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Are some plant life forms more effective than others in screening out ultraviolet-B radiation?

T.A. Day[1], T.C. Vogelmann[2], and E.H. DeLucia[3]

[1] Department of Biology, West Virginia University, Morgantown, WV 26506, USA
[2] Department of Botany, University of Wyoming, Laramie WY 82071, USA
[3] Department of Plant Biology, University of Illinois, Urbana, IL 61801, USA

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Summary. The unprecedented rate of depletion of the stratospheric ozone layer will likely lead to appreciable increases in the amount of ultraviolet-B radiation (UV–B, 280–320 nm) reaching the earth's surface. In plants, photosynthetic reactions and nucleic acids in the mesophyll of leaves are deleteriously affected by UV–B. We used a fiber-optic microprobe to make direct measurements of the amount of UV–B reaching these potential targets in the mesophyll of intact foliage. A comparison of foliage from a diverse group of Rocky Mountain plants enabled us to assess whether the foliage of some plant life forms appeared more effective at screening UV–B radiation. The leaf epidermis of herbaceous dicots was particularly ineffective at attenuating UV–B; epidermal transmittance ranged from 18–41% and UV–B reached 40–145 μm into the mesophyll or photosynthetic tissue. In contrast to herbaceous dicots, the epidermis of 1-year old conifer needles attenuated essentially all incident UV–B and virtually none of this radiation reached the mesophyll. Although the epidermal layer was appreciably thinner in older needles (7 y) at high elevations (Krumholtz), essentially all incident UV–B was attenuated by the epidermis in these needles. The same epidermal screening effectiveness was observed after removal of epicuticular waxes with chloroform. Leaves of woody dicots and grasses appeared intermediate between herbaceous dicots and conifers in their UV–B screening abilities with 3–12% of the incident UV–B reaching the mesophyll. These large differences in UV–B screening effectiveness suggest that certain plant life forms may be more predisposed than others to meet the challenge of higher UV–B levels resulting from stratospheric ozone depletion.

Key words: Conifer – Epidermis – Fiber-optic – Optical properties – Ozone depletion

Correspondence to: T.A. Day

Recent reports of increases in the amount of ultraviolet-B radiation (UV–B, 280–320 nm) reaching the earth's surface (Blumthaler and Ambach 1990) along with high concentrations of ozone destroying chemicals in the stratosphere at temperate latitudes (Kerr 1991; 1992) have prompted concerns, as it is well documented that UV–B is damaging to many biological systems. In plants certain reactions in the photosystem II center of photosynthesis appear to be partially inhibited by UV–B (Iwanzik et al. 1983; Bornman 1989). Similarly, intact-leaf photosynthesis and plant growth is depressed in some species under UV–B regimes predicted with continued depletion of stratospheric ozone (Caldwell et al. 1982; Barnes et al. 1987; Sullivan and Teramura 1989; Tevini and Teramura 1989), although no depressions in photosynthesis have been observed in many species under enhanced UV–B regimes (Caldwell et al. 1989). In addition to photosynthetic inhibition, UV–B can also alter the structure and function of nucleic acids in plants (Saito and Werbin 1969; Pang and Hayes 1991).

Plants may be particularly predisposed to enhanced UV–B damage because leaves are usually positioned to intercept large amounts of photosynthetically-active solar radiation. Potential damage from absorption of UV–B makes it essential that we know the UV–B screening ability of leaves if we are to predict changes in plant performance under future UV–B regimes. To date, however, there are no direct measurements of the amount of UV–B that reaches photosynthetic machinery and other chromophores such as nucleic acids in leaves. Leaf surface reflectance provides a first line of defense in preventing UV–B from reaching these chromophores, but in the vast majority of plants reflectance is low (<10%) and epidermal attenuation appears to be the dominant UV–B screening mechanism (Gausman et al. 1975; Robberecht et al. 1980; Caldwell et al. 1983b). Flavonoids and related phenolic compounds, which absorb strongly in the UV–B but transmit visible or photosynthetically-active radiation, appear responsible for epidermal attenuation. The importance of epidermal attenuation as a protective mechanism has been recently demonstrated by Tevini et

al. (1991) who found that UV–B-induced increases in concentrations of these compounds could prevent or reduce subsequent photosynthetic damage from UV–B.

