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Ultraviolet-B and visible light penetration into needles of two species of subalpine conifers during foliar development

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ABSTRACT

The depth of penetration of Ultraviolet-B (UV-B, 300 and 320 nm) and visible (680 nm) light was measured in foliage of *Abies lasiocarpa* and *Picea engelmannii* using a fibre-optic microprobe. Measurements were made on foliage at four times during development: needles were sampled from within expanding buds (*in bud*); within 72 h of emergence from the bud scales (*emergent*); from elongating branches (*elongating*); and from foliage that emerged the previous summer (*mature*). Light attenuation in pre-emergent needles of both species was steep and showed strong wavelength dependence. Short wavelength 300-nm light was attenuated strongly in the developing epidermal layer, but a significant proportion of this potentially damaging UV-B radiation penetrated into the mesophyll. For *A. lasiocarpa* and *P. engelmannii*, 99% attenuation of 300-nm light occurred at 51 and 96 μm, respectively, well within the mesophyll. At this stage, however, the bud scales were opaque to light below 400 nm. As the epidermal cell walls and cuticle continued to develop and chlorophyll accumulated following emergence from the bud scales, light attenuation, particularly of UV-B radiation, increased. Although no UV-B is transmitted through the epidermis-hypodermis of mature needles, small but measurable quantities of 300- and 320-nm light were measured in the photosynthetic mesophyll of post-emergent and elongating needles. Thus, shortly after emergence from the bud scales in mid-June to mid-July, when incident UV doses are highest, absorption of UV-B radiation by potentially sensitive chromophores in the mesophyll may disrupt physiological and developmental processes in these species. Soluble UV-absorbing pigments accumulated during needle maturation for *P. engelmannii* but not *A. lasiocarpa*, suggesting that, for *A. lasiocarpa* at least, the development of effective UV screening properties in the epidermis may not be related to the induction of soluble flavonoids.

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Key-words: Abies lasiocarpa; epidermis; fibre optic microprobe; foliage; light penetration; needle anatomy; ozone depletion; *Picea engelmannii*; ultraviolet-B radiation.

INTRODUCTION

Ultraviolet-B (UV-B, 280–320 nm) radiation is absorbed by a wide array of macromolecules and its ability to disrupt a number of physiological processes including photosynthesis is well established (Bornman 1989; Tevini & Teramura 1989). By selectively transmitting longer wavelengths, the leaf epidermis plays a major role in mitigating potential UV damage in the mesophyll. Reflectance of UV-B from leaf surfaces is generally low, approximately 10% of incident irradiation (Clark & Lister 1975; Gausman, Rodriguez & Escobar 1975), and most of the UV is screened within the epidermis by absorption. This absorbance is often attributed to UV-inducible flavonoids located in the vacuole of epidermal cells (Robberecht & Caldwell 1978; Robberecht, Caldwell & Billings 1980; Caldwell, Robberecht & Billings 1980). Sensitivity to UV damage is lower in species and populations from low latitudes and high elevations where annual UV-B doses are greatest, and part of this resistance is attributed to enhanced screening by the leaf epidermis (Caldwell, Robberecht & Nowak 1982).

Globally, solar UV-B radiation increases toward the equator where solar altitudes are the greatest and the stratospheric ozone column is the thinnest, and levels increase with increasing elevation (Caldwell 1968; Caldwell *et al.* 1980). A number of features contribute to high UV-B doses in montane and alpine regions. At mid-latitudes, the decrease in air mass with increasing elevation from 1500 to 3350 m above sea level contributes to a wavelength-dependent reduction of atmospheric scattering and a 32–42% increase in biologically effective UV-B radiation (Caldwell *et al.* 1980). UV-B levels are further enhanced by reflection from persistent snow and rapidly moving cumulus clouds (DeLucia, Day & Vogelmann 1991). In addition to chronically high UV doses, recent reports of an unprecedented depletion of stratospheric ozone caused by atmospheric pollutants may further increase incident UV-B radiation (Blumthaler &

Ambach 1990; Kerr 1991). Damage from UV-B radiation is a function of cumulative dose (Sisson & Caldwell 1976, 1977; Caldwell, Teramura & Tevini 1989; Tevini & Teramura 1989). The evergreen habit of high-elevation conifers, some of which maintain photosynthetically competent foliage for over 15 years, raises a question about the ability of these plants to minimize cumulative damage to the photosynthetic machinery.

Despite aspects of conifer needle morphology that may minimize UV damage (e.g. thick needles with highly developed cuticle and epidermal layers), Sullivan & Teramura (1988) found that five out of the 10 species of the *Pinaceae* surveyed were sensitive to supplemental UV-B radiation. The susceptible species were primarily in the genus *Pinus* and were from low elevation seed sources. Biomass of *Pinus taeda*, the most sensitive species, was reduced by 40% when grown for 22 weeks under supplemental UV-B radiation. Growth was actually enhanced for the subalpine species, *Picea engelmannii* and *Abies fraseri*, grown under the same conditions.

