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Long-term exposure to enhanced ultraviolet-B radiation in the sub-arctic does not cause oxidative stress in *Vaccinium myrtillus*

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SUMMARY

The aim of this work was to assess whether or not oxidative stress had developed in a dwarf shrub bilberry (*Vaccinium myrtillus* L.) under long-term exposure to enhanced levels of ultraviolet-B (u.v.-B) radiation. The bilberry plants were exposed to increased u.v.-B representing a 15% stratospheric ozone depletion for seven full growing seasons (1991–1997) at Abisko, Swedish Lapland (68° N). The oxidative stress was assessed on leaves and stems by analysing ascorbate and glutathione concentrations, and activities of the closely related enzymes ascorbate peroxidase (EC 1.11.1.11) and glutathione reductase (EC 1.6.4.2). The affects of autumnal leaf senescence and stem cold hardening on these variables were also considered. The results showed that the treatment caused scarcely any response in the studied variables, indicating that u.v.-B flux representing a 15% ozone depletion under clear sky conditions is not sufficient to cause oxidative stress in the bilberry. It is suggested that no strain was evoked since adaptation was possible under such u.v.-B increases. The studied variables did, however, respond significantly to leaf senescence and especially to stem cold hardening.

Key words: Antioxidants, bilberry, cold hardening, u.v.-B radiation, *Vaccinium myrtillus* L.

INTRODUCTION

Thinning of the stratospheric ozone layer increases the flux of ultraviolet-B radiation (u.v.-B; 280–320 nm) reaching the surface of the Earth. The ozone layer over the Northern Hemisphere is decreasing at a rate of 1% per year (Hofmann & Deshler, 1991). Consequently, a yearly increase in

u.v.-B of from 7% in summer to as much as 35% in winter was recorded between 1989 and 1993 in Toronto, Canada (Kerr & McElroy, 1993).

As on most living organisms, enhanced u.v.-B radiation is believed to have negative impacts on vegetation. Effects such as altered decomposition rate (Gehrke *et al.*, 1995; Rozema *et al.*, 1997), reduced growth (Johanson *et al.*, 1995a), impaired photosynthesis (Bornman, 1989) and damage to cellular macromolecules (Strid, Chow & Anderson, 1994) have been observed. One mechanism behind

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these changes might be oxidative stress arising from exposure to u.v.-B (Bornman & Sundby-Emanuelsson, 1995; Hideg & Vass, 1996; Ormrod, Schmidt & Livingston, 1997).

Most of the experiments on enhanced u.v.-B have been carried out in growth chambers and glass-houses. Responses of plants in controlled conditions, however, may differ from those in the field conditions, due to the presence of visible light and other inconstant environmental factors (Krupa & Kickert, 1989). Northern plants, for example, are expected to be weakly adapted to enhanced u.v.-B radiation because of their natural protection by the lower solar angle and thicker ozone layer compared with their counterparts at lower latitudes (Caldwell, Robberecht & Billings, 1980). A large quantity of the published u.v.-B research focusing on the plant kingdom has been mainly conducted using crops (Stapleton & Walbot, 1994; Liu, Gitz III & McClure, 1995; Takeuchi *et al.*, 1996; Dai *et al.*, 1997) and conifers (Sullivan & Teramura, 1992; Naidu *et al.*, 1993; Petropoulou *et al.*, 1995; Gordon, Percy & Riding, 1998). The aim of this investigation was to assess whether or not u.v.-B under field conditions causes stress in the bilberry (*Vaccinium myrtillus*), one of the main dwarf shrubs in boreal and sub-arctic ecosystems.

The antioxidant defence system of plants provides protection against increased free oxygen radical concentrations (i.e. oxidative stress) in tissues induced by unfavourable environmental conditions such as low temperatures, drought and high light intensities (Bowler, Van Montagu & Inzé, 1992; Allen, 1995). The ascorbate–glutathione cycle removes oxyradicals and their intermediates and is composed of both enzymatic and non-enzymatic mechanisms. The former include ascorbate peroxidase (APOD), dehydroascorbate reductase and glutathione reductase (GR), and the latter include ascorbate (ASA) and glutathione (GSH) (Smith, Vierheller & Thorne, 1989). The antioxidant system might also be one of the defence mechanisms protecting against u.v.-B stress. The ascorbate–glutathione cycle especially is reported to respond to enhanced u.v.-B in algae and herbs (Jansen *et al.*, 1996; Rao, Paliyath & Ormrod, 1996; Dai *et al.*, 1997; Hideg *et al.*, 1997). Therefore, we investigated the hypothesis that u.v.-B would modify the antioxidant metabolism in a shrub, *V. myrtillus*.