Measurements of epidermal peels from leaves using an integrating sphere reveal epidermal transmittance of UV–B ranging from < 1–25% (Caldwell et al. 1982; Robberecht et al. 1980; Caldwell et al. 1983b; Robberecht and Caldwell 1978; Robberecht and Caldwell 1983; Flint et al. 1985). While this technique provides useful information on the amount of UV–B reaching the epidermis/mesophyll interface, it is limited to species whose leaf anatomy facilitates obtaining an epidermal peel of sufficient size. Thus, such measurements are difficult to make on conifer needles where anatomical characteristics make it exceedingly difficult to obtain large epidermal peels. The optical properties of the leaf as a whole (i.e. intact leaves) may also be important in an analysis of radiation penetration since inhomogeneities within the leaf may cause substantial light scattering (Vogelmann 1989; Vogelmann et al. 1991; Bornman and Vogelmann 1988; Knapp et al. 1988). In addition, the epidermal peel technique gives no information on the amount of UV–B present at various depths in the mesophyll, which itself may have different screening ability. Ultraviolet-B penetration within the mesophyll is of particular interest since the vast majority of photosynthetic and other metabolic processes, and nucleic acids are largely confined to this tissue.

We used a fiber-optic microprobe to make direct measurements of UV–B penetration into intact foliage of a diverse group of plants. The microprobe was used to examine UV–B screening effectiveness in foliage from several herbaceous and woody dicots, grasses and conifers growing in the central Rocky Mountains. In addition, we predicted that (1) epidermal/cuticular thickness may be inversely correlated with UV–B screening ability, and (2) as a result of thin epidermal/cuticular layers in old needles and exposed needles of Krumholtz trees (Baig and Tranquillini 1976; DeLucia and Berlyn 1984; Hansen-Bristow 1986), these tissues will permit deeper UV–B penetration. To test these predictions we sampled needles of various ages from conifers along an elevational gradient.

Materials and methods

Plant material

Most plant material was collected from the Medicine Bow Mountains of southeastern Wyoming, USA. The two primary collection sites were a subalpine meadow (3310 m elevation) and a nearby (5 km) low elevation (2880 m) riparian community and adjacent open forest (Table 1). In order to assess the screening effectiveness of a deciduous conifer, we also sampled foliage of *Larix occidentalis*. As this species is not native to the Medicine Bow Mountains we collected needles of *Larix* from trees in Laramie, Wyoming (50 km east of the collection sites; 2195 m elevation) and Seattle, Washington state (30 m elevation). Material was collected from sunlight portions of unshaded individuals with the exception of *Mahonia repens*, *Heracleum lanatum* and *Orthilia secunda* in which material was taken from shaded individuals deep within the under-

Table 1. Most plant material was collected from individuals growing in a low-elevation riparian community (2280 m), mid-elevation meadow (3020 m) and a high-elevation subalpine meadow (3310 m) in the Medicine Bow Mountains, Wyoming.
* Foliage collected from individuals in Laramie, Wyoming (2195 m) and Seattle, Washington state (30 m). Most foliage was collected from sunlight portions of unshaded individuals
** Foliage collected from shaded individuals deep within the understory.
Nomenclature follows Dorn (1977)

Life form species	Species code	Collection site (elevation) Low	Mid	High
Conifers				
Larix occidentalis	a*			
Abies lasiocarpa	b	X	X	X
Pinus flexilis	c	X	X	X
Pinus contorta	d	X		
Picea engelmannii	e	X	X	X
Pinus ponderosa	f	X		
Pseudotsuga menziesii	g	X		
Juniperus communis	h	X		
Picea pungens	i	X		
Woody dicots				
Mahonia repens	j	X**		
Populus tremuloides	k	X		X
Salix planifolia	l			X
Grasses				
Phleum alpinum	m			X
Deschampsia caespitosa	n			X
Festuca ovina	o			X
Herbaceous dicots				
Rumex densiflorus	p			X
Sibbaldia procumbens	q			X
Polygonum bistortoides	r			X
Aster foliaceus	s			X
Gentianella detonsa	t			X
Heracleum lanatum	u	X**		
Orthilia secunda	v	X**		

story of the low elevation site. To examine the effect of elevation and age on needle epidermal thickness and the UV–B screening properties of the epidermis, we also collected 1-, 4- and 7-y old needles from individuals of *Abies lasiocarpa, Pinus flexilis* and *Picea engelmannii* from a mid-elevation (3020 m), open-forest site.

