UV-B radiation and acclimation in timberline plants

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More long-term field research is needed to assess the interaction of climate, soil and UV-B on timberline plants.

Abstract

Research has shown that some plants respond to enhanced UV-B radiation by producing smaller and thicker leaves, by increasing the thickness of epidermis and concentration of UV-B absorbing compounds of their surface layers and activation of the antioxidant defence system. The response of high-altitude plants to UV-B radiation in controlled conditions is often less pronounced compared to low-altitude plants, which shows that the alpine timberline plants are adapted to UV-B. These plants may have a simultaneous co-tolerance for several stress factors: acclimation or adaptation to the harsh climate can also increase tolerance to UV-B radiation, and vice versa. On the other hand, alpine timberline plants of northern latitudes may be less protected against increasing UV-B radiation than plants from more southern latitudes and higher elevations due to harsh conditions and weaker preadaptation resulting from lower UV-B radiation exposure. It is evident that more long-term experimental field research is needed in order to study the interaction of climate, soil and UV-B irradiance on the timberline plants.

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1. Introduction

The timberline ecotone is a latitudinal or altitudinal transition zone between continuous forest and treeless terrain, where trees struggle to survive by attempting to adapt their regeneration, growth forms, statures and physiological and genetic properties to the extreme conditions. Many theories and hypotheses have been suggested that climate is a determinant for the location of the timberline, or treeline, i.e. the extreme limit at which trees still achieve arboreal form and size (Stevens and Fox, 1991; AMAP, 1998; Veijola, 1998; Tuhkanen, 1999; Gervais et al., 2002). Theories also stress the significance of frosts, frost desiccation, wind and irradiance in the formation of this ecotone. In addition, topographic and edaphic factors, forest fires, plant diseases, insect infestations and human activities can be locally important (Tranquillini, 1979; Körner, 1998, 1999; Veijola, 1998; Wardle, 1998; Tuhkanen, 1999; Karlsson and Weih, 2001; Boyce et al., 2002; Vostral et al., 2002). The timberline is a heterogeneous boundary in both an ecological and a taxonomic sense, and therefore different tree species constitute the latitudinal and altitudinal timberlines in different parts of the world. Polar timberline is encountered in the Northern Hemisphere, and therefore terms like arctic or northern timberline are used. The northern timberline between the boreal...
coniferous forest and tundra zone is formed by about ten species of evergreen and deciduous conifers, especially in continental areas, and by ten broad-leaved species in oceanic areas (Apps et al., 1993; Veijola, 1998; Tuukkanen, 1999). Taxonomically the trees forming the northern timberline are different on the two sides of the Atlantic, although the habitats of the northern species and their responses to the environment are very similar. Latitudinal and altitudinal timberlines coincide in Polar Regions, so the timberlines in high latitudes can be characterized by the ecological factors of both high altitudes and arctic climates (AMAP, 1998; Veijola, 1998; Tuukkanen, 1999).

The climatic and edaphic conditions of the alpine timberline vary greatly depending on latitudinal location, topography, exposition and altitude, but in general, low temperatures, rapid temperature changes, poor soil, strong winds and extended periods of high irradiance levels are often characteristic of alpine timberline habitats. Alpine timberline vegetation is characterised by decreasing plant species diversity and abundance with increasing altitude (Körner, 1999). On the other hand, the biodiversity of the alpine timberline itself is wider than that in either of the ecosystems adjoining the ecotone. Isolated trees or small stands of trees on the alpine timberline are characterized by shapes ranging from tall and erect to short and thick trunks, to flagging crowns, and to hedge-like and prostate krummholz in response to increased exposure, and above the treeline they can often persist only as shrubs or thickets. Most importantly, alpine plant groups are of low stature or prostrate woody shrubs, graminoids such as grasses and sedges that mostly form tussocks, herbaceous perennials that often form rosettes and cushion plants of various types. In these ecosystems, plants are often adapted to harsh conditions by perennial, dwarfed, slow-growing characteristics, and by developing small and thick evergreen leaves. Cryptograms, bryophytes, lichens, algae and fungi may also play an important role (Baig and Tranquillini, 1976; Tranquillini, 1979; AMAP, 1998; Körner, 1998, 1999).