Besides responding to various stresses, the antioxidant system undergoes seasonal fluctuation in perennial species. Autumnal leaf senescence in deciduous species (Thompson, Legge & Barber, 1987; Taulavuori & Tolvanen, 1995; Bartoli *et al.*, 1996) and the cold hardening process (Esterbauer & Grill, 1978; Nakagawara & Sagisaka, 1984; Anderson, Chevone & Hess, 1992; Hausladen & Alschler, 1994; Taulavuori *et al.*, 1997a) may alter antioxidant metabolism. Thus this work was con-

ducted during the period of natural leaf senescence and stem cold hardening in order to relate the u.v.-B response of the antioxidant metabolism to its seasonal changes.

MATERIALS AND METHODS

Ultraviolet-B exposure system

To address the impact of u.v.-B radiation on a sub-arctic heath community, an experiment was established at Abisko, Swedish Lapland (68.35° N, 18.82° E, 360 m above sea level). The dominant species of the experimental site include *Empetrum hermaphroditum*, *Vaccinium myrtillus* L., *Vaccinium uliginosum* and *Vaccinium vitis-idaea* with a sparse cover of *Betula pubescens* ssp. *Czerepanovii*. A detailed description of the vegetation is given by Sonesson & Lundberg (1974). The experiment was based in open areas of the heath and concentrated on the impact of u.v.-B on the understorey shrub community. Established in the summer of 1990, the experiment has run for seven growing seasons (1991–1997), irradiation starting in the spring a few weeks before all the snow is gone (late April to mid-May) and running until mid-September. Enhanced u.v.-B radiation was supplied evenly from metal frames (2.5 × 1.3 × 1.5 m high) each with six fluorescent lamps (Q-PANEL UVB-313, Cleveland, OH, USA) as described by Johanson *et al.* (1995b). To derive biologically effective u.v.-B radiation (u.v.-B_{BE}), Caldwell's generalized action spectrum normalized at 300 nm was adopted (Caldwell, 1971). To filter u.v.-C radiation, u.v.-transmitting Plexiglass® (Roehm GmbH, Darmstadt, Germany) holding a cellulose diacetate filter (0.13 mm, Courtauld, Derby, UK) was placed beneath each of the lamps. For the control frames window glass was used rather than Plexiglass, which excluded all u.v.-B emission from the lamps. Ultraviolet-B radiation from the lamps was supplied daily, centred around noon and controlled by electronic timers providing stepwise 'square wave' increases. Exposure times were changed every second week to follow the seasonal change in natural u.v.-B radiation. The model developed by Björn & Murphy (1985) and Björn & Teramura (1993) was used to calculate the daily increase in u.v.-B radiation resulting from a 15% ozone depletion under clear skies at Abisko. The calculated ozone depletion, however, is actually closer to 18–19% once cloud effects are accounted for.

Harvesting, non-enzymatic and enzymatic antioxidant analysis

There were four replicate plots ($n = 4$) for both the u.v.-B and control treatments, harvesting of which was performed three times: on the 20 July, 10

August and 18 September 1997. Whole bilberry ramets were cut above ground level, frozen in liquid nitrogen and stored in a freezer (-70°C). Leaves and stems were separated and weighed. Immediately before analysis, samples were ground to a powder with a pestle and mortar in liquid nitrogen.

Total and reduced ascorbate (ASA) were analysed according to Nakagawara & Sagisaka (1984). This assay is based on the reduction of Fe^{3+} to Fe^{2+} by ASA in acidic solution, and a chelate compound formed by Fe^{2+} and bathophenanthroline is detectable at 534 nm. Total ascorbate was determined through a reduction of dehydroascorbate to ascorbate by dithiothreitol. After grinding, 3.75 ml of cold 5% (w/v) trichloroacetic acid was added to the resulting powder, and grinding was continued until the mixture was completely homogenous. The homogenate was then centrifuged at 16000 g (4°C) for 10 min. Ascorbate concentrations were calculated from standard curves. The proportion of reduced ascorbate (ASA %), which indicates the redox state of ascorbate, was calculated as a proportion of the total ascorbate concentration $(\text{ASA}/\text{total}) \times 100$.

