Trees (2001) 15:483–491
DOI 10.1007/s00468-001-0129-3

ORIGINAL ARTICLE

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Growth, structure, stomatal responses and secondary metabolites of birch seedlings (*Betula pendula*) under elevated UV-B radiation in the field

Received: 5 January 2001 / Accepted: 13 September 2001 / Published online: 26 October 2001

Abstract Three-year-old birch (*Betula pendula* Roth.) seedlings were exposed, in the field, to supplemental levels of UV-B radiation. Control seedlings were exposed to ambient levels of UV radiation, using arrays of unenergized lamps. A control for UV-A radiation was also included in the experiment. Enhanced UV-B radiation had no significant effects on height growth, and shoot and root biomass of birch seedlings. Leaf expansion rate increased transiently in the middle of the growing period in enhanced UV-B- and UV-A-exposed plants; however, final leaf size and relative growth rate remained unaffected. Leaf thickness and spongy intercellular spaces were increased in UV-B-exposed seedlings along with increased density of glandular trichomes. At the ultrastructural level, enhanced UV-B increased the number of cytoplasmic lipid bodies, and abnormal membrane whorls were found. Both enhanced UV-B and UV-A radiation induced swelling of chloroplast thylakoids. Stomatal density and conductance were significantly increased by elevated UV-B radiation. UV-A radiation increased the length and width of stomata, whereas UV-B radiation had only a marginal effect on stomatal size. UV-A and enhanced UV-B radiation attenuated the appearance of necrotic spots in autumn, probably caused by the fungus *Pyrenopeziza betulicola*, suggesting a direct harmful effect of UV on pathogens or reduced susceptibility to pathogens in UV-exposed seedlings. Secondary metabolite analysis showed increases in (+)-catechin, quercetin, cinnamic acid derivative, apigenin and pentagalloylglucose in birch leaves under enhanced UV-B radiation. Negative correlations between apigenin, and particularly quercetin concentrations and lipid peroxidation levels indicated an antioxidant role of secondary metabolites in birch leaves exposed to UV-B radiation.

Keywords *Betula pendula* · UV radiation · Structure · Secondary metabolites · Stomatal responses

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Introduction

Since approximately 90% of the total atmospheric ozone is in the stratosphere, changes in ozone concentration in this layer will strongly affect penetration of solar radiation at those wavelengths (UV-B 280–320 nm) where ozone is the principal absorber. Depletion of the stratospheric ozone layer results from emissions of halogenated chemicals, such as chlorofluorocarbons (CFCs). Despite reductions in emissions of O_3-depleting CFCs, levels of O_3 in the stratosphere will continue to fall, with especially severe declines occurring in the years 2010–2019 in the northern hemisphere, according to the global climate model that incorporates simplified ozone-depletion chemistry (Shindell et al. 1998). Recovery of stratospheric ozone to early-1980s levels is not predicted until roughly 2050.

UV-B radiation can induce a wide range of responses in plants, including increased concentrations of protective UV-B-absorbing pigments in leaves, and reductions in the rates of CO_2 assimilation and plant growth (Jordan et al. 1994; Teramura and Sullivan 1994; Nogues and Baker 1995).

Caldwell and Flint (1994) evaluated 300 papers from the past 20 years concerning plant exposure to UV-B. They concluded that this literature comprises mainly short-term chamber and greenhouse experiments that have limited value to solve ecological questions. In many growth chamber and greenhouse experiments the ratio of UV-B: photosynthetically active radiation (PAR) is considerably higher than in the field. Experiments

specifically designed to investigate the influence of PAR level on UV-B sensitivity showed that UV-B effects were less pronounced if plants were under higher PAR (Cen and Borman 1990; Kramer et al. 1991). As a result, insufficient PAR and, therefore, unrealistically high UV-B:PAR ratios in greenhouse and growth chamber studies have exaggerated UV-B effects. This also explains why plants growing in the field have been found to be much less sensitive to UV-B than those grown in greenhouses or growth chambers (Caldwell and Flint 1994; Antonelli et al. 1998).

Long-lived trees are subject to the accumulated effects of environmental stress in ways that annual plants are not. Sullivan and Teramura (1992) suggested that the effects of UV-B radiation may accumulate in trees and that increased UV-B radiation may significantly reduce the growth of loblolly pine over its lifetime. Studies on conifers and angiosperms, conducted in both greenhouses and the field, have demonstrated several growth and physiological responses to enhanced UV radiation (Lavola 1998; Nagel et al. 1998; Liakoura et al. 1999; Newsham et al. 1999). Our hypothesis tested in this study was that European silver birch seedlings show measurable growth, physiological and structural changes when exposed to realistic supplemental levels of UV-B radiation in the field.

Materials and methods

Experimental design

Three-year-old birch (*Betula pendula* Roth.) seedlings were exposed to supplemental UV radiation in the field for 16 weeks in 1999. The same seedlings had also been exposed to UV radiation in the preceding summer. Seedlings were grown in pots (diameter 25 cm, containing peat and sand in ratio 3:1), were fertilized regularly, and watered when needed. Treatments (control or ambient level of UV radiation, UV-A and enhanced UV-B radiation) were replicated four times, and each plot contained 8 seedlings providing in total 96 seedlings. A modulated UV exposure system that continuously tracks solar UV radiation and adds a constant proportion (30%) of supplemental UV was used. This UV system consisted of lamp arrays (3×1.2 m aluminium frames) hung above the plants. Each array consisted of six Phillips TL40/12 fluorescent tubes. A UV-B (280–320 nm) treatment was achieved by wrapping the lamps with cellulose diacetate film, which allowed the transmission of both UV-B and UV-A radiation. A control for the UV-A (320–400 nm) radiation was achieved by wrapping the lamps in UV-A treatment with polyester film, which excludes both UV-C and UV-B radiation. The ambient control array with unlit lamps provided the same shading as the actual UV-B or UV-A treatment. Lamp output was guided by an electronic controller for Osram HF 2×36 W DIM fluorescent tubes. Broadband, erythemally weighed solar sensors (Davies 7841) were situated in the middle of the exposure at the same height as the top of the canopy and were used for continuous computer control of lamp output. The distance between the canopy top and lamps was maintained at approximately 130 cm. Lamps were automatically switched off when irradiation fell below 10 mW m^{-2}. Exposure began in mid-May and continued until the birch senesced. Total effective erythemally weighed UV doses were 130 and 170 kJ m^{-2} for control, and 167 and 212 kJ m^{-2} for UV-B treatment in 1998 and 1999, respectively. Total daily erythemally weighed UV irradiance is shown in Fig. 1. Mean PAR level during the experiment was 318 and 338 µmol m^{-2} s^{-1} in 1998 and 1999, respectively.

