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THE RESPONSES OF ECTOHYDRIC AND ENDOHYDRIC MOSSES UNDER AMBIENT AND ENHANCED ULTRAVIOLET RADIATION

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Abstract

Previous reports on the effects of enhanced UV-B radiation on bryophytes have been equivocal. This study shows that mosses not only respond to enhanced UV-B, but they are affected by changes in ambient radiation. The studies were conducted with two model species common in northern environments; red-stemmed feather moss (*Pleurozium schreberi*) and juniper haircap moss (*Polytrichum juniperinum*).

Both species showed high concentrations of methanol-extractable UV-absorbing compounds (UACs) with high spring-time and early-summer UV, whereas in *P. juniperinum*, the concentration was affected by early-summer drought. The UACs of *P. juniperinum* increased again towards autumn suggesting a role in winter hardening. The (spring-time) cell wall-bound UV screen was important to both species. The fundamental adaptation of *P. juniperinum* to open and exposed environments was reflected in relatively higher concentrations of total UACs compared to *P. schreberi*.

The enhanced UV-B experiments *in situ* were conducted over two years in Oulu and six years at the FUVIRC site in Sodankylä. Some of the effects of UV-B were seen within the first years of the experiments, or even within hours, while others were observed after several years. Five or six years of enhanced UV-B treatment increased the methanol-extractable UACs of *P. schreberi* and decreased the green shoot growth of *P. juniperinum*. The immediate light environment was proposed to have an impact on the varying UAC concentrations. Some mitigating effects of UV-A were observed as well.

Off-site measured, reconstructed and modelled UV radiation data was used for comparisons of light environment *in situ*, or when performing a reconstructive research with historical samples. The environmental sample banks can provide a useful tool to study past environmental conditions, and even reconstruct past radiation levels.

It was shown in this study that UACs in *P. schreberi* and *P. juniperinum* have fundamental roles as UV-B screens in the cell walls, but there is also a variable response with the soluble fraction that reacts and adapts to the changes in UV radiation. The responses to increasing UV-B radiation vary in magnitude and in time. As *P. schreberi* and *P. juniperinum* possess circumboreal and cosmopolitan distributions, the effects of UV-B on these species and consequently on ecosystems has a broad application.

Keywords: cell wall, environmental specimen bank, experimental study, fluorescence microscopy, growth, long-term changes, methanol, mosses, natural conditions, photosynthesizing pigments, pleurozium schreberi, polytrichum juniperinum, ultraviolet radiation, UV-absorbing compounds

To my Family

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The author was the first author in papers I, II, and III, and co-author in writing paper IV. The author sampled, designed and participated in the chemical analyses of papers I, II and III, and performed the sampling and growth measurements in papers II and III. In the present work, the author performed the microscopy analyses and participated in the chemical analyses. The author was responsible for operating the UV experiment in Oulu (paper II), and calculated the radiation dosages in papers II and III. The author performed the statistics in papers II, III and in the present work, and part of the statistics in paper IV.

Oulu, April 2010 Niina Lappalainen

List of original papers

This work is based on the following papers, which are referred to in the text by their Roman numerals:

I Lappalainen NM, Hyyryläinen A & Huttunen S (2010) Seasonal and interannual variability of light and UV acclimation in mosses. In: Tuba Z, Slack NG, Stark LR (eds) Bryophyte Ecology and Climate Change. Cambridge, Cambridge University Press. In press.

II Lappalainen NM, Huttunen S, Suokanerva H & Lakkala K (2010) Seasonal acclimation of the moss *Polytrichum juniperinum* Hedw. to natural and enhanced ultraviolet radiation. Environmental Pollution 158: 891–900.

III Lappalainen NM, Huttunen S & Suokanerva H (2008) Acclimation of a pleurocarpous moss *Pleurozium schreberi* (Britt.) Mitt. to enhanced ultraviolet radiation *in situ*. Global Change Biology 14: 321–333.

IV Huttunen S, Taipale T, Lappalainen NM, Kubin E, Lakkala K & Kaurola J (2005) Environmental specimen bank samples of *Pleurozium schreberi* and *Hylocomium splendens* as indicators of the radiation environment at the surface. Environmental Pollution 133: 315–326.

In addition, unpublished data have been included in this thesis.

Contents

1 Introduction

Anthropogenic chemicals, especially chlorofluorocarbons (CFCs), have caused depletion of ozone (O_3) in the stratosphere. The ozone layer protects the Earth´s surface from the short wavelengths of the ultraviolet range of solar irradiation (review by Rowland 2006). These short wavelengths are harmful to living organisms.

The depth of the ozone layer varies seasonally and geographically, being naturally highest during spring and near the poles (I, Rowland 2006). The greatest negative trend in total stratospheric ozone has been detected during spring. The spring-time ozone hole was first reported over Antarctica by Farman *et al*. (1985). Depletion of Antarctic ozone during spring frequently reaches > 90% (Solomon *et al.* 2007). Besides the spring-time depletion, a significant decreasing trend of the total ozone column between the 1970s and the 1990s has been observed (WMO 2007, Weatherhead *et al*. 2005). Over the Northern hemisphere, ozone depletion was not verified until the 1990s due to the decreases in ozone concentration being less extreme, and variability between years and locations (review by Solomon 1999, Solomon *et al*. 2007). Arctic ozone depletion is less severe compared to Antarctic, but it affects some highly populated areas, especially the European sector (Solomon *et al*. 2007). Increases in UV radiation over the years have been observed over Northern Europe (I, Björn *et al*. 1998, Taalas *et al*. 2000), the spring-time increases being more severe during some years than others (Sinnhuber *et al*. 2000). In the Northern Hemisphere, decrease of the snow cover has also increased the exposure of groundlayer plants to UV-B radiation (IPCC 2007).

Of the ultraviolet (UV) wavelength region, the ozone layer absorbs all UV-C (100–280 nm), most of UV-B (280–315 nm), and very little UV-A radiation (315–400 nm). Visible light (400–700 nm, photosynthetically active radiation, PAR) which is essential to all photosynthesising organisms, passes through the ozone layer. Anthropogenic depletion of the ozone layer mostly affects the UV-B wavelength region, increasing the ratio of UV-B to UV-A and UV-B to PAR (Rowland 2006). UV-B radiation enables the human skin to produce vital vitamin D, but too much causes damage and can lead to skin cancer. Effects on plants and ecosystems have been reported as well (*e.g.* reviews by Rozema *et al*. 2002, 2005, Flint *et al*. 2003, Robinson *et al*. 2003, Caldwell *et al*. 2007). UV-A has not traditionally been considered to be harmful to living organisms (Rowland 2006), it has been observed rather to mitigate the negative effects of UV-B along with

visible and especially blue light (Caldwell *et al*. 1994, Flint & Calwell 1996). Recently however, UV-A has been reported to cause harmful effects as well (I and references therein). Besides ozone layer depth, latitude, season and time of day, the intensity of nearsurface solar UV is affected by the absorption and scattering of clouds, surface (albedo) and aerosols (I, WMO 2007).

The Montreal Protocol in 1987, with the Copenhagen amendments in 1992, has phased out the production of most of ozone-depleting compounds. Nevertheless, some of the compounds have long atmospheric lifetimes and the anthropogenic destruction of ozone will continue for decades to come (see Solomon 1999 and Rowland 2006 for review, WMO 2007).

The recovery process of the ozone layer is further complicated by other anthropogenic changes to the atmosphere (Weatherhead & Andersen 2006, WMO 2007). Climate change presents a challenge to the predictions of ozone recovery, since interactions with other changing atmospheric variables (like temperatures and cloudiness) are not well understood (Weatherhead & Andersen 2006, WMO 2007). In the Arctic, the interannual variability in ozone complicates the projection of future ozone depletion even more (Weatherhead *et al*. 2005). Models have predicted that the greenhouse gases, especially carbon dioxide, trap more heat in to the troposphere, which leads to cooling of the stratosphere (Weatherhead *et al*. 2005). Lower temperatures in the stratosphere will further increase polar ozone depletion – especially during spring – and the frequency of ozone holes (Weatherhead & Andersen 2006). In the Arctic, the most severe decreases in ozone have been detected at years of low spring-time stratospheric temperatures (*e.g.* see review by Solomon 1999). Any substantial recovery of the ozone layer to the pre-1980 levels cannot be expected until the 2050s in the Northern Hemisphere (Taalas *et al*. 2000). Additionally, future stratospheric ozone recovery is likely to occur in a very different atmosphere – compared to the atmosphere before the emergence of ozone-depleting substances – due to continuing anthropogenic impact (Weatherhead & Andersen 2006).

1.1 Effects of ultraviolet-B radiation on plants

Ecosystems at high northern latitudes are adapted to lower levels of ultraviolet-B radiation than ecosystem of middle and low latitudes, and therefore may be more vulnerable to increasing UV-B (I, review by Paul & Gwynn-Jones 2003). The changes in environmental conditions – such as increasing temperature – are more pronounced at high northern latitudes (IPCC 2007), where temperatures are low

and summer-time days are long. Snow cover impacts the UV-B radiation dose received as well, since reflectance of clean snow can be over 80% (Björn 1999, Rowland 2006, Weatherhead *et al*. 2005). The effect of surface reflectance of UV-B is pronounced during spring when there is simultaneously (patchy) snow and high UV-B radiation. The albedo of UV-B radiation for vegetation cover is low.

The UV-B wavelegth region is a small proportion of solar irradiation, but it´s biological effectiveness is high (Weatherhead *et al*. 2005). Action spectra are used to estimate the biological effectiveness of UV radiation. Action spectra are normally calculated separately, for example for DNA and human skin (the Comission Internationale de l'Eclairage, CIE; McKinlay & Diffey 1987). Caldwell (1971) developed a generalized plant action spectrum, from which modifications have been calculated (*e.g.* Caldwell 1986, Flint & Caldwell 2003a).

The effects of UV-B on native plants and ecosystems have been studied under natural solar irradiation, under filters blocking part of the natural solar UV-B, and under UV lamps simulating increasing ozone depletion (I, Flint *et al*. 2003). Enhanced UV-B treatment can be provided by a square-wave system (the lamps are switched on simultaneously and off again, burning for a period of time around noon, the time of highest natural solar UV-B radiation), a stepwise system (the irradiation output around noon is elevated and reduced in steps), or a modulated system. The modulated system allows a stable UV-B enhancement treatment by constantly following the natural solar irradiation and adapting the UV lamp output, offering the most realistic treatment (Rozema *et al*. 2005).

Plants have developed different strategies to protect themselves from UV-B radiation. The mechanisms that appear to inhibit UV transmittance into the mesophyll tissue involve cuticular waxes, hairs, increase in leaf thickness, decrease in specific leaf area, and induction of secondary phenolic metabolism provide protection to the plants (Caldwell 1971, Gwynn-Jones 2001). Besides the strategy of avoidance, species may have mechanisms for tolerating UV, for example their capacity for DNA repair (Caldwell *et al*. 2007). Rather than being just a damaging agent, it is now recognised that UV-B radiation is a specific regulator in coordinating plant growth and development (in a way similar to blue and red/far-red wavelengths) (Aphalo 2003).

Early studies found severe effects of enhanced UV-B on plants (Teramura 1983, 1990). UV-B radiation has been found to affect plants directly and indirectly, causing damage to DNA, proteins and membranes, alterations in transpiration and photosynthesis, and changes in growth, biomass accumulation, development and morphology (see Jansen *et al*. 1998). However, these early

studies were conducted mainly with crop plants in greenhouses and growth chambers with unrealistically low background UV-A and visible light, which has been observed to enhance the negative effect of UV-B on plants (Teramura 1983). Also lack of interaction with the surrounding ecosystem and environmental conditions can modify plant responses to UV-B radiation.

For estimating overall plant responses to changing UV-B radiation and predicting effects on ecosystems and vegetation processes, it has been suggested that the UV-B induced responses of plant functional types should be assessed (Li *et al*. 2010). Generalizing responses using plant functional types is not without its problems however, since species-specific responses have been observed (Dormann & Woodin 2002, Martínez-Abaigar *et al*. 2003). UV-B induced changes in biomass, morphology and physiology may affect the competitive interactions between species and ecosystem processes (Gehrke *et al*. 1995). Accumulation of secondary metabolites may affect the interactions between plants and their herbivores, pathogens and symbionts (Caldwell *et al*. 2007).

