SYMBIOSIS (2007) 43, 143–150 ISSN 0334-5114

Solar radiation screening in usnic acid-containing cortices of the lichen *Nephroma arcticum*

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(Received May 9, 2007; Accepted October 1, 2007)

Abstract

Three *Nephroma arcticum* populations were characterized with respect to cortical transmittance spectra and content of UV-B absorbing secondary compounds. Along a natural shade-sun gradient, the lichen acclimates by decreasing not only cortical UV-B transmittance, but also PAR-transmittance, from highly shaded lowland boreal *Picea abies* forests to open alpine/sub-alpine sites. The cortical UV-and PAR-transmittance decreases with increased concentration of the cortical dibenzofurane usnic acid and increased thickness of the upper cortex. Usnic acid provides solar radiation screening at the photobiont level. The presence of usnic acid as tiny crystals screens significant amounts of PAR, and reduces the UV-screening relative to the screening efficiency for the compound in the dissolved state. The UV-B absorbing medullary depsides nephroarctin and phenarctin showed highest concentration in the most shaded forests, consistent with functional roles other than sun-screening.

Keywords: Cortical transmittance, dibenzofurane, lichenized fungi, secondary compounds, UV-B

1. Introduction

A lichen is a symbiotic association between a mycobiont and a photobiont (green alga and/or cyanobacterium). The photobiont feeds both partners with organic carbon (as reviewed by Palmqvist, 2000). The mycobiont partner, encrusted by tiny crystals of secondary fungal compounds, encloses the symbiotic association, and frames the entire association with structural tissues facilitating water- and nutrient acquisition. Many secondary compounds have a high capacity for absorption of ultraviolet (UV) radiation (Huneck and Yoshimura, 1996; Hidalgo et al., 2002), and thus are considered to render protection against harmful levels of solar ultraviolet-B (UV-B) radiation (as reviewed by Rikkinen, 1995; Huneck, 1999). The various lichen substances have also been attributed biological functions other than solar radiation protection (e.g. Vicente, 1991; Fahselt, 1994; Boustie and Grube, 2005). The common faintly yellow UV-absorbing dibenzofuran, usnic acid, has for instance a high inhibitory effect on the activity of microfungi, bacteria, viruses, insect herbivores and even plants (Solhaug et al., 1995; Ingolfsdottir, 2002). Ecologically speaking usnic acid, with a carbonyl-orthohydroxyl chromophore unit is also interesting because it strongly absorbs in both the UV-B and UV-A regions (Quilhot et al., 1994; Rancan et al., 2002). This cortical compound correlates with light intensity (Rundel, 1969). Bjerke et al. (2002) and Buffoni-Hall et al. (2002) found positive correlations between UV-B radiation and usnic acid concentrations in *Flavocetraria nivalis* and *Cladonia arbuscula* ssp. *mitis*, respectively. On the other hand, BeGora and Fahselt (2001a; 2001b) and Bjerke et al. (2005) found that usnic acid levels dropped with exposure to UV-B. Therefore, studies carried out to date have been inconclusive regarding the relationship between usnic acid concentrations and ultraviolet radiation levels.

Nephroma arcticum (L.) Torss. is an usnic acid-containing lichen with the green alga *Coccomyxa* as the main photobiont and cyanobacteria localised in scattered cephalodia. This large foliose lichen is common on soils and rocks, particularly in northern forests, but also in mountainous areas above the tree line. Alpine populations are covered by snow during winter, but exposed to the sun with high ambient UV irradiation during summer.

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In the lowland near its southern distribution limit it is often restricted to shaded N-facing positions in old *Picea abies* forests (Hasselrot, 1953). Such wide ecological amplitude with respect to solar exposition makes this species suited for studying relationships between solar exposition and photoprotective traits. Furthermore, reflectance measurements of photosynthetically active radiation (PAR) and near infrared radiation suggested habitat-specific spectral characteristics of this lichen (Gauslaa, 1984).