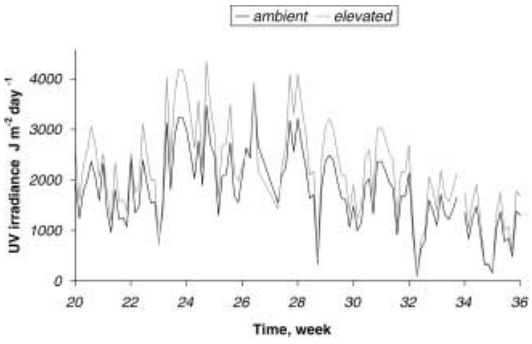

Fig. 1 Total daily erythemally weighed UV irradiance

Growth analyses and phenology test

Seedling height was measured every 3 weeks. Leaf growth expansion was measured every 3 days on one tagged leaf per seedling at two different periods (early June and early July). Relative growth rate (RGR) was determined with the following equation: RGR=(ln L_2–ln L_1)/ t_2–t_1, where L_2 and L_1 are leaf lengths at the times t_2 and t_1.

For specific leaf area (SLA) one leaf from each seedling was collected. A digital photo (Olympus C-4100) was taken and then analysed with an image processing programme (Adobe Photoshop) to calculate the surface area. Leaves were dried at 70°C and then weighed. SLA was determined with the following equation: SLA = dry weight /surface area.

After 13 weeks of exposure 3–4 birch seedlings per plot were harvested for root, leaf and stem biomass analysis. Samples were oven-dried at 70°C for 48 h, and then weighed.

Phenological observations of leaf senescence and pathogen-induced necrotic spots were evaluated from 14–15 seedlings per treatment every week from 10 August 1999. Regarding necrotic spots each seedling was assigned to one of two groups: 1= less than half of the leaves have necrotic spots and 2= more than half of the leaves have necrotic spots. Leaf senescence was classified into 5 classes: 1= all leaves green, 2= few yellow leaves, 3= half of the leaves yellow, 4=>90% of the leaves yellow, 5= all leaves yellow.

Stomatal responses and glandular trichome density

Stomatal conductance was measured on three fully expanded leaves per seedling every 3 weeks using a steady-state porometer (LI-1600, Li-Cor, USA). Fully expanded leaves (one per seedling) for the determination of stomatal and glandular trichome density were sampled every 3 weeks in connection with stomatal conductance measurements. A quick glue method resulting in leaf replicates of abaxial surface was used. Stomatal and glandular trichome densities were then counted under a light microscope from three frames (0.77×0.77 mm^2) per leaf using systematic uniform random sampling (Kubinova 1994). Results are expressed as means of counts per mm^2.

Microscopy

Samples for microscopy were collected after 12 weeks of exposure in early August before the appearance of necrotic spots. For scanning electron microscope (SEM) studies one fully expanded leaf from five seedlings per plot was sampled. The fresh leaf pieces (10×10 mm^2) were mounted on aluminium stubs and air-dried at room temperature for 24 h. Samples were then sputtered with gold-palladium under vacuum (Polaron Equipment, SEM Coating Unit E 5100) and were examined with SEM (JEOL JSM-35) at 400× magnification. The length and width of 20 randomly chosen stomata were measured on the abaxial surface of each leaf.

One leaf from eight seedlings per plot was collected for light and transmission electron microscopy. A 1.5 mm^2 square piece was cut with a razor blade from the basal area of each leaf under a drop of 2.5% glutaraldehyde in 0.1 M phosphate buffer. Leaf pieces were post-fixed in 1% osmium tetroxide solution, dehydrated in ascending ethanol series and embedded in LX 112 Epon. Sections for light microscopy were stained with toluidine blue and examined with a Zeiss (Germany) light microscope. Ultrathin sections were stained with uranyl acetate and lead citrate and were studied with a transmission electron microscope (JEOL JEM 1200 EX) operating at 80 kV at 7,500×.

Thickness of the whole leaf, the upper and lower epidermis, and the palisade and spongy parenchyma was measured from the light micrographs. In addition, point analysis was carried out to determine the relative (percentage) areas of epidermis, palisade and spongy parenchyma and intercellular spaces.

Ultrastructural measurements were made on five leaves per plot. Altogether 100 chloroplasts per plot were examined. The size of chloroplasts and starch granules was measured from digital electron micrographs using the Adobe Photoshop image-processing program. The presence of cytoplasmic membrane whorls was classified as 0= none, 1= present. The appearance of chloroplast thylakoids was classified as 0= normal, 1= swelling. Cytoplasmic lipid bodies were recorded as 0= no lipid bodies, 1= one or more lipid bodies per cell.

