The effects of UVB radiation on charophycean algae and bryophytes

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The temperate bryophyte *Syntrichia ruralis* var. *arenicola* grows more compactly upon enhanced UVB radiation, but does not produce more UVB absorbing compounds

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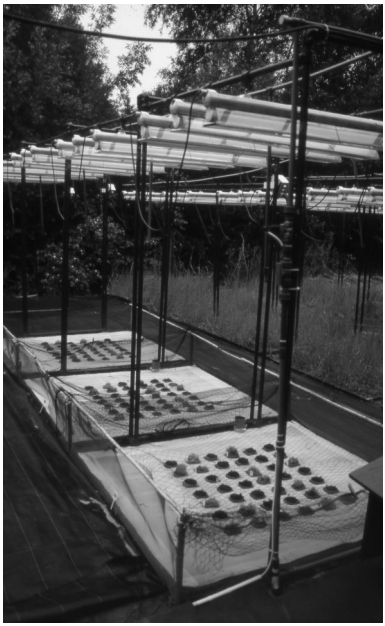

Abstract

In recent decades, stratospheric ozone depletion has resulted in higher levels of UVB radiation reaching the Earth's surface. Despite the fact that bryophytes cover large areas of terrestrial ecosystems, most research on the effects of and adaptations to UVB radiation has been performed on higher land plants. We investigated the response of the bryophyte *Syntrichia ruralis* var. *arenicola*, inhabiting open dune areas in the Netherlands, to ambient and enhanced levels of UVB radiation, during a 13 months study under semi-natural outdoor conditions.

Net growth was reduced upon enhanced UVB radiation. At the end of the experiment, total plant length was significantly decreased by UVB exposure, while there were no significant differences in branching pattern or length of the side branches among the different treatments. Since total dry weight was not affected by the treatments, plants under enhanced UVB exposure tended to be more compact.

The concentration of phenolic compounds was significantly increased by UVA exposure, but was not affected by enhanced UVB radiation. In contrast to vascular plants, but in common with other bryophyte studies, there were no significant differences in concentrations of UVB absorbing compounds among the different treatments. However, the levels of UVB absorbing compounds changed significantly within a growing season, independent of UV exposure levels. This indicates that these substances are constitutively abundant and that UVB exposure (alone) does not affect the amount of UVB absorbing compounds in this bryophyte. This suggests that no or another UVB defence mechanism might be acting in bryophytes than in higher plants. Since both UV exposure and desiccation may lead to oxidative stress, we hypothesise that possible protection of bryophytes against UVB radiation might be linked with their desiccation tolerance mechanism.

Introduction

During the last decades anthropogenic emissions of chlorofluorocarbon compounds and other ozone depleting substances have contributed to the global reduction of the thickness of the stratospheric ozone layer, which has led to enhanced levels of ultraviolet-B radiation (280-315 nm) (Madronich 1992, SPARC 1998). The thinning of the ozone layer is not restricted to Antarctica. Also in temperate regions in the Northern hemisphere the ozone layer thickness has decreased (Herman *et al.* 1996, Madronich *et al.* 1998, SORG 1999).

UVB radiation can influence biological processes in many ways, either directly or indirectly (Rozema *et al.* 1997). Research on the effects of enhanced UVB radiation on organisms has mainly focussed on higher land plants and marine organisms (Caldwell *et al.* 1998, Häder *et al.* 1998). These studies have shown that damage to functional structures, like membranes, proteins, DNA and pigments may occur. As a result, essential processes such as DNA replication are affected (references in Strid *et al.* 1994 and Caldwell *et al.* 1998). Another common feature observed in these studies is an altered morphology with increased branching or increased leaf thickness in higher plants under elevated UVB levels (references in Meijkamp *et al.* 1999). However, although damage may occur, vascular plants are able to protect themselves against UVB radiation (Rozema *et al.* 1997), e.g. via DNA repair mechanisms (Sancar and Sancar 1988), or via the induction of UVB absorbing secondary metabolites, such as (poly) phenolic compounds (Schnitzler *et al.* 1996, Meijkamp *et al.* 1999).