The objective of this study is to examine the role played by usnic acid in the prevention of UV- and PAR transmittance through the upper cortex of *N. arcticum*. Little is known about the UV- and PAR screening capacity of usnic acid-containing cortices, which may influence significant processes like light use efficiency in photosynthesis, as well as survival success under periods of extreme solar radiation. There are some measurements of internal UV-levels in the usnic acid-containing *Cladina arbuscula* ssp. *mitis* (Buffoni-Hall et al., 2002), but this fruticose species with complex three-dimensional structures producing internal shade lacks a cortical layer. In most lichens with an usnic acid-containing upper cortex, the cortex is too brittle to allow measurements, which is not the case for *N. arcticum*. Specifically, we aim to study spectral characteristics of transmitted UV and PAR reaching the upper part of the photobiont level in thalli sampled in habitats ranking from shaded boreal spruce forests to exposed sub-alpine and alpine habitats, and to relate transmittance to contents of UV-absorbing secondary compounds and thickness of the upper cortex.

To evaluate the screening role of usnic acid, our final objective is to study UV and PAR transmittance through a cover of usnic acid crystals. This is necessary because secondary lichen compounds in desiccated as well as hydrated thalli occur as crystals on the outer wall of fungal hyphae, whereas absorbance spectra are measured for compounds in solution.

2. Lichen Material

Many separate thalli were collected in each of three localities. After sampling, the lichens were air-dried and stored in the dark at –20°C for further analyses.

Alpine population

Thalli were sampled on the ground among bryophytes in a sun-exposed SE-facing site with no shading vegetation around. The collection sites were situated in E. Norway, Gausdal, Snuen, NN 417816, 1140 m, 16 June 2000. The habitat was the most extreme with respect to sun exposure. Thalli were sterile and small as is often the case in such locations (Sonesson et al., 1992).

Sub-alpine population

Sampled thalli grew in bryophyte carpets (mainly *Hylocomium splendens)* on the ground in NE-facing *Betula pubescens* forests with low and open canopy of scattered trees, E. Norway, Gausdal, Tortjørnhaugen, NN 339889, 910 m, 16 June 2000. This well-lit habitat was apparently optimal supporting large and richly fertile thalli.

Lowland population

Thalli were sampled in SE Norway, Ski, Kollåsen, PM 0925, 170–200 m, 29 August 2002. In this densely forested boreal habitat, *N. arcticum* was restricted to N-sloping rocks with thin cover of bryophytes. Due to dark evergreen *Picea abies* canopies, this habitat had the lowest PAR; some thalli experienced probably levels near the compensation point for net photosynthesis during the metabolically active part of the daylight period. Thalli were medium-sized and sparingly fertile, flat and shade-adapted.

3. Methods

Transmittance measurements of cortex pieces

The upper cortex of a small and smooth portion of an air-dry thallus was fixed to double-sided Scotch tape (type 665) under a dissecting microscope. The lower cortex and medulla were removed by scraping until the lower surface of the green photobiont layer was exposed. Thereafter, the sample was treated carefully by scraping gently, with the thin point of a glass Pasteur pipette until no more chlorophyll was visible. Finally, the cortex sample, area 2 mm^2, was carefully removed from the double-sided tape with a scalpel.

Each prepared cortex fragment was placed between two pieces of UV-transmitting cling-film that were in turn tightly taped down along the edges of a 2-inch integrating sphere (IS 270, Optronic Laboratories, Inc., Orlando, Florida, USA). UV and PAR radiation from a DH2000 (Ocean Optics) light source were applied through a 600-µm-thick optical fibre to the upper side of the lichen cortex. Transmittance was measured at two random positions per cortex fragment with a spectroradiometer (model: OL 756, Optronic Laboratories) connected to the output port of the sphere with an optical fibre. The percentage transmittance was calculated on the basis of a cling-film only measurement. Five separate cortex fragments were selected randomly from separate thalli from each of the three sites.

All samples were measured in an air-dry and a hydrated state. Hydrated cortices remained moist for the duration of the 15 min measurement period.

Spectral transmittance curves presented in the results were based on a total of ten measurements, i.e. two

measurements per cortex fragment, using five separate cortex fragments.

Cortex fragment dimension measurements

Following transmittance measurements, the dry weight of the cortex fragments was measured using a microbalance. The area of the fully hydrated fragments was determined by analysing digital photos (Nikon Coolpix 4500) using the Image Tool for Windows package (version 3.00, UTHSCA, USA).