Lipid peroxidation

The level of lipid peroxidation products in the leaves was expressed as thiobarbituric acid reactive substances (TBAS). The assay was carried out as described in Zwiazek and Shay (1988) with some modification. Leaf samples (0.1–0.3 g) were ground in boiling methanol (5 ml). After filtering, the volume was brought up to 3 ml with methanol. One millilitre samples were combined with 2 ml of trichloroacetic acid – *tert*-butyl alcohol – hydrochloric acid (TCA-TBA-HCl, 15% w/v TCA, 0.375% w/v TBA, 0.25 *N* HCl) and heated for 15 min in a boiling water bath. After cooling, the precipitate was removed by centrifugation at 1,000 *g* (2,700 rpm) for 5 min. The absorbance was determined at 535 nm with a spectrophotometer (UV-1201, Shimadzu) against blank samples. The values were corrected for unspecific turbidity by subtracting the absorbance at 600 nm. Malondialdehyde (MDA) concentration was calculated using the extinction coefficient 155 mM^{-1} cm^{-1} (Heath and Packer 1968).

Secondary metabolites

A pooled sample, containing either one fully expanded or one young, still expanding leaf per seedling, from eight birch seedlings per plot was collected for secondary metabolite analysis after 12 weeks of exposure in early August before the appearance of necrotic spots. A 0.5 cm^2 piece was cut from each of eight leaves (total about 15–20 mg) into an Eppendorf-tube and left to stand in 0.5 ml methanol on an ice-bath. After 20 min the sample was homogenized and centrifuged for 3 min at 13,000 rpm (Heraeus biofuge 13). The residue was extracted twice with 0.4 ml of methanol for 5 min and washed once with 0.4 ml of methanol. Supernatants were combined, dried under nitrogen and stored at –20°C until analysed. Dried leaf extracts were redissolved into 0.8 ml of 50% methanol and secondary metabolites were quantified by high performance liquid chromatography (HPLC) (Julkunen-Tiitto et al. 1996). Elution solvents were A (aqueous 1.5% tetrahydrofuran and 0.25% *ortho*-phosphoric acid) and B (100% methanol). The following gradient was used: at 0–5 min 100% of A, at 10 min 80% of A in 20% of B, at 20 min 60% of A in 40% of B, at 40–45 min 50% of A in 50% of B and rising to 100% of B, and equilibration to 100% of A. HPLC conditions were as follows: the column was HP Hypersil ODS II (3 µm), 60×4.6 mm ID 2 mm, flow rate 2 ml/min and oven temperature 22°C. The injected volume was 20 µl. Identification of phenolic compounds was based on comparison of retention times and UV-Vis spectra of the peaks with authentic reference compounds and results obtained from hydrolysis (Keinänen and Julkunen-Tiitto 1998).

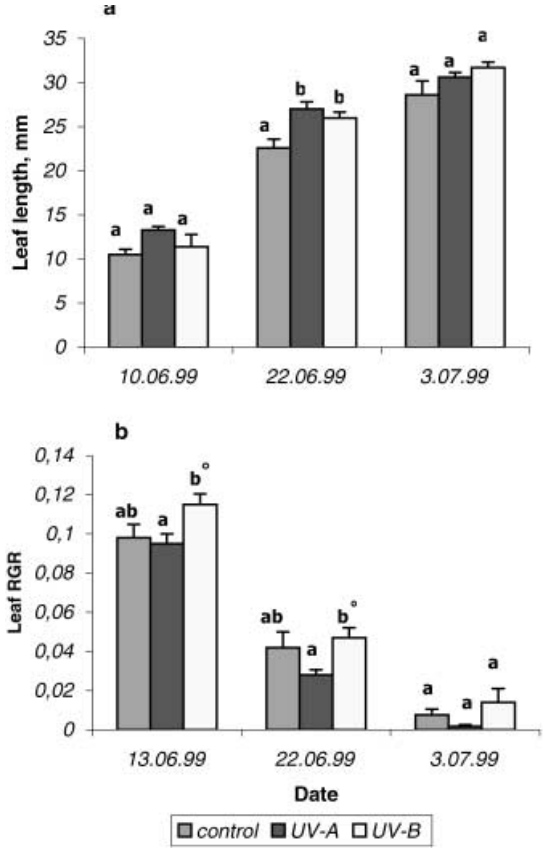

Fig. 2 Changes with time in leaf length (**a**) and relative growth rate (**b**) of *Betula pendula* leaves grown in the field under ambient or elevated UV radiation. Each *bar* represents the mean (±SE) of 4 replicates. Columns marked by a different letter are significantly different at $P \leq 0.05$. Letters followed by ° indicate columns which are significantly different at $P \leq 0.1$

Statistical analyses

The statistical analyses were carried out using SPSS 9.0 for Windows. Analysis of variance (Tukey's multiple range test) was used for comparing the group means between treatments. A chisquare test followed by a Mann-Whitney test was used for classified parameters. Pearson's bivariate correlation coefficients were calculated between stomatal conductance and both stomatal density and size, also between lipid peroxidation and concentration of apigenin and quercetin.