The amount of UV-B irradiance (ultraviolet-B, 280–315 nm) reaching the Earth’s surface is influenced by latitudinal gradients in total atmospheric ozone column thickness, solar zenith angles, elevation above sea level, albedo (surface reflectivity) clouds and aerosols (Blumthaler et al., 1997; Webb, 1997; AMAP, 1998; Björn et al., 1998; Gröbner et al., 2000). Due to high altitude, unshaded environments with sparsely-growing small trees and albedo from the long lasting snow cover and glaciers, irradiance levels on the alpine timberline during clear sky conditions can be high and stressful for plant life (Jokela et al., 1993; Blumthaler et al., 1997; Gröbner et al., 2000). The values for many irradiance parameters, e.g. annual global net radiation, UV-B, UV-A (ultraviolet-A, 315–400 nm), visible and PAR (photosynthetically active radiation) rise on many alpine timberlines and are associated with the zonation of the boreal and arctic vegetation (Blumthaler et al., 1997; Björn et al., 1998). The UV-B contribution to total solar radiation tends to increase with altitude because of a thinner, relatively unpolluted and more transparent atmosphere in many mountainous regions. Air pressure, i.e. the mass of air above, decreases in a vertical direction by about 125 millibar for one kilometre, which corresponds to about an 8% per km increase in PAR under summer conditions in Northern Scandinavia. For the first kilometre above sea level, the increase in biologically effective radiation is 15–20% depending on how clean the air is, and assuming there are no clouds below this elevation. However, the long-term means of UV-B radiation are strongly dampened by cloudiness, which also tends to increase with altitude (Körner, 1999). The combination of latitudinal and altitudinal trends yields maximum UV-exposure in tropical-alpine and minimum in arctic lowland plants. In arctic regions, UV-B radiation levels are lower than at temperate latitudes, but the relative ozone depletion and the relative increase in UV-B anticipated in the arctic are larger than at lower latitudes and this is probably more important for the timberline plant life than absolute radiation levels (Webb, 1997; AMAP, 1998; Björn et al., 1998). Recent overviews have been published on the research projects studying the response of terrestrial ecosystems to UV-B radiation from various climatic and geographical zones including the arctic and subarctic (Gwynn-Jones et al., 1999; Björn, 2002), grassland (Rozema et al., 1999; Campbell et al., 1999), Mediterranean (Manetas, 1999; Musil and Wand, 1999), South American (Ballare et al., 1999, 2001) and Antarctic ecosystems (Huiskes et al., 1999; Robinson et al., 2003). A number of reviews have discussed the direct and indirect effects of enhanced and ambient UV-B radiation on vascular plants (Jansen et al., 1998; Laakso and Hutton, 1998; Searles et al., 2001; Day, 2001; Day and Neale, 2002), some paying special attention to plant defence mechanisms against UV-B radiation (Jones and Hartley, 1998; Bornman, 1999; Cockell and Knowland, 1999; Meijkamp et al., 1999; Jordan, 2002). This work discusses the effects of UV-B radiation on the woody plants of the alpine timberlines, with special emphasis on the northern latitudes.1 We briefly review earlier research on UV-B transmittance and oxidative stress defence and then discuss the sensitivity of timberline plants to UV-B radiation and their possible co-tolerance with other stress factors.

1 The term “timberline” is used in a general sense to refer to the transition belt from forests to treeless vegetation, “northern timberline” referring to polar latitudinal timberline of the Northern Hemisphere and “alpine timberline” referring to timberline in accordance with increasing elevation.
2. UV-B transmittance in timberline plants

Timberline plants can avoid solar UV-B-irradiance stress by increased leaf-surface reflectance, thickness of the epidermis, the concentration and type of UV-B-absorbing compounds located in leaf epidermal cells, cuticle, waxes or hairs, by placing developing sensitive tissue below ground or enveloping it with bud scales or by phenological timing related to reproductive effort and biomass production (Caldwell, 1968; Ziska et al., 1992; Yang et al., 1995; Schnitzler et al., 1996; Lavola, 1998; Turunen et al., 1999a,b; Körner, 1999; Kinnunen et al., 2001; Jordan, 2002; Lavola et al., 2003). When UV-B radiation has penetrated through the epidermis into the photosynthesizing mesophyll, antioxidant systems (see “Oxidative stress defence”) and DNA repair processes play an important role in the defence against UV-B damage (Foyer et al., 1994; Jordan, 2002).

It has been shown that UV reflectance from the leaf surfaces depends on the optical properties of the surface, which is largely determined by the structure of the leaves, epicuticular wax and leaf hairs (Robberecht et al., 1980; Vogelmann, 1993; Holmes and Keiller, 2002). The reflectance of UV radiation from the leaf surface of most plant species studied so far has generally been below 10%, and therefore it is considered a less important defence mechanism against UV-B irradiance when compared to UV-B absorption (Caldwell, 1968; Robberecht et al., 1980; Vogelmann, 1993; Yang et al., 1995; Hoque and Remus, 1999; Holmes and Keiller, 2002). In an early work, Caldwell (1968) studied UV-A reflectance of 13 mountain and 18 alpine species in the Rocky Mountains of the United States, 1800 and 3750 m a.s.l. respectively, and found no difference between these two groups with reflectance ranging from 1.5 to 7%. In conifer species with a bluish appearance in the needles (glauccous blue due to epicuticular wax), e.g. in blue spruce (Picea pungens), UV-B radiation is reflected more effectively from the needle surface than in species with greenish appearance (Clark and Lister, 1975; Vogelmann, 1993). Reflectivity from Scots pine (Pinus sylvestris) needles was 5.5% at 315 nm, and it decreased slightly with increasing wavelength, being 3.3% at 400 nm (Hoque and Remus, 1999). Holmes and Keiller (2002) recently studied the total reflectance of ultraviolet (330 nm) and photosynthetically effective (680 nm) wavelengths for a range of different leaf types and concluded that both pubescence (presence of hairs) and glaucousness (presence of a thick epicuticular wax layer) had a marked effects on total reflectance. Glaucous leaves were very effective reflectors of both UV and longer wavelength radiation, whereas pubescent leaves tended to be more effective in reflecting longer wavelengths than ultraviolet radiation.