Simultaneously with the evolution of terrestrial plants the ozone layer has formed. It has been suggested that stratospheric ozone levels were lower in the past than at present and therefore UVB levels have been higher during the period that land plants evolved (see Rozema *et al.* 1997). Thus, adaptations and protection mechanisms to solar UVB radiation might have been very important in early land plant evolution. Since bryophytes and higher land plants are likely to have a common ancestor (Kenrick and Crane 1997) and UVB levels were higher during early land plant evolution (Rozema *et al.* 1997), similar adaptations to UVB radiation might be present in both plant types. Although cryptogams (both bryophytes and lichens) cover large areas of the terrestrial biosphere, only little attention has been paid to these plants with regards to responses and adaptations to UVB radiation.

This paper examines the effect of enhanced UVB radiation on the temperate bryophyte *Syntrichia ruralis* var. *arenicola*[1], a desiccation tolerant moss occurring in open dune areas in Western Europe (Westhoff 1947). We hypothesise that

this bryophyte responds in the same manner to enhanced UVB radiation as higher land plants do, i.e. by showing an increase in concentrations of UVB absorbing compounds and reduced growth and altered morphology. We examined this hypothesis by studying the effects of enhanced UVB radiation on the bryophyte *S. ruralis* during a 13 months study under semi-natural outdoor conditions.

Materials and methods

Collection of plant material

The bryophyte *S. ruralis* (Hedw.) F. Weber & D. Mohr var. *arenicola* (Braithw.) Amann[1] is a species that occurs in open dune areas along the West-European coasts from Spain to Denmark (Westhoff 1947). It colonises mobile sand and therefore stabilises sandy soils (Richardson 1981). As a coloniser, this species is fully exposed to solar radiation and therefore it is ecologically relevant to study the effects of and adaptations to enhanced UVB radiation.

A site homogeneously covered with *S. ruralis* was selected at the Noord-Holland dune area near Wijk aan Zee, the Netherlands (52°30' N; 4°36' E). From this site, 90 soil cores (height 9 cm; ø 7 cm) were taken in March 1999 and covered at the bottom with water permeable polyester tissue and capped with an open ring (Emergo, The Netherlands) to prevent sand loss during the experiment, while maintaining the natural vertical water flow as occurs in the dune system. The cores were transferred to the experimental garden of the VU University in Amsterdam, The Netherlands.

Experimental set-up

At the experimental garden, three treatments with different UV radiation levels were established by using frames with lamps. These treatments were: 1) ambient UV levels (Ambient); 2) an enhanced UVA control treatment (UVA); and 3) an enhanced UVA and UVB treatment (UVAB). The cores under the 'enhanced UVA and UVB' (UVAB) treatment were exposed to the ambient, natural background level of radiation and extra ultraviolet radiation (UV) that was provided by 3 fluorescent lamps (Philips TL 40). The added UVB radiation in this treatment simulated a reduction of the ozone layer of 15%. Weekly doses were calculated according to the model of Björn and Murphy (1985) for The Nether-

[1] *Syntrichia ruralis* var. *arenicola* is synonym for *Tortula ruralis ssp. ruraliformis* and *Tortula ruraliformis* (Dirkse *et al.* 1999). Here, *S. ruralis* refers to *Syntrichia ruralis* var. *arenicola*.

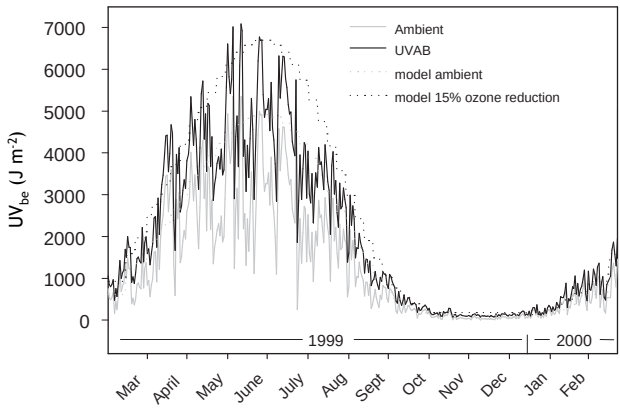

Figure 4.1: Caldwell weighed UVB radiation (UV_{be}) during the experiment for the different treatments. The background UV_{be} data were derived from the Royal Dutch Meteorological Institute (KNMI). These were the UVB levels in the Ambient and UVA treatment. The additional UV_{be} on top of this background UVB radiation in the UVAB treatment is based on the lamp-output and burning period of the TL tubes. The dotted lines represent the modelled UV_{be} doses for ambient ozone levels and 15% ozone reduction according to the model of Björn and Murphy (1985).