Results

Growth and phenology

UV radiation had no effect on seedling height, shoot, stem biomass and shoot to root ratio or SLA ($P \geq 0.344$). The leaf expansion rate increased by 20% in the middle of the growing period due to UV radiation, but the final leaf size remained unaffected (Fig. 2a). There was no

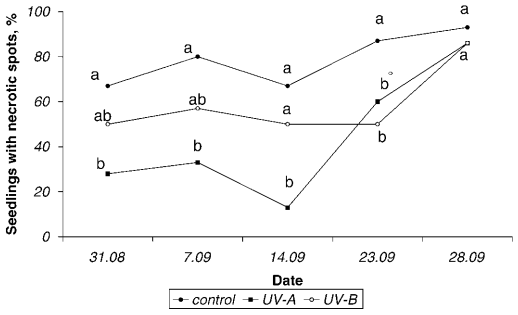

Fig. 3 Proportion of *B. pendula* seedlings with >50% leaves showing necrotic spots. Chi-square test followed by Mann-Whitney test, n=15. A different letter indicates significant difference at $P \leq 0.05$. Letters followed by ° indicate significant difference at $P \leq 0.1$

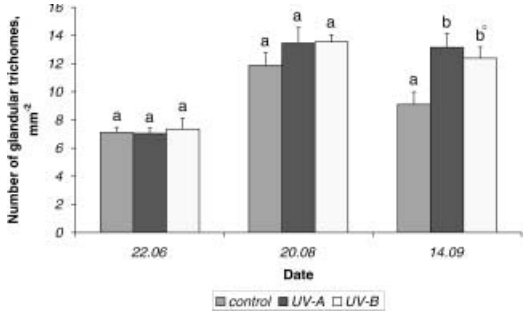

Fig. 5 Effect of exposure length on density of glandular trichomes in *B. pendula* grown in the field under ambient or elevated UV radiation. Each *bar* represents the mean (±SE) of 4 replicates. Columns marked by a different letter are significantly different at $P \leq 0.05$. Letters followed by ° indicate columns that are significantly different at $P \leq 0.1$

Table 1 Effects of UV radiation on leaf anatomy and on relative cross-sectional area (%) of *Betula pendula*. Values are means (±SD) of 4 replicates. ANOVA; Tukey's multiple range test. A different letter indicates significant difference at $P \leq 0.05$. Values followed by ° are significantly different at $P \leq 0.1$

Response	Control	UV-A	UV-B
Leaf thickness, µm	178.7 (8.6)a	190.1 (10)ab	195 (10)b°
Upper epidermis, µm	20 (0.6)a	21 (3.4)a	22 (1.4)a
Lower epidermis, µm	9.8 (1.1)a	10.3 (0.9)a	10 (0.4)a
Palisade parenchyma, µm	54 (6.7)a	60 (2.5)a	57 (4.5)a
Spongy parenchyma, µm	95 (12)a	98 (8.7)a	106 (7.8)a
Spongy intercellular space, %	23.1 (2.8)a	23.6 (1.4)ab	26 (0.6)b°

difference in RGR of leaves between the control and UV-exposed seedlings (Fig. 2b). However, UV-B marginally increased (P=0.096) leaf RGR compared to UV-A.

Supplemental UV radiation attenuated the appearance of necrotic spots in autumn (Fig. 3). Necrotic spots were significantly decreased in UV-A-exposed plants compared to control seedlings during the whole of September, whereas the difference between control and UV-B was significant only at the end of September. There was no difference in leaf senescence between the control and UV-exposed seedlings (data not shown).

Leaf anatomy and glandular trichomes

The exposure of seedlings to enhanced UV-B radiation resulted in an increase in leaf thickness (8%, P=0.06) (Table 1, Fig. 4b). This was associated with increases in thickness of the upper epidermis (10%, P=0.11) and spongy parenchyma (10%, P=0.18). The thickness of lower epidermal and palisade parenchyma cells was unaltered following supplemental UV-B radiation. Seedlings growing under UV-A radiation had a trend toward increased leaf thickness (6%, P=0.15) and palisade parenchyma (11%, P=0.11) while the upper and lower epidermis and spongy parenchyma were unaffected. The determination of the relative areas of tissue structures showed an increase in the spongy intercellular space (8%, P<0.1) under elevated UV-B radiation in comparison with the control treatment.

There was no influence of UV radiation on the glandular trichome density from June to August (Fig. 5). However, in the middle of September the glandular trichome density was increased by 40% and 35% due to UV-A and enhanced UV-B radiation, respectively.

Ultrastructure

At the ultrastructural level UV radiation had no effect either on chloroplast area or length (Table 2). However,

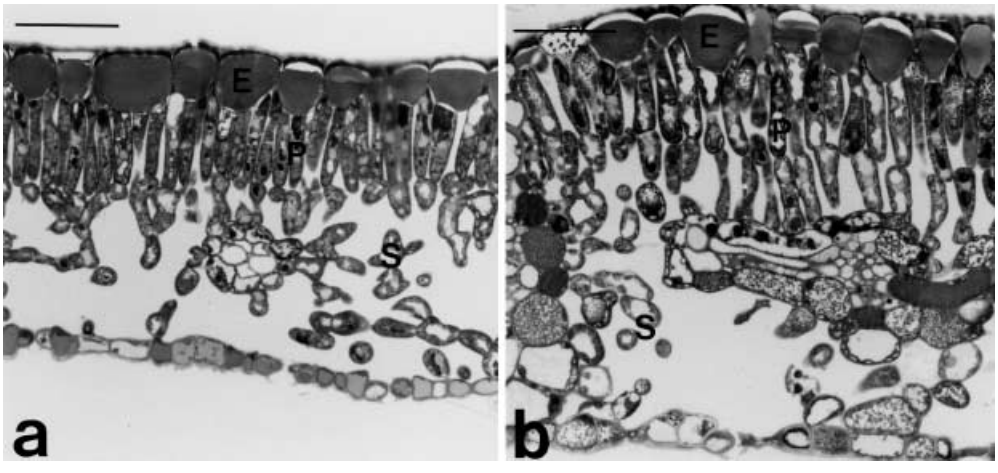

Fig. 4 Light micrographs of control (**a**) and UV-B-exposed (**b**) leaves of *B. pendula*. Note the UV-B-induced increase in leaf thickness. *E* epidermis, *P* palisade and *S* spongy parenchyma. *Bar* 50 µm