Quantification of lichen compounds

Lichen compounds were measured in a separate randomly selected set of thalli from the three populations, since those used for preparation of cortex fragments were severely broken and destroyed. The dry weight and hydrated area of four to five intact thalli from each habitat were recorded. After desiccation, they were rinsed air dry in 6 ml of acetone for 25 min. The rinsing procedure was repeated 4 times. The rinsed thalli were then ground up using a mortar and pestle and quartz sand with 2 ml of acetone. The mortar and pestle were washed 3 times with 2 ml acetone each time. The combined extracts prior to and after grinding were analysed separately on an ODS hypersil column, 60 × 4.6 mm, using a HP 1100 series HPLC (Aligent Technologies, Waldbronn, Germany).

The injection volume was 10 µl and the flow rate 2 ml min^{-1}. The mobile phase of A: Millipore water and 1% orthophosphoric acid, and B: 100% methanol were run following an adapted gradient of Feige et al. (1993). The run started at 30% B and increased to 70% over 15 min. After a further 15 min 100% B was reached and this continued isocratically for 5 min. The level of B then dropped to 30% over 1 min. A post-run of 30% B for 5 min was allowed. All compounds were detected at 245 nm. The identification of compounds was based on their retention times, UV spectra, comparison with an usnic acid standard (Apin Chemicals, Abingdon, UK) and the literature.

The results of the two extraction procedures were combined and the usnic acid values are expressed as percentage of dry weight (% w/w) and as weight per thallus area (mg cm^{-2}). Only relative values are given for nephroarctin and phenarctin (absorption units [AU] g^{-1} and [AU] cm^{-2}).

The lichen compound content of the cortex fragments was measured. The combined fragments from each habitat was ground up in a mortar and extracted with 1 ml acetone. The mortar with ground cortices was washed 3 times with 1 ml acetone each time. The combined extracts were concentrated to 0.3 ml before analysis.

UV and PAR transmittance measurements in crystals of usnic acid

Usnic acid (Apin Chemicals, Abingdon, UK) was dissolved in acetone and repeatedly applied on small UV-transmitting plexiglas pieces from disposable cuvettes (Cat. No. 759550, Brand GMBH, Wertheim, Germany) at ca. 50°C to enhance evaporation rate, and thereby reduce the size of precipitating crystals formed on the surface of the plexiglas. Few applications of dissolved usnic acid caused the formation of a few large and scattered crystals visible without any magnification, leaving a large portion of the plexiglas uncovered. Many applications caused a dense mat of tiny crystals to precipitate between the first few large crystals. After measuring the UV and PAR transmittance with the spectroradiometer (model: OL 756, Optronic Laboratories) with the same method as described above, the crystals were re-dissolved in ethanol and quantified in the spectrophotometer. The low application corresponded to the amount of usnic acid present per unit area in the *N. arcticum* thalli richest in usnic acid. The high application corresponded to an approximately five times higher content (0.5 mg cm^{-2}), a content that is slightly below normal values (0.6–0.8 mg cm^{-2}) in *U. longissima* (Nybakken et al., 2007). However, since only the high application resulted in tiny crystals, which is the situation in a lichen thallus, the high application was assumingly the most biologically relevant application.

4. Results

Cortical transmittance of air-dry cortices varied highly significantly between sampled habitats for the measured PAR and UV-A wavelength ranges ($P<0.0001$; one-way ANOVA; Fig. 1A–B), but not in the UV-B and UV-C ranges. Transmittance was very low in the UV-C and UV-B ranges, with a considerable increase in the UV-A range for the lowland spruce forest thalli (Fig. 1A). For hydrated cortices (Fig. 1 C–D), the difference between habitats was significant for all three UV-ranges ($P<0.0001$), as well as for the measured PAR-range ($P=0.0002$). Transmittance was clearly highest in thalli from shady lowland spruce forests, with few and less significant differences between the well-lit alpine and sub-alpine populations. Transmittance increased steadily with increasing wavelength into the visible range, reaching 35–40% at the longest wavelength measured (500 nm) in air dry thalli and 60–70% in hydrated thalli. The steep increase in transmittance started at lower wavelengths in hydrated compared to air dry cortices. Regardless of habitat, cortical transmittance of air-dry thalli was half or less than that of thalli in the hydrated state (Fig. 1 A,C).