lands (52° N), assuming cloudless sky conditions and using the Generalised Plant Action Spectrum (Caldwell 1971). The different light conditions were set by using a portable broadband UV-X radiometer with a UV-X 31 sensor (San Gabriel, CA, USA), which was calibrated with a double-monochromator spectroradiometer (Optronic Model OL 752, Orlando, Fl, USA). Lamp output was checked three times during the experiment by Optronic spectroradiometer. UV tubes were covered with 0.1 mm thick cellulose acetate foil (Tamboer and Co. Chemie B.V., Haarlem, The Netherlands), which absorbs radiation with wavelengths shorter than 290 nm. The 'enhanced UVA treatment' (UVA) had comparable conditions as the UVAB treatment, only here UVB radiation emitted by the UV tubes was filtered with 0.13 mm thick polyester foil (Mylar, Dupont Industries, USA), which absorbs radiation with a wavelength shorter than 313 nm. In the 'ambient' treatment (Ambient) no extra ultraviolet radiation was added. As day length and solar angle show seasonal changes, and therefore UVB radiation dose as well, burning periods of the UV tubes were adjusted twice a month. Both the Mylar and the cellulose acetate foils were changed regularly (every 2 weeks) to avoid changes in the radiation regimes

due to photodegradation of the foil. The experiment lasted from mid March 1999 till mid April 2000. Since this study was conducted in the temperate region, where bryophytes are generally year-round exposed to UVB radiation, UVB exposure was simulated year-round as well (Figure 4.1).

The cores were randomly assigned to the three treatments. Prior to the experiment, higher plants were removed. New growing higher plants during the experiment were cut at moss surface level once a month, to minimise shading effects. To reduce possible side effects of the location, e.g. differences in moisture status or solar radiation, the cores were rotated every two weeks within a treatment. Positions of complete treatments were shifted once per six weeks. During the summer period (June-July-August 1999), cores were automatically (tap) watered three times during the night.

Measurements and data handling

Growth and morphological characteristics

Mosses grow at the top and decay at the lower part of the plant tissue. Net growth was therefore measured as height increase of the vegetation using the 'cranked wire' method (Clymo 1970, Russel 1988). Measurements were performed once a week starting 12 May 1999. During the experimental period several cores had to be excluded from analysis, since the bryophytes in these cores had died.

At the end of the experiment, a sub-core with a diameter of 3 cm was taken from the central part of each core for measurements on total length and morphology. All moss plants within this sub-core were collected and rinsed. We considered green parts as alive and brown parts as decaying or dead. Measurements of the length of the green and brown parts were performed on the main shoot and side branches of each individual and the branching pattern was determined by counting the number of side branches on each shoot. Total fresh and dry weight of the green and brown parts of the bryophytes were determined and specific length, expressed as mg dry weight per cm length, was calculated. The average values of all plants per core of these parameters were used for further calculations.

Chemistry

Sampling for the UVB absorbing compounds was done twice during the experiment; in autumn (late September 1999) six cores per treatment were harvested and at the end of the experiment in spring (April 2000) the rest of each core, not used for morphology measurements, of the cores remaining were analysed

(n=13 to 16, Table 4.2). Only the green parts of the mosses were used for chemical analysis on concentrations of UV absorbing compounds and of total phenolic compounds. The green parts were separated and lyophilised, after which they were kept under vacuum till analyses were performed.

UVB absorbing compounds were extracted, with small modifications, according to the method of Caldwell (1968). Plants were extracted in acidified methanol (MeOH:H_2O:HCl in ratio 79:20:1) and heated for 105 minutes. After centrifugation at 2500 rpm, the absorbance of the supernatant was spectrophotometrically measured at 280-315 nm on a Shimadzu UV-1601PC spectrophotometer.

Phenolic concentrations were determined only in moss samples collected in April 2000 (n= 12 to 14, Table 4.2; Since limited plant material was available, the sample sizes deviate from the UVB absorbing compound analyses). Total phenolic compounds were analysed by extracting plant material in 50% MeOH, using the Folin-Ciocalteau method (Palm and Rowland 1997). This method measures the entire pool of (poly)phenols (low molecular weight phenols as well as hydrolysable and condensed tannins). A reference series with tannin (Merck) was used to calculate phenolic compound concentrations. Spectrophotometrical analyses were done at 760 nm (Waterman and Mole 1994).