Table 2 Ultrastructural observations of the palisade parenchyma cells of *B. pendula* seedlings under elevated UV radiation in an open field exposure. Values are means (± SD) of 4 replicates. ANOVA; Tukey's multiple range test. Chi-square test followed by Mann-Whitney test was used for observations of membrane whorls, lipid bodies and swelling of thylakoids in the studied leaves, n=20. A different letter indicates significant difference at $P \leq 0.05$

Response	Control	UV-A	UV-B
Chloroplast area, µm²	11.7 (1.0)a	12.1 (0.7)a	11.6 (1.1)a
Length of chloroplast, µm	5.1 (0.1)a	5.2 (0,2)a	5.4 (0.3)a
Volume of starch, µm²	5.8 (0.5)a	5.6 (0.7)a	4.8 (1.1)a
Starch: chloroplast, %	48.2 (0.9)a	44.6 (4.0)ab	39.3 (6.1)b
Membrane whorls, %	5a	15a	55b
Lipid bodies, %	5a	35ab	75b
Swelling of thylakoids, %	5a	60b	45bc

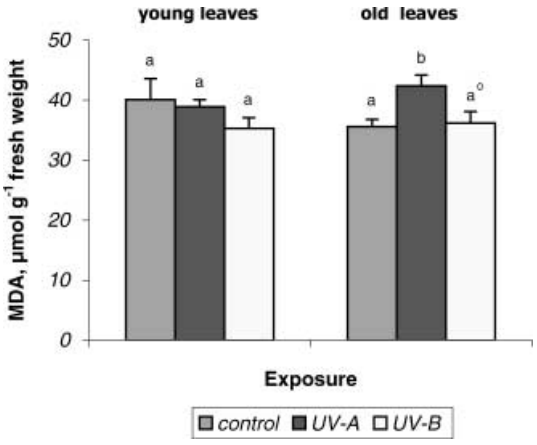

Fig. 7 The lipid peroxidation in young (last fully expanded) and old (first fully expanded, about 11 weeks old) leaves of *B. pendula* grown in the field under ambient or elevated UV radiation. Samples were collected on 9 August 1999. Each *bar* represents the mean (±SE) of 4 replicates. Columns marked by a different letter are significantly different at $P \leq 0.05$. Letters followed by ° indicate columns which are significantly different at $P \leq 0.1$

the ratio of starch to chloroplast area was significantly decreased under enhanced UV-B radiation (by 9%) due to the reduction in starch volume in UV-B-exposed seedlings (Table 2, Fig. 6c). Numerous membrane whorls were found in half of the studied leaves exposed to enhanced UV-B (Table 2, Fig. 6c), whereas in control plants membrane whorls were found in only 5% of studied leaves. Lipid bodies were present in 5% of control leaves and were significantly increased to 75% in UV-B-exposed leaves (Table 2, Fig. 6c). Thylakoid swelling occurred in 5% of studied control leaves and in 60% and 45% of UV-A- and UV-B-exposed leaves, respectively (Table 2, Fig. 6b, c). In addition, curling of thylakoid membranes was found under UV-A radiation (Fig. 6b).

Lipid peroxidation

UV-A radiation significantly increased (by 18%) lipid peroxidation, measured as TBAS, in the oldest fully expanded leaves (Fig. 7), but not in the young birch leaves. Enhanced UV-B exposure did not increase lipid peroxidation in fully expanded leaves compared with the control, while compared with UV-A, lipid peroxidation was diminished by 15% (P=0.061).

Secondary metabolites

The secondary metabolites were affected by UV radiation but only in the mature leaves (Table 3, data not shown for the young leaves). There was an increase in the concentration of (+)-catechin (30%, P=0.1) and pentagalloylglucose (about 50%, P=0.043) in plants grown

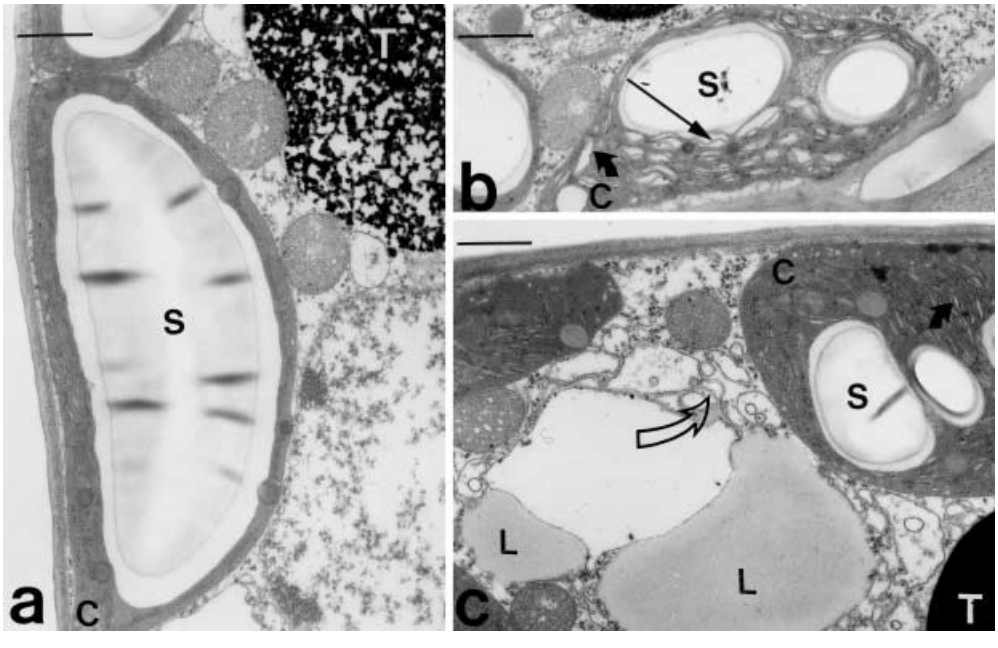

Fig. 6 Transmission electron micrographs of palisade mesophyll cells of *B. pendula* in control (**a**), UV-A- **(b)** and UV-B- (**c**) exposed leaves. *C* Chloroplast, *S* starch, *L* lipid, *T* tannin. **b** Severe curling (*long arrow*) and swelling (*short arrow*) of thylakoid membranes were typical symptoms for UV-A-exposed plants. **c** Note the decrease in starch to chloroplast area ratio, slight thylakoid swelling (*short arrow*) and the increases in lipids and membrane whorls (*curved open arrow*). *Bar* 1 µm