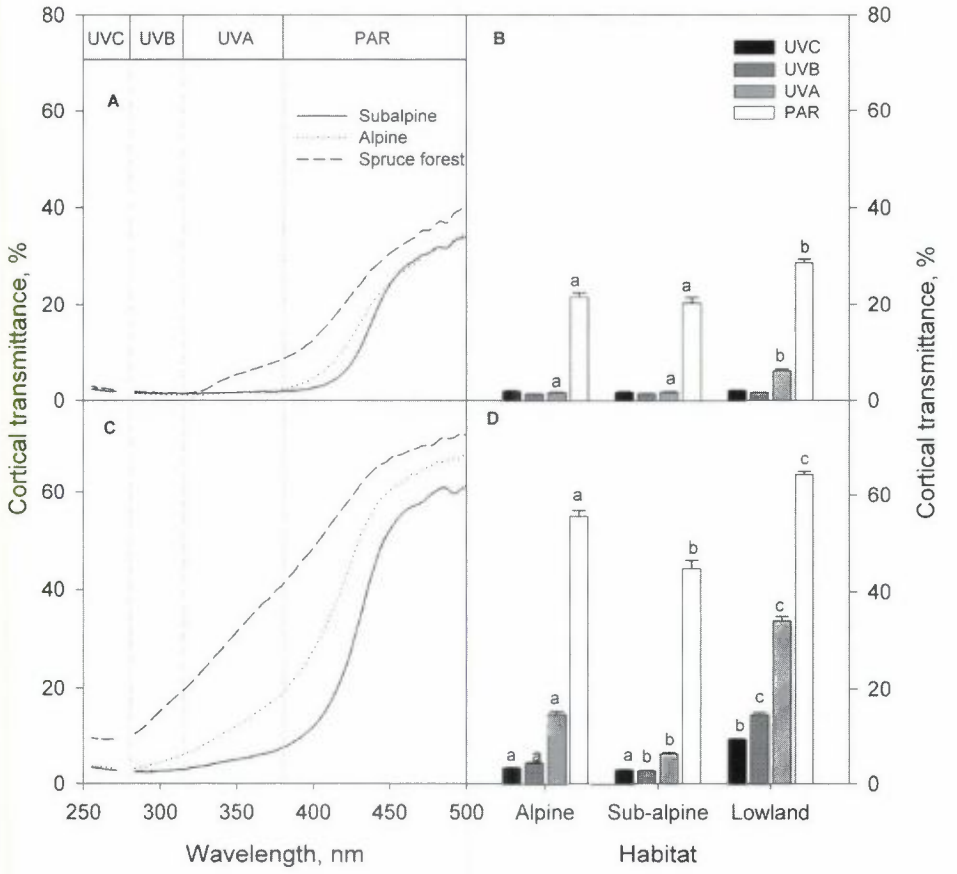

Figure 1. Percent average transmittance (n=5) of air-dry (A–B) and hydrated (C–D) upper cortices of *N. arcticum* sampled from 3 altitudinal different habitats. A one-way ANOVA showed a significant effect of habitat on transmittance in desiccated thalli in the UV-A (315–400 nm) ($P<0.0001$) and PAR (400–500 nm) ($P<0.0001$) ranges, but not in the UV-B (280–315 nm) ($P=0.491$) or UV-C (250–280 nm) ($P=0.694$) range. Habitat type had a highly significant effect on transmittance in wet thalli in the UV-C ($P<0.0001$), UV-B ($P<0.0001$), UV-A ($P<0.0001$), and PAR ($P=0.0002$) ranges. Means (in B and D) marked with the same letter within each individual irradiance range were not significantly different at $P<0.05$ by Student-Newman-Keul method.