Concentrations of total and oxidized glutathione were analysed according to Hausladen *et al.* (1990) from samples extracted with 2% (w/v) metaphosphoric acid, containing 2 mM EDTA. The assay is based on an enzymatic recycling procedure introduced by Griffith (1980). Glutathione concentrations were calculated from standard curves, reduced glutathione (GSH) obtained by subtracting oxidized glutathione (GSSG) from the total concentration. The proportion of reduced glutathione (GSH %), which indicates the redox state of glutathione, was calculated as a proportion of the total glutathione concentration $(\text{GSH}/\text{total}) \times 100$. Recovery of GSH and GSSG, added to the extraction medium before the extraction of leaf material, was 103 and 97%, respectively. Stem extraction recovered 96 and 92%, respectively.

Ascorbate peroxidase (APOD; EC 1.11.1.11) and glutathione reductase (GR; EC 1.6.4.2) activities were determined according to Anderson *et al.* (1992). Proteins were extracted with 5 ml of 50 mM PIPES buffer (pH 6.8), 6 mM L-cysteine hydrochloride, 10 mM D-isoascorbate, 1 mM EDTA, 0.3% Triton[®] X-100, 1% polyvinylpyrrolidone (mol. wt 10000), 1% polyclar-AT, and 1 drop of antifoam A emulsion (Sigma Biochemicals & Reagents). After centrifugation at 20000 g for 15 min (4°C) the remaining insoluble material was re-extracted with an additional 2 ml of extraction buffer and centrifuged as above. The supernatants of the two extractions were pooled. Immediately before the enzyme measurement, 3 ml of each extract was gel filtered with a Econo-Pac[®] 10DG column (Bio-Rad Laboratories, Hercules, CA 94547, USA) equilibrated with 50 mM Tris-HCl (pH 7.5), containing 1 mM EDTA and 1 mM D-isoascorbate. The APOD

activity was measured spectrophotometrically by monitoring the ascorbic acid-dependent reduction of H_2O_2 at 265 nm. Assays were performed at 25°C in a 3 ml reaction mixture containing 1.5 ml of 166 mM HEPES-KOH (pH 7.0), 300 μl 10 mM EDTA, 1.5 mM Na-ascorbate, and 1 mM H_2O_2 . The GR activity was measured in a 1 ml reaction mixture at 25°C as a function of the oxidation of NAD(P)H (170 mM) by GSSG (1 mM) in a Tris HCl-buffer (50 mM, pH 7.5), containing 3.0 mM MgCl_2 . The reaction was started by adding NAD(P)H and monitored by the decrease in absorbance at 340 nm.

Protein concentration in enzyme extracts was determined according to Bradford (1976).

Data analysis

The effects of treatment and harvest were tested with ANOVA repeated measures (SPSS, 1992).

RESULTS

The results showed that u.v.-B had only a minor (if any) effect on the antioxidant system of the bilberry. No differences in ascorbate concentrations (Fig. 1), ASA % (Table 1), APOD activities (Fig. 2) and glutathione concentrations (Fig. 3) were found between control and u.v.-B treated plants at $P < 0.05$. This holds true for both the leaf and stem data. The proportion of the reduced glutathione (GSH %), however, was lowered by u.v.-B treat-

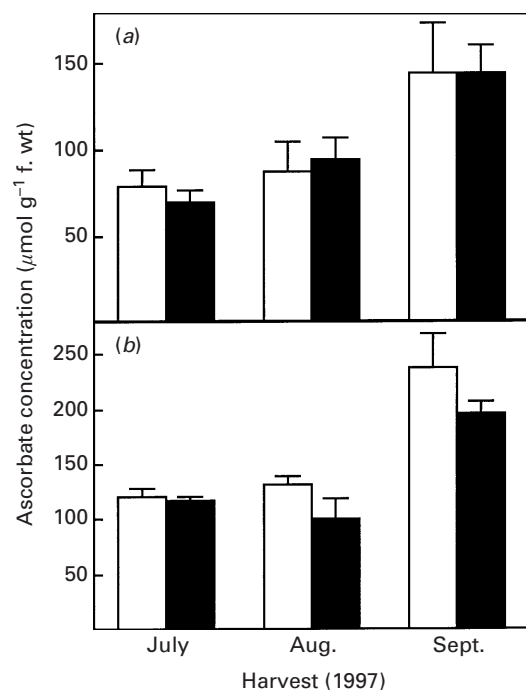


Figure 1. Total ascorbate concentration ($\mu\text{mol g}^{-1}$ f. wt \pm SE, $n = 4$) in bilberry (*Vaccinium myrtillus*) leaves (a) and stems (b) on three harvest dates (20 July, 10 Aug., 18 Sept. 1997). \square , controls; \blacksquare , treatment with u.v.-B representing a 15% ozone depletion.