Several meta-analyses of UV-B induced effects on plants have been published during the last decade (Searles *et al*. 2001, Newsham & Robinson 2009, Li *et al*. 2010). The most constant effect of enhanced UV-B has been found to be increase in the concentration of methanol-extractable UV-B-absorbing compounds. These compounds are considered to be the main protective mechanism against UV-B (Searles *et al*. 2001). Flavonoids absorb strongly in the UV wavelength region, but allow photosynthetically active radiation to pass through to the underlying cells (Day *et al*. 1992). They are accumulated mainly in the epidermis, attenuating over 90% of incident UV-B reaching the underlying cells (see Jansen *et al*. 1998). Free-radical scavenging activity of flavonoids may offer additional protection to the cells accumulating these compounds (Jansen *et al*. 1998, Tattini *et al*. 2004). Besides increases in UV-absorbing compouds, reductions in above ground biomass and height, and increased DNA damage were also observed in the meta-analysis (Newsham & Robinson 2009, Li *et al*. 2010).

1.2 Bryophytes as model plants

Bryophytes are the simplest land plants, and therefore have been used as slow-growing evergreen model plants in studies of plant acclimation (Cove *et al*. 1997). They are abundant ground layer plants in northern ecosystems (I), and can cover over 90% of the ground surface (Mäkipää *et al*. 2000). Bryophytes inhabit all environments and light conditions from shaded forests to open hills, from streams

to wet peatlands and dry environments. They form an important boundary layer between the atmosphere and the soil by keeping moisture and temperature of the underlying soil more stable (I). Mosses modify habitats creating seedbeds for vascular plants, a microclimate suitable for tree seedlings and other plants (Parker *et al*. 1997), and prevent erosion. Forest management and other changes in land use have altered the abundance of some species (Mäkipää & Heikkinen 2003). Despite their prevalence, knowledge about their responses to environmental changes is still insufficient (Gignac 2001).

Most bryophytes have simple structures, therefore they tend to respond to environmental stresses at the cellular level (Bates 2000, Christianson 2000). It has been suggested that lower plants, like mosses, express more basic and fewer defensive responses to UV-B than higher plants, and their responses are more rapid (Markham *et al*. 1998, Gwynn-Jones 2001). Mosses are poikilohydric, and their water content and metabolic activity depends on the surrounding water conditions. They also tolerate desiccation well (I, Proctor *et al*. 2007). During unfavourable conditions, for example drought and high light conditions, the metabolism is suspended and a rapid recovery follows when the conditions become more favourable (I, Vitt 1990, Oliver *et al*. 2005, Williams & Flanagan 1998). Mosses of high latitudes show seasonality in photosynthesis and growth (I, Callaghan *et al*. 1978, Davey & Rothery 1996), with the ability to photosynthesize during spring and autumn. Environmental conditions, like water availability, have a strong affect on the seasonality of bryophytes (I, Sonesson *et al*. 2002).

Mosses can be divided into ecto- and endohydric species on the basis of their water relations (Proctor 2000). Ectohydric species absorb and conduct water externally. By contrast to ectohydric species (like *Pleurozium schreberi*), bryophytes with endohydric characteristics (like *Polytrichum juniperinum*) can conduct water internally and control their water status to some extent. Some bryophytes seem to fall between the endo- and ectohydric types (mixohydric) (Proctor 2000).

1.2.1 Pleurozium schreberi *(Red-stemmed Feather-moss)*

Pleurozium schreberi (Britt.) Mitt. is one of the most common – if not the most common – forest floor mosses in Northern Europe, and is therefore highly important to northern forest ecosystems (III). *P. schreberi* is circumboreal (Vanha-Majamaa 2000) and it is adapted to moderate light environments (Dierßen 2001,

Sørensen *et al*. 2009), forming loose carpets on boreal and subarctic forest floors. It inhabits dry and moist forest heaths, thrives on dry and acidic soils, and avoids grass-herb forests. *P. schreberi* is a competitive perennial (Dierßen 2001) and is sensitive to heavy trampling (Sørensen *et al*. 2009).

Like most bryophytes, ectohydric *P. schreberi* has undifferentiated leaves of one cell layer, and no thick cuticles or epidermis to protect the underlying cells (I, III, Buck & Goffinet 2000). It has a thin, practically non-reflecting lipid layer and no waxes on laminal surfaces (Taipale & Huttunen 2002). This structural simplicity makes them vulnerable to environmental stresses and pollution (Bates 2000).

P. schreberi has a feather-like structure. During early summer the gametophyte continues to grow from the lateral branches formed the previous year, and the main shoot growth (a new "segment") occurs during autumn. It is pleurocarpous, forming sporophytes on the leaf axis. *P. schreberi* does not have root-like structures or below-ground storage to buffer above-ground stresses. As it is an ectohydric species, it is strongly affected by air humidity (Callaghan *et al*. 1978).

1.2.2 Polytrichum juniperinum *(Juniper Haircap Moss)*

Polytrichum juniperinum Hedw. is a common moss species in northern ecosystems with a wide, cosmopolitan distribution area (Mäkipää *et al*. 2000, van der Velde & Bijlsma 2003). In Finland, *P. juniperinum* has increased after the 1950s in some areas (Mäkipää *et al*. 2000). It is a pioneer species, occupying more open and disturbed habitats than most other moss species (Callaghan *et al*. 1978, Corradini & Clement 1999, Bradbury 2006, Botting & Fredeen 2006, Mäkipää & Heikkinen 2003). It colonizes environments of early successional stages, being a very common species on soil surfaces after disturbances like fire, in dry environments like rocky and fell areas, and in young forests. The populations are usually extensive. It can facilitate the growth of tree seedling and other plants (Groeneveld *et al*. 2007), thus competition between Polytricaceae and small vascular plants has been reported (Corradini & Clement 1999, Proctor 2005). The coverage of *P. juniperinum* is negatively affected by shading from other plants (Hedderson & Longton 2008, Sørensen *et al*. 2009), but it is not sensitive to trampling (Sørensen *et al*. 2009).

Among bryophytes, the leaf structure of endohydric *P. juniperinum* is closest to the function of the leaves of vascular plants (Clayton-Greene *et al*. 1985). *P.*

juniperinum has an epidermal wax layer, thick cuticles and differentiated leaves (II), with unistratose lamellae on the adaxial side of the leaf ('costa'), and remarkably curved leaf margins which provide protection to the underlying lamellae (Buck & Goffinet 2000). An internal water and carbohydrate conducting system allows it to be physiologically active to some extent during drought (Callaghan *et al*. 1978). *Polytrichum* species have two growing periods when the environmental conditions are favourable – spring and autumn (Corradini & Clement 1999). Seasonal variation in leaf morphology has been observed, with short leaves at the beginning and at the end of the season (see Callaghan *et al*. 1978). Due to their vertical growth pattern, the young leaves provide shade for the older leaves during wet conditions when the leaves are extended sideways. During drought, the leaves are situated along the stem. *P. juniperinum* is a dioecious species, producing sporophytes frequently. Gametangia are produced acrocarpously at the tips of the gametophytes. It also has a network of underground rhizomes to buffer aboveground stresses (Callaghan *et al*. 1978).

1.3 Bryophytes as indicators of radiation environment

Studies of bryophytes as indicators of their radiation environment – which refers to UV-B, UV-A and PAR – have been conducted mainly in boreal, Arctic and Antarctic regions (I). As in vascular plants, contradictory findings have been obtained (*e.g.* Martínez-Abaigar *et al*. 2003, Boelen *et al*. 2006). As the leaves of the majority of mosses – like *Pleurozium schreberi* – consist of only one cell layer, it is not possible to separate the photosynthesising cells, and the cells provoding protection to them. Nevertheless, in the leaves of the Polytrichales, non-photosynthetic cells cap the photosynthetic lamellae, giving the species a possibility to mimic the vascular plant location of UV-B screening compounds in the protective layer of epidermal cells (Raven 2002).

As in higher plants, UV-B-absorbing compounds have been proposed to indicate UV-B-induced responses in bryophytes (Gehrke 1998, Searles *et al*. 2001). Flavonoids – which are considered to be UV-B-absorbing compounds – are the most widespread phenolics in bryophytes, with over 350 different flavonoids identified in them (Mues 2000). For example apigenin and diosmetin glucosides have been detected in *P. schreberi* (Markham 1988), and benzonaphthoxanthenones called ohioensis and flavonones have been found in *Polytrichum* species (Seo *et al*. 2008, Fu *et al*. 2009). Most secondary metabolites can act as antioxidants (I, Basile *et al*. 1999, Grace 2005). Besides light, other

environmental factors like low temperatures can induce flavonoid biosynthesis (I, Núñez-Olivera *et al*. 2004).

Extractable free phenolic compounds are commonly used to estimate phenolic allocation in plant responses to environmental stresses (see Jones & Hartley 1998). Acidified methanol-extraction of the UV-absorbing compounds (UACs) is a suitable method for assessing the potential effects of UV-B radiation on the secondary chemistry of bryophyte species (Cornelissen *et al*. 2007, Seo *et al.* 2008).

The soluble UACs in bryophytes have been studied as bulk absorbance (*e.g.* Gehrke 1998), as concentration of individual compounds like apigenin, diosmin and luteolin, and as a ratio between different compounds (apigenin : diosmin) (see I for references, Markham *et al*. 1998). Besides the soluble UACs, attention has been drawn to cell wall-bound UACs as well (Taipale & Huttunen 2002, Clarke & Robinson 2008).

In addition, photosynthetic pigment composition and rate of photosynthesis, carbohydrates, morphological and ultrastructural features, biomass production, annual growth, turf surface reflectance, and plant coverage of bryophytes have been used as indicators of UV radiation (*e.g.* Gehrke *et al*. 1996, Johanson *et al*. 1995, Martínez-Abaigar *et al*. 2003, Newsham *et al*. 2002, Taipale & Huttunen 2002). Thick cuticles and surface wax may provide some UV protection through absorbance and reflection (Rozema *et al*. 1997a). Surface waxes of the leaves of *Polytrichum* mosses have been found to contain UV-B-absorbing compounds (Huttunen & Virtanen 2004).

In the Antarctic, the UV-absorbing phenolic compounds of herbarium moss samples and plant pollen and spores have been found to reflect past changes in ozone levels and UV radiation, and it has been suggested that frozen moss banks offer a valuable archive to study UV levels of the past (Markham *et al*. 1990, Rozema *et al*. 2001). Enhanced UV-B was found to increase the concentration of UACs in pollen (Rozema *et al*. 2001). Ground-based measurements of spectral UV irradiance in the high northern hemisphere have only relatively recently become available. In Sodankylä, for example, the measurements began in 1990 (Lakkala *et al*. 2008). Other methods for reconstructing past UV radiation environment must be used, in which case historical plant samples can be useful (Rozema *et al*. 2001).

Due to their poor protective capacity and limited assimilate availability, it has been suggested that the majority of bryophytes are susceptible to UV-B radiation (Gehrke *et al*. 1996, Gwynn-Jones 2001). Additionally, bryophytes commonly

tolerate desiccation well, but it has been argued that they are more vulnerable to UV-B radiation during dry periods, when they are in a desiccated state and inactive, therefore unable to activate repair mechanisms instantly (Takács *et al*. 1999, Proctor 2000, Gehrke *et al*. 1996). In short-term UV-B enhancement studies under laboratory and greenhouse conditions, bryophytes have shown changes in photosynthetic parameters (0/-), photosynthetic pigments (+/-), photosynthesis (-), UV-absorbing compounds (0/-), sucrose and glucose synthesis (+/-), cellular organelles (-), phonological development (+), and growth (+/-) (+/0/- refer to positive/no/negative effects of enhanced UV-B; I). In field conditions, plants are exposed to continuously varying levels of UV-B, and consequently their UV defence may be adjusting continuously as well (Jansen *et al*. 1998).