Calculations and statistical analysis

Length growth of the mosses for each treatment were determined by fitting a curve through the weekly measured 'cranked wire' data of each treatment. As the mosses shrink as they dry (Willis 1964) only data collected from bryophytes that were open, green and wet, were used for statistical analysis of length growth. To account for the seasonal dynamics in moss growth, a logistic curve was used. Fitting of the logistic curve and significance of this regression were analysed with Sigma Plot (version 9.01 for Windows, Systat software Inc. 2004).

The effects of treatments on all morphological (i.e. plant length of brown and green parts, plant density, length of branches, total dry weight, specific weights) and chemical characteristics (i.e. total phenolics), except for the amount of UVB absorbing compounds, were analysed by a one-way ANOVA with a priori comparisons (Sokal and Rohlf 1998). In this analysis, the ambient and UVA treatment were compared to test for the presence of an effect of the UVA radiation supplied by the fluorescent tubes, and the UVA and UVAB treatment were compared to isolate the effect of enhanced UVB radiation. The UVB absorbing compounds had been determined two times during the growing season and

were therefore tested with a two-way ANOVA with time during the season and treatment as fixed factors and by using the above described a priori comparisons. Prior to statistical analysis, we identified extreme values in the data with box plots in SPSS (version 10.1.0 for Windows, SPSS Inc. 2000). Values deviating more than three box-lengths from either the 25th or 75th percentile were excluded from analysis. Afterwards, normal distribution and homogeneity of variances of the data were tested with a Kolmogorov-Smirnov and Levene's test, respectively. To meet ANOVA assumptions, all data were transformed using a natural logarithm, except proportions of branching and length of the green part of the side branches which were transformed using rank numbers. Tests were performed using the program SPSS.

Results

Growth and morphology

The length growth of *S. ruralis* showed strong seasonal dynamics with hardly any net growth in spring (March) and summer (May - September) and a low net length increase in autumn that ceased again in winter. At the end of the experiment, total net length increase of about 2.2 to 1.8 mm was found under 'Ambi-

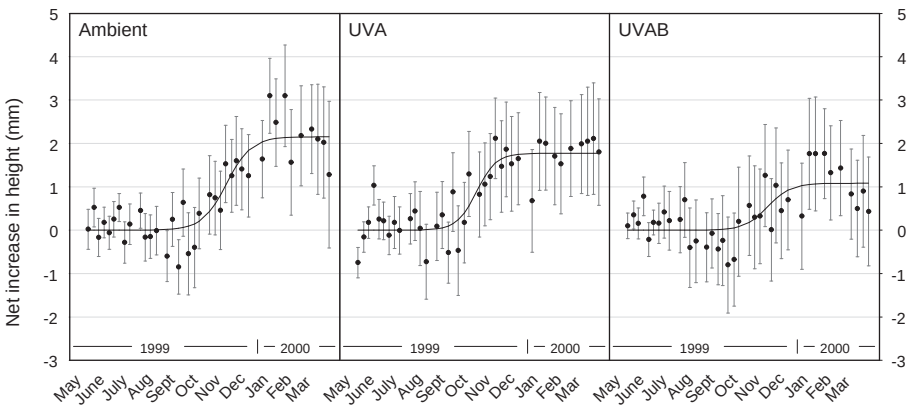

Figure 4.2: Increase in height in mm (average ± SE) of *S. ruralis* in response to UV treatments as measured using the 'cranked wire' method. Lines represent the fitted logistic curves with the following characteristics: Ambient r^2= 0.81; UVA r^2= 0.77; and UVAB r^2= 0.51 (P<0.001, n=39 for all treatments). Height increase is expressed relative to the lower asymptote of the fitted logistic curve, which is a more robust measure of the initial conditions than measurements related to any individual moment.

ent' and elevated UVA conditions (UVA), whereas only 1.1 mm net growth had occurred under elevated UVB radiation (UVAB) (Figure 4.2). The goodness of fit of the logistic function through the cranked wire growth data was high in the 'Ambient' and 'UVA' treatments (r^2 = 0.81 with P<0.001 and 0.77 with P<0.001, respectively), but in the 'UVAB' treatment only 51% of the variance was explained (P<0.001).