Material was collected from mid-July to early-September, 1990, after at least two days of predominantly sunny conditions. Herbaceous plants were collected with a substantial portion of the root system intact, while for woody plants the terminal 20–50 cm of a branch or stem was severed under water. Samples were placed in a humidified plastic container in an insulated box for transport to the laboratory. Microprobe measurements were made on fully expanded leaves and needles (at least 1-year old) within 8 h of field collection. Measurements were made promptly because the concentration of UV–B absorbing compounds such as flavonoids and related phenolics in leaves can change in response to UV–B levels (Tevini and Teramura 1989; Robberecht and Caldwell 1978; Caldwell et al. 1989), and other stresses (Tevini and Teramura 1989; McClure 1976). We also made microprobe measurements on plant material collected 10–14 d apart to determine the potential for relatively short-term changes in the UV screening properties of the epidermis in response to environmental variation. Within a species, the range in UV–B penetration at a given wavelength between sampling dates was no greater than that between leaves of different individuals on a given sampling date.

Microprobe measurements

Fiber-optic microprobes, which have recently been used to measure visible and UV–A (320–400 nm) radiation gradients within foliage (Vogelmann et al. 1991; Vogelmann 1989; Bornman and Vogelmann 1988; Knapp et al. 1988), were inserted into intact foliage to directly measure the penetration of UV–B in potentially sensitive photosynthetic tissue. Microprobes were fabricated from tapered fused-silica multimode step-index fibers and had a light-collecting tip of 10–15 μm in diameter. Unlike optical fiber made from glass, fused silica transmits well in the UV–B (Vogelmann et al. 1991). Microprobes were tapered by heating and stretching individual fiber-optic strands (125 μm OD) using a hydrogen-oxygen microtorch. The tapered region was coated with evaporated chromium and the tip was truncated with a diamond knife. Since both liquid and gaseous phases are encountered in foliage, the acceptance angles of the microprobes were measured in air and water. The microprobes used for our measurements had near-perfect Gaussian acceptance functions with 50% band widths of 16–28°. The amount of radiation measured by the fiber-optic microprobe depends on how much light enters its acceptance angle. As radiation migrates through plant tissue, some can be scattered so that it probably falls outside the acceptance angle of the microprobe (Vogelmann et al. 1991). While measurements of radiation gradients made at several different orientations can be used to derive actual radiant flux at a desired depth within foliage (Vogelmann et al. 1991), the low flux of UV–B within foliage did not allow us to make reliable measurements at orientations other than 0° (microprobe acceptance tip directly facing the radiation source). That UV–B flux was often too small to measure at other orientations is not surprising: since UV–B absorption within foliage was often very high a relatively small amount of these photons would be scattered. Thus, while the results we report are somewhat conservative estimates of UV–B penetration and in vivo fluxes, our reliance on measurements made at 0° orientation appears appropriate considering the largest UV–B flux moves through the leaf at this orientation.