The epidermal transmittance of UV-B radiation correlates with plant life forms, but it is still uncertain how well it is related to UV-B-mediated damage or damage-repair mechanisms (Day et al., 1992; Sullivan et al., 1996; Bilger et al., 2001). The UV-B-transmittance of Rocky Mountain plants varied from zero to 40%, being lower in the most typical timberline plants, e.g. conifers than in herbaceous dicots. In conifer needles, UV-B transmittance has varied in the range of 0.5–2.5%, depending on the altitude, age of the needles and trees, species and measurement techniques (Day et al., 1992, 1994; Sullivan et al., 1996; Turunen et al., 1999a). It has been shown that removal of epicuticular waxes does not significantly increase UV-B transmittance into needle mesophyll (Bornman and Vogelmann, 1988; Day et al., 1992). The reason for the low UV-B-transmittance in conifer needles is that the UV-B absorbing compounds are located in vacuoles as well as within epidermal cell walls, whereas in herbaceous dicots they are located primarily in the vacuole of the epidermal cell. The two primary groups of UV-B absorbing compounds, identified as soluble flavonoids and insoluble phenyl-propanoids of hydroxycinnamic acids, both have major absorption bands in the range of 304–350 nm and 352–385 nm respectively. Although these compounds absorb UV wavelengths effectively, they transmit visible or PAR into the mesophyll cells. Soluble flavonoids can be actively and rapidly mediated by UV-B exposure whereas cell-wall bound insoluble phenyl-propanoids represent a more passive UV screening mechanism (Schnitzler et al., 1996; Hoque and Remus, 1999; Turunen et al., 1999a,b; Jordan, 2002; Lavola et al., 2003). It is also possible that UV radiation could be converted into PAR by the epidermis (Hoque and Remus, 1999).

Tables 1 and 2 show that the increased UV-B absorption of plant foliage is often related to increased altitude and that different species, and populations of the same species and genus, can exhibit wide UV-B acclimation potential (Larson et al., 1990; Ziska et al., 1992; Sullivan et al., 1992; Wand, 1995; Rau and Hofmann, 1996). For example, significant altitude-dependent relationship with the UV-B absorbance of foliage was found in Hunsruck of Rhineland Palatinate (131–816 m a.s.l.) in Germany for the wood anemone (Anemone nemorosa), a spring geophyte, but for blueberry (Vaccinium myrtillus) and Fuchs grouse (Senecio fuchsiul), which are forest under storey species, no such relationship could be seen (Neitzke, 2002). Correspondingly, in the same region, the UV-B absorbance of the sun leaves of beech (Fagus sylvatica) increased with elevation but no such relationship could be seen in shade leaves (Neitzke and Therburg, 2000). In addition, the composition of flavonoids can vary according to altitude and latitude. For example, the Scots pine populations growing at high altitudes and latitudes were rich in prodelphinidin, whilst low-altitude and low-latitude populations were rich in taxifolin.
<table>
<thead>
<tr>
<th>Location</th>
<th>Altitude (m a.s.l.)</th>
<th>Sampling time</th>
<th>Origin of material</th>
<th>Response to altitude</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nederland, Colorado, Rocky Mountains, USA (40N, 10W)</td>
<td>1800 and 3750</td>
<td>Growing season</td>
<td>UV exclusion over the alpine tundra community</td>
<td>No difference in reflectance of the species from two altitudes</td>
<td>Caldwell, 1968</td>
</tr>
<tr>
<td>Patscherkofer Mountain (near Innsbruck), Austria (47N, 11E)</td>
<td>1000–2140</td>
<td>Winter</td>
<td><em>Picea abies, Pinus cembra</em> samples collected from wind-exposed and wind-protected branches from sun-exposed side</td>
