The effects of UVB radiation on charophycean algae and bryophytes

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Does habitat origin affect responses of temperate bryophytes to enhanced UVB radiation?

Nancy VJ de Bakker[1], Marianne Kosonen[1,2], Martin Stroetenga[1], Willem H van de Poll[3], Jelte Rozema[1], Rien Aerts[1] & Peter M van Bodegom[1]

[1]VU University, Institute of Ecological Sciences, Department of Systems Ecology, de Boelelaan 1085, 1081 HV Amsterdam, The Netherlands
[2]University of Oulu, Botany Division, Department of Biology, P.O.Box 3000, FIN-90 014, Finland
[3]University of Groningen, Department of Marine Biology, P.O. Box 14, 9750 AA Haren, The Netherlands

Abstract

Bryophytes occur in habitats that differ widely in radiation regimes. Due to thinning of the ozone layer, the exposure to harmful UVB radiation has increased over the last decades. Information on bryophyte responses to UVB is scarce, particularly for temperate bryophytes. Knowledge on the effect of habitat origin on UVB sensitivity is absent. Therefore, we investigated whether habitat origin affects the sensitivity to UVB radiation in bryophytes. Nine species were selected from three contrasting habitats - forests (low UVB exposure), bogs/fens (high UVB exposure and physiologically active) and dunes (high UVB exposure but at high UVB levels mostly physiologically inactive) - within the Netherlands. Growth rates, DNA damage and UVB absorbing compounds were measured in all species after exposure to different levels of UVB radiation and their control treatments in a climate-controlled greenhouse for ten weeks.

UVB radiation significantly increased DNA damage in most species and negatively affected growth rates in several species. UVB absorbing compounds were decreased in some of these temperate bryophytes. UVB responses were consistent within species from the same habitats. Species from the dune and the bog/fen habitat appeared to be more sensitive to UVB radiation, compared to species from the forest habitat in this experiment. Habitat origin thus seemed to influence the sensitivity of species against UVB radiation, with, paradoxically, increased sensitivity in species from those habitats that experience higher exposure to UVB under natural conditions. This may be related to the fact that other stresses than UVB may interfere with UVB tolerance.

Introduction

Due to thinning of the ozone layer, the level of harmful UVB radiation reaching the Earth surface has increased during the last decades. Many studies were conducted to elucidate the effects of higher UVB levels on different plant species. These studies showed that increased UVB radiation led to direct and indirect effects including damage to DNA, proteins and membranes, alterations in photosynthesis and changes in growth and morphology. However, plants are able to protect themselves against UVB radiation (Rozema *et al.* 1997, Jansen *et al.* 1998, Caldwell *et al.* 1998 and references therein), e.g. via DNA repair mechanisms (Sancar and Sancar 1988), or via the induction of UVB absorbing secondary metabolites (Schnitzler *et al.* 1996, Meijkamp *et al.* 1999).

In comparison with algae and vascular plants, only a limited number of studies have been conducted on bryophytes, of which most were conducted at high latitudes (both in the Northern and the Southern hemisphere) where UVB increments are highest (Boelen *et al.* 2006 and references therein). Like in vascular plants, exposure to UVB in bryophytes from (sub) polar regions showed reductions in growth, biomass and increases in DNA damage (Boelen *et al.* 2006 and references therein). However, in contrast to vascular plants, often no increase in UVB absorbing compounds was found (e.g. Niemi *et al.* 2002, Robinson *et al.* 2005), though Newsham *et al.* (2002) and Newsham (2003) found that natural radiation levels of UVB radiation were the best predictors for levels of UVB absorbing compounds.

Also in temperate regions UVB levels have increased since the early '80s (Herman *et al.* 1996, MNP 2007). Effects of UVB radiation on temperate bryophytes are relatively unknown, while bryophytes play an important ecological role in various temperate habitats.

In temperate regions, bryophytes occur in many different habitats; from open, scarcely vegetated areas in e.g. nutrient-poor dune grasslands and mountain slopes to shady places in forests and even in the water. This implies that different bryophyte species experience very different radiation regimes and this may, in turn, affect their sensitivity to elevated levels of UVB radiation. Earlier UVB research on marine algae revealed that habitat conditions such as UVB exposure influenced the sensitivity to UVB radiation; e.g. marine macro algae inhabiting deeper waters are more sensitive than littoral species (Van de Poll 2003, Bischof *et al.* 2006 and references therein). If this is a general rule, UVB sensitivity in bryophyte species may also be affected in accordance to the degree of UVB exposure at their respective habitats.

To evaluate the sensitivity to UVB radiation in bryophytes, the effects of different levels of UVB radiation on temperate bryophytes from different habitats were studied. Nine species were selected from three contrasting habitats (1) forests, (2) bogs/fens and (3) dunes within the Netherlands and effects of UVB radiation on growth, DNA damage and UVB absorbing capacity were measured. Species from forest areas are generally exposed to low UVB radiation levels, while species from the dunes and from bogs/fens occur in open areas, often fully exposed to UVB radiation. In fens and bogs, species are often physiologically active at high (UVB) irradiance levels, while dune species are often in a dry state due high evaporation, windy conditions and low water availability at high (UVB) irradiance levels. The aims of this study are therefore to reveal i) whether DNA damage, growth and UVB absorbing compounds of bryophytes is affected by UVB radiation and ii) if habitat origin affects the UVB responses consistently among these temperate bryophyte species.