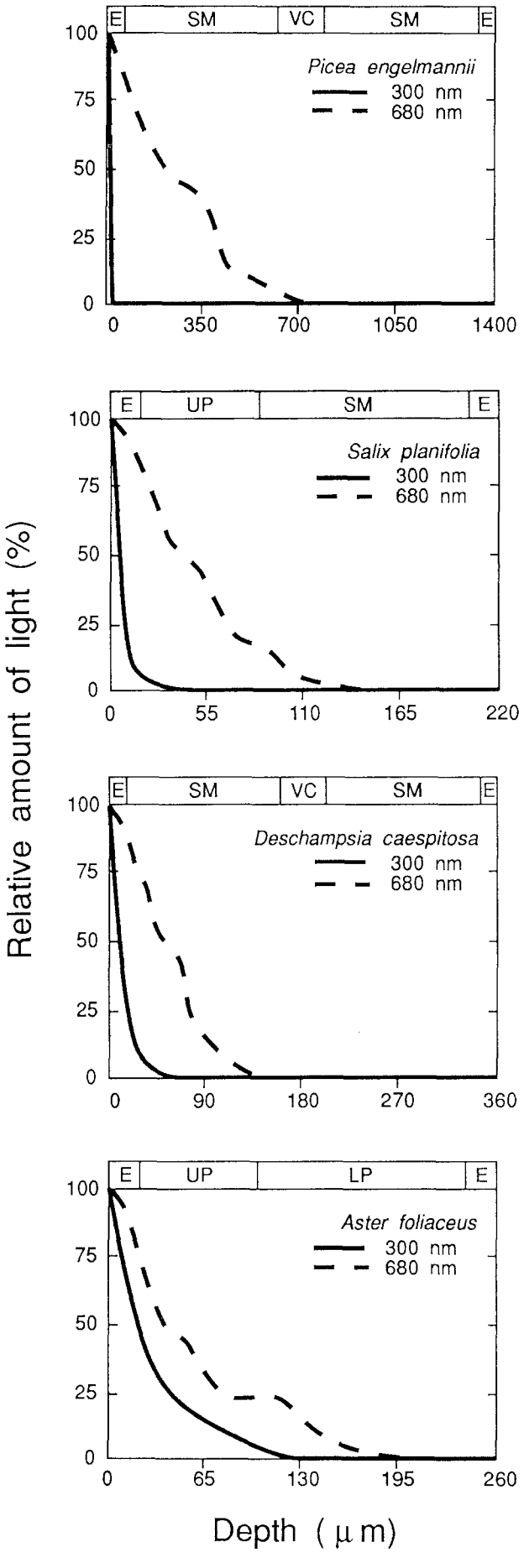
For measurements a microprobe was mounted on a stepper motor, positioned with a micromanipulator, and advanced at 12 μm/s through foliage that was irradiated with a broadband UV–B lamp. The lamp, a 150 W Hg-xenon arc lamp with a fused-silica filter, supplied a weighted irradiance at the foliage surface of 3,222 mW/m^2 biologically effective UV–B (UV–B_{BE}) using the generalized plant-damage action spectrum normalized to 280 nm (Caldwell et al. 1983a). This was measured with a UV actinic meter (International Light SED240/ACTS270/W) which was calibrated with an Optronics 752 UV/visible spectroradiometer (DeLucia et al. 1992). The UV actinic meter weights radiation in a manner similar to that of the generalized plant-damage action spectrum. This is a relatively conservative action spectrum; the biological effectiveness of UV–B decreases logarithmically as wavelength increases above 280 nm, becoming completely ineffective at 314 nm. The peak UV–B_{BE} irradiance around the summer solstice (1990) at the plant sampling sites was 121 Mw/m^2, measured with an Optronics 752 spectroradiometer (DeLucia et al. 1992). While the UV–B_{BE} irradiance at the foliage surface during microprobe measurements was substantially higher than this, a measurement was completed in 20–117 s. Examination of foliage following measurements did not reveal photobleaching or other visible changes.

The adaxial or upper surface of foliage was irradiated and the probe advanced through aeroles or vein-free regions toward the irradiated surface. In grasses and conifer needles the probe was passed through the vascular bundle or cylinder. As the microprobe passed through the foliage, radiation of a specific wavelength was measured with a spatial resolution of 1–2 μm with a UV/visible spectroradiometer (Optronics 742). With respect to reflectance, as the microprobe exits the irradiated surface of the foliage the radiation gradient measured is a result of attenuation by surface layers of the foliage as well as reflectance. Because of the very small spatial scales separating these two processes and the low leaf surface reflectance values reported by other researchers we did not attempt to distinguish between them. Since accumulation of material such as conifer resin on the tip of the probe would cause spurious results, the probe was examined microscopically as it exited foliage to confirm the tip was not occluded. Following each measurement the tip was cleaned with ethanol and rinsed with distilled water. Measurements were made on leaves or needles collected from 15 different individuals/species/site with penetration measured on each sample at 290, 300 and 310, as well as 680 nm. In contrast to UV–B, 680 nm light should not be attenuated by UV–B screening pigments in the epidermis and should penetrate into the mesophyll where it drives photosynthetic reactions. Clark and Lister (1975) suggested that surface or epicuticular waxes were important in reflecting UV–B in some conifer needles such as *Picea pungens*. We assessed the importance of epicuticular waxes in UV–B screening by measuring UV–B penetration after removing these waxes by immersing needles in chloroform for 10 min (Bornman and Vogelmann 1988). Following microprobe measurement we determined the thickness of various tissue layers by light and epifluorescence microscopic examination of needle and leaf fresh sections. The epidermal layer was considered to consist of the cuticle, epidermis and hypodermis (when present).