<td>Thickness of cuticle decreased at higher altitudes</td>
<td>Baig and Tranquillini, 1976</td>
</tr>
<tr>
<td>Peru (10S)</td>
<td>3000–4000</td>
<td>Spring and early summer</td>
<td>All species were collected from exposed habitats (43 species)</td>
<td>At higher latitudes, mean epidermal transmittance exceeded 5%, at low latitudes transmittance was less than 2%</td>
<td>Robberecht et al., 1980</td>
</tr>
<tr>
<td>Venezuela (10N)</td>
<td>3000–4000</td>
<td>Spring and early summer</td>
<td><em>Picea abies</em></td>
<td>No difference in thiol content, but the sulphydryl content was higher at higher altitude</td>
<td>Grill et al., 1988</td>
</tr>
<tr>
<td>Maui, Hawaii (20N)</td>
<td>2500–3000</td>
<td></td>
<td><em>Abies balsamea,</em> samples collected</td>
<td>Thickness of cuticle decreased in high altitude</td>
<td>DeLucia and Berlyn, 1983</td>
</tr>
<tr>
<td>Barrow and Atkasook, Alaska (71N)</td>
<td>2</td>
<td></td>
<td><em>Picea abies</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mt. Mooselauke (44N, 71E)</td>
<td>732–1455</td>
<td>Winter</td>
<td><em>Abies balsamea,</em> samples collected</td>
<td>Thickness of cuticle decreased in high altitude</td>
<td></td>
</tr>
<tr>
<td>Melcheben, Stubalpe (47N, 14E) and Tullnerbach near Vienna (48N, 16E)</td>
<td>400–1700</td>
<td>January and February</td>
<td><em>Picea abies</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Most northern location above Polar Circle in Sweden and most southern in Sierra Nevada Spain</td>
<td>Survey from different locations from 170 to 1700</td>
<td>Not mentioned</td>
<td><em>Pinus sylvestris</em> needles collected from nature or from plantations</td>
<td>Two flavonoid chemomorphs were found. The high altitude or latitude populations were almost entirely made up prodelphinidin at low altitude exhibits o-dihydroxylated flavonoids (quercetin and taxifolin)</td>
<td>Laracine-Pittet and Lebreton, 1988</td>
</tr>
<tr>
<td>Southern Appalachian Mountains, USA (37N, 80E)</td>
<td>Not mentioned</td>
<td>Plants potted in early spring</td>
<td>Phytotron studies on species from NE and SW slopes. <em>Rhododendron maximum</em> (valley), <em>R. periclymenoides</em> (NE slopes), <em>Kalmia latifolia</em> (SW slopes)</td>
<td><em>R. maximum</em> was the most sensitive to high radiation. Both <em>R. periclymenoides</em> and <em>Kalmia latifolia</em> improved their photosynthetic performance at high irradiance</td>
<td>Lipscomb and Nilsen, 1990</td>
</tr>
<tr>
<td>Mt. Mooselauke (44N, 71E)</td>
<td>760–1140</td>
<td>March 1989</td>
<td><em>Picea rubens,</em> samples collected from east and west sides</td>
<td>Cuticular resistance to water loss decreased with elevation. Sun-exposed shoots lost more water than shaded shoots</td>
<td>Herrick and Friedland, 1991</td>
</tr>
<tr>
<td>Wank mountain in the Bavarian Calcareous Alps</td>
<td>870–1700</td>
<td>May, mid-June, mid-September, early November and January</td>
<td>90 to 140-years-old <em>Picea abies</em></td>
<td>Ascorbate, GSH, GR, SOD levels were higher in needles obtained from high altitude</td>
<td>Polle and Rennenberg, 1992</td>
</tr>
<tr>
<td>Kleinrivier Mountains South Africa (34S, 19E)</td>
<td>100–824</td>
<td>From February towards the end of the summer</td>
<td>Sun and shade leaves collected from 38 species representing 17 families</td>
<td>UV-B absorbance (280 to 320 nm) was higher in leaves collected from high elevation</td>
<td>Wand, 1995</td>
</tr>
</tbody>
</table>
In addition, two other studies have reported significant differences in the characteristics of UV absorbing compounds among *Pinus* and *Betula* populations of different geographical origins (Kaundun et al., 1998; Lavola, 1998).