Materials and methods

Species collection and preparation

Nine bryophyte species were selected from three contrasting habitats (Table 5.1). Species selection was based on a) being a representative and abundant species for the specific habitat; and b) being of sufficient size for growth measurements. Based on these criteria we selected the dune-species *Campylopus introflexus*, *Polytrichum juniperinum*, *Syntrichia ruralis var. arenicola*[1], the forest species, *Dicranum scoparium*, *Mnium hornum*, *Polytrichum formosum*, the fen species *Polytrichum commune* and bog species *Sphagnum magellanicum* and *Sphagnum fallax*. Species were collected by taking soil cores (ø 9 cm, height 7 cm; for *P. commune* ø 11 cm, height 19 cm) from homogeneously covered sites. The cores were covered with polyester tissue at the bottom and capped with an open ring to prevent sand loss during the experiment, while maintaining the natural vertical water flow. For both bog species, *S. magellanicum* and *S. fallax*, sods were cut from homogeneously covered bogs. Individual plants from both species were excavated from the sods and cut to fixed lengths of 7 and 9 cm, respectively, and placed in small pots with similar density as in the field (55 individuals for *S. magellanicum* and 65 for *S. fallax* per pot; ø 9 cm, height 9 cm). These pots had holes at all sites to allow water movement around the plants.

[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*.

Table 5.1: The collected bryophyte species and their collecting location in The Netherlands.

Habitat origin	Species	Coordinates
Dune area	*Campylopus introflexus* (Hedw.) Brid.*Species*	N52°38; E4°39
Dune area	*Polytrichum juniperinum* (Hedw.)	N52°38; E4°39
Dune area	*Syntrichia ruralis* (Hedw.) F.Weber & D. Mohr var. *arenicola* (Braithw.) Amann	N52°30; E4°36
Forest	*Dicranum scoparium* (Hedw.)	N51°32; E5°08
Forest	*Mnium hornum* (Hedw.)	N53°06; E6°38
Forest	*Polytrichum formosum* (Hedw.)	N53°06; E6°38
Fen	*Polytrichum commune* (Hedw.)	N52°28; E4°46
Bog	*Sphagnum magellanicum* (Brid.)	N52°50; E6°26
Bog	*Sphagnum fallax* (H. Klinggr.)	N52°50; E6°26

The cores and the pots of the fen/bog species were placed in larger pots filled with artificially prepared rainwater (Lamers *et al.* 1999). Mylar foil transmitting UVA radiation was adjusted around the cores till the top of the plants for *P. commune*, *P. formosum*, and *S. fallax* to guide upward growth and prevent dehydration.

Experimental design and maintenance

The nine bryophytes species were exposed to seven different radiation regimes in a climate-controlled greenhouse for ten weeks in autumn/winter. The treatments covered: no UVB present, three different levels of UVB radiation, corresponding to UVB doses of ambient conditions and of 20 and 40% ozone reduction, and for each of these UVB treatments a UVA control treatment (Table 5.2).

170 µmol m^{-2} s^{-1} of photosynthetically active radiation (PAR) provided by Philips HPI/T lamps (400W) for seven hours a day was added to the natural background radiation (average of 232 µmol m^{-2} s^{-1} during the experiment, data from KNMI, the Royal Dutch Meteorological Institute) that passed through the clear green house windows. In the middle of this light period bryophytes were exposed to UV radiation for 4.5 hours a day. The ambient UVB levels were based on the average UV dose in early autumn (September) in the Netherlands. Doses were calculated using the model of Björn and Murphy (1985), assuming cloudless sky conditions. The simulated UVB levels in the experiment corresponded to average biologically effective UV doses (UV_{be}) of 0, 2.5, 3.6, 5.6 kJ m^{-2}

Table 5.2: Overview of PAR, UVA and Caldwell weighed biologically effective UVB radiation levels.

Treatments	PAR * (μmol m^{-2} s^{-1})	n	UVA ± SE (kJ m^{-2} day^{-1})		UV_{be} ± SE (kJ m^{-2} day^{-1})	
PAR	170	6	9.0	±0.5	0.0	±0.1
Ambient UVA	170	6	14.1	±0.7	0.0	±0.0
Ambient UVAB	170	7	17.9	±0.7	2.5	±0.1
20% - UVA	170	4	15.9	±0.6	0.2	±0.2
20% - UVAB	170	6	20.9	±1.1	3.6	±0.2
20% - UVA	170	4	20.7	±0.7	0.0	±0.0
20% - UVAB	170	4	29.3	±1.1	5.6	±0.2

** PAR levels were added to the natural background radiation (average of 232 μmol m^{-2} s^{-1} during the experiment, see text).*

day^{-1}, respectively, according to the Generalised Plant Action Spectrum of Caldwell (1971; normalised to 1 at 300 nm). Actual outdoor UV_{be} irradiance levels in early autumn measured by KNMI over the period 1999-2003 showed slightly lower levels of ambient UVB radiation (1.4 ± 0.6 kJ m^{-2} day^{-1}). The different radiation 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).

In the UVB treatments, Philips TL 40 UV tubes were covered with 0.1 mm thick cellulose acetate foil (Tamboer and Co., Haarlem, The Netherlands), which absorbs radiation with wavelengths < 290 nm. The three UVA control treatments were adjusted to the corresponding UVB level. After adjustment, the UV tubes of these treatments were covered with 0.13 mm thick polyester foil (Mylar, Dupont Industries, USA), which absorbs radiation < 313 nm. Mylar and cellulose acetate foils were changed once and twice a week, respectively, to avoid changes in radiation regimes due to photo degradation of the foils. In the 'PAR' treatment the UV tubes were set off.

