm was applied and the fluorescence of the products of the UV-absorbing compounds with Naturstoffreagenz A detected at 515 nm. Each section was imaged before and after staining. Red–green–blue colour mode was used for reproducing the fluorescence. Single images and sequences of equidistant images with 7 μm axial distance were taken from unstained and stained sections.

Statistical analysis

The mean amount of UV-absorbing compounds of three extracts per plot was calculated. This mean was then taken as the datum for that plot, giving four replicates for each treatment. These values were then used in a two-way analysis of variance. A *post-hoc* analysis applying the Least Significant Difference test was performed on the means for each species in order to identify significant differences. In order to assess the significance of bands in the difference spectra (Figs 1 & 2d–f), mean spectra from individual plots were normalized to 283 nm and the absorbance at peaks and troughs in the difference spectra analysed using a one-way analysis of variance.

RESULTS

Methanol-soluble, UV-absorbing compounds

The integrated absorbance of total methanol extracts in the spectral range 280–320 nm differed significantly between species (Table 1; $P < 0.01$). *Vaccinium myrtillus* extracts showed the highest absorbance, with that of *V. uliginosum* and *V. vitis-idaea* being approximately half that of *V. myrtillus* and not differing significantly from one another (Table 1).

The three species varied in the extent to which their methanol-extractable UV-absorbing compounds altered in response to enhanced UV-B. In *V. myrtillus,* UV-B absorbance was 21% greater ($P < 0.05$) under enhanced UV-B relative to control conditions (Table 1). In *V. uliginosum* absorbance tended to increase under enhanced UV-B but this was not significant. The absorbance of *V. vitis-idaea* methanol extracts was unaffected by enhanced UV-B.