**3. Oxidative stress defence**

Plants normally increase several components of the antioxidative system simultaneously in response to naturally occurring stresses such as stress at high altitude, chilling, drought, or nutrient deficiencies (Polle and Rennenberg, 1994; Tausz et al., 2003) (Table 1). However, there is no consensus about whether different stresses that generate active oxygen species (AOS) would cause a similar activation of AOS scavenging systems (Rao et al., 1996). Recently, Tegischer et al. (2002) found that the needles of mature trees had less photoprotective potential, but higher antioxidative potentials when compared with seedlings and saplings. A decreased capacity for photoprotection might impose a higher oxidative load on the radical scavenger systems because the production rate of AOS would increase and it must be counteracted with increased antioxidative defence involving glutathione, ascorbate and tocopherols (Tegischer et al., 2002).

In conifers, the glutathione (GSH) levels follow the diurnal and seasonal cycle (Polle and Rennenberg, 1994; Wildi and Lutz, 1996). A light-dependent increase in the GSH concentration of Norway spruce needles causes a diurnal cycle with high concentrations during the day and low concentrations at night (Schupp and Rennenberg, 1988). High wintertime levels of GSH (Esterbauer and Grill, 1978; Schupp and Rennenberg, 1988; Polle and Rennenberg, 1992) could mean that glutathione plays a role in the winter hardness of the foliage of evergreen plant by protecting it against winter injury. Thus, in the colder environment on the timberline, glutathione may have an even more important role in protection against the harsh climate. Streb et al. (1998) indicated that the leaves of high-mountain plants were highly resistant to photoinhibitory damage at low temperature and exhibited divergent strategies of photosynthetic adaptation. This could be due to higher levels of antioxidants observed in high altitude plants. For example, Norway spruces growing at high altitude have higher levels of GSH and higher glutathione reductase (GR) activities compared to ones growing at low altitudes (Grill et al., 1988; Polle and Rennenberg, 1992). The high levels of GSH and the high GR activity during periods of high light intensity around noon and at high altitudes could protect plants against UV-B radiation (Bielawski and Joy, 1986; Noctor et al., 1997). On the other hand, a low background UV-B level might stimulate the antioxidative systems and thereby mediate
<table>
<thead>
<tr>
<th>Location</th>
<th>Altitude (m a.s.l.)</th>
<th>Species and origin of material</th>
<th>Response</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>North Carolina, Oregon, Florida, Washington, USA</td>
<td>0–1524</td>
<td>Seven species of seed-grown conifer seedlings of high, intermediate and low altitude exposed to UV-B (0, 2.34, 4.78, 7.17, 9.56 W m⁻², 11 wk) in phytotron</td>
<td>Biomass production of high altitude species were least influenced by UV-B radiation</td>
<td>Kossuth and Biggs, 1980</td>
</tr>
<tr>
<td>36–41N (WY, NC, MD, USA)</td>
<td>30–3000</td>
<td>Ten species of seed-grown conifer seedlings of high, intermediate and low altitude exposed to UV-B (0, 12.4, 19.1 kJ m⁻², 22 wk) in greenhouse</td>
<td>Effects of supplemental UV-B dose were less for growth and stress symptoms of species native to higher elevations</td>
<td>Sullivan and Teramura, 1988</td>
</tr>
<tr>
<td>Not mentioned, probably Urbana, Illinois, USA</td>
<td>Alpine and non-alpine region</td>
<td>Seed-grown seedlings of alpine (<em>Aquilegia caerulea</em>) and non-alpine (<em>Aquilegia canadensis</em>) species of Colombine exposed to UV-B (19 kJ m⁻², 14 wk) in controlled conditions</td>
<td>Height growth was inhibited more in non-alpine species while the number of leaves increased faster in alpine species. No UV-B effects in photosynthetic capacity and stomatal conductance was found, but flavonoid contents increased</td>
<td>Larson et al., 1990</td>
</tr>
<tr>
<td>20N Maui, Hawaii, USA</td>
<td>0–3000</td>
<td>Seeds of 33 species collected, germinated and grown in greenhouse, 0, 15.5 or 23.1 kJ m⁻² UV-B for 12 wk</td>
<td>Large differences between species, but in general, high elevation species showed smaller response in plant height and biomass</td>
<td>Sullivan et al., 1992</td>
</tr>
<tr>
<td>20N Maui, Hawaii, USA</td>
<td>0–3000</td>
<td>Seeds of 33 species were collected, germinated and grown in greenhouse, 0, 15.5 or 23.1 kJ m⁻² UV-B for 12 weeks</td>
<td>UV-B resulted in e.g. earlier reproductive effort and increased photosynthesis in plants from high elevations. Increases in UV-B absorbing compounds for low elevation species, but not for high elevation species which had larger amounts even in the absence of UV-B</td>
<td>Ziska et al., 1992</td>
</tr>
<tr>
<td>52 N 4E Hoogezeand, Netherlands</td>
<td>0–2</td>
<td>Seed-grown <em>Silene vulgaris</em> seedlings and mature plants exposed to UV-B in greenhouse (0, 10, 16.2 kJ m⁻² UV-B, 18–20 d, two experiments)</td>
<td>Growth rates, gas exchange rates, transpiration and biochemical parameters did not show a difference in the response to enhanced UV-B</td>
<td>Van de Staaij et al., 1995</td>
</tr>
<tr>
<td>47N 15E Vorarlberg, Austria</td>
<td>1600</td>
<td>Seed-grown <em>Silene vulgaris</em> seedlings and mature plants exposed to UV-B in greenhouse (0, 6, 16.2 kJ m⁻² UV-B)</td>
<td>Lowland population showed a decrease in the number of seed producing flowers and the number of seeds per plant under elevated UV-B. Individual seed mass, seed germination percentages were unaffected</td>
<td>Van de Staaij et al., 1997</td>
</tr>
<tr>
<td>European Alps</td>
<td>&gt; 1200</td>
<td>Seed-grown plants from species pairs or triplets of 5 genera grown with (150 mW m⁻²) or without UV-B, then exposed to UV-B (600, 1000 mW m⁻²) 15 h d⁻¹ in growth chambers, many experiments during 3 yrs</td>
<td>In 2 genera the alpine species exhibited a better adaptation to UV-B pre-irrigation</td>
<td>Rau and Hofmann, 1996</td>
</tr>
<tr>
<td>Rocky Mountains, SE Wyoming, USA</td>
<td>2488</td>
<td>Potted seedlings (2488 m a.s.l) and natural field saplings (3567 m a.s.l.) of loddpole pine (<em>Pinus contorta</em> ssp. <em>latifolia</em>) grown under UV-exclusion 10 and 13 wk, respectively</td>
<td>No treatment effects in epidermal transmittance for naturally grown saplings at 3567 elevation</td>
<td>Turunen et al., 1999a</td>
</tr>
</tbody>
</table>
enhanced cross-tolerance to other environmental stress factors (Polle, 1997).