Results

There were large differences in the depth of penetration of UV–B in foliage of different species. Figure 1 shows typical UV–B (300 nm) and red light (680 nm) penetration into a conifer needle, and leaves of a woody and a herbaceous dicot, and a grass. Incident UV–B was attenuated by the epidermis of foliage much more readily than photosynthetically-active red light. While UV–B was completely attenuated by the epidermis of *Picea engelmannii* it penetrated 121 μm (1% of incident irradiance) into the leaf of *Aster foliaceus*.

When we expressed the results from all 22 species in terms of epidermal (including cuticle and hypodermis) transmittance of UV–B, values ranged from 0–42% (Fig. 2A). The epidermis of conifer needles was particularly effective at attenuating UV–B, and essentially none of this radiation reached the mesophyll. This was true not



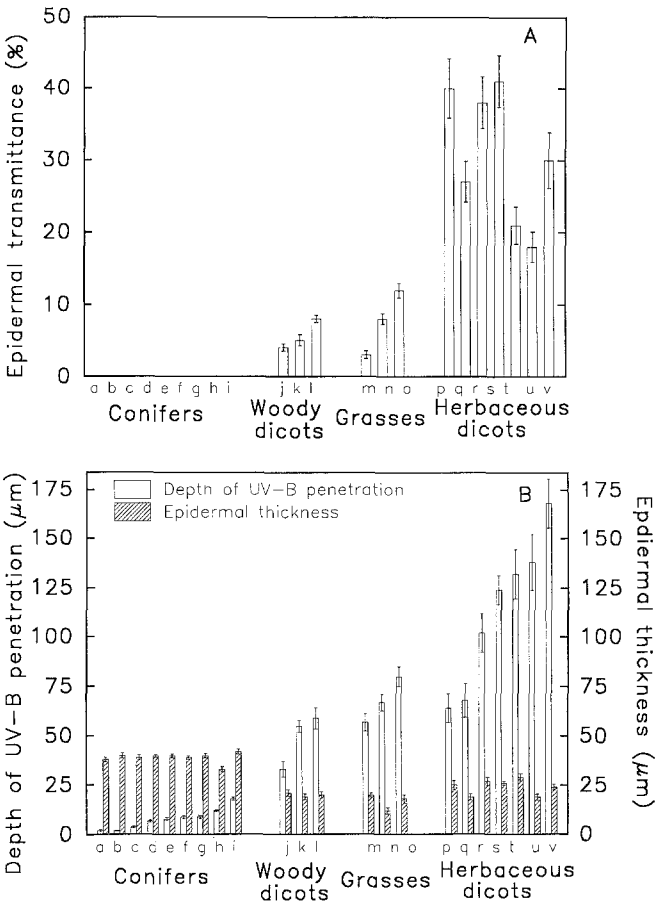


Fig. 2. A Epidermal transmittance of UV–B in foliage of 22 plant species (grouped by life form) growing in the Medicine Bow Mountains, Wyoming. Species are designated along the horizontal axis by the appropriate species code letter (see Table 1). Conifer needles (1-y old) were collected from the low elevation site. Results are means of microprobe measurements made at 290, 300 and 310 nm which were summarized with a weighted average, based on the generalized plant-damage action spectrum normalized to 280 nm (Caldwell et al. 1983a). Using this action spectrum, transmittance at 290, 300 and 310 nm was multiplied by 0.67, 0.28 and 0.05, respectively, and summed to give a weighted average. **B** Depth of UV–B penetration (1% of incident) and thickness of epidermis (including cuticle and hypodermis) in foliage of the above species. Depth of UV–B penetration was measured at 290, 300, and 310 nm and summarized with a weighted average as above. *Vertical lines* indicate SE ($n=15$); confidence intervals and means were back-transformed following arc-sine transformation