The apparent resistance of subalpine conifers to UV-B radiation results in part from the exceptional screening properties of the epidermis. Using a fibre-optic microprobe to measure the depth of penetration of light into mature (one-year-old) foliage, Day, Vogelmann & DeLucia (1992) and DeLucia *et al.* (1991) found that no measurable 300-nm light penetrates the needle epidermis. Most light does not penetrate the outer epidermal cell wall suggesting that pigments other than soluble flavonoids are responsible for UV absorption. However, the possibility that appreciable UV-B radiation may penetrate the epidermis to sensitive chromophores in the mesophyll during leaf development was not addressed. The objective of this study was to quantitate the depth of penetration of UV-B (300 and 320 nm) and visible light (680 nm) during needle development from just prior to emergence from the bud scales to maturation. These wavelengths were selected to bracket the major portion of UV-B radiation incident on foliage (300 and 320 nm) and to provide a comparison with the depth of penetration of photosynthetically active radiation (680 nm). Measurements were also made of ethanol-soluble UV-absorbing pigments to help clarify the potential contribution of soluble pigments to epidermal UV attenuation.

MATERIALS AND METHODS

Shoots of *Abies lasiocarpa* (Hook.) Nutt. and *Picea engelmannii* Parry were collected from trees in a subalpine forest at 3100 m above sea level in the Medicine Bow Mountains of Wyoming (41°21′N, 106°13′W). Trees were widely spaced and sun-branches with southern exposure were harvested from the lower third of the crown from five trees of each species. Branches were transported to the laboratory in humidified black-plastic bags where they were recut under water and stored in the dark at 4°C. Optical measurements were completed within 48 h of collection. Measurements were made on foliage four times during development beginning on 2 July 1991. Needles were sampled from within expanding buds (bud scales still completely enclosed the foliage), within 72 h of emergence from the bud scales, from elongating branches, and from foliage that had emerged the previous summer. Henceforth, developmental stages are referred to in the tables and figures as: *in bud, emergent, elongating*, and *mature*, respectively.

The penetration of 300-, 320- and 680-nm light into foliage was measured with a fibre-optic microprobe system as in Vogelmann, Bornman & Josserand (1989) and Vogelmann *et al.* (1991). Microprobes were fashioned from 125-μm diameter (OD) multimode step-index fibres made of fused silica (Polymicro Technologies, Phoenix, AZ, USA). Transmittance of fused silica fibres is uniformly high from 250 to 750 nm. While heated, fibres were drawn to a tip diameter of 5·3–9·8 μm. The tapered region of the probes were then coated with evaporated chromium and truncated with a diamond knife. Thus, light entry was confined to the tip of the probes that had near-perfect Gaussian acceptance angles (50% acceptance half width) of 27 to 34°. Photons captured by the microprobe were measured with a calibrated spectroradiometer (Model 742, Optronic Laboratories, Florida, USA), and data were captured and stored in a computer via an A/D converter. Measurements were made by clamping a needle of *A. lasiocarpa* or *P. engelmannii* between two plastic cover slips through which a small hole was drilled. A computer-controlled stepper motor (Stepper-mike Model 18515, Oriel, Stratford, CT, USA) was used to advance the microprobe through the foliage and toward the light at a rate of 6 μm s^{-1}. Depending on needle thickness, scan times ranged from 68 to 170 s. The needle was illuminated with a collimated beam from a 150-W Xenon arc lamp (Hanovia 901C-1).

For needles removed from closed buds or that had just emerged from the bud scales, the microprobe was advanced from the adaxial to the abaxial surface, and the needle was illuminated on the abaxial surface. During this stage of development, it was assumed that most light strikes the abaxial surface of the outer row of young needles. As the stem elongates proportionately more light strikes the adaxial needle surface. Thus, for needles on elongating stems and mature foliage, the probe was advanced from the abaxial toward the adaxial surface, and foliage was illuminated on the adaxial surface. The probe was advanced through the centre of the needle penetrating the vascular cylinder. Three to six scans were averaged for each developmental stage × wavelength. Fewer measurements were made on mature needles. Because of the toughness of this tissue it was not uncommon to break a probe during a measurement, most often as the probe penetrated the vascular cylinder. Light transmitted to the probe inside of foliage was expressed as the relative amount of light, calculated as

the ratio of light measured by the probe inside the tissue to light measured by the probe with no tissue (probe normal to incident light), as described in Vogelmann & Bjorn (1984).

Following the microprobe measurements fresh sections (ca. 25-μm thick) were prepared close to point of entry of the probe and foliage dimensions were measured with a calibrated ocular micrometer at 80×.