1.4 Aims of the study

Although plant responses to ultraviolet radiation have been studied for several decades now, there is no clear insight as to the reactivity of mosses, since the results have so far been somewhat contradictory. As mosses form an important part of the ground layer in northern ecosystems, and these ecosystems are exposed to increasing UV-B, moss responses to enhanced UV-B are of importance to the whole ecosystem. So far, only results from a few long-term UV-B enhancement studies have been reported (Phoenix *et al*. 2001, Rozema *et al*. 2006).

In the present study, enhanced UV-B radiation was expected to decrease the concentration of chlorophylls *a* and *b* and carotenoids, and thus the ability of these species to photosynthesize. The bryophytes were expected to enhance protection against increased UV-B radiation by increasing the concentration of the methanol-extractable UACs. Annual green shoot growth was expected to decrease, and this effect was expected to develop over time. At present, the knowledge of the relative importance of the soluble and cell wall-bound UV-protective compounds is limited. The cell walls, especially of sun-exposed *P. juniperinum*, were expected to contain a relatively high concentration of compounds, creating a uniform, stable and effective cell wall-bound UV-screen.

Since natural UV has been systematically recorded for only a few decades, the moss sample archives may provide an importat source of additional knowledge on the subject. The purpose of the few retrospective studies of the UACs has been to find indicators for past UV radiation. In the present study, connections between specimen bank samples and irradiation conditions were

expected. Storage time was not expected to cause any remarkable oxidation of the methanol-extractable UACs. Seasonality of the UACs has to be taken into account when using herbarium or environmental specimen bank samples. Seasonality was expected to be observed in the methanol-extractable UACs in relation with irradiation, *i.e.* higher concentration of compounds under high UV radiation during spring, and lower concentration under low UV during autumn, and under snow. On-site irradiation data is not always available, and therefore off-site measured irradiation, within reasonable distances, may be useful. Reconstructed, modelled and off-site measured UV, and global irradiation data were expected to have a corresponding connection with the methanol-extractable UACs of the mosses.

The analysis of the concentration of the UACs is a simple practical method that relates total irradiation to total UV protection. Including the radiation-exposed surface of samples to the calculations was expected to give additional value to the results.

The work is based on studies of two ecologically important but fundamentally different bryophyte species, *Pleurozium schreberi* (Britt.) Mitt. and *Polytrichum juniperinum* Hedw. They were studied under ambient UV and experimentally enhanced UV in boreal environments.

My aim was to study:

1. seasonality of the photosynthesising pigments and methanol-extractable UV-absorbing compounds (I–IV),
2. cell wall-bound UV-protecting capacity,
3. effects of enhanced UV radiation on photosynthesising pigments, methanol-extractable UV-absorbing compounds and shoot growth, and the usability of the species for bioindicator purposes (II, III),
4. environmental specimen bank samples as bioindicators of past irradiation climate (IV), and
5. the usability of off-site measured, reconstructed and modelled UV radiation, global irradiation data, and total radiation in correlation with the total UV-absorbance (II, IV).

2 Material and methods

2.1 Present responses – Seasonality and protective strategy (I–IV)

Seasonality of the methanol-extractable UACs in *Pleurozium schreberi* and *Polytrichum juniperinum* was studied with mosses growing *in situ* and with transplanted moss samples (I–III). The photosynthetive pigments of *Pleurozium schreberi* were studied as well (III). The summer-time samples were collected in northern Finland between the years 2000 and 2005. Winter-time seasonality of the UACs in *P. juniperinum* was studied in 2002–2003 in Oulu (II). Differences between the collecting months of the specimen bank samples of *P. schreberi* were compared in a retrospective study of the UACs (IV).

To study the methanol-soluble versus the cell wall-bound UV-protecting capacity of the species, *Pleurozium schreberi* and *Polytrichum juniperinum* were sampled twice from natural sites in Oulu during spring 2009 (results presented in this work). On 23rd April, samples of *P. schreberi* were collected from patches of *P. schreberi*, other mosses and shrubs just uncovered by melting snow in a forest. Mats of *P. juniperinum* on open sites at the Botanical Garden were sampled from two conditions; from plots situated at the edge of melting snow cover, and from plots which had been exposed to irradiation for several days. The sampling was repeated in June 23rd.

2.2 Simulation of ozone depletion and experimental design (II–III)

Ozone depletion was simulated in two enhanced UV-B experiments in Sodankylä (67º 22` N, 26º 38` E) and Oulu (65º 10` N, 27º 20` E). *Pleurozium schreberi* growing naturally on-site was sampled during years 2002 to 2005 in Sodankylä (III). In addition to paper III, *P. schreberi* was sampled at the end of the fifth treatment year on October 1st, 2006 (results presented in this work). *Polytrichum juniperinum* was sampled in 2002 and 2003 in Oulu (transplanted mosses), and after the sixth treatment year *in situ* in 2007 in Sodankylä (II).

A long-term enhanced UV-B experiment of six years *in situ* was performed at the Finnish Ultraviolet International Research Center (FUVIRC, http://fuvirc.oulu.fi/) in Sodankylä (II, III). The experimental site was situated in a dry pine forest with mosses, lichens and shrubs. About 20% ozone depletion was simulated in a modulated system. In the supplemental UV-B treatment, the UV

lamps were covered with cellulose diacetate filters which transmitted radiation above 290 nm. Since the mosses receive additional UV-A radiation under UV-B treatment, a UV-A control was conducted using polyester filters blocking radiation under 314 nm. In an ambient control, shading equal to the other treatments was provided using frames without lamps.

A two-year enhanced UV-B experiment was performed in Oulu (II). The experimental plots were situated at the edge of a forest on the experimental field of the University of Oulu. Supplemental UV-B treatment and UV-A control were achieved with UV lamps and filters in a stepwise system.

2.3 Past responses – Reconstruction of past light climate (IV)

Samples of *Pleurozium schreberi* and *Hylocomium splendens* have been routinely collected and stored in national and international environmental specimen banks to be used in monitoring metal deposition changes in the environment (Lippo *et al*. 1995, Kubin *et al*. 1997, Harmens *et al*. 2004). In Finland, moss samples have been collected since 1985 and stored in the Finnish Environmental Specimen Bank in Paljakka (Kubin *et al*. 1997). These specimen banks can be useful in the long-term study of environmental changes as well. The usability of specimen bank samples of *P. schreberi* to reconstruct past irradiation climate was studied (IV). For this study, samples were taken from the collections in two series. In the first series, samples collected from Southern and Central Finland in 1985 and 1995 were chosen. In the second series, specimen bank samples from two sites in Southern and Northern Finland from 1985, 1990, 1995 and 2000 were selected (IV).

2.4 Radiation and environmental data

In the UV experiment in Sodankylä, UV-B and UV-A radiation were measured with erythemally weighted (CIE; McKinlay & Differ 1987) sensors (II, III). The modulated system maintained the supplemental UV-B level at a constant 46% above the ambient level of UV-B, corresponding to ozone depletion of approximately 20%. Photosynthetically active radiation (PAR) was measured with a sensor as well, and the light conditions of each plot were compared with a portable PAR instrument (III).

A step-wise irradiation enhancement system was used in the UV experiment in Oulu (II). The UV lamps were burning for six hours every day. Three

irradiation steps were achieved with dimmers. A European Light Dosimeter Network device (ELDONET) measured UV-B, UV-A and PAR under the treatments.

Present and past solar UV radiation data was measured, reconstructed and modelled (II, IV). The MILOS weather station in Oulu measured solar incoming, reflecting, and net radiation with a solarimeter (II). In Sodankylä and Jokioinen, a Brewer MK II spectroradiometer (Kipp & Zonen) was used to measure UV-B and UV-A radiation, and the spectra were weighted with the plant damage action spectrum (biologically effective, BE; Caldwell *et al*. 1986) and the erythemal action spectrum (CIE) (II, IV). Long-term global radiation data was obtained from the statistics of the Finnish Meteorological Institute (IV). CIE-weighted UV has been reconstructed for the area of Sodankylä and Southern Finland (Kaurola *et al*. 2000, Lindfors *et al*. 2007) (II, IV), and modelled for the area of Oulu (STRÅNG data by Swedish Meteorological and Hydrological Institute, SMHI, produced with support from the Swedish Radiation Protection Authority and the Swedish Environmental Agency) (II).

Temperature and precipitation were measured at the Botanical Garden in Oulu (II), and at the experimental site in Sodankylä (II, III). Long-term temperature and precipitation data were obtained from the climatic reports of the Finnish Meteorological Institute (IV). Depth of snow cover was measured in Oulu (II).

2.5 Methods to measure functional responses

2.5.1 Photosynthesising pigments (III)

The content of chlorophylls *a* and *b* and the total amount of carotenoids were analysed from the UV-B-treated *Pleurozium schreberi* in 2002–2005 (III). About 100 mg of frozen sample was homogenized on ice in 80% acetone (v : v) and $MgCO_3$ under dim light conditions. The pigments were extracted from the samples for three hours, and the absorbances were analyzed with a spectrophotometer (Beckman, DU-64) at wavelengths 479, 646 and 663 nm. The contents of chlorophylls and carotenoid were calculated with formulae by Lichtenthaler and Wellburn (1983), and expressed per fresh mass.

2.5.2 The methanol-extractable UACs (I–IV)

The total methanol-extractable UV-B and UV-A-absorbing compounds (UACs) were used to study the responses of *Pleurozium schreberi* and *Polytrichum juniperinum* to enhanced UV-B radiation, and to assess their usability for indicating changes in ambient and past levels of UV-B.

The young, green tips of the air-dry bryophyte gametophytes (about 5 mg) were weighed. The surface area of the samples were measured with the ImageJ program (I–III), or a digital image analyzer (Microscale TM / TC) (IV). The specific leaf area (the one-side silhouette per dry mass, mm^2 mg^{-1}, SLA) was calculated for the samples.

The samples were ground, and the soluble UACs were extracted overnight in acidified methanol (MeOH : H_2O : HCl; v : v : v; 79 : 20 : 1). The absorbances of the extracts were analyzed with a spectrophotometer (Beckman Coulter Inc., DU-64) between wavelengths 280 and 360 nm (with an interval of 5, 10 or 20 nm). Total absorbances were calculated for UV-B and UV-A wavelength regions by summing the absorbances at separate wavelengths (between 280–315 nm and 320–360 nm, respectively). The content of UV-B and UV-A-absorbing compounds were expressed per specific leaf area (SLA), and per dry mass (DM).

From the samples of *P. schreberi* collected after the fifth UV-enhancement year, the methanol-extractable UACs were extracted from the youngest top and the following older green part of the moss shoots. The absorbances were measured between 280 and 360 nm, with an interval of 2 nm.

2.5.3 The cell wall-bound UACs

Acidified methanol-extraction does not extract all (if any) cell wall-bound UACs, which leaves a part of the protecting capacity undisclosed (Clarke & Robinson 2008). Therefore, the concentration of the cell wall-bound compounds of *Pleurozium schreberi* and *Polytrichum juniperinum* in relation to the methanol-extractable compounds was measured in an additional study.

The cell-wall bound UACs were studied with a method adapted from Clarke and Robinson (2008). Samples of *P. schreberi* and *P. juniperinum* collected in April 2009 were dried at 50 ºC, and 25–40 mg of young tips of dry gametophytes per sample was weighed (approx. five gametophyte tips per sample). Samples were frozen in liquid nitrogen, ground, and the soluble UACs were extracted in acidified methanol.

To extract the cell wall-bound UACs, the remaining cell debris was incubated for 20 minutes in 1 M NaCl, twice in 0.5% (w : v) sodium dodecyl sulphate, twice in chloroform : methanol (1 : 1, v : v), then washed in acetone and air-dried. A 10 mg sample of the cell debris was incubated in 1 M NaOH for approximately 16 h in the dark. 0.7 ml of 1.5 M formic acid was added to 0.7 ml of the supernatant, and the absorbances were measured between 280 and 360 nm (5 nm interval) with a spectrophotometer. Concentration of the UACs was calculated on the basis of dry mass, and the methanol-soluble and cell wall-bound UACs were compared.