Table 1. Proportions of reduced ascorbate (ASA, % \pm SE) and reduced glutathione (GSH, % \pm SE) in the leaves and stems of bilberry (*Vaccinium myrtillus*) on three harvest dates (20 July, 10 Aug. and 18 Sept. 1997); treatments are control and u.v.-B exposure representative of 15% ozone depletion

	July		Aug.		Sept.	
	Control	u.v.-B	Control	u.v.-B	Control	u.v.-B
ASA %						
Leaves	70 \pm 4	78 \pm 8	99 \pm 6	93 \pm 3	84 \pm 6	82 \pm 2
Stems	43 \pm 2	48 \pm 3	59 \pm 5	78 \pm 7	48 \pm 2	50 \pm 2
GSH %						
Leaves	83 \pm 1	80 \pm 1	82 \pm 1	85 \pm 1	83 \pm 1	78 \pm 2
Stems	89 \pm 1	88 \pm 1	92 \pm 2	95 \pm 1	97 \pm 1	94 \pm 1

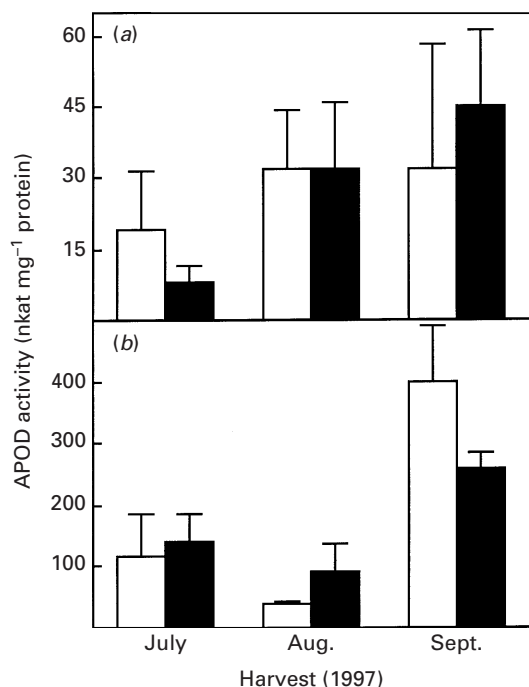


Figure 2. Ascorbate peroxidase (APOD) activity (nkat mg^{-1} protein \pm SE, $n = 4$) in bilberry (*Vaccinium myrtillus*) leaves (a) and stems (b) on three harvest dates (20 July, 10 Aug., 18 Sept. 1997). \square , controls; \blacksquare , treatment with u.v.-B representing a 15% ozone depletion.

ment in leaves ($P < 0.05$) and marginally in stems ($P < 0.1$) (Table 1). A marginal reduction ($P < 0.1$) in GR activities was also found in leaves, but not in stems (Fig. 4).

Leaf senescence significantly increased ascorbate concentration ($P < 0.001$) and ASA % ($P < 0.01$) (Fig. 1 and Table 1, respectively). No effect was observed in other variables studied.

Stem cold hardening had a positive impact on all the studied variables. It increased ascorbate concentrations (Fig. 1), ASA % (Table 1), glutathione concentrations (Fig. 3) and GSH % (Table 1) significantly at $P < 0.001$. In addition, APOD (Fig. 2) and GR (Fig. 4) activities increased significantly at $P < 0.01$.