Spectral transmittance of bud scales was measured by clamping a single scale in the holder, positioning the microprobe on the adaxial surface, and illuminating the abaxial surface which collimated broad-band light. Following replicate spectral scans from 280 to 680 nm (2-nm interval), the bud scale was removed and the probe was repositioned prior to scanning the lamp. Transmittance was calculated by dividing the mean bud scan by the mean lamp scan and was expressed as 'apparent transmittance' because no corrections were made for surface reflection or scattered light.

UV-absorbing pigments were extracted from dried needle samples in 99:1 (v/v) ethanol:acetic acid under diffuse subdued light as in Flint, Jordan & Caldwell (1985). Combined samples of foliage from five branches, each from a different tree, were dried at 70°C to constant mass. Needles were ground with a mortar and pestle, subsampled (ca. 25 mg), and ground again in 2 cm^3 of the extraction medium. Samples in 5 cm^3 of extraction medium were then boiled for 6 h at 78°C and incubated overnight at 25°C. Following extraction the samples were centrifuged and the supernatant was decanted into a 10 cm^3 volumetric flask. The pellet was then resuspended in 5 cm^3 of extraction medium, centrifuged, and the supernatant was added to the volumetric flask giving a final extraction volume of 10 cm^3. Absorbance from 250 to 400 nm in 2-nm intervals was measured with a computer-controlled single-beam spectrophotometer (Ultraspec II, LKB). Absorbances were calculated relative to an ethanol-acetic acid blank and were normalized by dry mass of the tissue.

RESULTS

Needles removed from the closed but expanding buds of *A. lasiocarpa* had poorly developed cuticle, no hypodermis, and the differentiation of resin ducts had just initiated and was variable (data not shown). The thickness of the abaxial epidermis and mesophyll was 20 and 76 μm, respectively (Table 1). Light attenuation in these pre-emergent needles was steep and showed a strong wavelength dependence, with shorter wavelengths being most rapidly attenuated (Fig. 1). The 300-nm light was attenuated 50% by 20 μm and 99% by 51 μm into the abaxial needle surface (Table 2). The 320-nm light penetrated significantly deeper into the mesophyll, attaining 99% attenuation by 82 μm. Thus, appreciable levels of UV-B radiation penetrated into the mesophyll at this early stage of development. As with UV light, attenuation of 680-nm light was nonlinear (Fig. 1). However, in contrast to UV light, attenuation of 680-nm light was less steep and a significant portion was transmitted through the entire needle (Table 2). Needles at this stage were pale green in appearance.

Following emergence from the bud scales, needles became darker green, indicating an increase in chlorophyll concentration. Resin ducts also became apparent at this time. By the time the twig had begun to elongate, a well-defined hypodermal layer had formed (Table 1). Shortly after emergence from the bud scales, and at later developmental stages, differences in attenuation through the epidermis-hypodermis of 300- and 320-nm

	epid.	hypo.	mes.	vas. cyl.	mes.	hypo.	epid.	tot.
Abies ladiocarpa								
In bud	20	–*	76	199	94	–	23	411
(n=16)	(1)	–	(6)	(25)	(21)	–	(5)	(16)
Emergent	16	–	72	192	102	–	20	330
(n=16)	(4)	–	(8)	(14)	(12)	–	(5)	(17)
Elongating	25	12	134	264	139	13	27	614
(n=21)	(5)	(4)	(23)	(22)	(18)	(4)	(4)	(47)
Mature	24	16	201	300	205	23	24	793
(n=10)	(5)	(5)	(25)	(23)	(24)	(8)	(4)	(63)
Picea engelmannii								
In bud	27	–	96	188	125	–	31	466
(n=17)	(3)	–	(17)	(17)	(23)	–	(6)	(29)
Emergent	29	11	253	316	268	12	27	915
(n=12)	(2)	(3)	(35)	(17)	(41)	(4)	(5)	(54)
Elongating	28	20	259	364	256	24	25	974
(n=18)	(4)	(4)	(16)	(47)	(26)	(6)	(5)	(47)
Mature	21	26	291	348	301	26	26	1039
(n=13)	(4)	(4)	(26)	(44)	(51)	(7)	(6)	(77)

*Dashes indicate the designated tissue or cell type was not apparent.