More attention has been paid over the past five years to the effects of UV-B radiation on oxidative stress as well as to the role of phenylpropanoids as antioxidants in plants. UV-B radiation may produce such extreme stress in terms of active oxygen production that the local antioxidative systems are overwhelmed (Foyer et al., 1994). In laboratories, UV-B exposure can increase the activity of several oxidative stress defence systems, but the effect is less clear under field conditions (Day, 2001; Tausz et al., 2003). Enhanced UV-B exposure has increased oxidative stress in crop plants (Takeuchi et al., 1996; Dai et al., 1997; Kalbin et al., 1997), herbaceous plants (Hideg and Vass, 1996; Rao et al., 1996; Hideg et al., 1997) and aquatic organisms (Malanga and Puntarulo, 1995; Jansen et al., 1996) in laboratories. A phytochamber study on the interactive effects of ozone (O₃) and UV-B on oxidative stress in Norway spruce and Scots pine (Pinus sylvestris) indicated that pine needles exposed to UV-B and elevated O₃ levels had elevated lipid peroxidation and a 5-fold increase in dehydroascorbate, suggesting that pine was less protected and suffered greater oxidative stress than spruce. UV-B radiation with ambient O₃ levels increased the total superoxide dismutase activity in spruce, but no changes in superoxide dismutase, ascorbate, glutathione or peroxidase activities were seen in pine (Baumbusch et al., 1998). As shown by several authors (i.e. Mirecki and Teramura, 1984; Flint et al., 1985; Cen and Bornman, 1990; Fernbach and Mohr, 1992; Jordan et al., 1992; Mackerness et al., 1996) the effects of UV-B in laboratory experiments are more pronounced and not relevant to natural environments.

A long-term UV-B field experiment was established in Oulu, Northern Finland to study more realistic responses of the oxidative stress of mature Scots pines to UV-B radiation. In this study, UV-B stress was observed in the two-year-old needles of mature Scots pines as a degradation of total glutathione, and a bigger proportion of oxidized glutathione in July during the third UV-B exposure season. In current-year needles, no increase in oxidized glutathione was seen in either the first or the third season. After the third experimental season in September, the total glutathione levels decreased in UV-B-treated current-year needles. These results suggested that the effect of UV-B on the oxidative stress of mature Scots pines could be duration-dependent and cumulative (Laakso et al., 2001). This is consistent with previous short-term experiments on Norway spruce and Scots pine needles (Baumbusch et al., 1998) and pear shoots (Predieri et al., 1995), which did not show any UV-B effects on glutathione status. No evidence for cumulative UV-B effects on oxidative stress was found in the deciduous dwarf shrub bilberry (Vaccinium myrtillus L.) after seven
growing seasons of UV-B exposure at Abisko, Swedish Lapland, where it was shown that there was scarcely any response in the oxidative stress in ascorbate and glutathione concentrations, and activities of the closely related enzymes ascorbate peroxidase and glutathione reductase (Taulavuori et al., 1998). More field studies are needed to find out the long-term effects of UV-B radiation on oxidative stress of the plants, particularly in the stressful environment of the timberline.

4. Is UV-B radiation stressful to timberline plants?

A meta-analysis of 62 field-based research papers published over 20 years indicated that the effects of enhanced UV-B were most apparent for UV-B absorbing compounds, with an increase in concentration of approximately 10% across all studies when comparing ambient solar UV-B control to treatment (ambient UV-B plus supplemental UV-B), but little or no response was found for plant height and leaf mass per area (Searles et al., 2001). Another recent meta-analysis reviewed 36 Arctic field studies, and concluded that Arctic plants are not affected in the short term by increases in UV-B radiation — instead, temperature elevation, increases in nutrient availability and major decreases in light availability were more meaningful for plant-growth and nutrient cycling (Dormann and Woodin, 2002). However, how will timberline plants react to increasing UV-B radiation? It has been shown that natural plant populations exhibit wide variation in UV-B radiation sensitivity, which is related to the ambient UV environment in which these plants grow and therefore reflects the long-term adaptation of plants to radiation conditions (Larson et al., 1990; Sullivan et al., 1992; Van de Staaij et al., 1997).

Table 2 indicates that enhanced UV-B radiation in controlled conditions has resulted in less pronounced effects of UV-B irradiance on high-altitude plant species versus low-altitude species, which shows that alpine plants are adapted to higher UV-B levels. This was shown by Larson et al. (1990), who exposed seedlings of alpine (Aquilegia caerulea) and non-alpine (Aquilegia canadensis) species of Aquilegia to enhanced UV-B radiation in controlled conditions. They found that height growth was inhibited in all non-alpine species while the number of leaves increased faster in alpine species. No UV-B effects in photosynthetic capacity and stomatal conductance were found, but flavonoid contents increased. Sullivan et al. (1992) collected seeds of 33 species along a 3000 m a.s.l. altitudinal gradient in Hawaii, exposed seedlings to UV-B (15.5 or 23.1 kJm$^{-2}$ for 12 weeks weighted with the generalized plant response action spectrum) in controlled conditions, and found that the response to UV-B varied among species. In general, high elevation provenances again showed smaller response in plant height and biomass.