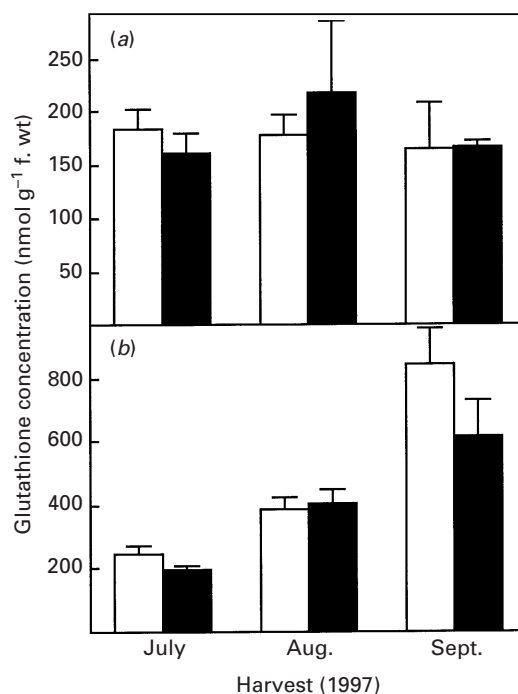


Figure 3. Total glutathione concentration (nmol g^{-1} f.wt \pm SE, $n = 4$) in bilberry (*Vaccinium myrtillus*) leaves (a) and stems (b) on three harvest dates (20 July, 10 Aug., 18 Sept. 1997). \square , controls; \blacksquare , treatment with u.v.-B representing 15% ozone depletion.

DISCUSSION

Varying responses in antioxidants under u.v.-B exposure have been reported, depending on species and intensity of radiation (Strid, 1993; Rao *et al.*, 1996; Takeuchi *et al.*, 1996; Dai *et al.*, 1997), revealing no uniform response pattern. For example, increased APOD activity was reported in *Arabidopsis thaliana* under enhanced u.v.-B radiation at the level of 18 $\text{kJ m}^{-2} \text{d}^{-1}$ (Rao *et al.*, 1996). By contrast, u.v.-B exposure at the level of 13 $\text{kJ m}^{-2} \text{d}^{-1}$ decreased APOD, catalase and superoxide dismutase activities and ascorbate level of *Oryza sativa*, but increased glutathione level (Dai *et al.*, 1997). Enhanced u.v.-B in the current work (max. 2.5–5.8 $\text{kJ m}^{-2} \text{d}^{-1}$ vs. 2.0–4.6 $\text{kJ m}^{-2} \text{d}^{-1}$) had scarcely any effect on the

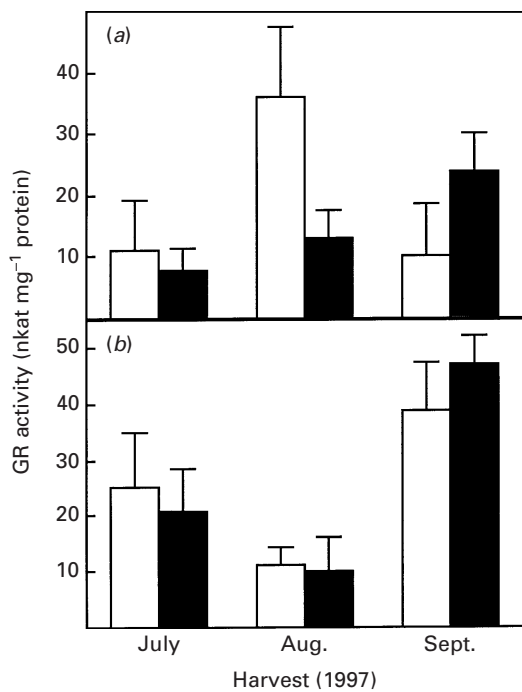


Figure 4. Glutathione reductase (GR) activity (nkat mg⁻¹ protein \pm SE, $n = 4$) in bilberry (*Vaccinium myrtillus*) leaves (a) and stems (b) on three harvest dates (20 July, 10 Aug., 18 Sept. 1997). \square , controls; \blacksquare , treatment with u.v.-B representing 15% ozone depletion.

antioxidant defence system (ASA, APOD, GSH, GR) of the bilberry which might suggest that the level of radiation used was not sufficient to induce any overall changes.