Table 1. Thicknesses (μm) of various tissue layers during needle development of *A. lasiocarpa* and *P. engelmannii*. Developmental stages are described in the Methods. Foliage was either enclosed in the bud scales (*in bud*), emerging from the bud scales (*emergent*), expanding and on a branch that was rapidly elongating (*elongating*), or had emerged during the previous growing season (*mature*). Reading from left to right in the table are thicknesses of adaxial to abaxial tissues. The tissue layers are: epid., epidermis; hypo., hypodermis; mes., mesophyll; vas. cyl., vascular cylinder; and tot., total needle thickness. Values are means, and the standard deviations are shown in parentheses

Table 2. Depth (μm) to 50, 90 and 99% attenuation of incident light of 300, 320, and 680 nm in needles of *A. lasiocarpa* and *P. engelmannii* of different developmental stages. Developmental stages are described in the 'Methods' and in the legend for Table 1. Each value is a mean and the standard deviation is shown in parentheses. Sample sizes are the same as in Figs 1 and 2

		In bud	Emergent	Elongating	Mature
Abies lasiocarpa					
300 nm	50%	20 (18)	13 (1)	19 (8)	11 (4)
	90%	28 (5)	26 (4)	34 (7)	15 (1)
	99%	51 (9)	49 (4)	47 (7)	19 (7)
320 nm	50%	21 (6)	11 (4)	23 (11)	15 (7)
	90%	46 (18)	25 (6)	31 (12)	18 (9)
	99%	82 (22)	50 (2)	50 (10)	22 (13)
680 nm	50%	47 (15)	48 (15)	49 (11)	75 (70)
	90%	274 (57)	299 (26)	129 (69)	119 (102)
	99%	–*	–	509 (106)	203 (106)
Picea engelmannii					
300 nm	50%	11 (5)	25 (6)	27 (5)	3 (2)
	90%	41 (5)	51 (16)	68 (7)	4 (3)
	99%	96 (19)	100 (24)	124 (12)	8 (5)
320 nm	50%	16 (6)	22 (13)	36 (22)	13 (8)
	90%	62 (20)	42 (13)	67 (34)	14 (7)
	99%	141 (21)	79 (9)	112 (71)	16 (4)
680 nm	50%	59 (7)	26 (7)	57 (53)	86 (7)
	90%	257 (49)	217 (69)	256 (121)	212 (57)
	99%	–	573 (53)	745 (180)	451 (90)

*Dash indicates that $I/I_O<0{\cdot}01$.

light were no longer evident (Fig. 1). As needles began to elongate they decreased in thickness from 411 to 330 μm (Table 1). Although the absolute depth of penetration of 320-nm light was not different for emergent needles and needles still enclosed in the bud scales, and the absolute depth of penetration of 300-nm light was actually reduced, both wavelengths penetrated further into the mesophyll in emergent needles because of the transient decrease in needle thickness.

The combined thickness of the adaxial epidermis and hypodermis for needles on elongating twigs was 37 μm (Table 1). At this stage, UV-B radiation was attenuated more than 90% within these layers. However, a small amount of UV-B radiation entered the mesophyll; 99% attenuation occurred at 50 μm which was ca. 22 μm into the mesophyll. Tissue became darker green, and a dramatic decrease in penetration of 680-nm light was evident at this stage. Attenuation (90%) of visible light decreased from >270 μm at earlier developmental stages to 129 μm (Table 2).

Attenuation of UV-B radiation in one-year-old needles of *A. lasiocarpa* was striking. The relative amount of UV-B decreased by 99% within the first 22 μm; a depth within the adaxial epidermis (Fig. 1, Table 2). No measurable UV-B radiation reached potential chromophores in the mesophyll. The steepening of the visible light gradient observed for elongating needles continued, such that by one year 680-nm light was completely attenuated in the adaxial mesophyll (Fig. 1, Table 2).

Needle development had similar effects on the UV and visible light penetration in foliage of *P. engelmannii*; striking decreases in the depth of penetration of UV-B and visible light were evident as foliage matured (Fig. 2). However, the depth of penetration for all wavelengths tended to be slightly greater in *P. engelmannii* than *A. lasiocara* needles, particularly at early developmental stages. For needles still enclosed in bud scales, 300- and 320-nm light penetrated the entire mesophyll and into the vascular cylinder (Fig. 2, Table 2). The hypodermis formed earlier in development in *P. engelmannii* than *A. lasiocarpa* (Table 1), but despite the presence of this additional cell layer, significant quantities of UV-B were transmitted into the mesophyll of emergent and elongating needles. The 300- and 320-nm light was 99% attenuated at depths greater than 112 μm in elongating needles, a depth well within the mesophyll (Fig. 2, Table 2). For one-year-old foliage, however, UV-B radiation did not penetrate the epidermis of *P. engelmannii*. Attenuations of 99% of 300- and 320-nm light occurred in 8 and 16 μm, respectively (Table 2).

As was observed for *A. lasiocarpa*, during development, the decline of visible light in needles of *P. engelmannii* became progressively steeper as needles accumulated chlorophyll (Fig. 2). However, the penetration of 680-nm light was slightly greater in *P. engelmannii* than *A. lasiocarpa* needles. A small amount of visible light passed through the adaxial mesophyll and into the vascular cylinder of mature *Picea* needles (Fig. 2, Table 1).