Bryophyte cores were randomly allocated to the seven different treatments. Six replicate pots were used in each treatment, except for *P. juniperinum* where only five replicates were used. Air temperature in the greenhouse was kept at 15.5 ± 2.0°C. After two weeks of adjustment, the air humidity was kept constant at 96.5 ± 5.9 %. To avoid any effects of differences in climate or light conditions in the greenhouse the cores were rotated within a treatment twice a

week. In addition, complete treatments were rotated within the greenhouse once a week.

Plants were sprayed with a nutrient solution once a week. Nutrients were supplied with a N:P:K ratio of 10:1:7. The total amount of nitrogen corresponded to 4 g N m^{-2} $year^{-1}$, which is equal to the annual nitrogen deposition in the Netherlands (Aerts and Bobbink 1999). The amounts of added water corresponded to the average annual precipitation in the Netherlands (800 mm yr^{-1}). Nutrients were added as KNO_3, NH_4Cl, NH_4HPO_4 to artificial rainwater (Lamers *et al.* 1999).

Growth measurements

Three methods were applied to measure growth rates, dependent on the size of the bryophytes. For the larger *Polytrichum* species, *P. formosum* and *P. commune*, small wires were knotted around the plants. Length growth from the knot to the top was measured for five plants per core every two weeks. 'Cranked wires' (Russel 1988) were used to measure growth in the smaller species (*S. ruralis*, *C. introflexus*, *P. juniperinum*, *M. hornum* and *D. scoparium*). Length increments were found in the cranked wire measurements from about week two onwards. Both *Sphagnum* species were cut at fixed length at the start of the experiment (7 cm for *S. magellanicum* and 9 cm for *S. fallax*). After ten weeks the average length of 25 randomly selected plants per pot was used as a measure for growth during the experiment. The growth rates for non-*Sphagnum* species were derived via regression analysis from the linear part of the growth curves. For *P. formosum* and *P. commune* all data were included, for the other species linear growth was found from about week 3-5 till the end of the experiment.

M. hornum and *C. introflexus* showed altered growth under the conditions in the experiment. The newly grown material was long, thin and pale and could be clearly distinguished from the starting material collected from the field. *S. ruralis* turned brown within a very short period half way the experiment, leaving only little green, living, material for analysis. The rapid decay of *S. ruralis* indicated presence of fungi (Dr. Kruyer, personal comment).

Harvest and chemical analysis

At the end of the experiment, samples for DNA damage were taken directly after the UV treatment by randomly cutting tops of bryophyte plants from each core. Samples were immediately frozen into liquid nitrogen and stored at -80°C until further analyses. For analysis of UVB absorbing capacity samples were also

collected from the upper top part of the plants, freeze-dried and stored under vacuum until analysis.

Plant DNA extraction and CPD quantification

DNA from the bryophytes was extracted by grinding the material in liquid nitrogen and applying the protocol from DNAzol (Molecular Research Center, Inc. Cincinnati, OH, USA Doc. Rev. 04/23/01). RNase was added to the lysis buffer at the start of the protocol. DNA concentrations were quantified fluorometrically with Picogreen (Molecular Probes, Eugene, OR, USA) using a 1420 Victor multilabel counter (Wallac, Inc. Gaithersburg, MD, USA). Subsequently, 100 ng denatured DNA per sample was used to detect cyclobutane pyrimidine dimers (CPDs) according the method of Van de Poll *et al.* (2002). CPD concentrations were estimated by comparison with calibration series with known amount of CPDs. CPDs are, with 6-4 Photoproducts, the most common types of UVB induced DNA damage (Britt 1999).

UVB absorbing capacity

UVB absorbing compounds were extracted according to Caldwell (1968) with small modifications. Bryophytes were extracted in acidified methanol (CH_3OH: demineralised water: HCl in ratio 79:20:1) for 105 minutes at 90°C. After centrifugation at 2500 rpm, the absorbance of the supernatant was measured spectrophotometrically from 280 to 315 nm on a Shimadzu UV-1601PC spectrophotometer. The total absorbance from 280 to 315 nm was used as a measure for UVB absorbing capacity.

Statistical analysis

The effects of UVB and UVA radiation on growth rates, DNA damage (as expressed through levels of CPDs) and UVB absorbing capacity in bryophytes were statistically tested by applying a one-way ANOVA with a priori contrasts. In the comparisons the results found in the UVA control treatments were tested together against the PAR treatment to investigate the effects of UVA radiation emitted by the TL-tubes. In addition, within each ozone reduction scenario the results of the UVB treatment were tested against the UVA treatment for the effect of UVB radiation. Prior to statistical analyses, normality and homogeneity of variances were tested by using Kolmogorov-Smirnov and Levene's test, respectively. Since analysis of variances is robust to considerable heterogeneity of variances as long as samples sizes are nearly equal (Zar 1999), only for *P. formosum* and *P. commune* ^{10}log transformed CPD data were used.

These statistical analyses were done in SPSS version 10.0; significance level was 0.05.

Testing the consistency of the effects among species from the same habitat and analysis of the UVB dose-response relation was done by applying meta-analysis with the log natural of response ratios. The natural log of the response ratio (lnRR) and its standard deviation (Hedges *et al.* 1999) of growth rates, DNA damage and UVB absorbing capacity for the individual UVB treatments over the PAR control were calculated as a measures of the magnitude of the responses to UVB (n=27). Positive lnRR values indicate higher values for growth rates, DNA damage and UVB absorbing capacity under UVB exposure than in the PAR control treatment and vice versa. Class means were calculated for each combination of habitat and UVB treatment as the weighted mean of the individual lnRRs using the reciprocal of the standard deviation as the weight. The effects of habitat and the effects of different doses of UVB on class means of lnRR were tested in an analysis using the QB statistic, assuming a χ^2 distribution test. Confidence intervals were derived by a bootstrap analysis as a robust non-parametric measure of uncertainty. Deviations of the confidence intervals from zero and among classes other show significant effects. 'PAR' as control gave similar results as the average PAR and UVA treatments as control. Therefore only the results of UVB treatments against PAR are projected in the figures. The meta-analysis was carried out in MetaWin 2.0, a statistical software package for meta-analysis (Rosenberg *et al.* 1997).