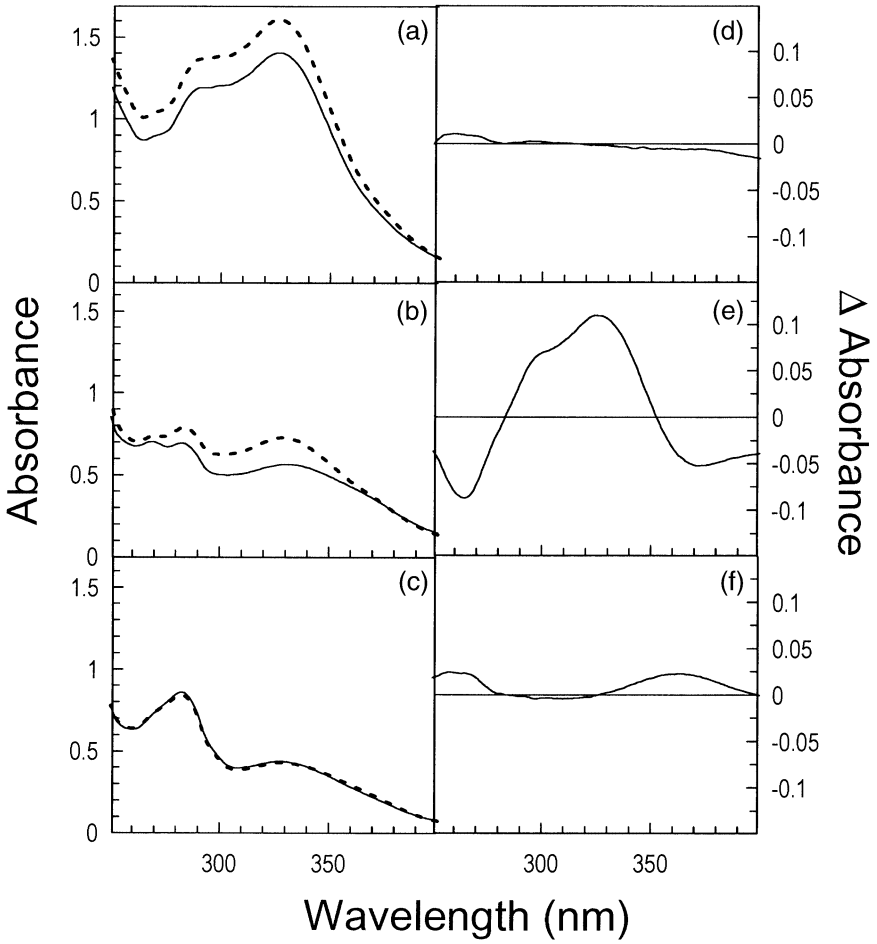

Figure 1. (a)-(c); Mean absorption spectra of methanol extracts from leaves of *V. myrtillus* (a), *V. uliginosum* (b) and *V. vitis-idaea* (c) grown under control (solid line) and enhanced UV-B (dotted line) conditions. (d)–(f); Normalized absorption difference spectra (enhanced UV-B minus control) for methanol extracts from *V. myrtillus* (d), *V. uliginosum* (e) and *V. vitis-idaea* (f). Extracts used were on the basis of 100 mg fresh weight per cm^3 extract. Spectra were normalized to absorbance at 283 nm prior to calculating difference. Significance of differences: (d), not significant; (e), $P = 0.098$ for difference at 324 nm; (f), not significant.

The spectra of the methanol extracts of the three species differed, indicating that the composition of UV-absorbing compounds varied (Fig. 1a–c). In *V. myrtillus* and *V. uliginosum* there were two peaks – one in the UV-B region between 280 and 290 nm and another in the UV-A region around 330 nm. In *V. myrtillus* the peak at the longer wavelength was more prominent, whereas in *V. uliginosum* the two peaks were of similar magnitude. In *V. vitis-idaea*, there was one sharp peak at 280 nm and a broad shoulder at 330 nm.

In *V. uliginosum* there were substantial qualitative differences in the absorbance spectra of the methanol extracts between plants grown in control and UV-B enhanced plots, as indicated by the large positive and negative peaks in the normalized difference spectrum (although these were only marginally significant, $P = 0.098$; Fig. 1e). Plants from the UV-B plots showed higher absorbance in the waveband 280–350 nm than plants from control plots, and lower absorbance at wavelengths shorter than 280 nm and longer than 350 nm. In *V. vitis-idaea*, slight differences in the absorption spectra of the methanol extracts were also apparent, although these were not significant (Fig. 1f). Plants from the UV-B-treated plots showed relatively higher absorbance at short wavelengths (< 280 nm) and at long wavelengths (> 330 nm). Such changes suggest variation in the concentrations of the flavonoids, that have absorption maxima in the region of 280 nm and 350 nm, relative to simpler phenolics. There was no qualitative difference between the spectra of control and UV-B-treated plots in *V. myrtillus* (Fig. 1d).

Alkali-extractable cell wall-bound phenolics

The integrated absorbance in the UV-B region of the cell wall extracts differed significantly between the three species ($P < 0.01$). *Vaccinium uliginosum* showed the highest UV-B absorbance, *V. myrtillus* showed approximately two-thirds and *V. vitis-idaea* approximately half the absorbance of *V. uliginosum* (Table 2).

The three species showed different responses to enhanced UV-B, both in terms of magnitude and direction. Thus, UV-B absorbance was significantly lower in cell wall extracts from *V. uliginosum* grown under enhanced UV-B ($P < 0.05$). In *V. vitis-idaea* the UV-B absorbance of the cell wall-bound phenolic extracts was 40% higher under enhanced UV-B ($P < 0.05$). The absorbance of the cell wall extracts of *V. myrtillus* was largely unaffected by enhanced UV-B.

The spectra of the alkali-extractable cell wall-bound phenolics of the three species were similar (Fig. 2a–c), showing