There were no significant differences in any plant morphological parameter between the 'Ambient' and 'UVA' treatment. Any significant effects found in the 'UVAB' treatment can therefore be ascribed to extra UVB supplied during the experiment. Indeed, under enhanced UVB radiation the average height of the main shoot of the bryophyte plants was significantly lower (about 2 mm) compared to plants under enhanced UVA radiation (Figure 4.3a, P< 0.05). There was no significant difference in the length of the green (living) part of the plants, but the length of the brown (decaying) part was significantly reduced under enhanced UVB radiation (Figure 4.3a, P<0.05). In all treatments, the majority of the plants (about 73%) did not form lateral branches (Figure 4.4). Neither was there a significant difference in the average number of side branches (P=0.31). When branches were formed, the total length nor the length of green and brown parts of the side branches separately were significantly affected by enhanced UVB radiation (Figure 4.3b).

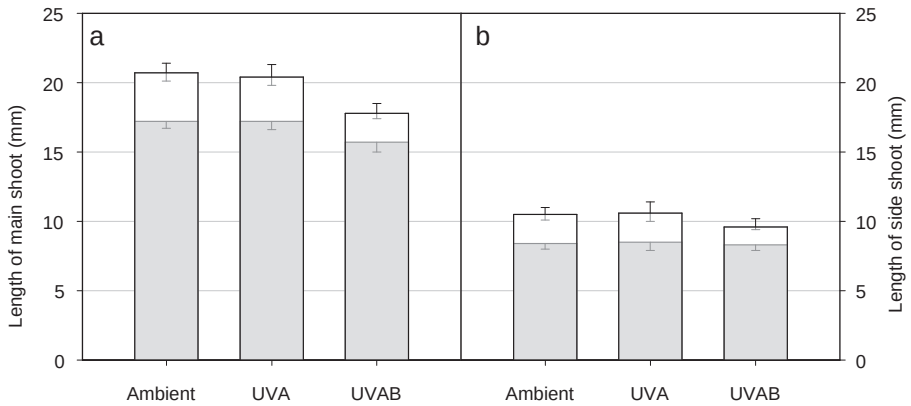

Figure 4.3: Average length in mm (+ SE in black) of the main shoots (a) and side branches (b) of *S. ruralis* under different UV treatments. The white and gray bars represent, the average length of the green and brown parts of the shoots (- SE in gray), respectively. The number of replicates is 15 (Ambient), 17 (UVA), 15 (UVAB) for the different treatments.

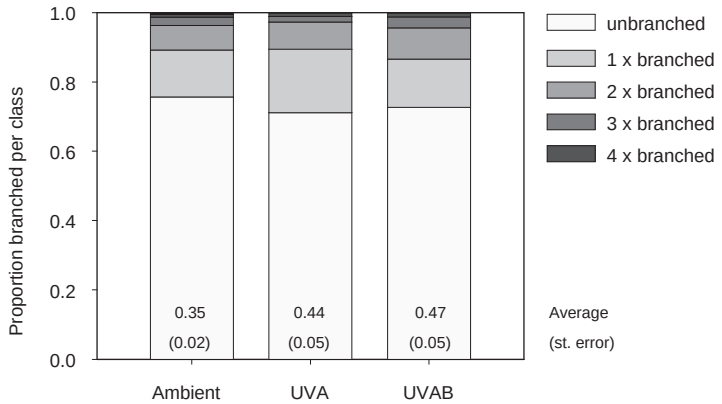

Figure 4.4: Branching pattern in *S. ruralis* cores in response to UV treatments. Bars represent the proportion per branching class. The numbers in the bars represent the average (± SE) number of branches (the higher the number, the more branched the plants are on average).

Although the length specific weight, which is a measure for plant compactness similar to e.g. leaf thickness in higher plants, of the green and brown parts separately were not significantly different upon UVB exposure (P= 0.82, and 0.17 respectively), the length specific weight of total plants was positively affected by enhanced UVB, though only marginally significant (Table 4.1, P=0.08). Total dry weight of the moss plants per core was, however, not affected by UVB (Table 4.1, P=0.36).