The location of the UACs in *P. schreberi* and *P. juniperinum* was studied with fluorescence microscopy. Samples of *P. schreberi* collected in April (dry samples) and June 2009 (fresh samples), and *P. juniperinum* collected in June 2009, were embedded in paraffin wax (Merck) (Karppinen *et al*. 2008). The samples were sectioned with a microtome (Minot-Mikrotom Type 1212, Ernst Leitz GMBH Wetzlar, Germany) to a thickness of 10 µm and spread on glass slides. Paraffin was removed from the cross-sections by rinsing them twice for 15 minutes in Histochoice (Sigma). The samples were immediatelly stained for 5 min with 0.5% (w : v) Naturstoff reagenz A (diphenylboric acid 2-aminoethyl ester, Carl Roth GmbH + Co.KG, Germany) in methanol, washed in methanol three times and covered with cover slides. Naturstoff reagenz A is a specific stain for flavonoids and vegetable acids. A fluorescence microscope (Optiphot-2-EF-D, Nikon Corporation, Tokyo, Japan) was used to locate the blue-green fluorescence in the cross-sections with magnifications of 10x, and autofluorescence was detected as well. Images were taken with a digital camera (Infinity 1, Lumenera Corporation, Ottawa, Canada), the iSolution Lite image program (IMT i-Solution Inc., Canada) was used to add contrast and brightness. Exposure time was enhanced under UV (about 750 ms).

2.5.4 Shoot growth (II, III)

The heights of the *Polytrichum juniperinum* segments were measured from the lowest green leaves to the tip of the gametophytes (II). The dry mass or the density of the green leaves were determined. The annual segment growth of *Pleurozium schreberi* gametophytes was determined (III). The stem height and the dry mass of the segments were measured, and the ratio of dry mass to height was calculated.

2.6 Statistical analyses (II–IV)

The data of the papers was tested using the T-test (II, III), one-way and two-way ANOVA (II–IV), repeated measures multivariate ANOVAs (III), and Pearson´s Correlation Test (II). If the assumptions of the parametric tests were not met originally and after log-transformation, the Kruskal-Wallis Rank Test (II, III, this work), and Spearman's rank correlation test were used (II–IV). Regression coefficients (R^2) were presented for the correlations (II). Differences between treatments were tested with *post hoc* multiple comparison tests (Least Significant Difference test in Papers II, III, and Tukey's HSD and Bonferroni tests in Paper IV). No statistical analyses were performed in Paper I. Sample size represent the number of sampling plots, as an average value per plot was used in the tests. The errors were calculated as standard deviation from the mean. The statistical analyses were performed using SPSS for Windows (versions 10.0, 15.0 and 16.0 were used in Papers IV, III, and II and this work, respectively; SPSS Inc., Chicago, IL, USA) statistical package.

Experimental designs, variables studied and the main results obtained here have been collected into Tables 1 and 2.

3 Results and discussion

3.1 Present responses – Seasonality and protective strategy (I–IV)

The two common moss species at northern latitudes, *Pleurozium schreberi* and *Polytrichum juniperinum*, differ from each other in morphology and in habitat preferences. Among bryophytes, endohydric *P. juniperinum* is anatomically and functionally close to vascular plants. As a pioneer species, it occupies open habitats and young forests (Botting & Fredeen 2006). At open sites, plant cover receives natural solar irradiation directly without alteration of the intensities of UV-B, UV-A and photosynthetically active radiation, PAR. Ectohydric *P. schreberi* has anatomical and functional characteristics typical for the majority of bryophytes. It is a common forest species present in a wide range of successional stages (Botting & Fredeen 2006). Under the forest canopy, the total amount of irradiation and the relative proportions of UV-B, UV-A and PAR received by a plant are influenced by absorption and reflection of radiation by other plants (Flint & Caldwell 1998). Knowing the plant responses under normal irradiation conditions is of importance, if the aim is to gain a good understanding of the effects of enhanced UV-B (Aphalo 2003).

3.1.1 Seasonality in photosynthetic pigments (III)

At northern latitudes, changing seasons have unquestionable influences on plants, ecosystems and their functions. In *P. schreberi*, the concentration of clorophylls *a*, *b* and carotenoids was observed to follow the intensity of PAR, decreasing towards autumn (III; Table 1). Nevertheless, the ratio of total chlorophylls to carotenoids increased with decreasing PAR, and this was primarily due to a proportionally greater reduction in carotenoids than in chloropylls over this time. This implies stronger protection from light-mediated stress during high irradiation conditions (Gehrke 1999). It has been hypothesized that chlorophylls are more sensitive to UV-B than carotenoids (Martínez-Abaigar *et al*. 2003). In this study the chlorophylls were observed to be rather insensitive to UV-B, since the reduction in total chlorophylls only occurred at the end of September (III).

Table 1. Overview of the experimental designs of *Pleurozium schreberi* (Britt.) Mitt., variables studied and methods used, the main results and references.

Experiment	Variable and Method[1]	Main results[2]	Ref
In situ / transplanted	Soluble UACs (MeOH)	Seasonality; varied between years, generally high during early summer (*)	I
In situ	Soluble UACs (MeOH) vs. Wall-UACs (Alkali)	Approx. 1:10 (^, during spring)	Thesis
In situ	UACs, fluorescence microscopy (NA)	Green fluorescence in the leaf cell walls, blue in stem and leaves close to it	Thesis
UV-B-experiment, four years	Soluble UACs (MeOH)	Increased (compared to UVA-tre) in 1st year (*, ^), positive corr. between UVB-comp – UVBR and UVA-comp – UVAR under UVB-tre during 4 years (*)	III
UV-B-experiment, 5th year	Soluble UACs (MeOH)	Increased mean and variation under UVB-tre (*, ^)	Thesis
Environmental specimen bank	Soluble UACs (MeOH)	Negative corr. with UVBR (off-site, ^), positive corr. with global radiation (*), decreased in time (*, ^), negative corr. with temperature and precipitation (*)	IV
UV-B-experiment, four years	Chl *a*, *b* + Car	Increased with PAR, Chl *a*: *b* and Chl:Car increased during 4 years, negative corr. between Chl:Car – PAR	III
UV-B-experiment, four years	Annual shoot growth	Increased under UVA-tre in 2nd year, decreased under UVB-tre and UVA-tre in 3rd	III

[1] UACs, UV-absorbing compounds, in the UV-B wavelength range unless otherwise stated; MeOH, acidified methanol; NA, Naturstoff reagenz A; Chl *a*, *b* + Car, chlorophylls *a* and *b*, and carotenoids,
[2] (*) UACs calculated per specific leaf area (SLA, $mm^2\ mg^{-1}$); (^) UACs calculated per dry mass (mg); results of the enhanced UV-B experiments presented under +UV-B treatment unless otherwise stated, UVB-tre, UVA-tre (tre = treatment); UVBR, UVAR, UV-B and UV-A radiation (R = radiation); PAR, photosynthetically active radiation; off-site, UV-B radiation modelled, reconstructed, or measured off-site

Table 2. Overview of the experimental designs of *Polytrichum juniperinum* Hedw., variables studied and methods used, the main results and references.

Experiment	Variable and Method[1]	Main results[2]	Ref
In situ / transplanted	Soluble UACs (MeOH)	Seasonality; high during autumn, commonly high during early summer (*)	I
In situ	Soluble UACs (MeOH)	Seasonality; negative corr. with UVBR (off-site, *), low during mid-summer (*, influence of unfavourable condition), relatively high under snow (*, ^)	II
In situ	Soluble UACs (MeOH) vs. Wall-UACs (Alkali)	Approx. 1:12 (^, during spring)	Thesis
In situ	UACs, fluorescence microscopy (NA)	Faint blue fluorescence in lamellae	Thesis
UV-B-experiment, two years (transplanted)	Soluble UACs (MeOH)	Decrease or inhibition after daily UVB-tre in times and positive corr. with UVBR at mornings during early stages of experiment (*)	II
UV-B-experiment, 6th year	Soluble UACs (MeOH)	Increased variation under UVB-tre	II
UV-B-experiment, 2nd year (transplanted)	Green shoot growth	No	II
UV-B-experiment, 6th year	Green shoot growth	Decreased under UVB-tre, increased under UVA-tre	II

For [1] and [2]; see Table 1

3.1.2 Seasonality in methanol-extractable UACs (I–IV)

Plants are reactive to alterations in UV-B within the ambient range (Paul & Gwynn-Jones 2003 and referenced therein). Seasonality in the methanol-extractable UACs of the mosses was expected to follow the intensity of irradiation, *i.e.* higher concentration of compounds under high UV radiation during spring, and lower concentration under low UV, and winter-time (I–III). The summer-time seasonality pattern in the methanol-extractable UV-B-absorbing

compounds of *P. schreberi* per SLA acted approximately as predicted (I, III; Table 1). The compound concentration was generally higher during early summer with high UV-B conditions compared to late summer with low UV-B. However, differences between years in seasonal behaviour of *P. schreberi* were observed (I). High autumnal absorbances were observed at the seashore (I), and both early and late summer peaks in compound concentration have been observed in retrospective studies of *P. schreberi* (IV, Huttunen *et al*. 2005).

Apart from *P. schreberi*, similar seasonal pattern in the compounds has been observed in *Hylocomium splendens* (I, Taipale & Huttunen 2002) and *Dicranum polysetum* (I). High autumnal absorbances were observed in *Dicranum scoparium*, *Pohlia nutans*, and *Racomitrium canescens* at the seashore (I). Antarctic mosses *Andreaea regularis*, *Bryum pseudotriquetrum*, and *Sanionia uncinata* have been shown to respond to seasonal changes in UV radiation, with highest methanol-extractable UV-B-absorbing compound concentration measured during austral spring (I, Newsham 2003, Dunn & Robinson 2006, Newsham *et al.* 2002).

Compared to *P. schreberi*, *P. juniperinum* showed considerably higher concentration of methanol-extractable UV-B-absorbing compounds per SLA (I) and per DM (Fig. 1). Unexpectedly, the seasonality pattern in the methanol-extractable UAC concentration of *P. juniperinum* (per SLA and DM) was the opposite, with an increase in the concentration towards autumn (I, II; Table 2). The minimum values were observed in July, which had the highest irradiation levels. Unfavourable environmental conditions, *i.e.* a period of simultaneous drought, high temperatures and high irradiation during early summer, had a negative effect on the concentration of the soluble compounds, but the effect was not statistically significant (II). In two lichen species, a long period of drought and high irradiation has been suggested to have caused a decrease in usnic acid which absorbs effeciently in the UV-B range of the spectrum (Bjerke *et al*. 2005). Decrease in polyphenols under drought stress has been observed in sun-exposed higher plants as well (Tattini *et al*. 2004). On the other hand, simultaneous enhanced UV-B and water stress increases the UV-absorbing compound and flavonol glycoside concentrations in *Trifolium repens* (Hofmann *et al*. 2003).