Table 3 Secondary metabolite concentrations in mature leaves of UV-exposed *B. pendula* seedlings. Values represent the mean (±SD) of 4 replicates. ANOVA, Tukey's multiple range test. A different letter indicates a significant difference at $P \leq 0.05$. Values followed by ° are significantly different at $P \leq 0.1$. (*RT* retention time, min)

Metabolite, mg·g^{-1} dry weight	RT	Control	UV-A	UV-B
(+)-Catechin	8.0	0.93 (0.22)a°	0.87 (0.09)a	1.2 (0.15)b
Quercetin-3-arabinopyranosid	19.7	3.48 (0.41)ab	2.92 (0.42)a	3.73 (0.61)b°
Cinnamic acid derivative	25.3	0.40 (0.12)a	0.63 (0.14)b°	0.52 (0.15)ab
Cinnamic acid derivative	25.9	0.46 (0.09)a	0.19 (0.13)b	0.32 (0.07)ab
Cinnamic acid derivative	31.0	0.38 (0.05)a	0.17 (0.08)a	0.24 (0.1)b°
Apigenin derivative	44.8	0.49 (0.09)ab	0.38 (0.14)a	0.63 (0.08)b
Pentagalloylglucose	20.6	0.4 (0.02)a	0.41 (0.14)a	0.61 (0.11)b

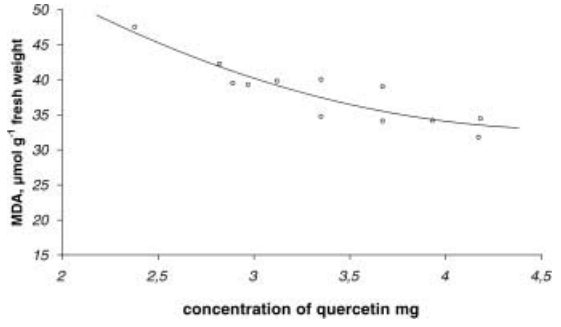

Fig. 8 The relationship between the concentration of quercetin and lipid peroxidation in fully expanded leaves of *B. pendula* ($y=2.7x^2-24.8x+90.7$, $R^2=0.83$)

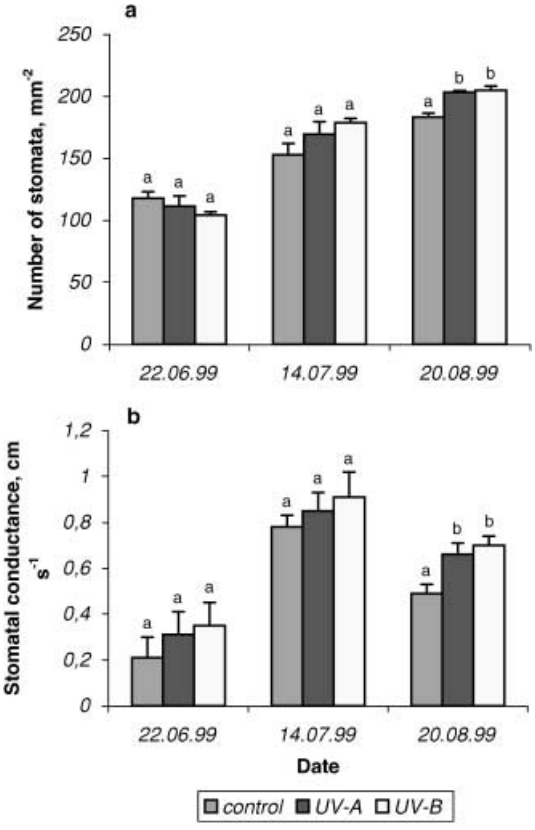

Fig. 9 Changes with time in the density of stomata (**a**) and stomatal conductance (**b**) in *B. pendula* grown in the field under ambient or elevated UV radiation. Each *bar* represents the mean (±SE) of 4 replicates. Columns marked by a different letter are significantly different at $P \leq 0.05$

under elevated UV-B radiation compared to control plants. There was also a difference between plants grown under enhanced UV-B and UV-A: concentrations of (+)-catechin and pentagalloylglucose were 38% (P=0.044) and 50% (P=0.048) higher due to UV-B radiation, respectively. Levels of apigenin and quercetin derivatives were 66% (P=0.023) and 28% (P=0.1) higher under enhanced UV-B radiation, respectively, than under UV-A arrays, but there was no difference between UV treatments and the control. Significant negative correlations between the concentration of quercetin and lipid peroxidation (r=–0.888, P=0.000; Fig. 8), as well as between apigenin and lipid peroxidation (r=–0.697, P=0.012, data not shown), were found for mature leaves. Cinnamic acid derivatives were mainly affected by UV-A radiation: some were significantly decreased by about 60% (P=0.012), but the others marginally increased (P=0.097).