Fig. 1. Typical amount of radiation at 300 and 680 nm as a function of depth in a 1-y old needle of a conifer *(Picea engelmannii)*, and a leaf of a woody dicot *(Salix planifolia)*, a grass *(Deschampsia caespitosa)* and a herbaceous dicot *(Aster foliaceus)* growing at the high elevation (3310 m) site. The horizontal bar at the top of each graph denotes the thickness of various tissue layers: E (cuticle, epidermis and hypodermis), SM (spongy mesophyll), VC (vascular cylinder or bundle), UP and LP (upper and lower palisade). The scale of the horizontal axis differs with each species because of differences in foliage thickness

only within the UV–B waveband, but up to 340 nm within the UV–A as well (not shown). In contrast, foliage of herbaceous dicots was relatively ineffective at screening UV–B; 18–41% of the incident UV–B reached the mesophyll. The leaves of woody dicots and grasses were intermediate in their UV–B screening abilities with 3–12% of the incident UV–B reaching the mesophyll. The relative ineffectiveness in UV–B screening of herbaceous dicots was even more evident when we examined the depth of UV–B penetration (expressed as depth of 1% of incident irradiance; Fig. 2B). Ultraviolet-B penetrated 60–170 μm into leaves of herbaceous dicots, but only 2–18 μm into conifer needles.

Although our sampling size was somewhat limited, there was a significant plant life form effect on both epidermal UV–B transmittance and depth of penetration ($P<0.001$ using a one-way ANOVA and a general linear model approach; $n=22$; transmittance data were analyzed following arc-sine transformation) (SAS 1990). This trend in UV–B screening and life form does not appear to be solely attributable to epidermal thickness since there was only a marginal correlation between epidermal thickness and epidermal transmittance ($r=-0.37$; $P=0.054$ that $r\neq 0$, $n=22$).

As expected the needle epidermis of *Abies lasiocarpa*, *Picea engelmannii* and *Pinus flexilis* were thinner in high elevation individuals and in older needles ($P<0.05$, one-way ANOVA; $n=20$; for both elevation and age effects) (Fig. 3). However, we found no significant difference in the depth of UV–B penetration within these species across elevation or age treatments, and essentially all UV–B was attenuated before it reached the mesophyll ($P>0.40$, data not shown). We assessed the relative importance of epicuticular waxes in UV–B screening in all eight conifer species by measuring UV–B penetration after epicuticular wax removal with chloroform. Removal of waxes had no significant effect on depth of UV–B penetration in any species ($P>0.20$, data not shown). Since conifer foliage attenuated all UV–B in the epidermis in all treatments, we had no information on the relative screening effectiveness of the mesophyll. To evaluate the screening effectiveness of the mesophyll we removed a strip (≈50 μm wide) of epidermis from 1-yr old *Picea engelmannii* needles from the low elevation site and measured UV–B penetration below the stripped area. The mesophyll of these needles was much less effective in screening UV–B than epidermal tissue (Fig. 4).