Chemistry

Neither in autumn 1999 nor in early spring 2000, significant differences in total UVB absorbing compounds were found between the different treatments

Table 4.1: Plant density, total dry weight and length specific weight of the bryophyte *S. ruralis* in response to UV treatments (n=15, 17, 15 respectively). None of the treatment effects were significant at P<0.05, although there was a trend for the specific weight of total plants (P=0.08).

	Ambient	SE	UVA	SE	UVAB	SE
Plant density (shoot numbers cm^{-2})	6.8	±0.2	7.3	±0.4	6.9	±0.4
Specific weight brown plant parts (mg cm^{-1})	1.64	±0.07	1.69	±0.07	1.76	±0.08
Specific weight green plant parts (mg cm^{-1})	2.29	±0.16	2.42	±0.17	2.8	±0.38
Specific weight total plant (mg cm^{-1})	1.73	±0.05	1.76	±0.06	1.85	±0.08
Total dry weight (mg cm^{-2})	31.34	±1.60	33.81	±2.34	30.93	±2.02

Table 4.2: Amounts of UVB absorbing compounds (averages on an area basis, integrated from 280-315 nm; recalculated per mg dry weight) and total phenolic compounds (averages expressed as mg tannin per gram dry weight) of the bryophyte *S. ruralis* in response to UV treatments. The numbers in brackets indicate the number of replicates. Different letters per parameter indicate a significant difference among treatments ($P<0.05$). In addition, the effects of time during the season were highly significant ($P<0.001$), but the interaction with treatment was insignificant ($P=0.68$).

	Ambient	SE	UVA	SE	UVAB	SE
UVB absorbing compounds						
September 1999	25.84^{a} (6)	±0.69	26.30^{a} (6)	±0.52	27.46^{a} (5)	±0.39
April 2000	30.89^{b} (15)	±0.62	31.65^{b} (16)	±1.24	32.24^{b} (13)	±1.29
Total phenolic compounds	1.71^{a} (14)	±0.05	1.91^{b} (13)	±0.06	1.86^{b} (12)	±0.08

(P=0.60). However, there was a strong difference in total absorption between these sampling periods (Table 4.2, $P<0.001$). The interaction between time and treatment was insignificant ($P=0.68$), indicating that the absence of treatment effects occurred at both times. There were spectral differences in the absorption curves between the samples from autumn 1999 and early spring 2000 (Figure 4.5). Both in autumn 1999 and in early spring 2000 a peak with an absorbance optimum around 279 nm was found in all treatments. However, in April 2000 this peak was present in a more pronounced way (Figure 4.5). This resulted in an

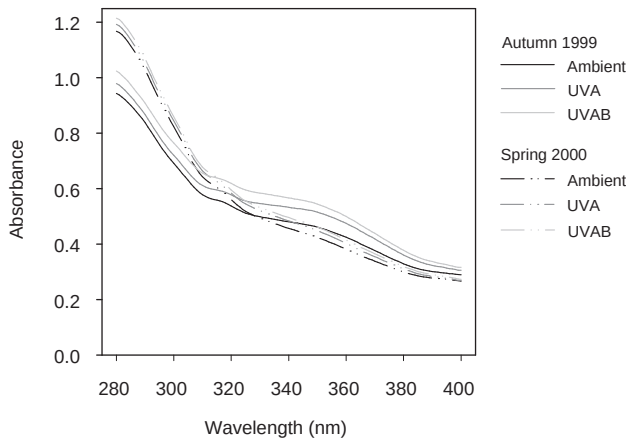

Figure 4.5: Average absorption curves of acidified methanol extracts of *S. ruralis* in response to the different UV treatments in September 1999 (n=6) and April 2000 (n=13-16; see Table 4.2).

increased total area under the absorption curves for April (Table 4.2). In summer 1999, there was a higher absorbance in the UVA region of the spectrum (Figure 4.5).

At the end of the experiment, the total phenolic compounds showed a significant effect of the UVA radiation treatment ($P<0.05$), but no effect of the UVB treatment was found (Table 4.2).