The result could also be explained by the adaptability of bilberry to slightly enhanced u.v.-B, especially as the studied plants had experienced it for seven growing seasons. Enhanced u.v.-B radiation representing at 15% ozone depletion increases u.v.-B absorbing pigments of a subarctic grass *Calamagrostis lapponica* (Gwynn-Jones & Johanson, 1996). Such a pigmentation response might well be the particular adaptation mechanism adequate to cope with a subtle u.v.-B increase, allowing the plant to avoid the oxidative stress condition and consequent response in antioxidant metabolism. Consistent with this view, no antioxidant response was found in this *Calamagrostis* study (unpublished data). In addition, being a perennial species, bilberry may have greater adaptability to environmental stressors compared with annual species: in the context of cold hardening dehydration of cells may pre-adapt plants to u.v.-B stress, since drought stress can ameliorate u.v.-B radiation effects (Tevini, Iwanzik & Teramura, 1983; Teramura, Sullivan & Lydon, 1990). Irrespective of the mechanism, the bilberry is apparently quite tolerant of enhanced u.v.-B radiation representing a 15% ozone depletion.

Leaf senescence significantly increased ascorbate concentrations and ASA% in this study. Ascorbate is needed in the detoxification of H₂O₂ produced in

senescing tissue. It is also a reductant in the regeneration of α -tocopherol and in the zeaxanthin cycle, both of which are part of the protective system in chloroplasts (Alscher, Donahue & Cramer, 1997). The marked increase in total ascorbate concentration and ASA% observed in this investigation thus reflects an enhanced protection against increased level of reactive oxygen species generated during senescence.

All components of the antioxidant system studied generally increased during cold hardening of the stems, indicating enhanced requirements for protection against oxidative stress during this process (Esterbauer & Grill, 1978; Nakagawara & Sagisaka, 1984; Anderson *et al.*, 1992; Hausladen & Alscher, 1994; Polle & Morawe, 1995; Taulavuori *et al.*, 1997a; Taulavuori *et al.*, 1997b). This result highlights the main outcome of the present investigation; although no response to u.v.-B was obtained, the methods used were shown to be sensitive enough to respond to seasonal changes. According to general stress concepts (e.g. Lichtenthaler, 1996) the response may alternate between positive and negative. In the present work, however, there were no difficulties in interpreting the data, since no particular response to u.v.-B was detected, although several variables and the tissues of two separate organs were studied.

CONCLUSIONS

To our knowledge, this is the first investigation of the antioxidant status of a plant species within a natural community exposed to long-term enhanced u.v.-B radiation conditions. The antioxidant metabolism responded only slightly to u.v.-B exposure, varying responses occurring in GSH% and GR activity, depending on harvest date. In addition, senescence induced varying responses in components of the antioxidant system, and cold hardening almost exclusively induced an increase in the levels of non-enzymatic and enzymatic antioxidants. Thus, it is concluded that the antioxidant system of bilberry is capable of resisting the harmful impact of enhanced u.v.-B radiation at the level imposed in this experiment.