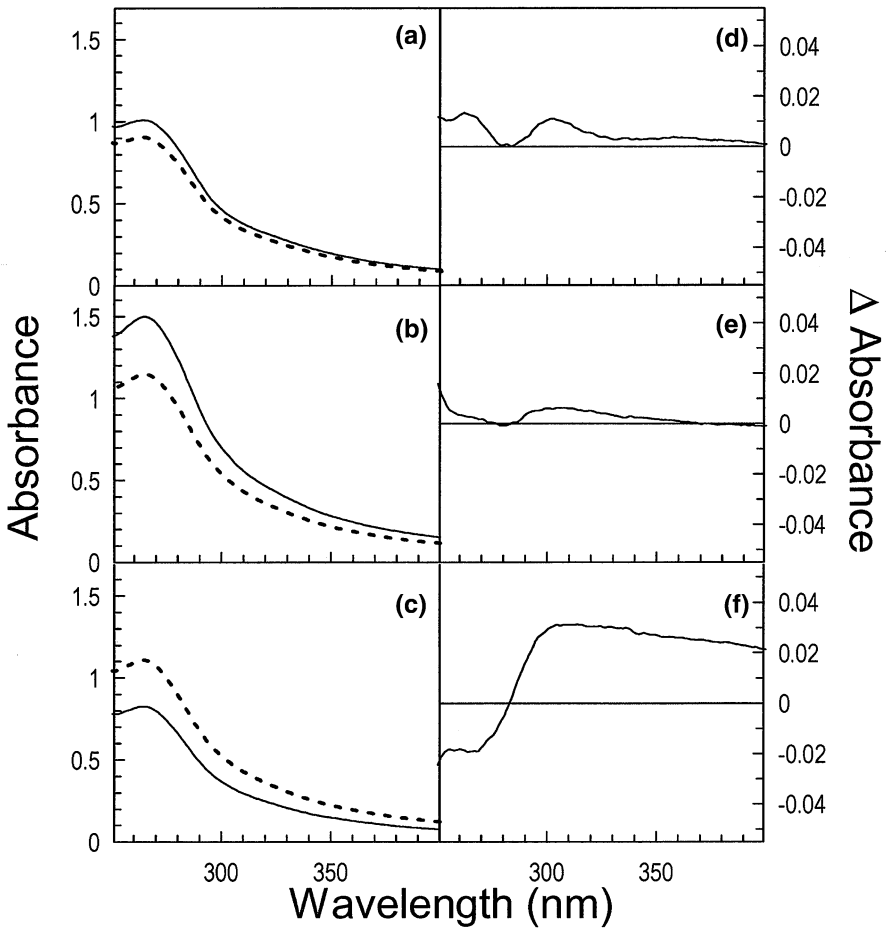

Figure 2. (a)–(c); Mean absorption spectra of cell wall alkali extracts from leaves of *V. myrtillus* (a), *V. uliginosum* (b) and *V. vitis-idaea* (c) grown under control (solid line) and enhanced UV-B (dotted line) conditions. (d)–(f); Normalized absorption difference spectra (enhanced UV-B minus control) for methanol extracts from *V. myrtillus* (d), *V. uliginosum* (e) and *V. vitis-idaea* (f). Extracts used were on the basis of 1 mg cell wall material per cm^3 extract. Spectra were normalized to absorbance at 283 nm prior to calculating difference. Significance of differences : (d), not significant; (e), not significant; (f), $P = 0.013$ for difference at 314 nm, $P = 0.051$ at 266 nm.

peak absorbance in the UV-C region, between 260 and 270 nm. Qualitative differences between extracts of plants taken from control versus UV-B enhancement plots were most apparent in *V. vitis-idaea*, with highly significant differences in the normalized difference spectrum (Fig. 2f), in which there was a relative increase in compounds absorbing at wavelengths longer than 280 nm.

Localization of UV-absorbing compounds

In *V. myrtillus* the UV-absorbing compounds were present throughout the leaf (Fig. 3a). In the epidermis, these were apparent in the vacuoles, sometimes as a fine deposit on the inside of the tonoplast, and in the nuclear region (not shown). Stomatal guard cells contained more UV-absorbing compounds than the achlorophyllus epidermal cells, as judged by the high fluorescence intensity of the former (not shown). Large amounts of UV-absorbing compounds were also present in the palisade cells, particularly associated with the chloroplasts (not shown). Confocal laser scanning fluorescence microscopy indicated that, when the entire depth of the leaf was scanned by means of equidistant images with a step of 7 μm, the greatest fluorescence was observed in the palisade mesophyll, between 30

Table 1. UV-B-absorbing capacity of methanol extracts, expressed as the integrated area between 280 and 320 nm per mg leaf weight ± SE

Species	Control	UV-B	Treatment difference (%)	Interspecific difference (%)
V. myrtillus	459 ± 26	557 ± 24	+21[a]	100[b]
V. uliginosum	224 ± 26	273 ± 18	+22	49
V. vitis-idaea	222 ± 6	217 ± 8	–2	48

Treatment difference percentage denotes the percentage difference in the mean absorbance comparing control and treatment extracts in each species. Interspecific difference percentage denotes the difference in the integrated areas of control extracts between different species, where the largest area is assigned 100%. [a]Denotes statistically significant treatment effect at $P < 0.05$; [b]denotes a species differing significantly from the others in absorbance of control plots.