Discussion

Growth and morphology

The bryophyte *S. ruralis* showed a significantly reduced average total plant length under enhanced UVB conditions at the end of the experiment. This reduced growth was also apparent from the 'cranked wire' growth data, where net length had increased much less in the enhanced UVB treatment compared to either control treatment. A reduced length growth in response to enhanced levels of UVB radiation has been reported in higher plants as well (Caldwell *et al.* 1998). Searles *et al.* (2001) performed a meta-analysis of 26 field studies on higher plants and found a slight (but significant) height reduction due to enhanced UVB radiation which did not only occur in greenhouse experiments, but also in outdoor experiments. Also in the scarce bryophyte outdoor studies on UVB effects (Rozema *et al.* 2005, Boelen *et al.* 2006) significant growth reductions have been found under higher UVB radiation levels. In a subarctic heathland supplementation of UVB radiation significantly reduced growth in the bryophytes *Hylocomium splendens* after two and of *Polytrichum commune* after three years (Gehrke 1999). Searles *et al.* (2002) found significant growth reduction in *Sphagnum magellanicum* in response to higher UV conditions in the second growing season, while after the first year no effects of UVB on growth were found. In the third year height reductions were comparable with the second year, though marginally significant ($P=0.07$, Searles *et al.* 2002). Ballaré *et al.* (2001) argued that the effects of UVB radiation might only become visible after several years, since bryophytes are generally slowly growing plants. However, in this study, like in the *Sphagnum* study from Gehrke (1998), the effects were already found after one year of enhanced UVB exposure. This suggests that *S. ruralis* is rather sensitive to enhanced UVB radiation, although the climate conditions of the temperate oceanic region may also have caused higher growth rates, thus allowing detecting negative effects more quickly.

Though length growth was significantly affected, total dry weight of the plants was not affected by the different treatments in this study. Plants tended

to be more compact under elevated UVB conditions (increased length specific weight, Table 4.1), explaining the difference in effects between length and weight. The absence of differences in biomass, while plant length was shorter under enhanced UVB radiation, suggests that photosynthesis was not affected. Consistent with this, chlorophyll fluorescence measurements showed that photosystem II was not affected by the different treatments in this study (data not shown). Therefore it is likely that UVB negatively affects length through other processes regulating length growth, e.g. by causing DNA damage or by altering levels of growth hormones. Both in terrestrial higher plants (Mazza *et al.* 1999) and in algae (Buma *et al.* 1995, Van de Poll *et al.* 2001), UVB induced growth reductions could be related to UV induced DNA damage. Lud *et al.* (2002) showed the occurrence of UV induced DNA damage also in bryophytes under field conditions. UVB exposure has also been shown to coincide with altered levels of the phytohormone indoleacetic acid (IAA) (Jansen 2002), which are also present in bryophytes (Christianson 2000). This may lead to inhibition of cell expansion and thereby affecting plant length. The exact process remains unknown, since DNA damage and expression of growth hormones were not measured in this study.

Chemical protection

Plants can protect themselves to potentially damaging levels of UVB radiation by damage repairing mechanisms or by reducing incoming radiation by morphological and/or chemical adaptations. Many bryophytes, like higher plants, do have mechanisms to avoid or reduce some damage e.g. by the presence of waxes on leaves, by increased reflection of solar radiation by glass hairs (Proctor 2000) and by rolling, curling and folding of leaves while dehydrating to reduce absorption of irradiance (Willis 1964, Alpert 2000). In vascular plants, phenolic and UVB absorbing compounds may contribute to reduce damaging effects of UVB radiation (Meijkamp *et al.* 1999 and references therein). However, in this study only exposure to UVA radiation resulted in higher total phenolic concentrations in the bryophyte *S. ruralis*, while no significant effects of UVB radiation were found on UVB absorbing and total phenolic compounds. Most other bryophytes studies on responses to UVB radiation did not show an increase in leaf UV absorbing compounds either (Gehrke 1998, 1999, Searles *et al.* 1999, Lud *et al.* 2002, Niemi *et al.* 2002a). The only difference found once was a significant reduction (!) in the amount of UVB absorbing compounds in *Polytrichum commune* in response to enhanced UVB radiation after three years of UVB exposure (Gehrke 1999).