Results

UVB effects on DNA damage, growth and UVB absorbing compounds

The UVA radiation control treatments did not lead to significant effects compared to the PAR control treatment on any of the measures in any of the bryophytes, whereas the UVB treatments did lead to significant effects.

Most species showed significant increments in DNA damage, measured as cyclobutane pyrimidine dimers (CPDs), after 10 weeks of daily UVB exposure (Figure 5.1). Levels of CPDs were significantly increased in most species in the highest UVB treatment, but often also at the lower UVB doses. In *S. ruralis*, *M. hornum*, *S. magellanicum*, *S. fallax* and *P. commune* the lowest, ambient UVB doses already led to significant levels of DNA damage. Only in *D. scoparium* and *P. formosum* no significant effects were found in any treatment. In *D. scoparium*, UVB exposure led to higher CPD levels. However, due to large variation within the treatments, this effect was not significant.

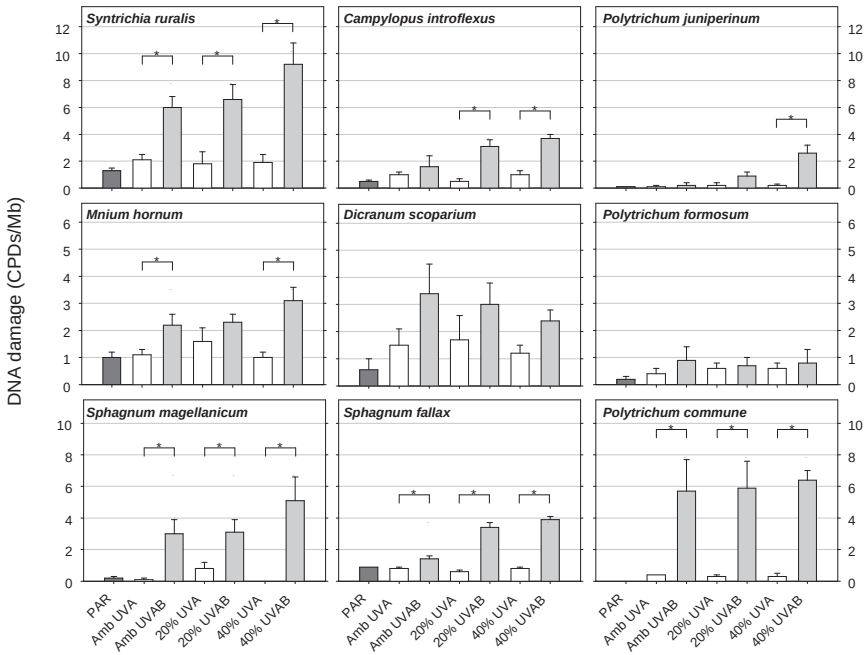


Figure 5.1: Average (+ SE) of DNA damage (cyclobutane pyrimidine dimers (CPDs) per 10^6 nucleotides (Mb)) of nine temperate bryophytes after exposure to seven different UV treatments. Species from the same habitat are depicted in the same row with the first row those from dune habitats, the second row from forest habitats and the third row from bog/fen habitats. The dark grey bar represents the PAR treatment, white bars 'UVA' and grey bars 'UVAB' treatments. ⌐*¬ refers to significant difference according the ANOVA analysis with contrasts ($P<0.05$). N equals 5 to 6 for each species-treatment combination.

Growth rates varied from less than 0.1 mm day^{-1} in *S. ruralis* and *C. introflexus* to almost 1 mm day^{-1} in *S. fallax* (Figure 5.2). While UVB radiation affected the levels of CPDs in most species, only in some species growth rates were significantly affected by UVB radiation: the highest UVB treatment, simulating 40% ozone reduction, significantly affected growth negatively in *S. magellanicum*, *S. fallax* and *P. commune*, all species from bog/fen habitats. For both *Sphagnum* species the intermediate UVB treatment showed already a marginally significant effect on growth ($P=0.08$ and 0.10 for *S. magellanicum* and *S. fallax*, respectively). The ambient UVB treatment significantly affected growth negatively in *P. commune*. In