The total thallus concentration of the UV-absorbing cortical pigment usnic acid decreased significantly from the sun-exposed alpine and sub-alpine populations to the shaded lowland forest population (Table 1). This trend was also found for the usnic acid content in isolated cortical fragments (Table 1), but since all fragments in each habitat had to be combined due to the small size, no statistical test could be made. Pigment extraction revealed that on average, only 20–39% of the usnic acid was extracted from intact thalli using the acetone rinsing method, with the highest extractable percentage from the lowland population characterized by the lowest concentration (Table 1).

The remaining major fraction, 61–80%, was extractable only after grinding. Measurements of cortical fragments showed that a large fraction (32–74%, depending on habitat and thus sun exposure) of the total usnic acid content lies in the thin cortical layer. In addition to the dibenzofuran usnic acid, two didepsides nephroarctin and phenarctin were detected in full-thallus extracts from all populations (Table 1). The later two compounds were efficiently extracted from intact thalli by regular acetone rinsing (94–99%; Table 1) with minor further quantities detectable after grinding. There was a significant effect of habitat on the content of the two depsides (nephroarctin, $P=0.028$; phenarctin $P=0.035$), with decreasing altitude/irradiance leading to increasing concentration, the opposite of the usnic acid trend. The two depsides were closely correlated across all habitats and thalli ($r=0.964$; $n=13$; $P<0.0001$), but showed no correlation with the usnic acid.

Habitat had a significant effect on thallus thickness (dry weight per area; $P=0.006$; Table 1). Alpine thalli were significantly thicker than sub-alpine and lowland spruce forest thalli. Trends in the specific weight of cortex fragments had a less obvious trend ($P<0.036$), with the sub-alpine fragments significantly thicker than the spruce forest but not the alpine cortices. Alpine cortices were not significantly thicker than those from the spruce forest.

In the dataset comprising all measured variables, the population means were the only available observations (n=3). In a search for the best relationships in this small

Table 1. Total thallus content of usnic acid, nephroarctin and phenarctin, as well as specific weight of both intact thallus and isolated cortex fragments in *Nephroma arcticum* thalli (mean values ± 1 SE) collected along an altitudinal and solar radiation gradient. The ANOVA column shows *P*-values (one-way ANOVA). Means followed by the same letter in a row were not significantly different at $P<0.05$ by Student-Newman-Keul method. *:) Phenarctin concentrations were log-transformed to meet the requirements of the ANOVA.

	Alpine (1140 m) exposed heath	Subalpine (910 m) open birch forest	Lowland (200 m) spruce forest	ANOVA *P*-level
Entire thalli (n=4–5):				
Usnic acid				
% w/w	0.737 ± 0.12 (a)	0.838 ± 0.24 (a)	0.179 ± 0.11 (b)	0.037
mg cm^{-2}	0.118 ± 0.01 (a)	0.113 ± 0.03 (a)	0.023 ± 0.02 (b)	0.013
% extracted prior to grinding	21.1 ± 4.1	20.2 ± 6.4	39.1 ± 11.6	ns
Nephroarctin				
AU mg^{-1}	195 ± 20 (a)	226 ± 17 (a)	370 ± 71 (b)	0.028
AU cm^{-2}	3,194 ± 296	3,083 ± 191	4,521 ± 950	ns
% extracted prior to grinding	96.9 ± 0.7	97.4 ± 0.9	99.4 ± 0.1	ns
Phenarctin				
AU mg^{-1}	320 ± 71 (a)	397 ± 33 (a/b)	646 ± 155 (b)	0.035*
AU cm^{-2}	5,282 ± 410	5,418 ± 376	7,974 ± 2201	ns
% extracted prior to grinding	95.5 ± 1.7	94.4 ± 2.1	97.8 ± 1.0	ns
Dry weight per area (mg cm^{-2})	16.6 ± 1.2 (a)	13.7 ± 0.3 (b)	11.6 ± 0.8 (b)	0.006
Cortices only:				
DW per area (mg cm^{-2}; n=6)	1.7 ± 0.2 (ab)	2.1 ± 0.2 (a)	1.4 ± 0.1 (b)	0.036
Usnic acid in cortices (% w/w; n=1)	2.2	3.9	0.56	