Ethanol-acetic acid extracted pigments from needles of *A. lasiocarpa* had broad absorbance that gradually decreased from 250 to 400 nm (Fig. 3). Relative absorbance above 300 nm increased as needles emerged from the buds but then decreased in mature foliage. In contrast there was a consistent increase in UV-B (280–320 nm) absorbance by pigments extracted from needles of *P. engelmannii*. Moreover, pigments from mature *Picea* needles had a broad absorbance peak at 273 nm.

The thickness and composition of bud scales during bud expansion were highly variable for both species. As buds of *A. lasiocarpa* expanded regions of the scales became thinner and translucent. Eventually, the needles emerged through the distal portion of the bud scales. Similar changes in the bud scales were evident for *P. engelmannii*, but on emergence the distal portion of the bud scales was retained as a 'cap' on the emergent foliage. Prior to needle emergence bud scales of both species transmitted no light in the UV and blue portions of the spectrum (Fig. 3). However, even for relatively thick scales of closed buds there was significant transmittance of red light. As the bud scales thinned transmittance increased, but especially for *P. englemannii*, transmittance increased more in the red than in the blue portion of the spectrum. No UV-B penetrated bud

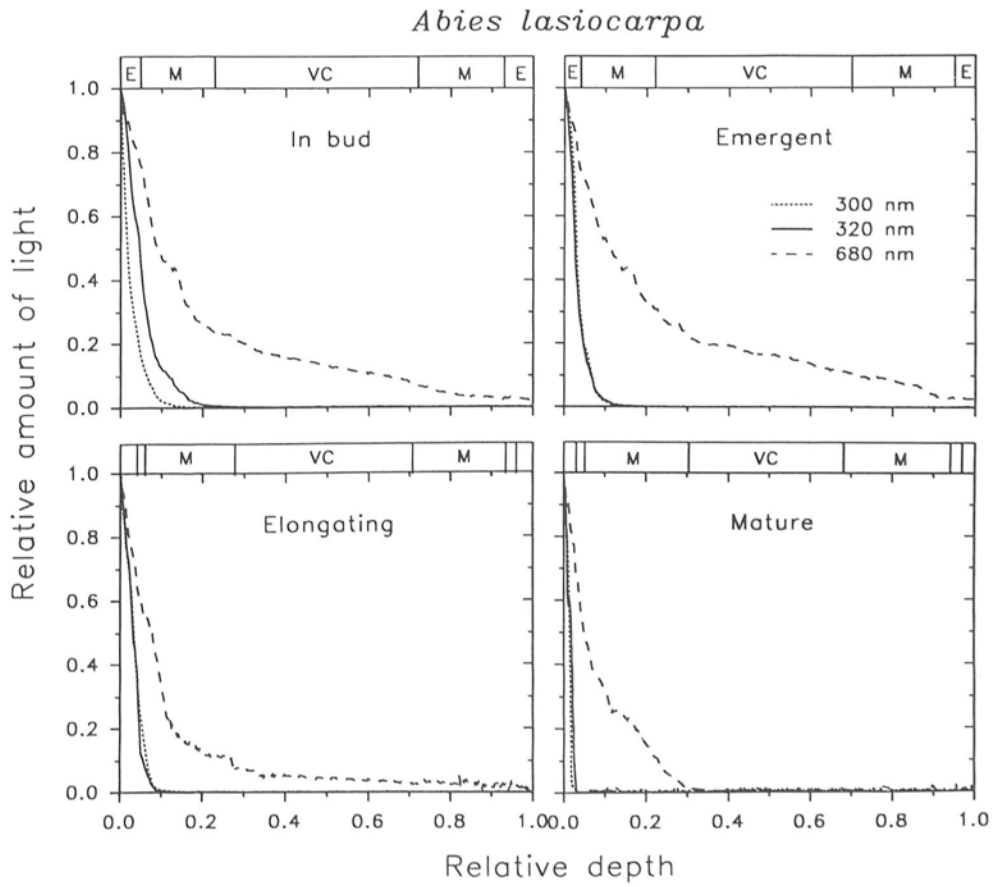

Figure 1. Penetration of UV-B and visible light into needles of *A. lasiocarpa* during development. The relative amount of light (incident/measured in needle) is shown as a function of the relative depth in the needle for 300- (short dashes), 320- (solid line), and 680-nm (long dashes) light. Foliage was either enclosed in the bud scales (*in bud*), emerging from the bud scales (*emergent*), expanding and on a branch that was rapidly elongating (*elongating*), or had emerged during the previous growing season (*mature*). The bar at the top indicates the relative thicknesses of different tissue layers. The different tissues are designated as follows: E, epidermis; M, mesophyll; VC, vascular cylinder. Where a hypodermal layer was evident, the E was omitted and the thickness of the hypodermis is shown by an additional line. Needles still in the bud scales (*in bud*) and just emerging from the bud scales (*emergent*) were illuminated on the abaxial surface, and the abaxial epidermis is on the left. Needles on elongating twigs (*elongating*) or mature needles (*mature*) were illuminated on the adaxial surface, and the adaxial epidermis is on the left. Each line is mean of six measurements, with the exception of scans of mature needles which were means of two to three measurements.

scales of *P. engelmannii* at any developmental stage. During later stages of expansion, bud scales of *A. lasiocarpa* transmitted light well into the UV-B.