Thus, the chemical responses of bryophytes and higher plants to UVB radiation differ markedly. This difference in response is not likely an artefact caused by the use of bulk parameters as a measure for the chemical response. In higher plants the induction of specific UV absorbing compounds, e.g. flavonoids, generally occurs in the epidermal layer (Schnitzler *et al.* 1996, Meijkamp 2006), thereby protecting the lower leaf tissue to some extent from direct UV damage. This direct UV damage includes damage to DNA and photosystems in mesophyll, parenchyma and lower epidermis (Schnitzler *et al.* 1996 and references therein, Shirley 1996). In contrast to higher plants, bryophyte leaves are undifferentiated and consist often of only one cell layer without a cuticle (Gehrke 1998). The use of bulk parameters comprising total leaf material in bryophytes is therefore a valid method to measure chemical changes to UVB radiation. Thus, a change in UV absorbing compounds, if present, is likely to be found. In this study, cell-wall-bound phenolic compounds (Harborne 1989), were probably not extracted with the method we used. Therefore, the amount of phenolic and UV absorbing compounds might be underestimated. However, Searles *et al.* (1999, 2002) did not find any significant difference in UV absorbing compounds in bryophytes exposed to different UV levels either, even when using a method by which cell wall-bound compounds were extracted as well.

This study showed that within one growing season levels of UVB absorbing compounds significantly differed, independent of the UV treatments (Figure 4.4 and Table 4.2). In most other studies, UVB absorbing compounds were measured only once per growing season and therefore possible seasonal differences had not been detected. These seasonal effects, in combination with the fact that different studies measured UVB absorbing compounds at different times in the year, hamper analysis of quantitative differences among bryophyte species. Only Niemi *et al.* (2002b) found significant differences in UVB absorbing compounds during the season under ambient outdoor conditions in *Sphagnum angustifolium*, while this effect was absent in *Sphagnum papillosum* and in any UV supplementation treatment. The seasonal differences may also put other results in a different light. For instance, Newsham *et al.* (2002) reported on synthesis of UVB screening pigments in two Antarctic bryophytes in response to changing UVB radiation in situ. They found that the ratio of UVB to PAR irradiance was a good predictor for the concentrations of UVB screening compounds in bryophyte species in Antarctica, but these effects might also have been due to seasonal differences, independent of UVB exposure. Also the lack of response of UV absorbing compounds in bryophytes to any artificially applied UVB treatment suggests that other factors than the ratio UVB to PAR irradiance levels (alone)

influences levels of UVB absorbing compounds. The absence of UVB treatment effects on levels of UVB absorbing compounds suggest that either the level of UV absorbing compounds is constitutively high as suggested earlier by Lud *et al.* (2002), or that there is no induction of UV absorbing compounds at all. Therefore, defence or protective mechanisms to UVB radiation in bryophytes may differ from those in higher plants or are even absent. This raises the question how bryophytes are protected against high levels of UVB radiation? Is chemical defence constitutively high or are there other mechanisms acting?

Alternative UVB defence mechanisms for bryophytes?

A possible alternative defence mechanism against UVB radiation in bryophytes might be related to the way they cope with and recover from fluctuations in water availability. Many bryophytes are desiccation tolerant (Richardson 1981). Desiccation and subsequent rehydration damages membranes, proteins and DNA due to oxidative stress (Smirnoff 1993, Alpert and Oliver 2002). The presence of constitutive protection to this oxidative stress, e.g. antioxidants and enzymes, but also additional repair mechanisms, give bryophytes the ability to cope with oxidative stresses (Alpert and Oliver 2002). Exposure to UVB radiation also leads to oxidative stress and damage (Brosché and Strid 2003, Blokhina *et al.* 2003). As damage due to oxidative stress might be the effect of both desiccation and/or UVB radiation, not only the repair mechanisms might be shared, but also the constitutive protection mechanisms (Smirnoff 1993).

This hypothesis is supported by Takács *et al.* (1999) who reported a positive correlation between tolerance to desiccation and tolerance to UVB radiation in bryophytes. A consequence of this mechanism could be that due to this chemical defence against oxidative stress in desiccation tolerant bryophytes, there is no direct need for an induction of UV absorbing compounds under UV stress. This might explain the lack of response we found in this study.

In conclusion, this study has shown that enhanced levels of UVB radiation significantly reduced length growth of the bryophyte *S. ruralis*, as has been found in higher plants. However, there was no induction of UV absorbing compounds in response to enhanced UVB radiation. This contrasts with the response of higher plants. The mechanisms associated with the ability to cope with desiccation might be linked to chemical protection against UVB stress in mosses. However, this topic clearly needs further study.

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