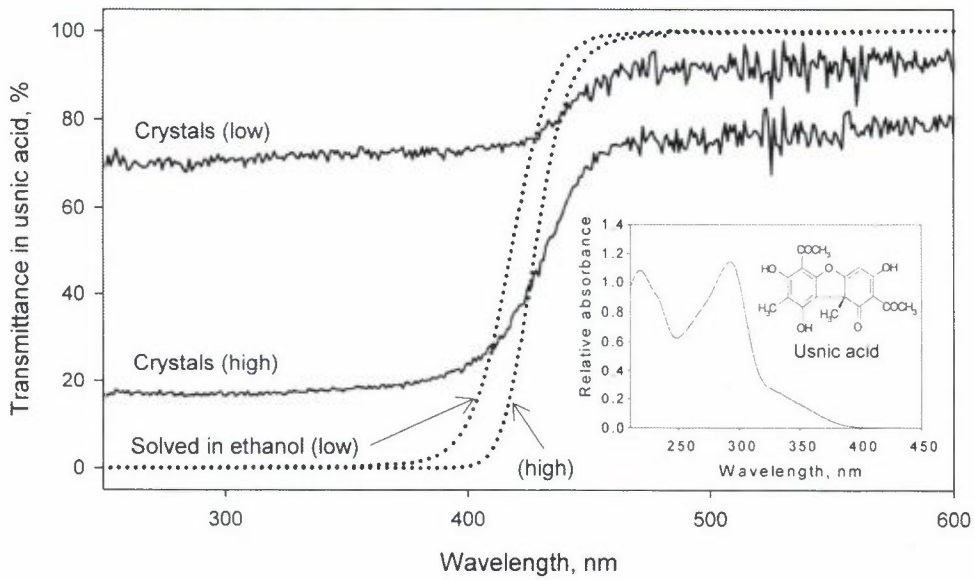

Figure 2. Transmittance through a cover of few large (low) and many small (high) crystals of usnic acid formed on a plexiglass (solid lines). Dotted lines show the transmittance for the same two amounts of crystals dissolved in methanol. See further explanation in methods. The insert shows the UV spectrum of dissolved usnic acid and the molecular structure of usnic acid. The main absorption peaks occur in the UV-C range (λ_{max}=220) and the UV-B range (λ_{max}=290).

dataset, the UV-B (r=–0.999) and UV-A transmittances (r=–0.987) for hydrated cortices decreased linearly with usnic acid concentration, whereas the PAR transmittance (r=–0.999) most closely followed the mass per area of cortex fragments. However, for desiccated cortices, the PAR transmittance closely followed the usnic acid concentration (r=–0.999; the primary data used for computing these relationships are shown in Fig. 1 B,D and Table 1).

Usnic acid in solution transmitted hardly any UV (close to 0%) at all UV wavelengths (<380 nm), and transmitted 100% of PAR with wavelengths longer than 450 nm (Fig. 2). A high usnic acid concentration displaced the steep

transition to slightly higher wavelengths. Transmittance was significantly changed when measured through a layer of precipitated crystals. Crystals allowed a consistently higher transmittance of UV, even for the high application; whereas they screened a significant part of the PAR also at wavelengths beyond 450 nm. The absolute levels of crystal spectra in Fig. 2 should be taken cautiously, since crystals formed on fungal hyphae may not necessarily show the same size and/or density as those measured.

5. Discussion

This study shows habitat-specific differences in cortical screening of solar radiation in *N. arcticum* (Fig. 1). A recent eight years reciprocal transplantation of sub-alpine and alpine *N. arcticum* thalli suggested a weak ecotypic differentiation, although not supported by differences in two internal transcribed DNA spacers (Sonesson et al., 2007). Due to the limited positive evidence of ecotypic differentiation (Sonesson et al., 2007) as well as documented environmental regulation of screening pigments (Solhaug and Gauslaa, 2004) and thallus thickness (Gauslaa et al., 2006b) in foliose lichens, we consider a major part of the responses in Fig. 1 and Table 1 to reflect acclimation.