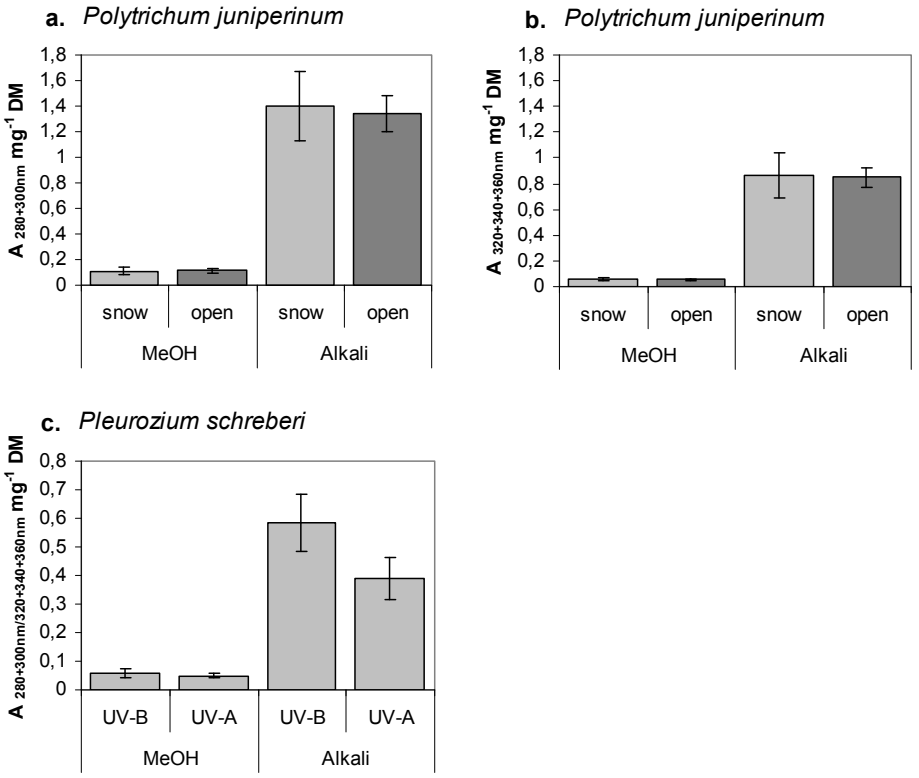

Fig. 1. The methanol-soluble (MeOH) and cell wall-bound (Alkali) UV-B (a, c) and UV-A (b, c) absorbing compounds of *Polytrichum juniperinum* (a, b) and *Pleurozium schreberi* (c) per dry mass in Oulu on April 23rd 2009. Light gray bars represent samples just uncovered by melting snow (snow), and dark gray bars represent samples exposed to direct solar irradiation for several days (open). Means and standard deviations were calculated for five sampling plots.

Both UV-B and desiccation can cause oxidative stress (*e.g.* Takács *et al*. 1999 and references therein). As mosses often experience periods of drought, they may have adaptations against oxidative stress (Lud *et al.* 2002). UV-B tolerance and desiccation tolerance are suggested to involve similar defence mechanisms (Takács *et al*. 1999, Hofmann *et al*. 2003), some of which have been proposed to be constitutive (Takács *et al*. 1999). The effects of drought and temperature has been observed in the moss *Bryum pseudotriquetrum*, as the degree of DNA damage increased with decreasing temperature and turf water content under

Antarctic conditions, indicating a lower rate of DNA repair with less favourable conditions (Turnbull & Robinson 2009).

During drought, the leaves of desiccated *P. juniperinum* gametophytes are appressed to the stem. In hydrated conditions, the leaves are extended sideways. This altering leaf orientation may offer the plant additional protection from UV-B, since the upward orientated leaves receive less irradiation than the sideways extended leaves. Wax on the surface of the leaves may also reflect the incoming radiation. It is possible, that during dryer periods the gametophytes have received less UV-B under enhanced UV-B treatment than was anticipated, thus contributing to the lack of treatment effect.

Under snow, the monthly compound concentrations of *P. juniperinum* were comparable to the spring and autumn-time concentrations, the concentration being especially high during low air temperatures in January (II). Increase in the methanol-extractable UACs per area due to low temperature (typical for winter-time) has been observed in aquatic moss *Fontinalis antipyretica* (Núñez-Olivera *et al*. 2004). The ratio between UV-B and UV-A-absorbing compounds varied, being at it's highest in December and lowest in July, mainly due to changes in the concentration of UV-B-absorbing compounds. During early summer in June, weak but statistically significant positive correlations between UV radiation and compound concentration per SLA and DM were observed on four consecutive days. These findings suggest that the soluble UACs are affected by environmental factors other than UV radiation alone (II), and that they have a role in winter hardening as well (II). On the other hand, the rate and rapidity of transformation of the UACs from soluble to cell wall-bound form is not known in mosses. In conifers, certain flavonoids have been found to translocate soon after formation from the soluble pool into the cell wall-bound pool (Strack *et al*. 1989, Kaffarnik *et al*. 2006). The translocation may occur more rapidly in certain conditions than in others.

The UV-B and UV-A-absorbing compound concentrations of *P. juniperinum* collected from the Botanical Garden in Oulu were correlated with three alternative UV data sets since there were no UV measurements conducted at the site (II). Even with a gap in the radiation data, the off-site measured UV_{BE} (Brewer data from Sodankylä) showed the strongest negative correlation with the compounds ($P < 0.001$ or $P < 0.01$). Reconstructed (for the area of Sodankylä) and modelled (for the area of Oulu) UV_{CIE} data did not have as strong a correlation, but they were still highly significant (the significance level $P < 0.05$ or higher) (II). The modelled UV data had the weakest correlation with the

compounds. This may be partly explained by the STRÅNG-model being Swedish, and Oulu is at the edge of the area of their modelling interests. The best correlations were gained with radiation data from three days prior to the time of sampling.

It is clear that off-site measured, reconstructed and modelled radiation data cannot take into account the local changes in cloudiness and other variables affecting the radiation dosages received by the plants. Also, UV_{CIE} data is meant for human skin and not for plants like UV_{BE} data. Nevertheless, the correlations were significant which supports the usability of this kind of radiation data as a rough estimation of irradiation in cases where of no local radiation measurements have been made.

3.1.3 Visualization of cell wall-bound compounds

Both *P. schreberi* and *P. juniperinum* show evidence of high alkali-extractable UV-screening capacity in the cell walls in April 2009 (Table 1, Table 2). Compared to the methanol-extractable compounds (per DM), the concentrations were 10 and 12 times higher, respectively (Fig. 1). In *P. juniperinum*, the mean concentration of soluble and cell wall-bound compounds did not differ between the samples which had been exposed to direct sunlight for a few days and the samples which situated at the edge of melting snow cover, but the variance was higher in the samples most recently uncovered by the protecting layer of snow. This suggests that the gametophytes were reacting to the recent change in their light environment, as has been observed in higher plants (see Caldwell 1971 for reference). On a dry mass basis, *P. juniperinum* has roughly twice as high concentration of soluble and cell wall-bound UACs than *P. schreberi*. This reflects the differences in habitat preference of the species. As *P. juniperinum* has more soluble and cell wall-bound UACs than *P. schreberi*, it may be better protected against UV-B radiation to begin with (Arróniz-Crespo *et al*. 2004). In both species, the total UV-B-absorbance (methanol-soluble and cell wall-bound together) was higher than the total UV-A-absorbance (Fig. 1). It has to be considered, that the alkali extraction may have extracted other compounds from the cell walls besides those absorbing UV.

The cell walls of *P. schreberi* are relatively thick, 1.5–2 µm (Taipale & Huttunen 2002), containing polymerized lipids, hydroxyl acid, dicarboxylic acids, fatty acids, fatty alcohols, and some unidentified components (Kälviäinen *et al*. 1985). Caffeine has been used to distinguish phenolic compounds in plant cells

(Charest *et al*. 1986). In higher plants, the intracellular flavonoids have been found to accumulate mainly within the cell walls (Charest *et al*. 1986). Caffeine stained cell walls of *P. schreberi* show a staining pattern similar to those of higher plants (Taipale & Huttunen 2002). Localization of phenolic compounds in the tips of *P. schreberi* and *P. juniperinum* gametophytes was studied with Naturstoff reagenz A which is a specific stain for flavonoids (Markham *et al*. 2000, 2001).

Many fluorescent substances have been reported in plants (Rost 1995). For example, hydroxycinnamic acid derivatives like ferulic acid and caffeic acid, and flavonoids can act as fluorophores, emitting blue-green autofluorescence under UV irradiation (excitation wavelengths 337 and 310 nm) (Morales *et al*. 1996, Lichtenthaler & Schweiger 1998). Blue-green fluorescence can be used to measure plant stress (see Johnson *et al*. 2000 for references). In *P. schreberi*, the Naturstoff reagenz A strongly stained the cell walls of the moss a green colour (Fig. 2a-d). Naturstoff reagenz A stains flavonoids specifically and fluoresces yellow to green under UV irradiation (Markham *et al*. 2000, 2001). The green fluorescence in the cell walls of *P. schreberi* indicates the existence of UACs, and more specifically, flavonoids. Interestingly, leaves of samples collected in April (dry) and in June (fresh) expressed strong green fluorescence, but in the samples collected in April, the leaves closest to the stem and the stem itself had a faint blue fluorescence. In June, this blue fluorescence was not observed. The results suggest that the stable cell wall-bound UV screen in *P. schreberi* is located where it is most needed, in the outer and therefore most UV-exposed leaf layers, and is expressed as green fluorescence. An explanation for the blue fluorescence observed in the Naturstoff reagenz A stained samples was not found in the literature. Structural differences in the phenolic compounds of bryophytes and higher plants may have contributed to the fluorescence. The proximity of winter, the spring-time high UV radiation levels and recent exposure to direct sunlight may have influenced the occurrence of the blue fluorescence but this can not be verified. This phenomenon needs further study.

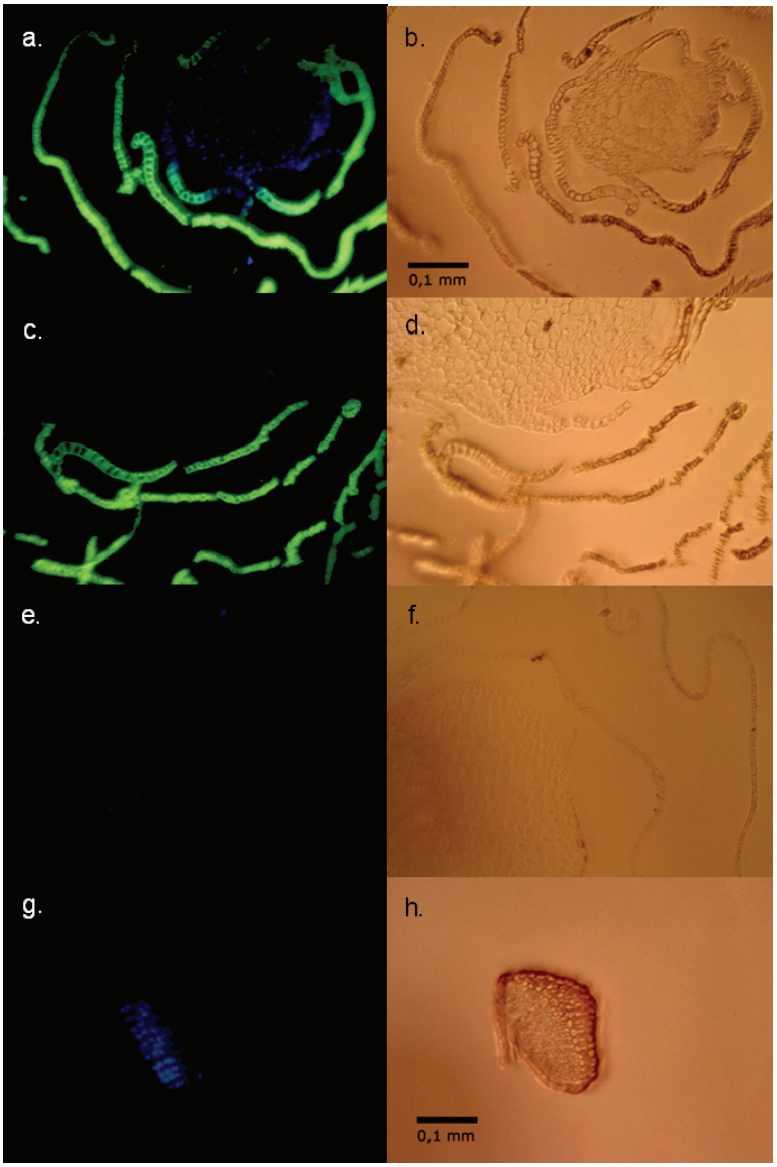

Fig. 2. Cross-sections of the tips of *Pleurozium schreberi* (a–f) and *Polytrichum juniperinum* (g–h) gametophytes collected in April (a–b) and in June (c–h), 2009. The samples (a–d, g–h) were dyed with Naturstoff reagenz A for UV-absorbing compounds (flavonoids) and the samples were studied under UV (left, excitation wavelength 365 nm) and visible (right) light. Autofluorescence was not detected in the control samples (e–f).