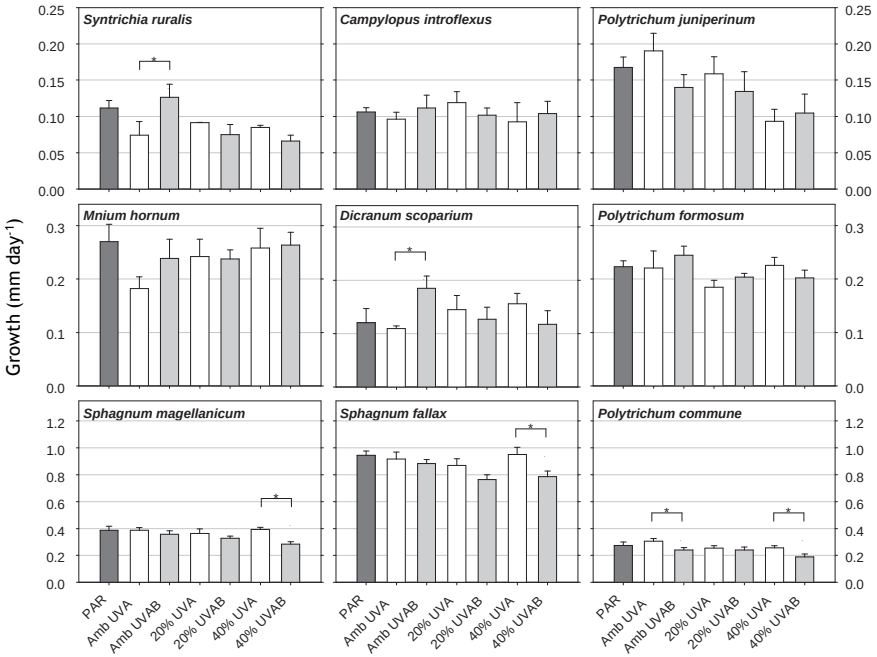


Figure 5.2: Average (+SE) of growth rates (mm day^{-1}) of nine temperate bryophytes after exposure to seven different UV treatments. Species order, colour schemes and statistical analysis are as in Figure 5.1. N equals 3 to 5 for *C. introflexus* and *S. ruralis* due to mortality; and 5 to 6 for all other species treatment combinations.

contrast, growth of *S. ruralis* and *D. scoparium* was positively affected by the ambient UVB levels. None of the other species showed significant effects of UVB radiation on growth.

The basal UVB absorbing capacity, i.e. the total absorption from 280–315nm per mg dry material under PAR exposure (in absence of UVA and UVB radiation), differed much among the different bryophytes (Figure 5.3). This basal UVB absorbing capacity was significantly higher in all *Polytrichum* species and *M. hornum* than in the other bryophyte species. *Polytrichum* species and *M. hornum* had around two to three times higher levels than *C. introflexus* and both *Sphagnum* species. Exposure to the different UVB levels resulted in significant, negative, effects in only two species (Figure 5.3). In *S. fallax*, the UVB absorbing capa-

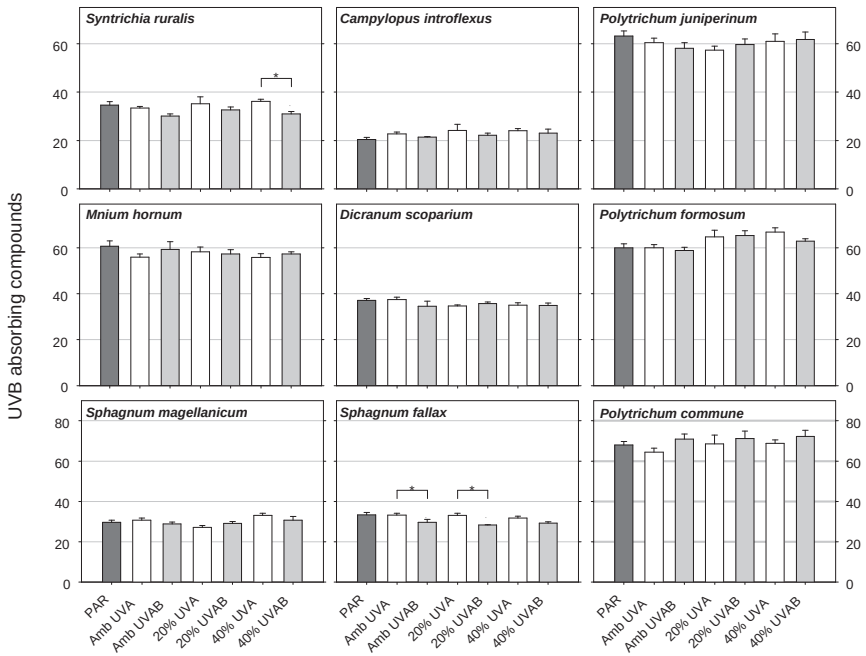


Figure 5.3: Levels of UVB absorbing compounds (absorption 280-315nm per mg dry weight; +SE) of nine temperate bryophytes after exposure to seven different UV treatments. Species order, colour schemes and statistical analysis are as in Figure 5.1. N equals 5 to 6 for all species treatment combinations, except for *S. ruralis* (N equals 3 to 6) due to mortality.

city was significantly reduced under UVB exposure in all UVB treatments (though marginally significant in the highest; P=0.06), while in *S. ruralis* only the highest UVB levels, simulating 40% ozone reduction led to a significant reduction.

Habitat consistency of UVB effects

The consistency of UVB effects on DNA damage, growth and UVB absorbing capacity across habitats and UVB doses was evaluated through a meta-analysis. The lnRRs for levels of DNA damage were significantly higher than zero after exposure to UVB in all treatments and all habitats (Figure 5.4a), indicating a significant increase in DNA damage upon UVB exposure. The average increase in CPDs, as a measure of DNA damage, after UVB exposure varied between doubling (ambient UVAB levels in the bog/fen habitat), to an over 7 times in-

crease (40% ozone reduction UVAB levels in the dune habitat) compared to the control. In the dune and in the bog/fen habitats an increase in UVB radiation led to an increase in CPDs, though this dose-response relation was only significant for the dune habitat. In the bog/fen habitat the confidence intervals were large, mainly due to high amounts of CPDs in *P. commune* in all UVB treatments independent of the UVB levels. Though confidence levels are smaller when excluding *P. commune* from the analysis, the trend was the same and again not significant. Mosses from the forest habitat differed in their response by showing significantly lower CPDs than mosses from other habitats at the 20% and 40% ozone reduction scenarios and not having a dose-response relation with increasing UVB doses.