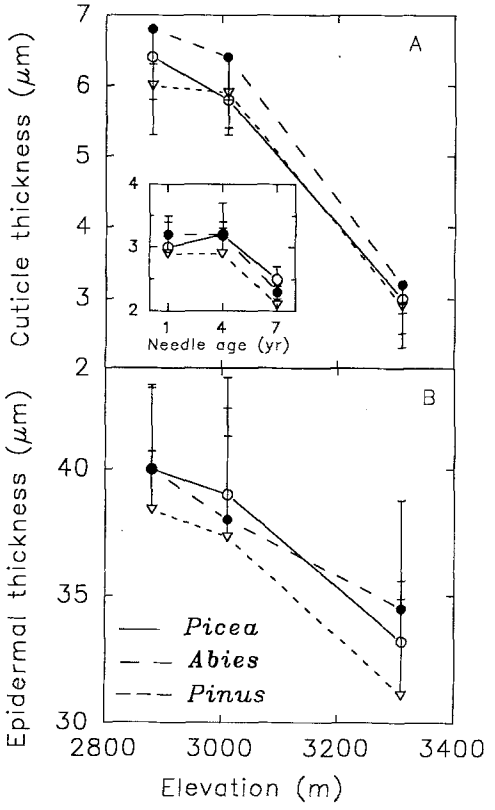


Fig. 3. **A** Changes in needle cuticle thickness with elevation in *Picea engelmannii*, *Abies lasiocarpa* and *Pinus flexilis* in the Medicine Bow Mountains. *Insert:* Changes in cuticle thickness with needle age. One-year old needles developed over the previous growing season. **B** Changes in needle epidermal thickness (cuticle, epidermis and hypodermis) with elevation. *Vertical lines* indicate SE ($n=15$)

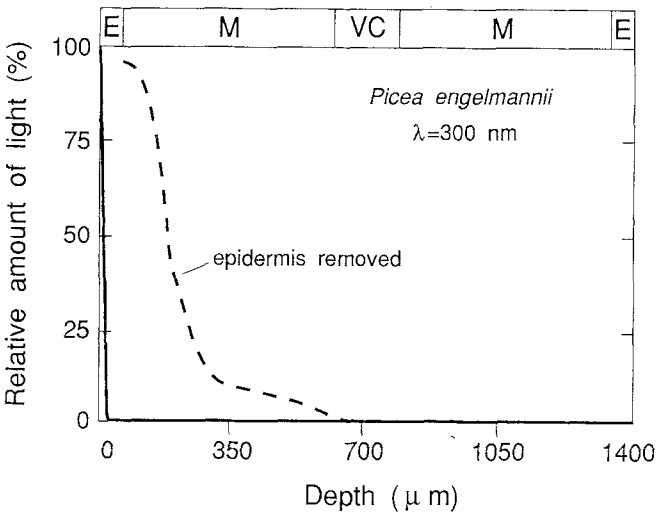


Fig. 4. Typical amount of UV–B (300 nm) as a function of depth in a *Picea engelmannii* needle (collected from the low elevation site) before and after a strip of epidermis (≈50 μm wide) was removed. Results were similar on six replicates

Discussion

We found a surprisingly large range in both epidermal transmittance and the depth to which UV–B penetrates into the mesophyll of foliage of different life forms. Previous researchers have noted a larger range in UV–B transmittance (through epidermal peels) among temperate than among equatorial, tropical, or arctic species (Robberecht et al. 1980; Robberecht and Caldwell 1978). However, we found a much greater range in epidermal transmittance among our species (0–41%) than previous researchers (< 1–25%) (see Introduction). The lower values of epidermal transmittance in previous studies may be the result of epidermal cell damage during peeling. It has been suggested that the majority of flavonoids and

other UV–B screening compounds are located in the vacuoles of epidermal cells (Caldwell et al. 1983b), and this may be particularly true in herbaceous dicots (see below). Epidermal cell disruption during peeling may have allowed UV–B screening compounds to leek out along the bottom of the peel and attenuate UV–B which normally penetrates through anticlinal cell-wall regions of the epidermis.

The difference in UV–B screening effectiveness that we observed among different life forms appears primarily the result of different optical characteristics of their epidermis and not thickness per se. There was only a weak correlation between epidermal thickness and UV–B transmittance ($r = -0.37$). In addition, although the needles of the conifers we studied had a relatively thick epidermal layer (30–45 μm), attenuation of UV–B *within* their epidermis was much greater relative to foliage of other life forms. For example, conifer needles attenuated essentially all UV–B in the outer 2–18 μm of their epidermis (Fig. 2B). All other species had epidermal layers > 18 μm thick, except the grass *Deschampsia caespitosa* (12 μm thick), but none attenuated all incident UV–B.