Table 2. UV-B-absorbing capacity of cell wall-bound, alkali extractable phenolics expressed as the integrated area between 280 and 320 nm per mg weight ± SE

Species	Control	UV-B	Treatment difference (%)	Interspecific difference (%)
V. myrtillus	203 ± 14	182 ± 13	–10	67
V. uliginosum	303 ± 27	208 ± 21	–31[a]	100[b]
V. vitis-idaea	161 ± 16	226 ± 13	+40[a]	53

Treatment difference percentage denotes the percentage difference in the mean absorbance comparing control and treatment extracts in each species. Interspecific difference percentage denotes the difference in the integrated areas of control extracts between different species, where the largest area is assigned 100%. [a]Denotes statistically significant treatment effect at $P < 0.05$; [b]denotes a species differing significantly from the others in absorbance of control plots.

and 50 μm from the adaxial surface. In *V. uliginosum* the fluorescence was also visualized throughout the leaf, although the intensity was much lower than in *V. myrtillus*, reflecting the lower levels of UV-absorbing compounds in this species (see Table 1). The greatest fluorescence of all layers examined was emitted from the outer epidermal cell walls (Fig. 3b) and from the trichomes. In *V. vitis-idaea* the fluorescence was localized predominantly in the anticlinal and periclinal cell walls of the epidermis (Fig. 3c) and in the cell walls of the vascular tissues.

DISCUSSION

The three species examined in this study are closely related; all belong to the genus *Vaccinium*, co-exist in the same ecosystem and have a similar overall growth form. In spite of this, they were found to differ in terms of their intrinsic content of UV-B-absorbing phenolic compounds and the distribution of those compounds through the leaf. These differences were reflected in the responses observed in UV-absorbing compounds, comparing plants grown under control and UV-B enhanced conditions.

None of the species studied here showed any response to UV-B in terms of changing leaf thickness or general morphology (Semerdjieva *et al*. 2003), so comparisons of data on a leaf mass basis give a reasonable estimate of changes in the UV-B screening potential of these plants. The most striking differences were between *V. myrtillus* and *V. vitis-idaea*. Comparison of these two species indicates that they are adopting very different strategies in terms of their use of phenolic compounds as protection against UV-B. *Vaccinium vitis-idaea* seems to adopt a strategy of exclusion, with high amounts of UV-B-blocking compounds in the epidermal cell walls but little throughout the rest of the leaf (cf. Day 1993). This would seem to represent an optimal strategy for avoiding the damaging effects of UV-B, as it would largely prevent exposure of sensitive chromophores to such radiation. The strategy adopted by *V. myrtillus* seems intuitively less obvious. This deciduous species has much less substantial cell walls than *V. vitis-idaea* (approx. 50% less per unit fresh weight; Semerdjieva 2002) perhaps placing a limit on its capacity to produce a protective surface layer of phenolic compounds. It does contain phenolic compounds within the cells of the epidermis, as indicated by staining with Naturstoffreagenz A, however, these are mostly associated with guard cells (not shown). Phenolic compounds were also found in substantial quantities throughout the leaf, especially in the palisade cells. This distribution seems to reflect an association with sites sensitive to UV-B. The photosynthetic apparatus is thought to