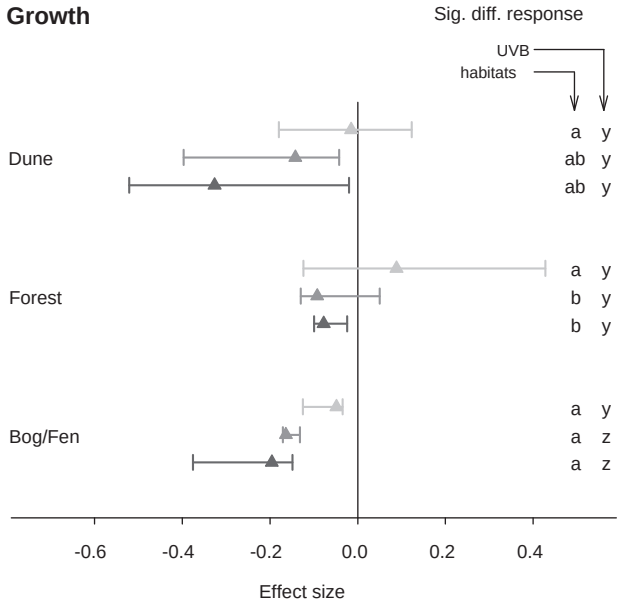
Growth rate responses to UVB radiation as a function of habitat and UVB dose showed a comparable pattern to that of DNA damage (Figure 5.4b), although the effects were less pronounced. In all habitats, exposure to UVB radiation resulted in significant growth reductions. However, the UVB dose at which the growth rates were significantly reduced differed among the habitats. Forest species showed smallest reductions, which were significant from zero only at the highest UVB level. In the dune habitat significant growth reduction occurred at the UVB treatments simulating 20% and 40% ozone reduction, while in the bog/fen habitat significant growth reductions were found at all UVB levels. In both dunes and bog/fen habitats the growth reductions were significantly stronger at higher UVB doses. Species from the forest habitat responded least to UVB exposure, both in growth reduction and in dose response, while the bog/fen habitat differed most in effect size and in dose-response effects. The dune habitat species responded intermediately.

Exposure to UVB did not lead to significant effects in the UVB absorbing compounds, except for the ambient UVB treatment in the forest species (Figure 5.4c), where a reduction was found. In addition, there was no dose-response relation in UVB absorbing compounds, nor any significant differences among habitats. When analysing all classes together, there was a small but significant decrease in the amounts of UVB absorbing compounds after exposure to UVB radiation.

Discussion

Species-specific UVB effects

Through this greenhouse experiment, we profoundly extended the available information on the effects of UVB on bryophytes. So far only a limited number of


Growth
Sig. diff. response
UVB
habitats
Dune
Forest
Bog/Fen
a y
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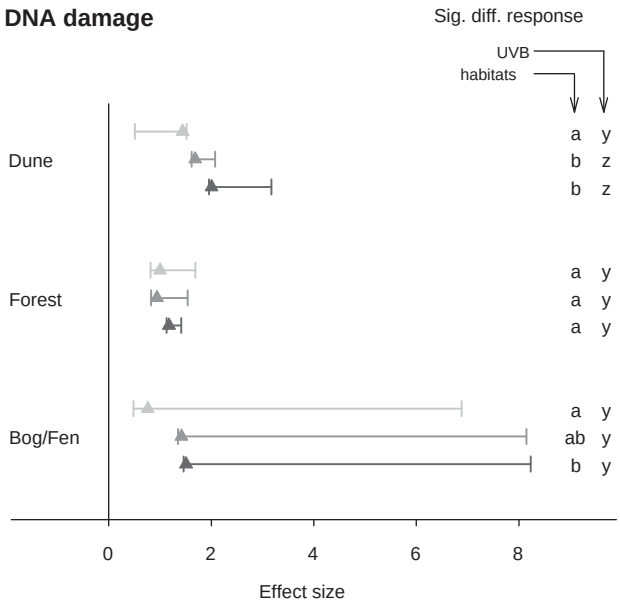
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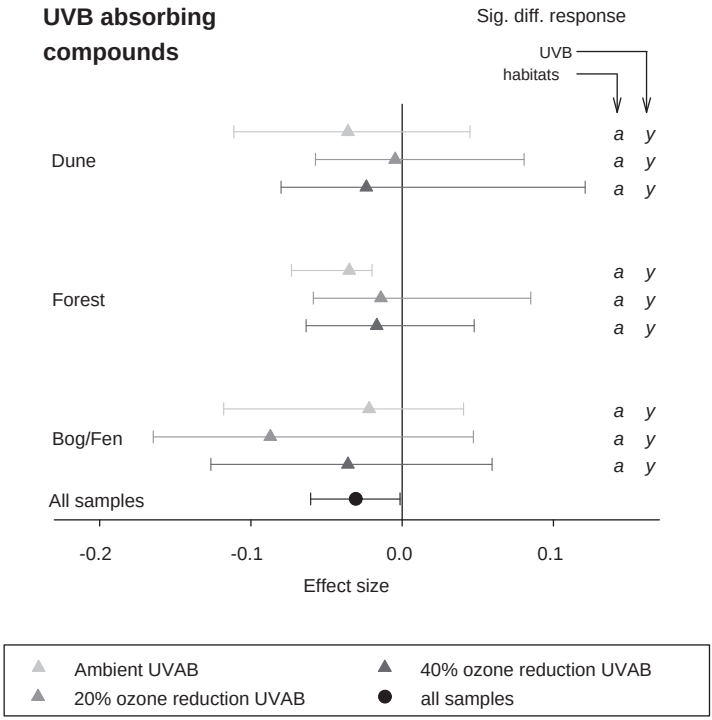