DISCUSSION

The UV-screening properties of mature needles of *A. lasiocarpa* and *P. engelmannii* were exceptional. No 300- or 320-nm light penetrated the epidermal and hypodermal cell layers to reach the photosynthetic mesophyll (Table 2). DeLucia *et al.* (1991) and Day *et al.* (1992) found that, out of 11 conifer species from six genera growing on the west slope of the Rocky Mountains, no measurable UV-B radiation penetrated the epidermis of mature needles. Effective screening of UV-B by the epidermis may be an adaptive feature related to leaf longevity and the potential for accumulating large life-time doses of UV-B of 'evergreen' gymnosperms from high-light habitats. For herbaceous and woody species with mesophytic leaves, previous studies using epidermal peels (Robberecht & Caldwell 1978; Caldwell, Robberecht & Flint 1983) or direct measurement with a fibre-optic microprobe (Day *et al.* 1992; DeLucia *et al.* 1991) show a range of epidermal transmittances from less than 5% to rare instances of transmittances greater than 25%. The majority of species studied have epidermal transmittances of less than 10%, and the lowest epidermal UV-transmittances are for the sclerophyllous evergreen foliage of conifers. Low epidermal transmittance suggests that mature foliage of subalpine conifers may be resistant to damage from UV-B radiation. However, efficient screening properties of the

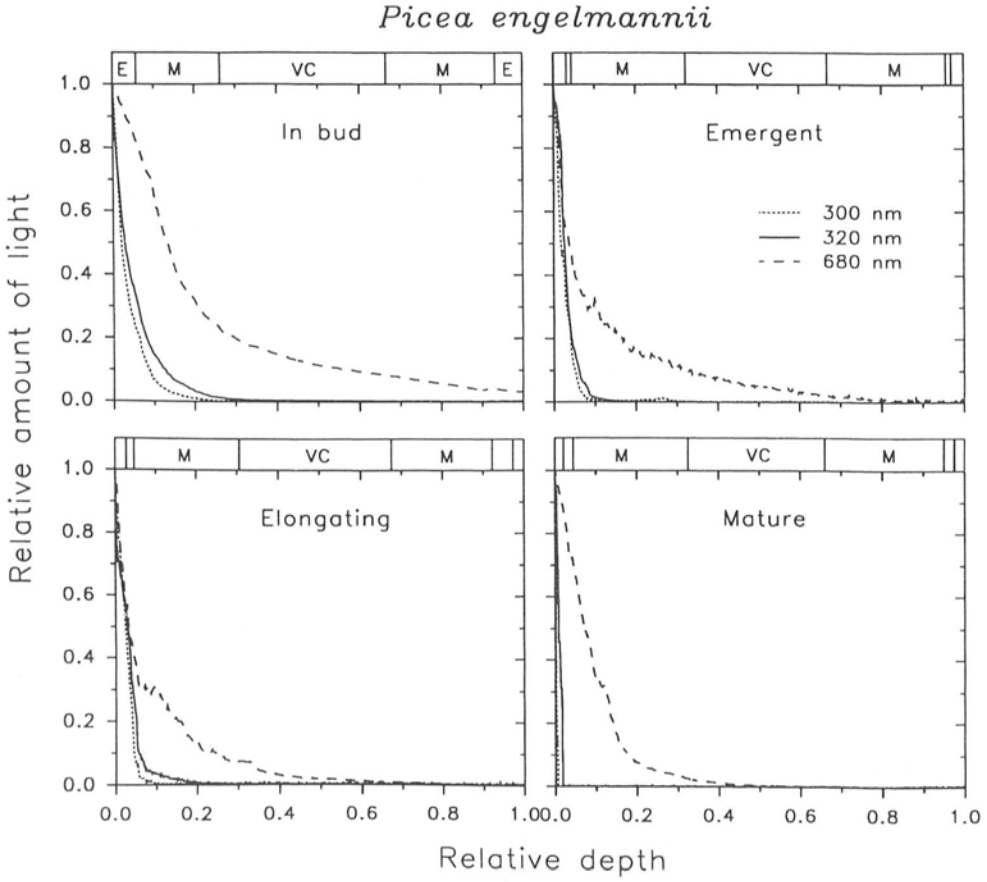

Figure 2. Penetration of UV and visible light into needles of *P. engelmannii* during development. Symbols and tissue designations are described in the legend for Fig. 1.

epidermis do not seem to be in place at earlier stages of needle development.