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REFERENCES

- Allen RD. 1995. Dissection of oxidative stress tolerance using transgenic plants. *Plant Physiology* **107**: 1049–1054.
- Alscher RG, Donahue JL, Cramer CL. 1997. Reactive oxygen species and antioxidants: relationships in green cells. *Physiologia Plantarum* **100**: 224–233.
- Anderson JV, Chevone BI, Hess JL. 1992. Seasonal variation in the antioxidant system of Eastern white pine needles. *Plant Physiology* **98**: 501–508.
- Bartoli CG, Simontacchi, M, Montaldi E, Puntarulo S. 1996. Oxidative stress, antioxidant capacity and ethylene production during ageing of cut carnation (*Dianthus caryophyllus*) petals. *Journal of Experimental Botany* **47**: 595–601.
- Björn LO, Murphy TM. 1985. Computer calculations of solar ultraviolet radiation at ground level. *Physiologie Végétale* **23**: 555–561.
- Björn LO, Teramura AH. 1993. Simulation of day light ultraviolet radiation and effects of ozone depletion. In: Young AR, Björn LO, Moan J, Nultsch W, eds. *Environmental UV photobiology*. New York, USA: Plenum Press, 41–71.
- Bornman JF. 1989. Target sites of UV-B radiation in photosynthesis of higher plants. *Journal of Photochemistry and Photobiology B: Biology* **4**: 145–158.
- Bornman JF, Sundby-Emanuelsson C. 1995. Response of plants to UV-B radiation. In: Smirnoff N, ed. *Environment and plant metabolism: flexibility and acclimation*. Oxford, UK: BIOS Scientific Publishers Limited, 245–262.
- Bowler C, Van Montagu M, Inzé D. 1992. Superoxide dismutase and stress tolerance. *Annual Review of Plant Physiology and Plant Molecular Biology* **43**: 83–116.
- Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* **72**: 248–254.
- Caldwell MM. 1971. Solar UV irradiation and the growth and development of higher plants. In: Giese AC, ed. *Photophysiology*. New York, USA: Academic Press, Vol 6, 131–177.
- Caldwell MM, Robberecht R, Billings WD. 1980. A steep latitudinal gradient of solar ultraviolet-B in the Arctic-Alpine life zone. *Ecology* **61**: 600–611.
- Dai Q, Yan B, Huang S, Liu X, Peng S, Miranda MLL, Chavez AQ, Vergara BS, Olszyk DM. 1997. Response of oxidative stress defence system in rice (*Oryza sativa*) leaves with supplemental UV-B radiation. *Physiologia Plantarum* **101**: 301–308.
- Esterbauer H, Grill D. 1978. Seasonal variation of glutathione and glutathione reductase in needles of *Picea abies*. *Plant Physiology* **61**: 119–121.
- Gehrke C, Johanson U, Callaghan TV, Chadwick D, Robinson CH. 1995. The impact of enhanced ultraviolet-B radiation on litter quality and decomposition processes in *Vaccinium* leaves from the Subarctic. *Oikos* **72**: 213–222.
- Gordon DC, Percy KE, Riding RT. 1998. Effects of u.v.-B radiation on epicuticular wax production and chemical composition of four *Picea* species. *New Phytologist* **138**: 441–449.
- Griffith OW. 1980. Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. *Analytical Biochemistry* **106**: 207–212.
- Gwynn-Jones D, Johanson U. 1996. Growth and pigment production in two subarctic grass species under different UV-B irradiation levels. *Physiologia Plantarum* **97**: 701–707.
- Hausladen A, Alscher RG. 1994. Purification and characterization of glutathione reductase isozymes specific for the state of cold hardiness of red spruce. *Plant Physiology* **105**: 205–213.
- Hausladen A, Madamanchi NR, Fellows S, Alscher RG, Amundson RG. 1990. Seasonal changes in antioxidants in red spruce as affected by ozone. *New Phytologist* **115**: 447–458.
- Hideg É, Mano J, Ohno C, Asada K. 1997. Increased levels of monodehydroascorbate radical in UV-B irradiated broad bean leaves. *Plant and Cell Physiology* **38**: 684–690.
- Hideg É, Vass I. 1996. UV-B induced free radical production in plant leaves and isolated thylakoid membranes. *Plant Science* **115**: 251–260.
- Hofmann DJ, Deshler T. 1991. Evidence from balloon measurements for chemical depletion of stratospheric ozone in the Arctic winter of 1989–1990. *Nature* **349**: 300–305.
- Jansen MAK, Babu TS, Heller D, Gaba V, Mattoo AK, Edelman M. 1996. Ultraviolet-B effects on *Spirodela oligorhiza*: induction of different protection mechanisms. *Plant Science* **115**: 217–223.
- Johanson U, Gehrke C, Björn LO, Callaghan TV. 1995a. The effects of enhanced UV-B radiation on the growth of dwarf shrubs in a subarctic heathland. *Functional Ecology* **9**: 713–719.
- Johanson U, Gehrke C, Björn LO, Callaghan TV, Sonesson M. 1995b. The effects of enhanced UV-B radiation on a subarctic heath ecosystem. *Ambio* **24**: 106–111.