Stomatal responses

Under UV radiation stomatal density marginally increased from the middle of July (13%, P=0.12) and significantly increased from the end of August (10%, P=0.001) (Fig. 9a). There were no differences between UV-A- and UV-B-exposed plants. Stomatal conductance was unaltered following UV exposure until August when it was significantly increased under UV-A (35%, P=0.039) and enhanced UV-B radiation (43%, P=0.015) (Fig. 9b). Again there was no difference between UV treatments. UV radiation also affected the size of abaxial stomata. Compared to the control, UV-A radiation increased both the length and width of stomata (by about 30%, P<0.01; Fig. 10b), while enhanced UV-B radiation showed only a marginal increase in the length of stomata (18%, P=0.085; Fig. 10c). Stomatal conductance showed a positive correlation with the number and length of stomata in August (r=0.74, P=0.006 and r=0.63, P=0.028, respectively).

Discussion

Growth and phenology

The responses of birch seedlings to UV radiation (UV-A and enhanced UV-B) were studied in an outdoor experi-

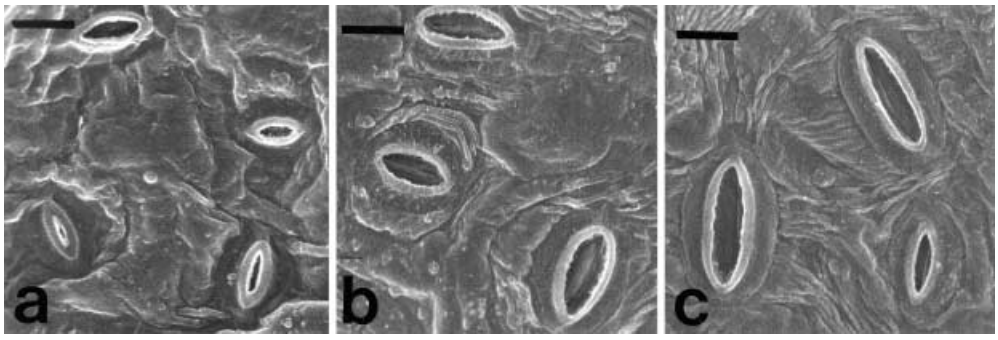

Fig. 10 Scanning electron micrographs of control (**a**), UV-A- (**b**) and UV-B- (**c**) exposed *B. pendula* leaves. Note the increase in stomatal size due to UV-A and UV-B radiation. *Bar* 20 µm

ment during the summer of 1999. In our study, UV radiation had no effect on height or biomass of birch seedlings. Similar results have been found for *Olea europaea* (Liakoura et al. 1999) and *Liquidambar styraciflue* L. in the field (Sullivan et al. 1994). The shoot growth of birch seedlings in a growth chamber experiment was also unaffected (Lavola 1998). Likewise shoot to root ratio was also unaffected by enhanced UV-B radiation, corroborating previous studies (Newsham et al. 1999). It might be that 2 years of exposure is insufficient to induce any change in growth parameters of birch, since UV-B radiation damage in woody plants develops slowly and accumulates over time (Sullivan and Teramura 1992).

Leaf expansion rate was increased by both UV-A and enhanced UV-B radiation, but the final leaf size remained unaffected. The increase in leaf expansion due to UV treatment here supports the theory that suggests a positive effect of UV-A radiation on plant growth. Middleton and Teramura (1993) reported that UV-B radiation was deleterious to plant function, whereas the UV-A radiation produced positive effects. Newsham et al. (1996) reported that supplemental UV-A radiation, even at low levels relative to ambient UV-A, could affect plant responses in the field. Similarly, after 19–21 days, cucumber grown in the absence of UV-B but exposed to UV-A had on average 34%, 55% and 40% greater biomass of leaves, stems and roots, respectively, 27% greater stem height, and 35% greater leaf area than those grown under ambient UV-B (Krizek et al. 1997). Supplementation of UV-A also promoted seedling growth of *Cyamopsis tetragonoloba* L. and enhanced the synthesis of chlorophyll and carotenoids, which accompanied relatively high photosystem I activity (Lingakumar and Kulandaivelu 1998).

In late August and September we followed the appearance of necrotic spots, a typical phenomenon of the autumnal senescence process and most probably involving the fungus *Pyrenopeziza betulicola* (Paavolainen et al. 2000). Both enhanced UV-B and UV-A radiation delayed the appearance of necrotic spots on birch leaves suggesting direct harmful effects of UV on pathogens or improved tolerance against pathogens in UV-exposed seedlings. Many studies, using a wide range of pathogens, have shown that UV can kill fungal spores and inhibit germination or germ tube growth (reviewed by Paul 2000). Changes in host plants, such as increasing flavonoids, inhibition of photosynthesis and therefore reduction in the availability of photoassimilates, may also help to reduce infections by pathogens in UV-B-exposed plants. Also UV-B-induced synthesis of phenolic compounds would give rise to the production of plant metabolites with antibiotic activity against pathogens (Manning and Tiedemann 1995).

Leaf anatomy and ultrastructure

Birch seedlings grown under elevated UV-B radiation in the present study had thicker leaves than those of control plants. The thick leaves were associated with increases in the thickness of the upper epidermis, spongy parenchyma and also spongy intercellular space. Our results corroborate previous studies where, in outdoor experiments, leaves of oak saplings (*Quercus robur* L) exposed to UV-B treatment had thicker and smaller leaves relative to those exposed to ambient levels of radiation (Antonelli et al. 1998; Newsham et al. 1999). Supplemental UV-B radiation was also found to increase the thickness of palisade parenchyma in *Populus trichocarpa* and *Q. rubra* growing in a greenhouse, but did not induce any leaf epidermal changes (Nagel et al. 1998). At the structural level, increased length of inner leaf cells or increases in cell number, both palisade and spongy mesophyll, influence the penetration and spectral distribution of UV radiation across a leaf (Caldwell et al. 1995). Thicker leaves enable plants to attenuate more UV-B radiation and hence to protect palisade layers from deleterious effects of irradiation (Newsham et al. 1998). Nevertheless, UV-induced ultrastructural changes in birch indicate that increase in leaf thickness did not completely provide protection for palisade mesophyll against UV-B radiation.