Figure 5.4: Effects of UVB treatment and of habitat on temperate bryophytes and its effects on a) DNA damage (CPDs/Mb), b) growth (analysed in mm day^{-1}) and c) levels of UVB absorbing compounds (analysed as absorption 280-315nm per gram dry weight). Effects were analysed through the natural logarithm of response ratios ('effect size') calculated for each individual species. Effect sizes larger than zero indicate higher values upon UVB treatment compared to the PAR control and vice versa. Means for species from the same habitat and 95% confidence intervals based on a bootstrapping procedure are indicated. For each bryophyte species, three situations were analysed corresponding to three levels of UVB exposure. Different letters for habitat and UVB treatment indicate significant differences in response among different habitats for the given UVB exposure or within a habitat for different UVB exposures, respectively, analysed using the QB statistic assuming a χ^2 distribution. 'All samples' refers to means and 95% confidence intervals calculated when combining all species and UVB exposure combinations.

studies on the UVB effects on bryophytes were available in literature and none of the studies screened a relative large number of bryophyte species simultaneously on the here studied parameters. Moreover, most studies on UVB effects in bryophytes were conducted in Arctic and (near) Antarctic regions with species from open and exposed habitats that are often wet from (snow) melt and with short growing seasons during the short summer period when snow is melting. In contrast, in the temperate Atlantic region from which the bryophytes of this study originated, bryophytes are generally year-round exposed to solar radiation. A different sensitivity to UVB radiation may therefore be expected. All bryophytes in this study, except *P. formosum* and *D. scoparium*, were significantly negatively affected by UVB radiation. UVB exposure led to increased levels of DNA damage in most species, growth reductions in some species and reduced UVB absorbing capacity in two species.

The few studies available that focussed on UVB induced DNA damage in bryophytes were all conducted under field conditions and showed either accumulation of cyclobutane pyrimidine dimers (CPDs) in the DNA (Lud *et al.* 2002), or no increase in CPDs (Boelen *et al.* 2006) under higher levels of UVB radiation. Here, in a controlled environmental study, we showed that out of the nine bryophyte species included, seven were prone to DNA damage upon UVB exposure. These results reflect the pattern found in higher plants and marine organisms that enhanced UVB radiation induces damage to functional structures, like membranes and proteins, DNA and pigments and therefore essential processes, like DNA replication are affected (references in Strid *et al.* 1994 and Caldwell *et al.* 1998).

For various plant species, including vascular plants, mosses and charophytes, higher levels of damaging UVB radiation often results in higher amounts of damage (e.g. Tackeuchi *et al.* 1996, Lud *et al.* 2001, De Bakker *et al.* 2005). In our experiment (Figure 5.1) similar trends in dose-response patterns were observed, although, for a given habitat, dose- response patterns were not significant. Within each habitat, however, significantly higher CPD levels were found at UVB exposure compared to the control PAR treatment (Figure 5.4a). Of all species, *P. commune*, showed the most remarkable response with constantly much higher CPD levels at any UVB exposure level compared to the PAR treatment. These results suggest that even low UVB levels induce significant levels of DNA damage in *P. commune*.

In general, damage in DNA and essential proteins and pigments may influence cellular processes, such as nutrient uptake, DNA transcription and replication of photosynthesis, which may then lead to growth reductions or impaired

reproduction (references in Boelen *et al.* 2006). In this study, growth rates were significant reduced only in the three species from fen/bog areas *P. commune*, *S. magellanicum* and *S. fallax*. Here, growth rates could be significantly correlated to levels of DNA damage (Pearson correlation $P<0.05$). The significant growth reductions found for these moss species here, coincide with significant growth reductions upon increased UVB levels found in *P. commune* (Gehrke 1999) and *S. magellanicum* (Searles *et al.* 2002) under field/outdoor conditions after the third and second growing season respectively. These responses may thus indicate a trend for moss species from this habitat. In the other species, coming from the dune and forest habitats, no significant UVB growth responses were found. In another study in similar temperate habitats with *S. ruralis* (De Bakker *et al.*, in preparation) we found a significant growth reduction under outdoor conditions after one year with enhanced UVB exposure.

UVB absorbing compounds are considered to act as protective screens against UVB radiation, by absorbing UVB, involved in scavenging of free oxygen radicals (Cockell and Knowland 1999). Bryophytes lack other means to minimise the damaging effects of UVB, like increasing leaf thickness (Bornmann and Vogelmann 1991) as they have undifferentiated leaves of one cell layer thick and no protective layer to attenuate UVB (Gehrke 1999). Nevertheless, increasing levels of UVB in general did not lead to change in UVB absorbing compounds. This coincides with results found in most other bryophyte (field) studies (e.g. Searles *et al.* 1999, Searles *et al.* 2002, Niemi *et al.* 2002, Robinson *et al.* 2005). In this study, the only exceptions to this rule were *S. ruralis* and *S. fallax* in which UVB absorbing compounds were reduced at high and medium UVB levels, respectively. Barsig *et al.* (1998) and Gehrke (1999) reported reduced levels of UVB absorption after UVB exposure in *P. commune* before.

There was a large difference in the 'basal' levels of UVB absorbing compounds in the bryophytes. All *Polytrichum* species and *M. hornum* had significantly higher UVB absorbing compounds than the other species. Also Dunn and Robinson (2006) reported significant differences in UVB absorbing compounds between *Bryum pseudotriquetrum*, *Ceratodon purpureus*, and *Grimmia antarctica*. In these bryophytes the chemical composition of compounds absorbing in the UVB region is largely unknown. Differences of constitutive levels of UVB absorbing compounds between species may be related to this.