The response of lodgepole pine (Pinus contorta ssp. latifolia Engelm.) needles to natural sunlight was studied by employing two different UV-exclusion experiments at high elevation field sites in the Rocky Mountains (potted seedlings at 2488 m a.s.l, and natural field saplings at 3567 m a.s.l.). No differences among treatments were found in epidermal transmittance (<2.5%) for naturally-grown saplings at 3567 elevation (Turunen et al., 1999a), which agrees with earlier research reporting an increase in UV-B absorbing compounds after exposure to supplemental UV-B radiation in plants grown at low elevation but not in those grown at high elevation (Larson et al., 1990; Sullivan et al., 1992; Van de Staaij et al., 1997).

Probably the most stressful time for the timberline plants is the spring, when the solar radiation dose, and particularly the UV dose received by conifers and alpine plants emerging from the snow cover, may be particularly high. The plants may experience strong, but often short radiation stress at the start of their growing season due to reflectance of irradiance from the surrounding snow cover (albedo) and are therefore exposed to the direct effects of UV-B radiation. UV-B albedos for most ground covers are <0.1, but much higher values can be encountered for snow, typically 0.8 for fresh and dry snow, and lower values for old, wet and dirty snow. On a clear day, new snow can reflect over 90% of all the incoming UV radiation on vertical surfaces, and thin cloud cover can cause the multiple reflections of UV rays between snow and clouds, increasing the UV dose in all directions (DeLucia et al., 1991; Jokela et al., 1993; Björn et al., 1998; Körner, 1999; Gröbner et al., 2000). Within two days, or within a few hours, when snow melts, plant tissue may become exposed to high intensities of solar radiation. It is likely that mature and hardened evergreen conifers and alpine plants can defend their photosynthesis from direct radiation stress by increasing concentrations of compounds absorbing visible UV-A and/or UV-B radiation, or by increasing the thickness of their leaves. Recently, Shi et al. (2004) exposed Saussurea superba and Gentiana straminea to UV-B$_{0.25}$ radiation for one growing season (15.80 kJm$^{-2}$ d$^{-1}$), at an alpine meadow on the Qinghai-Tibet Plateau in China (3200 m a.s.l) and found evidence that the increase in leaf thickness in both species could compensate for the photodestruction of photosynthetic pigments when light passes through the leaf.

It is not known, however, if still dormant and/or premature tissue with undeveloped epidermis, cuticle or epicuticular wax, due to a short and cold growing season, suddenly released from snow, can defend against radiation stress. During the spring, undeveloped epidermal cells in expanding conifer needles are not sufficiently able to absorb UV radiation and it can therefore
penetrate into the mesophyll (DeLucia et al., 1992). The fascicular sheath protects the developing needle but it probably does not block all gas exchange and the penetration of PAR and UV-B radiation (Chabot and Chabot, 1975; Dvora´k and Stokrova´, 1993). It has been suggested that mesophyll cells may become photosynthetically active while they are still within the fascicular sheaths, since the chlorophyll (a + b) content was high and the necessary gas exchange was possible due to the well-developed stomatal complexes (Chabot and Chabot, 1975; Dvora´k and Stokrova´, 1993). Thus, particularly in emerging and expanding leaves, increased penetration of UV-B radiation into the mesophyll may disrupt physiological and developmental processes (DeLucia et al., 1992).

5. Co-tolerance for several stress factors?

It is interesting to speculate whether structural and physiological acclimation or adaptation to the harsh climate, particularly drought and low temperatures, and poor soil also provide protection against increasing UV-B radiation. On the other hand, are the plants in the northern timberline environment more sensitive to UV-B radiation due to harsh conditions and weaker preadaptation due to naturally lower UV-B radiation compared to plants from more southern latitudes and elevations? And vice versa: does defence against increased UV-B radiation, such as thicker epidermal layer and cutin, increased concentration of UV-B absorbing compounds in the epidermal cells, and higher levels of glutathione (Laakso et al., 2000, 2001; Turunen et al., 1999a,b; Kinnunen et al., 2001), increase the plants’ tolerance against another environmental stresses such as drought and low temperatures? Most probably all these statements are true.

Many xerophytic plants with thick leaves and an epidermal cell layer are adapted to both water stress and high irradiance. It has been shown that many drought tolerators are generally resistant to UV-B damage and that enhanced UV-B radiation can induce xeromorphic characteristics, such as a thicker cutin layer, in northern conifers (Laakso et al., 2000) or in Mediterranean species (Manetas, 1999). Latola et al. (2001) found that increased UV-B decreased the cross-sectional needle area of fully-grown fascicle needles of Scots pines. Since no decrease in needle length was seen, it was suggested that the smaller cross-sectional area was due to the more xeromorphic structure of UV-B-treated needles. In primary needles of Scots pine seedlings, UV-B increased the thickness of the cutin layer and the outer and inner periclinal walls and the anticlinal wall thickened to fill cell lumen, which are common features in xeromorphic needles (Laakso et al., 2000). Thus, the xeromorphic structure might give better protection also against UV-B radiation and on the other hand, the enhanced UV-B radiation may cause a more xeromorphic structure in evergreens.