It has been assumed for some time that usnic acid offers protection against harmful UV-B radiation (Rundel, 1978; Rikkinen, 1995; Quilhot et al., 1998), but there is inconclusive experimental evidence for this claim. Fernández et al. (1996) and Rancan et al. (2002) have demonstrated that extracted usnic acid offers comparable UV-B protection to that of commercial sun-blocks. However, BeGora and Fahselt (2001b), for example, failed to find a positive correlation between enhanced UV irradiation and the concentration of usnic acid in *Cladonia uncialis*. The strong coupling between transmittance means for the three populations and their respective usnic acid concentrations suggests that the usnic acid located in the upper cortex and in the upper parts of the photobiont level shades and protects underlying photobiont cells from excess solar radiation. The strongest habitat-specific effect of screening in hydrated cortices occurs in the UV-ranges (Fig. 1 C–D), which is the expected trend, considering the spectral characteristics of usnic acid (Fig. 2). The UV-B screening is still substantial in shade-adapted lowland populations fairly low in usnic acid, suggesting that additional unknown UV-B absorbing compounds may occur in the lichen cortex. In the cortex of *Lobaria pulmonaria*, the UV-B transmittance in pale shade-adapted cortices remained low even after acetone rinsing (Gauslaa and Solhaug, 2001). The relative cortical transmittance of UV-A, -B and -C (Fig. 1 B,D) apparently do not fully match the usnic acid crystalline transmittance spectrum (Fig 2). This can be explained by the fact that the covering of crystals on the plexiglas pieces was incomplete. Spectroradiometer emission light that did not hit any crystals passed straight through the plexiglas, reaching the detector as a full spectrum of light, resulting in a uniform UV-transmittance curve.

Cockell and Knowland (1999) proposed the following set of criteria to determine whether a compound has a screening role: 1) the compound should absorb the relevant radiation; 2) biosynthesis of the pigment must be inducible by the radiation; 3) screening activity should be demonstrated *in vivo,* and 4) enhanced survival under elevated radiation should be shown to be due to the compound. Usnic acid absorbs strongly in the UV-C and UV-B ranges of the spectrum, with absorption maxima at 220 and 290 nm (Fig. 2 insert). In contrast to phenarctin and nephroarctin, usnic acid also absorbs strongly in the UV-A range (Bjerke et al., 2005). We recently demonstrated that induction of usnic acid synthesis in *Xanthoparmelia stenophylla* exclusively occurs with UV-irradiation, although artificial addition of photosynthates boosted the synthesis (McEvoy et al., 2006). We now provide evidence (Fig. 1 and Table 1) that usnic acid present in the cortical layer screens the underlying photobiont layer from UV-irradiation. The final requirement relating to protection afforded by the presence of usnic acid in the cortex is difficult to prove because it is not possible to efficiently extract the pigment from living, intact cortical fragments (McEvoy et al., 2006). *Nephroma arcticum* is relative to other species highly sensitive to prolonged acetone rinsing (Solhaug and Gauslaa, 2001).

The clear habitat-specific effect in the PAR-range was unexpected. However, in intact lichen cortices, usnic acid is deposited as dense layers of tiny crystals outside fungal hyphae. Since crystals scatter and reflect visible wavelength (Fig. 2), usnic acid *in situ* screens longer wavelengths than when dissolved. Although the level of screening due to crystals of usnic acid cannot be accurately assessed, a 30% screening of PAR (Fig. 2) is not unrealistic, implying that usnic acid may account for a significant part of the PAR screening in hydrated cortices (Fig. 1 C–D). In addition, habitat-specific differences in cortex thickness (weight per area unit) contribute to the PAR screening in hydrated thalli. Our data are consistent with previous studies showing that lichen cortices from sun-exposed habitats had reduced transmittance compared to those from shaded habitats (Ertl, 1951; Büdel and Lange, 1994; Dietz et al., 1999). The effect of excess irradiance on poikilohydric lichens depends on their hydration status (Gauslaa and Solhaug, 1996). Hydrated thalli are often more susceptible than desiccated thalli because desiccation e.g. reduces the transmittance of solar radiation through the upper cortex (Ertl, 1951; Gauslaa and Solhaug, 2001). Our results show that exposed populations of *N. arcticum* are able to maintain a fairly high level of cortical screening in the hydrated state.