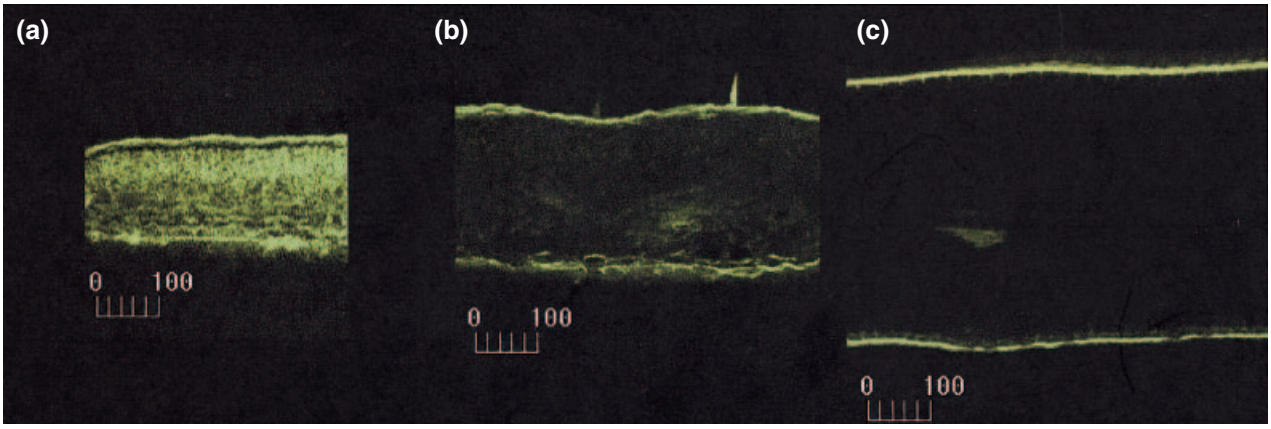

Figure 3. Leaf cryosections of *V. myrtillus* (a), *V. uliginosum* (b) and *V. vitis-idaea* (c) stained with Naturstoffreagenz A to visualize phenolics; confocal laser scanning microscopy. Figures shown are from leaves from control plots (differences between control and treatment are not apparent from visual examination). Scale bar represents 100 μm.

be particularly sensitive to UV-B (Wilson & Greenberg 1993) and the localization of UV-absorbing compounds in photosynthetically active cells suggests that this species is adopting a strategy of protection where it is needed, rather than producing an overall screen. Although this seems likely to be less efficient, it may represent a better investment for a plant in which the leaves are relatively short-lived. The phenolic compounds produced by *V. myrtillus* remain in the soluble phase so it might be speculated that this pool is recovered by the plant, prior to leaf drop. A substantial investment in cell wall-bound material would be more cost-effective in *V. vitis-idaea,* in which the leaves are retained for more than one season.

The strategy adopted by *V. uliginosum* is less clearly defined than that of the other two species, perhaps not surprisingly given its nature. Like *V. myrtillus*, this is a deciduous species, however, its leaves are much more lignified, similar to those of *V. vitis-idaea*. In terms of the distribution of phenolic compounds between methanol soluble and cell wall bound, it is more like *V. vitis-idaea*, although the amounts of cell wall-bound phenolics is by far the greatest of the three species. Its response to enhanced UV-B is, however, more similar to that of *V. myrtillus*. The methanol-soluble pool of phenolics was higher (although not significantly) in plants grown under enhanced UV-B. The cell wall-bound pool was significantly and substantially depleted under enhanced UV-B. This response might reflect a change in the distribution of resources under UV-B treatment, reflecting a move towards investment in local protection instead of global screening.

The phenolic composition of soluble extracts differed substantially between the three species but there was no indication of gross differences in composition of cell wall-bound phenolics. The composition of phenolic compounds in the three species will be discussed in detail elsewhere (Semerdjieva 2002), but broadly, the spectra of soluble compounds are characteristic of complex phenolics, notably flavonoids and hydroxycinnamic acids, whereas the spectra of the cell wall extracts are more characteristic of simple phenols or phenolic acids (Harborne 1989). Based on the difference spectra of cell wall extracts, there were significant changes in the composition of cell wall-bound phenolics in *V. vitis-idaea* but not in the other two species. *Vaccinium uliginosum* was the only species in which there was any indication of a change in the composition of methanol-soluble phenolic compounds (although these were of only marginal significance). In both these cases, the increase in relative absorbance in the region 300–350 nm is consistent with an increase in flavonoids relative to simple phenolics. The small spectral changes seen in the other two species are indicative of the opposite trend. In all three species, the changes in the composition of the cell wall extracts would result in increased relative absorbance in the (possibly more biologically relevant) long wavelength region of the UV-B spectrum.

Taken overall, the observations made for the three *Vaccinium* species fit into the pattern proposed by Day and others (Day *et al*. 1992, 1993; Day 1993), who observed a trend of increasing UV-B screening efficiency moving from herbaceous to evergreen woody species. However, as far as we are aware, this is the first time that such striking differences in UV-B protective strategies have been observed in closely related species. Such differences and the possible strategic plasticity observed in *V. uliginosum* imply that there is a great deal of flexibility available to plants, both in developmental and adaptive terms, in determining how resources are distributed between different pools of phenolic compounds. Clearly other factors, not considered here (shade, climatic factors), will have a substantial influence on the efficacy of these different strategies and need further investigation before we gain a full picture of how plants use phenolic compounds to protect themselves from UV-B stress.

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