Significant fluxes of 300 and 320-nm light penetrated the epidermis into the mesophyll of needles still enclosed by the bud scales. At this stage, however, needles were effectively protected from the potentially damaging effects of this radiation by the bud scales. Even at late stages of bud expansion the bud scales of *A. lasiocarpa* and *P. engelmannii* attenuated light strongly below 400nm (Fig. 4). Although light transmission through bud scales varies considerably throughout the year, it typically remains low at shorter wavelengths (Pukacki & Giertych 1982). However, foliage may be vulnerable to UV-induced damage immediately following emergence from the bud scales and during needle expansion. At this time, 300- and 320-nm light was attenuated by more than 50% in the epidermis, but 90 and 99% attenuation occurred in the developing mesophyll tissue. These values may be undertestimates of the actual UV penetration because the narrow acceptance angle of the microprobes misses much of the scattered light. At high elevation sites, bud burst occurs from mid-June to mid-July, and the high solar altitude and persistent snow can contribute to high incident fluxes of UV-B radiation at this time (Day, DeLucia & Smith 1989; DeLucia *et al.* 1991). A small but measurable percentage of incident UV-B radiation was still detected in the mesophyll of maturing foliage on elongating twigs.

The production in the epidermis of soluble flavonoids, which absorb strongly in the UV-B and UV-C (wavelengths <280nm) portions of the spectrum, is purported to convey resistance to UV damage. Several key enzymes in phenylproponoid metabolism leading to the production of flavonoids are induced by UV light (Ebel & Hahlbrock 1982; Wellman 1975; McClure 1975; Wellman 1982), and resistance to UV-B is often correlated with the production of these compounds (Murali & Teramura 1986). Soluble UV-absorbing pigments account for 20–57% of the UV-B attenuation in the epidermis of herbaceous plants (Robberecht & Caldwell 1978), and pre-irradiation with low doses of UV-B to induce flavonoid production substantially decreases damage to the photosynthetic apparatus by subsequent exposure to high UV-B doses (Tevini, Braun & Fieser 1991). However, changes in penetration of UV-B into foliage of *Abies lasiocarpa* appeared to be uncoupled

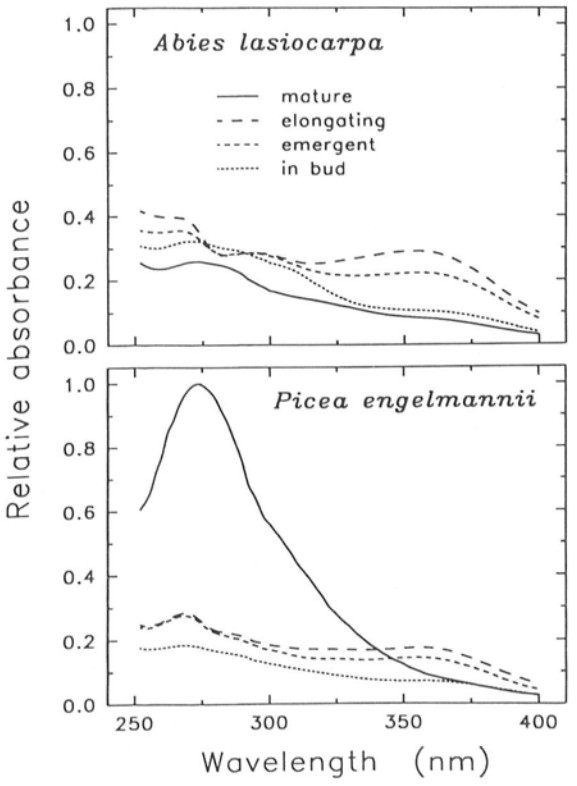

Figure 3. Relative absorbance of UV-absorbing pigments extracted (ethanol:acetic acid, 99:1 v/v) from needles at different stages of development of *A. lasiocarpa* and *P. engelmannii*. Foliage was either enclosed in the bud scales (*in bud*), emerging from the bud scales (*emergent*), expanding and on a branch that was rapidly elongating (*elongating*), or had emerged during the previous growing season (*mature*). Scans were normalized to the peak absorbance of mature *P. engelmannii* needles.

from the production of UV-absorbing pigments, and these compounds may be of secondary importance in protecting subalpine conifers from UV radiation.