In *Pinus* species, excitation with UV induced fluorescence in naturally occurring fluorescent substances, observed as blue fluorescence in the inner layers, and green fluorescence in the outer layers (surface) of the needles (Johnson *et al*. 2000). In higher plants, cell walls generally show blue autofluorescence, and flavins show green fluorescence (Rost 1995). Autofluorescence was not detected in the samples studied (Fig. 2 e–f).

Green fluorescence was not observed in *P. juniperinum* in June (Fig. 2 g–h). This was a surprising result, since it was expected that the leaf ('costa') surrounding lamellae would express strong fluorescence, and thus provide protection to the lamellae. Faint blue fluorescence was observed only in the lamellae. Because staining with Naturstoff reagenz A was successful in *P. schreberi*, the results imply that the UACs in the cell walls of *P. juniperinum* are something other than flavonoids. The structure of flavonone-styryl hybrid molecules (communins) and benzonaphthoxanthenones (ohioensis) which have been found in *Polytrichum* species (Seo *et al*. 2008, Fu *et al*. 2009) may differ from the structure of flavones (apigenin and diosmetin glucosides) detected in *P. schreberi* (Markham 1988) enough to affect the staining of the *P. juniperinum* cell walls.

Altogether, the alkali-extractable cell wall-bound UACs evidently provide a uniform and effective (sping-time) UV protective screen in both *P. schreberi* and *P. juniperinum*. In *P. schreberi*, the screen is located in the outer leaf layers of the gametophyte which are more exposed to irradiation. This cell wall-bound UV screen has been presumed to be stable, but possible seasonal changes are not known.

In Antarctica, varyious protective strategies against UV-B radiation in the cosmopolitan species *Bryum pseudotriquetrum* and *Ceratodon purpureus*, and endemic *Schistidium antarctici* have been reported. *B. pseudotriquetrum*, which has been shown to react to changing UV-B through methanol-extractable UV-B-absorbing compounds, had equal proportions of soluble and cell wall-bound compounds (Dunn & Robinson 2006, Clarke & Robinson 2008). In the other two species, the majority of the UV-screening capacity was situated in the cell walls (Clarke & Robinson 2008). The endemic *S. antarctici* has only a half of the total UV-screening capacity of the other mosses, and it has been concluded to be the most vulnerable species to increasing UV-B (Robinson *et al*. 2005, Dunn & Robinson 2006, Clarke & Robinson 2008). In the aquatic moss *Fontinalis antipyretica*, enhanced UV-B was observed to change the colour of the cell walls while the protoplasts remained green (Martínez-Abaigar *et al*. 2003). This

phenomenon was suggested to indicate increased UV-B protection in the cell walls, or degradation of the cell wall compounds.

Our results showed notably higher concentrations of the cell wall-bound compounds in proportion to the soluble ones in boreal mosses, compared to those in the Antarctic. This may be due to differences in timing and environmental conditions of the sampling, differences between Arctic and Antarctic conditions (Rozema *et al*. 2005), or the differences in the methods. Also, *P. juniperinum* shows higher UV-protecting capacity than *P. schreberi*, suggesting that *P. juniperinum* is better protected from UV-B.

3.2 Long-term simulation of ozone depletion (II, III)

The responsiveness of *Pleurozium schreberi* and *Polytrichum juniperinum* to environmental changes – to enhanced UV-B in this case – is particularly important, since these very common species have an unquestionable role in the functions of northern ecosystems. In addition, arctic areas of Scandinavia are predicted to be the most affected by changes in UV-B radiation in the Northern Hemisphere (Björn *et al*. 1998, Taalas *et al*. 2000). Previous studies of several bryophyte species have shown variability in their responsiveness and the direction of the response to enhanced UV-B radiation (I and references therein).

In Arctic plant communities, experimental manipulation studies of four years or less in duration are relatively short in terms of observing plausible treatment effects (Dormann & Woodin 2002). To our knowledge, results of enhanced outdoor UV experiments of five years or more have been reported so far for four moss species, *Hylocomium splendens*, *P. schreberi*, *Polytrichum hyperboreum*, and *P. juniperinum* (II, III, Gehrke *et al*. 1996, Björn *et al*. 1998, Phoenix *et al*. 2001, Rozema *et al*. 2006, Lappalainen *et al*. 2008). For *H. splendens* and *P. schreberi*, the results have been reported annually. Age of an individual moss gametophyte is limited – a mean age of three years of living vegetative shoots has been determined for *P. commune*, reaching up to 7 years (Callaghan *et al*. 1978) – but in *P. juniperinum* rhizoids connect separate gametophytes. Average age of the gametophytes in the UV experiments was not determined.

3.2.1 Effects on photosynthetic pigments (III)

The chlorophylls and carotenoids of *P. schreberi* did not show any response to the enhanced UV-B treatment, and thus the potential to photosynthesize was not affected (III; Table 1). A meta-analysis of Arctic and Antarctic bryophytes and angiosperms showed no UV-B induced effects in photosynthetic pigments or photosynthetic parameters (Newsham & Robinson 2009). In bryophytes, photosynthetic pigments have, in general, either expressed no (I, Gehrke *et al*. 1996, Lud *et al*. 2002, Sonesson *et al*. 2002) or reversible responses to UV-B (Takács *et al*. 1999). Nevertheless, enhanced UV-B can reduce the ability to photosynthesize, as has been observed in aquatic moss *Fontinalis antipyretica* (Martínez-Abaigar *et al*. 2003, Núñez-Olivera *et al*. 2004), and in two lichen species (Bjerke *et al*. 2005). UV-A treatment increased the concentration of photosynthetic pigments in *Sphagnum magellanicum* (Niemi *et al*. 2002b) but this was not seen in *P. schreberi* (III).

Even though no treatment effect was observed, the ratios between chlorophyll *a* to chlorophyll *b*, and chlorophylls to carotenoids increased during the four study years (III). These ratios can indicate stress responses (Martínez-Abaigar *et al*. 2003), and increase in the ratios may be due to adaptation of *P. schreberi* to stress.

On the basis of photosynthetic responses to enhanced UV-B, Takács and others (1999) divided bryophytes into four groups; the most desiccation tolerant species tolerate UV-B well, the desiccation tolerant forest species undergo acclimation after transient responses, and species from moist and cool habitats suffer irreversible damage, or recover only after the stress is over. Facilitative effects of UV-B were also found, as the overall photosynthetic rate of desiccation-tolerant *Dicranum scoparium* increased temporarily (Takács *et al*. 1999). Fewer accumulated DNA photoproducts in desiccated mosses compared to hydrated mosses supports the division, suggesting that screening and passive defence mechanisms are effective in bryophytes adapted to dry conditions (Turnbull *et al*. 2009).

In nature, several factors influence the performance of plants. Enhancing UV-B radiation has been found to decrease the photosynthesis of some species. Climate change scenarios predict increases in carbon dioxide (CO_2) concentration (IPCC 2007), which generally has been found to have a positive influence on photosynthetic capacity of plants. These contrary responses are likely to have interactions (Sonesson *et al*. 1996).

3.2.2 Effects on methanol-extractable UACs (II, III)

Formation of soluble UACs was expected to be induced as a direct response to enhanced UV-B. Treatment effects within hours, transient effects, and effects only after several treatment years were observed in *P. schreberi* and *P. juniperinum* (Table 1, Table 2).

In *P. juniperinum*, reversible responses within hours were observed under the stepwise irradiation enhancement system at the early stages of the experiment (II). On some days, six hours treatment of enhanced UV-B was found to inhibit UV-B-absorbing compound formation or decrease the content (per SLA). Inhibiting effects of UV-B treatment on the compounds have been observed before in *Polytrichum commune* (Gehrke 1999) and in *Sinapis alba* (Buchholz *et al*. 1995). Inhibition of flavonoid biosynthesis may have caused a decrease in UV-B-absorbing compound concentration (Buchholz *et al*. 1995, Gehrke 1998). Also, high doses of UV-B (Barsig *et al*. 1998), and high doses in general (*e.g.* in toxicity studies) have shown to have inhibiting effects on metabolism, development and growth (Calabrese & Blain 2009). In this study, the effect was transient and was reversed during the night (II). Reversible effects have been observed in *Sanionia uncinata*, as DNA damage induced by day-time UV-B treatment was repaired during the night (Lud *et al*. 2002). Nevertheless, before the UV lamps were turned on in the mornings, the compounds of the UV-B-treated *P. juniperinum* gametophytes (per SLA and DM) correlated positively with the UV radiation of the previous 2–3 days (II). Regardless of the occasional reversible inhibiting effect of high irradiation treatment, the response of the gametophytes to the amount of UV-B radiation received during the previous 2–3 days can be seen in the mornings. The formation of UV-absorbing pigments has been found to be induced after 7 hours of UV exposure in *Arabidopsis thaliana* (Lois 1994). With bryophytes, it has been suggested that soluble UV-B-absorbing compounds are able to respond to changes in UV-B radiation within 24 hours (Newsham *et al*. 2002). It is possible, that the six hour treatment was not sufficient to cause constant effects, since effects were not observed after every daily UV treatment during the first weeks (II). After two years, no UV-B treatment effect was discovered. On the basis of the results discussed earlier, performing regular samplings during mornings may have had an affect on the UV-absorbing compound concentration and furthermore on the lack of trend. Veit *et al*. (1996) reported diurnal changes in flavonoids of higher plants, but stable diurnal

concentrations of flavonoids have also been observed (Ken Ryan, personal comm.).

Dicranum elongatum and *D. polysetum* were observed to express no significant treatment effects in their UACs after one or two years of exposure to UV-B (I, Sonesson *et al*. 2002). *Dicranum* mosses fall between ecto- and endohydric species. On the other hand, bryophytes from moist conditions, like *Sphagnum* species and aquatic moss *Fontinalis antipyretica*, have shown responses to enhanced UV-B within months (Niemi *et al*. 2002ab, Martínez-Abaigar *et al*. 2003, Núñez-Olivera *et al*. 2004). These mosses do not suffer from water stress in the same way that forest mosses do, which may give them the ability to respond more rapidly to changes in UV-B. However, contradictory responses to enhanced UV-B have also been observed in *Sphagnum* species (Niemi *et al*. 2002b).

In *P. schreberi*, UV-B induced enhancement in soluble UACs (per SLA) was observed after the first year when compared to the UV-A control, but not to the ambient control (III). Enhanced UV-A radiation in the UV-A control may have mitigated the effect of ambient UV-B on the UAC concentration. During the three following years, there were no detectable treatment effects in the UACs. Nevertheless, during the four study years, the UAC concentration of the UV-B and UV-A treated gametophytes correlated with UV-B and UV-A radiation levels of the previous 2–3 days, respectively (III). Enhanced UV-B was found to increase the concentration of methanol-extractable UV-B-absorbing compound (per DM) after three months in *Hylocomium splendens* (Taipale & Huttunen 2002).

After the fifth treatment year, higher mean concentrations of UV-B-absorbing compounds in *P. schreberi* – and higher variance among the treatment replicates as well – was observed under enhanced UV-B compared to UV-A and ambient controls (per DM $\chi^2 = 7.329$, df = 2, P = 0.026; per SLA P = 0.05; Fig. 3a, c). This increase was observed in the young top segments of the gametophytes. No significant treatment effects were found in the UV-A-absorbing compounds (Fig. 3b, d), and no cumulative accumulation of compounds was observed in the older green parts of the gametophytes. In *Polytrichum commune*, decrease in the soluble UACs under enhanced UV-B was also observed only after the third treatment year (Gehrke 1999).