Slightly lower, but non-significant usnic acid levels in the fully exposed alpine heath compared to the sub alpine-forested habitat may have been a consequence of suboptimal environmental conditions resulting in small, sterile and stunted thalli in alpine sites. According to the transplantation study of Sonesson et al. (2007), a sub-alpine habitat is the optimal habitat for both sub-alpine and alpine *N. arcticum* populations. The fully exposed alpine thalli may have received less hours of light during hydration periods which determines lichen growth (Dahlman and Palmqvist, 2003) and presumably reduces the production of photosynthates that can be used in secondary metabolism. Furthermore, the slightly reduced levels of usnic acid in the most exposed alpine population is consistent with the results of BeGora and Fahselt (2001a; 2001b) suggesting a UV-B induced degradation of usnic acid in lichens subjected to the highest UV exposures.

Nephroma arcticum is restricted to northern latitudes in Europe (Poelt, 1969) and America (Brodo et al., 2001). The studied lowland locality is situated in the hemiboreal region near the southern distribution range of the species. In such areas it is often restricted to shaded sites in old spruce forests, different from the preferred open sub-alpine habitats in central parts of its distribution area. The reason for this shift in habitat preference is not known. Southern populations may have a lower ability to produce usnic acid because of genetic and/or environmental factors. However, this alpine-boreal lichen may have low tolerance to high temperatures, like the boreal-temperate *Lobaria pulmonaria* (Gauslaa and Solhaug, 1999; McEvoy et al., 2007) showing a similar pattern with a preference for more shaded sites in warmer climate zones.

Thalli in dark spruce forests need to maximize PAR at the algal level, since light is often a limiting factor for lichen growth in spruce forests (Gauslaa et al., 2006b). Consequently, the cortical transmittance needs to be high. Usnic acid may reduce UV-cortical transmittance with less shading of PAR (Fig. 1) than melanins (Gauslaa and Solhaug, 2001) and parietin (Gauslaa and Ustvedt, 2003; Vráblíková et al., 2006) that screen wider wavelength ranges of PAR. The light compensation point of CO_2 exchange has been shown to be consistently lower in low elevation populations of *N. arcticum* compared to alpine populations (Sonesson et al., 1992). Minimizing light losses in the cortex is important for shade populations with low light compensation levels in order to obtain a long-term positive net assimilation (Dietz et al., 1999).

Nephroma arcticum populations may acclimate to their specific light environments not only by variations in cortical screening. Sonesson et al. (1992) reported on acclimation of various photosynthetic characteristics in *N. arcticum*. Chlorophyll levels in alpine sites were approximately half those of sub-alpine sites and the light compensation point was consistently higher in alpine populations, reflecting differences in light regimes.

The opposite trends of usnic acid and the two medullary compounds phenarctin and nephroarctin in relation to habitat/sun-shade gradient may appear surprising given that all these compounds have their absorbance maxima in the UV-ranges of the spectrum. However, given their exclusive medullary location, nephroarctin and phenarctin are unlikely to have a screening role in *N. arcticum*. McEvoy et al. (2007) and Nybakken et al. (2007) found no correlation between light exposure and the content of six of seven UV-absorbing medullary depsidones in two independent transplantation studies with *Lobaria pulmonaria* along a sun-shade gradient. Also in *Usnea longissima*, the cortical usnic acid and the medullary diffractaic acid showed inverse relationships (Nybakken et al., 2007; Nybakken and Gauslaa, 2007). The inverse relationship between the concentration of medullary compounds and altitude may be consistent with a herbivore defence role of such compounds (Pöykkö and Hyvärinen, 2003; Gauslaa, 2005; Pöykkö et al., 2005). Grazing marks by molluscs are common in *N. arcticum* growing in low-altitudinal southern forests (Gauslaa, pers. obs.), suggesting a spatial variation in the frequency of lichen-feeding snails and slugs, as was the case for the old forest lichen *Lobaria pulmonaria* (Gauslaa et al., 2006a). Therefore, the elevated concentrations of phenarctin and nephroarctin in shaded spruce forests may be a response to increased pressure from invertebrate herbivores at low altitudes.

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