Effects of habitat origin on UVB responses

Although the number of studied bryophyte species per habitat was relatively small, this is the first study to show the differential effects of UVB radiation on

growth rates, DNA damage and UVB absorbing compounds in bryophytes across different temperate habitats. So far, the information on UVB responses of bryophytes had been too sporadic to analyse general trends. The UVB responses in bryophytes reported in literature are variable and differ among species, but also between experiments or experimental duration with the same species (e.g. Gehrke 1999, Sonnesson *et al.* 2002, Phoenix *et al.* 2001, Robinson *et al.* 2005). Different responses in the same species are often explained by temperature differences among years (Searles *et al.* 2002, Sonesson *et al.* 2002), precipitation (Phoenix *et al.* 2001, Searles *et al.* 2002), local conditions and altered habitat conditions (Robinson *et al.* 2005). This study with a broader screening at identical conditions showed, however, that the effects found are consistent among the species from the same habitat. Habitat origin therefore, seems to influence the sensitivity of species against UVB radiation.

Comparing the three different habitats showed that species from the forest habitat had in general less DNA damage, had least reduced growth rates compared to species from dune and bog/fen habitats and showed no dose-response relations with UVB radiation. Therefore, species from the forest habitat seemed to be least sensitive to UVB radiation, compared to species from the dune and bog/fen habitats in this experiment.

Under natural conditions, the forest bryophytes cover the forest floors and are mostly shaded by trees and therefore exposed to lower UVB and PAR levels throughout the year than species from open, both dry and wet habitats, like the dune and bog/fen habitats. It was therefore expected that bryophytes from forests would have been more sensitive to UVB radiation in this experiment with relatively high PAR and UVB levels compared to what is experienced naturally by moss species in forests. Remarkably, the obtained results contrast with these expectations. If any effect, our experimental conditions should have led to an overestimation of the UVB effects (references in Searles *et al.* 2001).

In this experiment, conducted under climate controlled conditions, all species were kept at higher PAR irradiance levels than in northern hemisphere winters (400 compared to 232 $\mu mol^{-1}\ m^{-2}\ s^{-1}$), while the experimental PAR/UVB ratios ranged from 70-160 (compared to a natural PAR/UVB ratio of 165 over the experimental period in open areas like dunes, fens and bogs). As a consequence of the added high air humidity, all mosses were physiologically active during UVB exposure. This represents the natural habitat conditions in fens and bogs where *Sphaghum* species are physiologically active throughout most conditions (Porley and Hodgetts 2005). The high sensitivity of bryophytes from fens and bogs is thus not an artefact of this experiment, but suggests that these spe-

cies systematically lack adaptations to UVB. Dune species, however, are at natural conditions often in a dry state at high (UVB) irradiance levels due high evaporation, windy conditions and low water availability. These species show most growth in autumn and spring when UVB levels are lower than in summer. Gehrke (1998) argued that bryophytes might be more sensitive to UVB radiation when physiologically inactive, since no direct repair of UVB induced damage could take place. Own field observation for *S. ruralis* did not show DNA damage after UVB exposure for two years under natural conditions (data not published). However, presence of glass hairs at the leaf tip to increase albedo and by curling leaves against the stems to reduce surface area (Proctor 2000) may reduce harmful UVB. This experiment therefore provides a measure of potential sensitivity to UVB for these species, while they may be less sensitive to UVB at natural conditions.

Takács *et al.* (1999), Csintalan *et al.* (2001) and Dunn and Robinson (2006) pointed that adaptations to other stress factors in bryophytes, like the ability to cope with desiccation, might interfere with the UVB tolerance in bryophytes. If this is a general rule, not habitats, but the ability to cope with different stresses may determine differences in tolerance to UVB. Both desiccation and UVB exposure may lead to oxidative stress (Brosché and Strid 20003, Blokhina *et al.* 2003). Ranking the bryophytes from this study according the oxidative stress encountered - following the differences in wetness of the respective habitats and their the ability to cope with desiccation (Landwehr 1966, Takács *et al.* 1999 and Dr. B. van Zanten, personal comment) - leads to the following order: *S. ruralis*, an extreme desiccation tolerant species and *P. juniperinum* occur at dry places, *C. introflexus*, *D. scoparium*, *P. formosum*, *M. hornum* and *P. commune* form an intermediate group. Both *Sphagnum* species occur in wet habitats and are least adapted to desiccation. Only the more sensitive species from fens/bogs (*P. commune* and both *Sphagnum* species) showed significant effects on DNA damage and growth. The coincidence of UVB tolerance and tolerance to oxidative stress shows that it would be interesting to study the relation of multi-stresses and UVB tolerance.

Thus, the mechanisms through which species from different temperate habitats differ in sensitivity to UVB are unknown and will have to be evaluated in a screening involving more bryophyte species. Such a validation is preferably carried out in outdoor conditions specific for each habitat, given that greenhouse experiments with different radiation conditions compared to the field could have modified UVB responses, as Robinson *et al.* (2005) showed that altering the PAR radiation conditions of *Grimmia antarctica* affected the UVB response

in this bryophyte. Alternatively, transplantation experiments with addition of UVB radiation in the field, may be used to validate the impact of habitat origin on species sensitivity to UVB radiation.

In conclusion, our study showed that UVB radiation significantly affected growth rates, DNA damage and UVB absorbing compounds in bryophytes from temperate regions. The sensitivity of species against UVB radiation consistently differed with habitat origin: species from the dune and bog/fen habitat appeared to be most sensitive to UVB radiation, compared to species from the forest habitat in this experiment. Ideally, the response to elevated UVB of the nine bryophyte species from dune, fen/bogs and forest ecosystems should be studied under field conditions as well, to see if habitat origin similarly affects sensitivity of moss species to UVB under natural conditions.

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