At the ultrastructural level enhanced UV-B exposure gave rise to numerous cytoplasmic lipid bodies and abnormal membrane whorls. Similar symptoms were found in birch seedlings exposed to UV-B radiation in a glasshouse experiment (Wulff et al. 1999). The lipid accumulations may indicate accelerated cell senescence (Wulff et al. 1996). In the present study enhanced UV-B and particularly UV-A radiation increased thylakoid swelling. Dilation of thylakoid membranes in the chloroplast has been shown for *Pisum sativum* exposed to UV-B radiation (He et al. 1994). Curling of thylakoid membranes due to UV-A radiation may indicate breaks in previous structural

contacts with stroma, the formation of free radicals, and photodestruction/regeneration of membrane components (Selga et al. 1998).

Secondary metabolites and lipid peroxidation

Enhanced UV-B radiation increased secondary metabolites, such as (+)-catechin, quercetin, apigenin, pentagalloylglucose and cinnamic acid derivatives in birch leaves. This increase was accompanied by a control level of lipid peroxidation. By contrast, UV-A radiation had no effect on secondary metabolites, or even reduced some (cinnamic acid derivatives) compared to control seedlings. Such increases in cinnamic acid and specific flavonoids (myricitin and quercetin) in response to UV-B radiation were found earlier in birch leaves (Lavola 1998). It is suggested that the overall antioxidant effect of flavonoids on lipid peroxidation may be due to the scavenging properties of their hydroxyl and superoxide radicals, directly related to the number of hydroxyl groups substituted at ring B (Husain et al. 1987). Quercetin can prevent lipid peroxidation of thylakoid membranes exposed to UV-B radiation in wheat seedlings (Dawar et al. 1998). Flavonols and flavones including quercetin and apigenin are likely to be responsible for protection against lipid peroxidation and active radicals in *Ginkgo biloba* leaves (Joyeux et al. 1995). We also detected significant increases in pentagalloylglucose, one of the main hydrolysable tannins found in *Betula* species. This component also could be a potential UV-protecting component based on its fairly intense UV absorption and its richness in OH groups indicating potential antioxidative activity.

In the present study, enhanced UV-B radiation did not affect lipid peroxidation. This agrees with a previous study where birch seedlings were exposed to UV-B in a glasshouse (Wulff et al. 1999). In contrast, lipid peroxidation products were increased under UV-B treatment in cucumber and tomato in a greenhouse (Kramer et al. 1991) and in the field (Balakumar et al. 1997). Interestingly, however, in our experiment UV-A radiation increased (19%) lipid peroxidation in the oldest fully expanded leaves. This supports a study where wheat leaves exposed to UV-A radiation showed an increase in lipid peroxidation (Joshi et al. 1991). In conclusion, UV-A exposure caused a low level of secondary metabolites and an increase in lipid peroxidation whereas enhanced UV-B increased secondary metabolite production and maintained a control level of lipid peroxidation. Strong negative correlations were found between concentrations of quercetin and apigenin, and lipid peroxidation indicating antioxidant role of secondary metabolites in birch leaves exposed to UV-B radiation.

Glandular trichomes

After 15 weeks of exposure we found that both UV-A and enhanced UV-B radiation increased the glandular trichome density in birch leaves. Similarly, the frequency of glandular trichomes (no. mm^{-2}) was considerably higher in sun leaves than in shade leaves of *Phillyrea latifolia* (Tattini et al. 2000). We suggest that glandular trichomes of birch contain flavonoids (supported by previous studies of *B. pendula* (Keinänen and Julkunen-Tiitto 1998) and *P. latifolia* surfaces (Tattini et al. 2000), and that they are capable of protecting the leaf against UV radiation similarly as non-glandular trichomes (Karabourniotis and Bornman 1999).

Stomatal responses

During first 2 months of exposure there were no UV-induced changes in stomatal responses but by August stomatal conductance was significantly increased under both UV-A and enhanced UV-B radiation. This increase was associated with increasing stomatal size and density, suggesting increased activity of the photosynthetic system. This can be interpreted as a compensatory mechanism since more efficient photosynthesis facilitates protective reactions, resulting, for example, in an increase in UV-absorbing pigments, leaf thickness and glandular trichomes. Moorthy and Kathiresan (1997) showed a 47% increase in stomatal conductance for *Rhizophora apiculata* after a 10% increase in UV-B radiation. A recent study of *Vicia faba* (Jansen and Noort 2000) showed a dependence of UV-B-induced stomatal closing and opening on the PAR level: an increased level of PAR stimulated stomatal opening. Increased stomatal density under UV-B radiation was found in *Pinus banksiana* (Stewart and Hoddinott 1993) and *Pisum sativum* (Nogues et al. 1998).

In conclusion, despite the absence of effects on birch growth and biomass, changes in stomatal and ulrastructural parameters, and activation of defensive mechanisms, such as increases in concentration of secondary metabolites, density of glandular trichomes and leaf thickness were found to be due to enhanced UV-B radiation. Negative correlations between some flavonoids and lipid peroxidation levels indicate a protective role of secondary metabolites against oxidative damage due to enhanced UV-B in birch seedlings.

Acknowledgements Research was supported by a Centre for International Mobility, CIMO (Finland), grant to Elena Kostina. We thank the staff at the Laboratory of Electron Microscopy and also Mr. Timo Oksanen and Mr. Topi Kuronen for their technical help. Thanks are due to Dr. Lucy J. Sheppard for invaluable comments on the manuscript and revising the language.

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