The epidermis of conifers is exceptionally thick and lignified and obliterates most of the cell lumen (Esau 1977). Attenuation of UV-B is precipitous in the outer cell wall of the epidermis of *A. lasiocarpa* and *P. engelmannii* (Figs 1 & 2) and for other conifers examined (Day *et al.* 1992; DeLucia *et al.* 1991). Thus, it appears that much UV-B attenuation occurs in the epidermal cell wall of conifer needles. Unbound flavones and flavonols, with major absorption bands from 304 to 350 and 352 to 385 nm, respectively, occur in the waxy coating on leaves (Wollenweber 1982). However, neither removal of epicuticular waxes (Bornman & Vogelmann 1988) or extraction of intact needles with a variety of polar solvents (E.H. DeLucia, unpublished results) significantly increased the depth of penetration of UV-B radiation into conifer foliage. Compounds such as ferulic acid absorb strongly from 260 to 280 nm and are co-polymerized with cutin and lignin in the cell wall (Wollenweber 1982). Ferulic acid and other bound phenylproponoids may play a dominant role in UV screening in the cell wall of conifer needles. Although cell-wall-bound compounds appear important in UV-screening in conifer needles, conclusions regarding the primary structures involved in UV-B absorption based on light gradients measured with fibre optic microprobes must be treated with caution. When the microprobe penetrates and exits the epidermis, particularly in the thick and heavily lignified epidermis of conifer needles, bulging and other distortions of this tissue may occur. These distortions reduce the depth resolution necessary to identify the contribution to light absorption of cell wall versus other cellular components (Vogelmann *et al.* 1991).

In a greenhouse study with seedlings grown under supplemental UV-B radiation simulating a 40% reduction in stratospheric ozone, Sullivan & Teramura (1988) found that *P. engelmannii* and *A. fraseri* were resistant to UV-damage. Efficient screening of UV-B radiation in the epidermis, possibly by constituents in the epidermal cell wall, effectively protects sensitive chromophores in

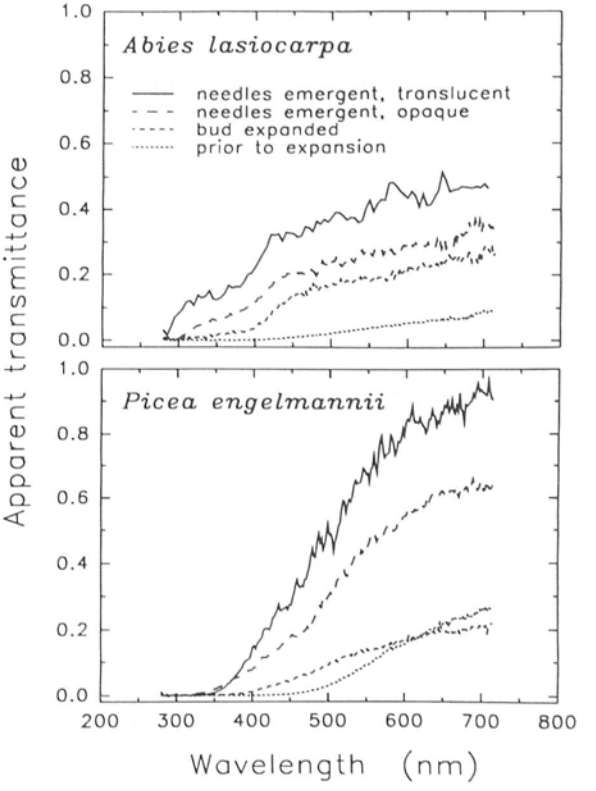

Figure 4. Apparent spectral transmittance of bud scales of *A. lasiocarpa* and *P. engelmannii* during different stages of emergence of foliage from the bud. Bud scales were illuminated on the abaxial surface, and data were not corrected for reflectance. Scans were made on bud scales prior to needle emergence (*prior to expansion*), at full bud expansion (*bud expanded*), and on translucent or opaque portions of the bud scale following needle emergence (*needles emergent, translucent* or *opaque*, respectively). Data are for representative scans.

the mesophyll of mature foliage of subalpine conifers from UV-B damage. This screening may come at a cost, however. The thick cell walls of *A. lasiocarpa* and *P. engelmannii* probably scatter a large proportion of collimated visible light incident on the foliage and significantly attenuate photosynthetically active wavelengths (in this study 680-nm light) before it is absorbed by chlorophyll (Figs 1 & 2). The question remains whether penetration of small amounts of UV-B radiation through the epidermis into the photosynthetic mesophyll during early stages of needle development will impair growth and photosynthetic competence of foliage of these species under field conditions. Significant reduction in net photosynthesis for *Oenothera stricta* exposed to enhanced levels of UV-B levels comparable to those predicted by future reductions of stratospheric ozone, occurred in spite of 95% attenuation of UV-B by the epidermis (Robberecht & Caldwell 1983).

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