In *P. juniperinum*, six years of constantly enhanced UV-B did not affect the mean concentration of methanol-soluble UACs (per SLA or DM), but the variance among the treatment replicates was significantly larger compared to UV-

A and ambient controls (II). This may be due to induction of UACs in some gametophytes but not in all (Gehrke 1999). Another possible explanation is uneven shading in the forest, which may have affected especially the UV-B radiation dose received by the UV-B treated gametophytes. The position of mosses relative to the canopy causes variance in the UV-B exposure and in the proportion of UV-B to PAR received by individual gametophytes (Flint & Caldwell 1998). As shading by neighbouring plants has been found to affect coverage of some bryophyte species (Sørensen *et al.* 2009), shading may also decrease the UV-B treatment dose enough to reduce the UV-B induced effect in bryophytes (Gehrke 1998, Deckmyn *et al*. 2001). The acidified methanol-soluble UV-B-absorbing compounds of Antarctic moss *Sanionia uncinata* were found to increase with increasing ratio of UV-B to PAR (Newsham *et al*. 2002). Shading by neighbouring shrubs alters this ratio (Flint & Caldwell 1998), and this may have caused the enhanced variation in the methanol-extractable UACs detected in the UV-B treated *P. schreberi* and *P. juniperinum* (II, this work). The light conditions on the basis of PAR measurements varied among the individual plots, but no clear relationship between the amount of light and the UACs at the UV-B treatment plots was observed (II, III). The low sample size under field conditions may have hindered the observation of statistically significant effects of enhanced UV-B (Stephen *et al*. 1999).

On the basis of these results, the effects of five or six years of exposure to enhanced UV-B were not apparent. Treament effects have been found in several measured variables of the mosses, but on foundation of soluble UACs only, distinct conclusions are hard to make. High variance in the compounds among the individual study plots was observed in both species. The source of variation may have been differences in adaptation between gametophytes to enhanced UV-B radiation, or varying conditions between the study plots. The effect of varying micro-irradiation climates may have influenced the outcome of field studies reporting no statistically significant effects. At an open site, without shading by other plants, the effects in the compounds might have been more constant for the pioneer *P. juniperinum*. The proportion of the cell wall-bound UV-screening compounds may vary as well. In the sub-Arctic dwarf shrub *Vaccinium vitis-idaea*, enhanced UV-B was observed to increase the concentration of epidermal cell wall-bound UACs (Semerdjieva *et al*. 2003). It was suggested that evergreen *V. vitis-idaea* would have a strategy of exclusion, while deciduous *V. myrtillus* invests in soluble UACs throughout the leaf (Semerdjieva *et al*. 2003).

Shade gametophytes of Antarctic *Bryum subrotundifolium* (Green *et al*. 2005) and aquatic *Fontinalis antipyretica* (Núñez-Olivera *et al*. 2005) have been shown to be more sensitive to UV-B and UV-A radiation than sun-exposed gametophytes. Nevertheless, it has been suggested that the sun and shade gametophytes are equally tolerant in bryophyte species with high UV-B tolerance (Núñez-Olivera *et al*. 2005). *P. juniperinum* populations in open habitats experience different selective pressures than populations in more closed surroundings (Hedderson & Longton 2008). Even if *P. juniperinum* is adapted to sites of high irradiation and has more total UACs than the forest species *P. schreberi*, different populations may still have slightly differing responses to environmental stresses. However, the genetic diversity within *P. juniperinum* populations is not very high (van der Velde & Bijlsma 2000).

According to a meta-analysis with polar bryophytes and angiosperms, UV-B radiation induces the production of UV-B-absorbing compounds, with a mean increase of 7.4% on a dry mass basis (Newsham & Robinson 2009). The increase was observed under decreased (screens) and unmanipulated natural UV-B radiation, but not under enhanced UV-B radiation (UV lamps). Undetectable treatment responses under UV lamps were partly explained by unstable outputs from the lamps at low polar temperatures (Johanson & Zeuthel 1998, Newsham & Robinson 2009), since responses have been observed under UV lamps at temperate regions (Searles *et al*. 2001). Unstable lamp outputs may have influenced our experiment as well. Also the generalized action spectrum (Caldwell 1971), used to weight the UV-B radiation, has been suggested to result in unrealistically low UV-B doses applied from UV lamps to vegetation (Flint & Caldwell 2003b, Newsham & Robinson 2009). Additionally, with slow processes of polar ecosystems taken into account, some of the species in the meta-analysis may still have been adjusting to the higher levels of UV-B emitted by the lamps, and the more significant effects of enhanced UV-B would have been still to come. This may be the case for *P. schreberi* and *P. juniperinum*, assuming sufficient longevity of the gametophytes at the northern dry pine forest research field.

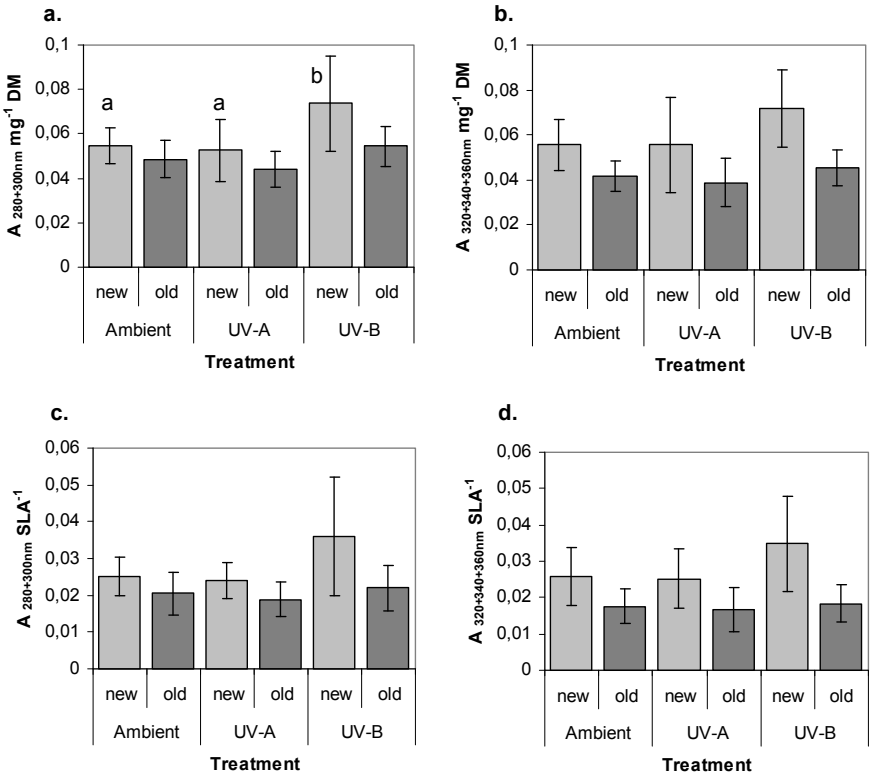

Fig. 3. The methanol-soluble UV-B (a, c) and UV-A (b, d) absorbing compounds of *Pleurozium schreberi* after fifth year in enhanced UV-B experiment in Sodankylä on October 1st, 2006. Results are presented for the young, top part of the gametophyte (new) and for the lower older part (old). Means with standard deviations are presented on dry mass (a, b) and on specific leaf area (mm^2 mg^{-1}, SLA; c, d) basis. For each bar, N = 7.

The crude methanol-extraction of the UACs may not reveal all there is to know about the compounds. Even if no significant changes can be observed in the bulk compound concentration, enhanced UV-B may still change the proportions of individual compounds. This has been observed with flavonoids of the liverwort *Marchantia polymorpha*; enhanced UV-B did not show a clear effect on luteolin or apigenin individually, but the luteolin : apigenin ratio increased, suggesting that luteolin is more effective against UV-B (Markham *et al*. 1998). Compound extraction with methanol : chloroform : formic acid extraction media has given

higher yields and a more complex pattern of extractable phenolics (with HPLC) than 80% aq. methanol (Strack *et al*. 1989). It would be useful to study the individual compounds of *P. schreberi* and *P. juniperinum* with HPLC.

It has been suggested that mosses may have other mechanisms to tolerate UV-B in addition to the accumulation of methanol-extractable UACs, such as efficient DNA and oxidative damage repair system and structural protection through leaf overlapping (Arróniz-Crespo *et al*. 2004). Several or all these protecting mechanisms are likely to act simultaneously, at least to some extent.

3.2.3 Effects on shoot growth (II, III)

In *P. juniperinum*, six years of enhanced UV-B in Sodankylä decreased the density of green leaves on a gametophyte stem (II; Table 2). At the same time, the gametophytes under the UV-A control showed taller green-leaved segments. Increased stem growth under UV-A has been observed in *Polytrichum commune* as well but – contrary to our results – the leaf density increased under enhanced UV-B (Gehrke 1999). Investing in UV tolerance and repair affects growth over time (Laakso *et al*. 2000, Gwynn-Jones 2001). Initiation of reproduction in mosses is likely to be dependent on size rather than age of the gametophyte (Stark *et al*. 2000, Hedderson & Longton 2008). Therefore UV-B induced reduction in the (green) shoot growth (II) may, in the long run, influence the reproduction capability of *P. juniperinum*, which normally produces sporophytes frequently. Growth effects during the first or second treatment year in Oulu were not observed.

In *P. schreberi*, UV-B induced effects on annual segment growth were observed early in the treatment programme (III; Table 1). After the second year of enhanced UV-B treatment in Sodankylä, the height and dry mass of the newly grown segment were found to be higher under the UV-A control. These increased growth responses under the UV-A control in both species may be due to mitigation of ambient solar UV-B effect by enhanced UV-A radiation. After the third year, dry mass of the annual segment, and the ratio between dry mass and height, had decreased under both enhanced UV-B and the UV-A control, in comparison to ambient. Besides mitigating UV-B induced effects, UV-A radiation can also have inhibiting effects. Differences between the treatments were not observed after the fourth year. It has been suggested that mosses like *P. schreberi*,

may entirely escape from accumulating growth effects due to their simple morphology and relatively short life-time (Gehrke *et al*. 1996).

Growth of the aquatic moss *Fontinalis antipyretica* has been found to decrease under enhanced UV-B as well, with an increase in schlerophylly, *i.e.* thickening of leaves (Martinez-Abaigar *et al*. 2003). Decreased height increment and dry mass production has been observed in *Hylocomium splendens*, *Polytrichum commune*, and some *Sphagnum* species (Gehrke *et al*. 1996, Gehrke 1998, 1999, Niemi *et al*. 2002a, Sonesson *et al*. 2002). In the liverwort *Marchantia polymorpha*, enhanced UV-B decreased the growth and increased the amount of dead tissue in a growth chamber experiment (Markham *et al*. 1998). Ambient UV-B in Antarctica was observed to increase branching of *Sanionia uncinata,* but it did not affect the biomass production in comparison with reduced ambient UV-B (Lud *et al*. 2002). On the other hand, in a five-month growth chamber experiment, enhanced UV-B increased the length and dry mass of *Hylocomium splendens*, but since the dose of (photorepairing) UV-A is higher under enhanced UV-B treatment compared to ambient, it has been suggested that this had an influence on the results (Sonesson *et al*. 1996). Positive effects of UV-A on growth were also seen in our results (II, III).

It is worth remembering that *P. juniperinum* grew more or less as single shoots in Sodankylä, which made the number of available samples low. In the case of *P. schreberi*, the heterogeneity in abundance of the species under the treatment plots was high – it was very abundant under some plots, and scarce under others. Growing as single shoots compared to turfs has an entirely different consequence for *P. schreberi* than *P. juniperinum* (Callaghan *et al*. 1978, see Bates 1988). The former is more dependent on the neighbouring gametophytes in water relations than the latter. Photosynthesis is dependent on water availability in *P. schreberi* and on intensity of PAR in *P. juniperinum* (Callaghan *et al*. 1978).

A meta-analysis of polar bryophytes and angiosperms showed negative UV-B induced effects on biomass and growth, but since the data on biomass and growth consisted mainly of angiosperms, it is difficult to draw conclusions on effects on bryophytes (Newsham & Robinson 2009). Since biomass production is a slow process in the subarctic, long-term exposure to enhanced UV-B has been suggested to have a cumulative effect on plant growth (Newsham & Robinson 2009). Long-term effects of enhanced UV-B were observed in *Polytrichum hyperboreum*, as the length of male gametophytes was reduced after seven years of UV-B treatment (Rozema *et al*. 2006).