One such optical property which may explain some of the differences among life forms in screening effectiveness is the location of UV–B absorbing compounds within the epidermis. In herbaceous dicots these screening compounds appear to occur primarily in the vacuole of epidermal cells (Weissenböck et al. 1984; 1986; Schnabl et al. 1986; Schmelzer et al. 1988), while in conifers they are located in vacuoles as well as within epidermal cell walls (Strack et al. 1988; 1989). Thus, the epidermis of conifer needles may provide a much more laterally complete or uniform UV–B screen than that of herbaceous dicots.

The trend between UV–B screening effectiveness and life form may partly reflect differences in selective forces associated with leaf longevity. Needles of the conifers that we sampled may remain on trees for 5–> 25 y, depending on the species. In general, the woody dicots retain their leaves for a complete growing season while leaves of the grasses and herbaceous dicots remain active for only part of the growing season. More effective UV–B screening in long-lived conifer needles may be an evolutionary response to potentially large life-time doses of UV–B. Alternatively, the exceptional screening properties of the epidermis of conifer needles may result from selection for photosynthetic organs which can withstand other stresses such as mechanical abrasion and winter desiccation.

Along these lines, from a cost-benefit standpoint we would predict that deciduous conifers such as *Larix* would not invest in large amounts of UV–B screening compounds and be relatively ineffective UV–B screeners since foliage is only retained for one growing season. The epidermis of *Larix* needles from individuals at both Laramie, Wyoming and Seattle, Washington state were as effective in UV–B screening as needles of the evergreen conifers. Ultraviolet-B penetrated 3 ± 1 μm (mean ± SE) in *Larix* needles collected from both locations, being attenuated well within the epidermal layer (38 ± 1 μm thick).

A prominent anatomical surface feature of needles in many of the conifer species we sampled was large deposits of crystalline epicuticular waxes. These waxes do not appear critical in UV–B screening, as their removal had no affect on depth of UV–B penetration. Similarly, Bornman and Vogelmann (1988) found little change in UV–A (360 nm) penetration following removal of epicuticular waxes from *Picea engelmannii* and *Abies lasiocarpa* needles. We observed crystalline epicuticular waxes on needles of *Picea engelmannii* and *pungens, Abies lasiocarpa, Pseudotsuga menziesii, Pinus flexilis* and *Juniperus communis;* in each case these waxes were largely confined to stomatal regions of the needle (stomatal "valleys" running parallel to the needle axis), and were particularly well developed over the stomatal antechambers. These waxes may therefore be important in (1) conserving moisture through increased boundary layer/diffusional resistance and/or (2) enabling CO_2 uptake to occur when foliage is wet by preventing water droplets from blocking stomata. Clark and Lister (1975) found that these waxes occurred over the entire surface of *Picea pungens* needles and suggested that waxes were an adaptation for UV–B protection in this species. However, the crystalline epicuticular waxes on the needles of *Picea pungens* we sampled were largely confined to stomatal valley regions. As with other conifers, we found no changes in UV–B penetration gradients following wax removal. Although the value of these waxes in mesophyll UV–B protection appears limited, they may protect potentially sensitive surface targets such as guard cells or cells lining the substomatal cavity.

While the UV–B fluxes or thresholds required for photosynthetic damage are not known and actual fluxes at the site of photosynthetic damage can not be derived from our data, the deep penetration of UV–B into the photosynthetic tissue of certain species, particularly herbaceous dicots, is somewhat unexpected given this radiation is quite damaging to photosynthetic reactions (Iwanzik et al. 1983; Bornman 1989). This is surprising considering that many of the herbaceous plant species examined to date have not exhibited reductions in intact-leaf photosynthesis under enhanced UV–B levels (Caldwell et al. 1989). An explanation for this disparity is that the photosynthetic machinery may be protected by UV–B absorbing compounds at the organelle level. Flavonoids have been detected in the outer membrane of chloroplasts in some species (Haupt and Scheuerlein 1990) and may provide a final means of photosynthetic protection from UV–B. Alternatively, large amounts of UV–B may reach sensitive photosynthetic machinery and nucleic acids leading to enhanced rates of repair. Either case should ultimately lead to a diversion of resources from growth and may result in lower productivity.

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