There are several other examples of the existing co-tolerance to UV-B radiation and drought, UV-B radiation, and low temperatures. The fact that UV-B radiation can act as an environmental signal to induce tolerance to high light and drought stress in Douglas fir (Pseudotsuga menziesii) seedlings was demonstrated by Poulson et al. (2002). They investigated the effects of an ambient dose of UV-B radiation on chamber-grown seedlings and found that the presence of ambient UV-B radiation in the growth light regime induced the production of UV-B absorbing pigments and that the seedlings had 30% fewer stomata per unit leaf area compared with control seedlings. Reduced stomatal frequency in response to UV-B radiation may limit water loss by seedlings and/or decrease the potential for leaf cooling through evapotranspiration. UV-B radiation has also been reported to increase cold tolerance in the plant species typical to timberline regions (Dunning et al., 1994; Mendez et al., 1999). Both cold stress and UV-B exposure are known to increase synthesis and the accumulation of phenolic compounds (Bäck et al., 1993; Jones and Hartley, 1998) and, for example, increased anthocyanin concentrations may play a role in protection against photoinhibition, UV-B radiation, desiccation and freezing resistance (Chalker-Scott, 1999; Hoch et al., 2001).

Timberline plants may be poorly protected against UV-B radiation due to the harsh climate. Table 1 shows that, depending on microclimatic and edaphic conditions, cuticle thickness, the amount of epicuticular waxes and wettability of alpine timberline plants, particularly conifer needles, either decrease, increase or remain unchanged with increasing altitude. A decrease in cuticle thickness with increasing altitude has been reported for Balsam fir (Abies balsamea) Norway spruce (Picea abies) and Stone pine (Pinus cembra). Gunthardt and Wanner (1982) found an increase in the total amount of waxes in high-elevation Stone pine and Norway spruce trees and Sase et al. (1998) showed a similar trend in Japanese-cedar (Cryptomeria japonica). On the other hand, Anfodillo et al. (2002) studied minimum cuticular conductance and cuticle features of Norway spruce and Stone pine needles along an altitudinal gradient of 1050–2170 m a.s.l. of the Dolomites (NE Italian Alps), where the treeline is seldom subjected to strong winds. They found no effect of altitude in minimum cuticular conductance in either species. In this study, the wettability of the needle surface was lower in high-altitude needles than it was in low-altitude needles, the cuticle was thicker in needles of high-altitude trees than in needles of low-altitude trees, and there was no correlation between minimum cuticular conductance and cuticle thickness, meaning that the desiccation resistance did not decrease with altitude in either species.
Short and cool growing seasons may delay the development of protective epidermis, cuticles and the epicuticular wax of evergreen foliage (Turunen and Huttunen, 1990, 1991, 1996; Kinnunen et al., 2001), which may then contain less UV-B-absorbing compounds, and at the same time, low temperatures may slow down the enzymatic and non-enzymatic UV-B repair processes (Foyer et al., 1994). This may predispose timberline plants to additional stresses particularly during the early spring, such as photoinhibition due to high irradiance levels and winter desiccation (Tranquillini, 1979; Strand and Öquist, 1985; Ottander and Öquist, 1991). Classical studies in Europe and the USA have showed that the role of protective surface layers in woody plants may be vitally important in alpine timberline conditions. The decreased cuticular transport of water vapour in conifer needles due to the reduced allocation of carbon for the development of cuticle increased the risk of winter desiccation in the Alps of Central Europe (Baig and Tranquillini, 1976; Tranquillini, 1979), and direct mechanical damage to the surface layers and stomatal dysfunction due to windblown snow and ice contributed more to conifer winter deaths in the alpine treeline in Scotland (Grace, 1990) and the Rocky Mountains of the United States (Hadley and Smith, 1990). Thin cuticles and the low amount of wax in timberline plants could also mean less protection against UV-B radiation. Epicuticular wax itself may not play a major role in the UV-B screening of the timberline plants, however. The cuticular layer has been found to thicken due to increased UV-B radiation, thus protecting photosynthetic tissue, mesophyll, against harmful radiation. In Scots pine for example, both the well-developed wax layer and thick epidermis prevent UV-B penetration. Kinnunen et al. (2001) found that young developing Scots pine needles with undeveloped epicuticular waxes had a higher amount of UV-absorbing compounds than fully-grown needles with well-developed wax indicating that the defence mechanisms vary with the needle age. This shows that if one protection mechanism is unable to function then other mechanisms take over.

6. Conclusions

It has been shown that many plants respond to enhanced UV-B radiation by producing smaller and thicker leaves, by increasing the thickness of the cutin layer and the epidermal wall, and by increasing concentrations of UV-B absorbing compounds in the epidermal cells, waxes and leaf hairs, and activation of the antioxidant defence system. Alpine timberline plants may have a simultaneous co-tolerance for several stress factors, such as drought, high UV-B, UV-A, PAR or low temperatures. On the other hand, they may be worst protected against increasing UV-B radiation than plants from southern latitudes and higher elevations due to harsh conditions and weaker preadaptation resulting from comparatively lower UV-B radiation exposure. This review suggests that more long-term experimental field research is needed in order to study the interaction of harsh climate, poor soil and UV-B irradiance on the ecophysiology and defence mechanisms of timberline plants.

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References