3.3 Past responses – Reconstruction of past irradiation climate (IV)

Herbarium and specimen bank samples provide a possibility to study changes in the historical concentration of UACs. Relationships between these compounds and past UV-B radiation levels have been studied (*e.g.* Huttunen *et al*. 2005). The possibility of using herbarium and specimen bank samples to reconstruct past irradiation conditions is interesting, since UV radiation measurements only began relatively recently.

Variations between sampling years in the methanol-extractable UACs per DM were observed in *P. schreberi* (IV; Table 1). During the years studied, the compounds per DM were found to have weak but statistically significant negative relationships with reconstructed UV radiation (IV). A similar relationship has been observed with herbarium samples of liverwort *Jungermannia exsertifolia* subsp. *cordifolia* (Otero *et al*. 2009). It has been suggested that cold temperatures during spring and early summer may hinder the formation of the UACs, thus evoking the negative relationship (Otero *et al*. 2009). On the other hand, the total flavone concentration and the ratio of luteolin to apigenin of herbarium samples of the Antarctic moss *Bryum argenteum* have shown to increase with increasing UV-B radiation or decreasing ozone concentration (Ryan *et al*. 2009). The other species studied, *Hylocomium splendens*, showed positive relationships between the compounds per sample surface area and global irradiation (IV). In another study, the UV-B-absorbing compound concentration of *P. schreberi* and *Polytrichastrum alpinum* increased between the 1920s and 1990s, and the compounds of *Sphagnum capillifolium* increased with global radiation (Huttunen *et al*. 2005).

The daily UV radiation data used in the study (IV) was calculated from reconstructed monthly values (Kaurola *et al*. 2000). Using monthly values decreased the significance of the correlations, since the UACs have been observed to react to changes in UV-B radiation within hours or days (II, Lois 1994, Newsham *et al*. 2002). In addition to this, the decomposition rate of UACs under natural conditions is unclear (Newsham *et al*. 2002). Nevertheless, correlations could be detected (IV).

According to our results, specimen bank storage time does not affect compound concentrations substantially (IV). This validates the use of specimen bank samples. However, one has to be careful when sampling the herbarium or specimen bank collections and interpreting the results. For example, moss seasonality may affect the results and where possible, samples should be collected

at similar dates in different years. This is not always possible, but it is an important factor to keep in mind. In our study, the sampling month did not statistically influence the results, even though the sampling dates varied widely between the years sampled, spring being emphasized during one year and autumn during another (IV). Nevertheless, it is possible that the subtle changes in concentrations between samples collected in different summer months may affect the outcome of the study.

Differences in the methanol-extractable UV-absorbing compound concentrations per SLA between Southern and Northern samples of *P. schreberi* were not detected (IV). In *H. splendens*, the compound concentration was higher in the Northern samples compared to Southern ones during the years 1990 and 2000, but the collecting dates of these samples were concentrated in June and August in 1990, and September in 2000 (IV). In *P. schreberi*, seasonal patterns in the methanol-extractable UACs (per SLA) varied between years (I). In the specimen bank samples, the UAC concentration varied between the studied years as well (IV). The concentration of UACs was smaller in 2000 compared to 1985 and 1990. Additionally, the compound concentration correlated negatively with temperature and precipitation in the Northern locations (IV).

Environmental conditions during the sampling time are likely to affect the results as well, causing variation in the concentration of UACs. For example, reduced UV-B at the sampling time due to cloudiness, spring-time snow cover, environmental stressors besides UV-B, such as water stress and temperature (Björn *et al*. 1998, Caldwell *et al*. 2007) – all these subtle and insignificant effects can accumulate into significant ones.

The methods used in this study can give indicative results of the connections between historical moss samples and concurrent radiation levels. It has been suggested for herbarium samples that analysing the more stable flavonoids, instead of the bulk absorbance of UACs, would give a better approximation for the reconstruction of past UV-B levels (Otero *et al*. 2009, Ryan *et al*. 2009). In previous studies, the UV-B to PAR ratio has been shown to have high correlations with methanol-extractable UACs and flavonoids (Newsham *et al*. 2002, Newsham 2003, Ryan *et al*. 2009).

3.4 Possible effects on ecosystem processes

Increasing UV-B radiation may affect the ecosystem decomposition processes directly and indirectly via the moss layer in several ways. Accumulating UACs (phenolics) may slow down the decomposition rate (Rozema *et al*. 1997b, Gehrke *et al*. 1995, Paul & Gwynn-Jones 2003), which means less available nutrients for primary production in already nutrient-limited Northern ecosystems (Gehrke 1998). According to our results, UV-B radiation may increase the concentration of secondary metabolites, but at least in *Pleurozium schreberi*, no cumulative accumulation of these metabolites into the older parts of the gametophytes was detected (this work).

Production and decomposition are slow processes in Northern environments, due to low temperatures and short growing seasons. Over the long term, changes in biomass accumulation and morphology due to UV-B radiation can affect the competition between plants and alter the community composition. Growth of other plants may be affected by changes in the depth of the moss layer (van der Wal *et al*. 2001). Additionally, bryophytes have an important role in nutrient cycling (Brown & Bates 1990). UV-B-induced changes in the concentration of UACs can change the tissue quality and thus affect nutrient cycling through degradation (Rozema *et al*. 1997b, Selås 2005).

Negative effects of increasing UV-B on moss production may lead to thinning of the moss layer, and to more light-exposed soils. Direct UV-B radiation may increase the degradation of organic litter by breaking down chemical compounds, and possibly decrease degradation by affecting the decomposer community and microbial activity (Newsham *et al*. 1997, Gehrke 1998). Thinning of the moss layer can lead to lower insulation capacity and to increases in soil temperature (Gehrke 1998, van der Wal *et al*. 2001, van der Wal & Brooker 2004). Increase in the soil temperature would stimulate microbial activity and carbon dioxide release into the atmosphere, therefore contributing further to climate change and breakdown of stratospheric ozone (Gehrke 1998, Karhu *et al*. 2010). In *Polytrichum juniperinum*, the density of green leaves had decreased after five years under enhanced UV-B (II). This may lead to lower insulation capacity. UV-B induced decreases in shoot biomass have been found to be dependent on the degree of simulated ozone depletion (Searles *et al*. 2001).

Increased UV-B has shown only minor effects on morphological traits and biomass of vascular plants (Searles *et al*. 2001). In polar bryophytes and angiosperms, decreases in height and biomass were observed (Newsham &

Robinson 2009). Since plant species differ greatly in their growth responses to UV-B it is anticipated that a reduction in productivity of one species will probably lead to increased productivity of another, more UV-tolerant species (Caldwell *et al*. 1995). In the case of no UV-B-induced effects on vascular plant biomass and negative effects on moss biomass, vascular plants may increase in dominance, especially under a warming climate (Lenoir *et al*. 2008).

In nature, plants are affected by several stress factors at the same time. The other stress factors can modify the effectiveness of UV-B radiation a great deal (Caldwell *et al*. 1995). Several stress factors together can intensify, weaken, hide or even eliminate the response of a plant to one of the factors. In *Hylocomium splendens*, increased precipitation together with UV-B radiation inverted the negative effect of UV-B on biomass (Gehrke *et al*. 1996, Phoenix *et al*. 2001).

Species have been observed to be more influenced by varying environmental conditions between years than by enhanced UV-B treatment (Bjerk *et al*. 2005). Ectohydric mosses, like *Pleurozium schreberi*, do not have underground storages to buffer environmental fluctuations between years (Callaghan *et al.* 1978). The clonal structure of *Polytrichum* mosses, on the other hand, offers an advantage of sharing resources between gametophyte stems (Callaghan *et al.* 1978, see Corradini & Clément 1999). In sub-arctic heath ecosystems, four years of enhanced UV-B treatment did not cause detectable changes in the species composition (Gehrke 1998). Nevertheless, future changes in the species composition cannot be excluded. It has been suggested, that even a six year experiment is too short in duration for detecting all effects under field conditions, as some of the effects are small but cumulative with time (Aphalo 2003).

4 Conclusions

This study shows that increasing UV-B radiation has an effect on mosses. Some of these effects can be seen within the first few years of experimentally enhanced UV-B, or even within hours, whereas other effects can only be seen after several years. The enhanced UV-B experiments were conducted over six years.

The two mosses studied, *Pleurozium schreberi* and *Polytrichum juniperinum*, represent mosses of different characteristics and habitat preferences. Differences in their protective strategy and responses to changes in the levels of UV-B radiation were observed. A high spring-time concentration of the methanol-extractable UACs with high UV radiation was observed in *Pleurozium schreberi*. In *Polytrichum juniperinum*, the UV-absorbing compound concentration was reduced by unfavourable weather conditions during early summer, but the UV-absorbing compound concentration increased again towards autumn and was suggested to have a role in winter hardening as well. The spring-time cell wall-bound UV screen was important to both species. The fundamental adaptation of *Polytrichum juniperinum* to open and exposed environments was reflected in the relatively higher concentrations of soluble and cell wall-bound UACs, compared to *Pleurozium schreberi*.

In the UV experiment, negative effects of UV-B and the mitigating effects of UV-A were evident during the first and second treatment year in *Pleurozium schreberi*. In this species, the soluble UACs increased under enhanced UV-B compared to the UV-A control, while the annual shoot growth increased under UV-A. After the third year, both U-B and UV-A radiation had reduced annual growth. UV-B treatment caused an increase in UACs again after the fifth treatment year. In *Polytrichum juniperinum*, the daily UV-B treatment of six hours had an effect on the soluble UACs on some days. UACs were observed to decrease compared to the control, or remain at the same concentration level while an increase was observed under the control. This occasional treatment effect was reversed during night, however, and no effect was observed in the mornings. High variance in the UV-absorbing compound concentrations and decreased green shoot growth was observed after the sixth year of UV-B treatment. A coinciding positive effect of UV-A on shoot growth was also observed. The immediate light conditions of the individual gametophytes varied due to uneven shading, which may have affected the UV-B dose received, and thus the reactions of the individual gametophytes. Additionally, the leaf orientation of *P. juniperinum* during drought may offer supplemental protection to the plant from UV-B. If the

plants experienced sustained dry conditions during the experiment, it may have decreased the UV-B dose received under UV-B treatment.

The methanol-extractable UACs of *Polytrichum juniperinum* under natural conditions correlated with the short-term solar UV radiation even when the UV data had been measured off-site. Since on-site radiation measurements are typically not available in natural conditions, this correlation with radiation measured within reasonable distances from the sampling plot is useful when studying the effects of ambient UV, or performing a study with historical samples. The environmental sample banks and herbaria can provide a useful tool to study past environmental conditions, and even reconstruct past radiation levels. Indicative results of the connections between historical moss samples and the concurrent radiation levels can be achieved with the methods used in this study. The methanol-extractable UACs of the historical samples were stable, without notable deterioration during storage.

Choosing to study UACs rather than specific phenolic compounds shows the usefulness of these species to be commonly used as indicators of changing UV-B radiation. The methanol-extraction of UACs is a relatively simple and inexpensive method to learn and use, and therefore available for wide utilization in monitoring studies. Conversely, studying specific compounds could have revealed more significant treatmet effects and correlations.

It was shown that both *Pleurozium schreberi* and *Polytrichum juniperinum* have a fundamental UV-B screen in their cell walls, but that they can also use the compounds present in the soluble fraction to react and adapt to the changes in UV radiation. They respond to increasing UV-B radiation, but the effects vary in magnitude and in time. As indicators of the light environment, these species can reflect both UV radiation conditions of the sampling time and the cumulative effects of the preceding radiation environment. The effects of enhancing UV-B on these mosses growing more or less as mats can affect the interactions between the moss cover and other components of the ecosystems, for example through competition and degradation. As *Pleurozium schreberi* and *Polytrichum juniperinum* possess circumboreal and cosmopolitan distributions, the effects of UV-B on these species and consequently on ecosystems has a broad application. Further research is required to understand the impacts of heterogenous light, UV-A / blue light and UV-B on bryophytes as a part of their environment under climate change. The effects of UV-B radiation on individual UACs, cell wall-bound UACs, decomposition rate of bryophytes, and the consequent effects on other organisms and the whole ecosystem are of importance.

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