- Kerr JB, McElroy CT. 1993. Evidence for large upward trends of ultraviolet-B radiation linked to ozone depletion. *Science* **262**: 1032–1034.
- Krupa S, Kickert RN. 1989. The greenhouse effect – impacts of ultraviolet-B (UV-B), carbon dioxide (CO₂), and ozone (O₃) on vegetation. *Environmental Pollution* **61**: 263–293.
- Lichtenthaler HK. 1996. Vegetation stress: an introduction to the stress concept in plants. *Journal of Plant Physiology* **148**: 4–14.
- Liu L, Gitz III DC, McClure JW. 1995. Effects of UV-B on flavonoids, ferulic acid, growth and photosynthesis in barley primary leaves. *Physiologia Plantarum* **93**: 725–733.
- Naidu SL, Sullivan JH, Teramura AH, DeLucia EH. 1993. The effects of ultraviolet-B radiation on photosynthesis of different aged needles in field-grown loblolly pine. *Tree Physiology* **12**: 151–162.
- Nakagawara S, Sagisaka S. 1984. Increase in enzyme activities related to ascorbate metabolism during cold acclimation in poplar twigs. *Plant and Cell Physiology* **25**: 899–906.
- Ormrod DP, Schmidt A-M, Livingston NJ. 1997. Effect of UV-B radiation on the shoot dry matter production and stable carbon isotope composition of two *Arabidopsis thaliana* genotypes. *Physiologia Plantarum* **101**: 497–502.
- Petropoulou Y, Kyparissis A, Nikopoulos D, Manetas Y. 1995. Enhanced UV-B radiation alleviates the adverse effects of summer drought in two Mediterranean pines under field conditions. *Physiologia Plantarum* **94**: 37–44.
- Polle A, Morawe B. 1995. Seasonal changes of the antioxidative systems in foliar buds and leaves of field-grown beech trees (*Fagus sylvatica*, L.) in a stressful climate. *Botanica Acta* **108**: 314–320.
- Rao MV, Paliyath G, Ormrod DP. 1996. Ultraviolet-B and ozone induced biochemical changes in antioxidant enzymes of *Arabidopsis thaliana*. *Plant Physiology* **110**: 125–136.
- Rozema J, Tosserams M, Nelissen HJM, van Heerwerden L, Broekman RA, Flierman N. 1997. Stratospheric ozone reduction and ecosystem processes: enhanced UV-B radiation affects chemical quality and decomposition of leaves of the dune grassland species *Calamagrostis epigeios*. *Plant Ecology* **128**: 284–294.
- Smith IK, Vierheller TL, Thorne C. 1989. Properties and functions of glutathione reductase in plants. *Physiologia Plantarum* **77**: 449–456.
- Sonesson M, Lundberg B. 1974. Late quaternary forest development of the Torneträsk area, North Sweden. I. Structure of modern forest ecosystems. *Oikos* **25**: 121–133.
- SPSS. 1992. SPSS Base System: Syntax Reference Guide. Release 5.0, SPSS Inc., Chicago, IL, USA.
- Stapleton AE, Walbot V. 1994. Flavonoids can protect maize DNA from the induction of ultraviolet radiation damage. *Plant Physiology* **105**: 881–889.
- Strid Å. 1993. Alteration in expression of degenase genes in *Pisum sativum* after exposure to supplementary ultraviolet-B radiation. *Plant and Cell Physiology* **34**: 949–953.
- Strid Å, Chow WS, Anderson JM. 1994. UV-B damage and protection at the molecular level in plants. *Photosynthesis Research* **39**: 475–489.
- Sullivan JH, Teramura AH. 1992. The effects of ultraviolet-B radiation on loblolly pine. 2. Growth of field-grown seedlings. *Trees* **6**: 115–120.
- Takeuchi Y, Kubo H, Kasahara H, Sakaki T. 1996. Adaptive alterations in the activities of scavengers of active oxygen in cucumber cotyledons irradiated with UV-B. *Journal of Plant Physiology* **147**: 589–592.
- Taulavuori E, Taulavuori K, Laine K, Pakonen T, Saari E. 1997a. Winter hardening and glutathione status in the bilberry (*Vaccinium myrtillus*) in response to trace gases (CO₂,

- O₂) and nitrogen fertilization. *Physiologia Plantarum* **101**: 192–198.
- Taulavuori E, Tolvanen A. 1995.** Glutathione status of the bilberry (*Vaccinium myrtillus*) as a function of plant phenology and artificial herbivory. *Canadian Journal of Botany* **73**: 1838–1841.
- Taulavuori K, Laine K, Taulavuori E, Pakonen T, Saari E. 1997b.** Accelerated dehardening in bilberry (*Vaccinium myrtillus* L.) induced by a small elevation in air temperature. *Environmental Pollution* **98**: 91–95.
- Teramura AH, Sullivan JH, Lydon J. 1990.** Effects of UV-B radiation on soybean yield and seed quality: a 6-year field study. *Physiologia Plantarum* **80**: 5–11.
- Tevini M, Iwanzik W, Teramura AH. 1983.** Effects of UV-B radiation on plants during mild water stress. II. Effects on growth, protein and flavonoid content. *Zeitschrift der Pflanzenphysiologie* **110**: 459–467.
- Thompson JE, Legge RL, Barber RF. 1987.** The role of free radicals in senescence and wounding. *New Phytologist* **105**: 317–344.