UV-B Effect on Constituents of *Azolla caroliniana*

Mohamed M. Ibrahim and Eazaz M. Mostafa*

Botany Department, Faculty of Science, Alexandria University, 21511, Alexandria, Egypt. Fax: +203-3911794. E-mail: eazazmouhamed@yahoo.com

* Author for correspondence and reprint requests

Z. Naturforsch. **62c**, 246–252 (2007); received August 28/November 23, 2006

Changes in growth and ultrastructure of *Azolla caroliniana* in response to elevated UV-B radiation were investigated. Exposure of plants to UV-B radiation for 1, 8, 16, 24 and 48 h exhibited a significant decrease in biomass and relative growth rate. This decrease resulted in an increase in doubling time over the control. Also, Chl a and b contents were significantly decreased especially after 16 h. The reduction was accompanied by a decrease in 5-aminolaevulinic acid content (precursor of chlorophyll). On the other hand, contents of carotenoid and UV-absorbing phenolic compounds (flavonoids and anthocyanins) were increased.

Key words: Doubling Time, Relative Growth Rate, UV-Absorbing Compounds

Introduction

Increases in natural UV radiation due to decreased stratospheric ozone concentrations have stimulated research on mechanisms and maximum capacities for protection against UV exposure. Plants exposed to UV-B radiation exhibit physiological, biochemical, morphological, and anatomical changes with the level, duration of exposure and other environmental conditions closely related to the response and complicate the effect of UV-B radiation on plants (Wetzel, 2001). As UV-B exposure time increases, a biochemical limitation of the photosynthetic process is observed, interpreted as a limitation in the transfer of CO_2 from the intercellular air spaces to the stroma in the chloroplasts inducing photoinhibiton (Chaves *et al*., 2003). In fact, absorbed energy, unable to be used in carbon reduction, could lead to the formation of singlet oxygen from the energy transfer at the level of PSII complexes and also to superoxide at the electron acceptor side of PSI, both known to induce oxidative stress and eventually cause great damage to the thylakoid membranes, and photosynthesis becomes limited from irreversible damage (Britvec *et al*., 2001). Also, UV-B exposure resulted in decreased contents of photosynthetic pigments and altered thylakoid integrity (Nogues and Baker, 1995).

Microscopic studies could serve as an additional and sensitive tool in the assessment of plant responses to UV-B radiation. By means of electron microscopy, early stress responses can be observed in the cell structure before the first visible symptoms occur (Holopainen *et al*., 1992). Special emphasis was put on the chloroplast ultrastructure because alterations in the chloroplast could lead to changes in carbon assimilation and biomass accumulation. Furthermore, it is well established that chloroplast structure is altered by UV-B radiation, usually before other cell organelles (Holopainen *et al*., 1996).

Photosynthetic organisms, however, have evolved mechanisms to protect themselves against oxidative stress, by decreasing the excitation pressure on PSII reaction centers, repairing damaged complexes, and transforming or eliminating the toxic species produced by stress. One of the most studied forms of protection is the chlorophyll concentration change in order to reduce the extent of the absorbed light (Murchie and Horton, 1997) by chloroplast movements, reducing the organelle and photosynthetic complexes exposure to light (Haupt, 1990), and increasing in the capacity for scavenging the active oxygen species (Foyer *et al*., 1994). The green pigments enable plants to response rapidly to changes in the spectral environment, as well as to exploit rich habitats. Equally they provide some protection against photoinhibition and photooxidation, the damaging effects excess quanta.

Phytochemicals, especially phenolics, UV-absorbing compounds such as flavonoids and anthocyanins, in fruits and vegetables have received great deal of attention because of their antioxidant activity. Anthocyanins have been implicated in tol-

erance to different stresses by absorbing high-energy quanta; anthocyanic cell vacuoles protect chloroplasts from the photoinhibitory and photooxidative effects of strong light, and prevent the catabolism of photolabile defence compounds (Chinnici *et al.*, 2004). Flavonoids and anthocyanins also mitigate photooxidative injury by serving as a UV-B filter and react with reactive oxygen species, thus protecting the cells against UV-induced oxidative damage; these pigments may to some extent be critical for plant survival (Gould, 2004). Similarly, Coleman and Day (2004) found that more than 70% of ambient UV-B radiation induced the production of soluble UV-B-absorbing compounds in sorghum and cotton plants.

Effect of UV-B radiation on the ultrastructure of aquatic plants and symbiotic systems has been scarcely reported. Hence an attempt has been made to evaluate the influence of UV-B radiation (280–320 nm) on the vegetative growth, pigment contents and ultrastructure of chloroplasts in the aquatic fern *Azolla caroliniana*.

Materials and Methods

Plants and cultivation

Azolla caroliniana Wild. (known as water velvet) was provided by Prof. C. Van Hove, Catholic University of Lauvain, Belgium. The plants were acclimated in the greenhouse of the Faculty of Science, Alexandria University, Egypt, in 2500 cm^3 polyethylene vessels which were filled with a nitrogen-free, 2/5 modified [KNO_3 and $Ca(NO_3)_2$ were replaced by KCl and $CaCl_2$, respectively] Hoagland solution (pH 5.1). About 5 g fresh mass (FM) of *Azolla* from the stock material were inoculated in each vessel to make a new subculture, and so on.

The plants were freed from epiphytic microorganisms by thorough washing with distilled water. The cultures were grown in a growth chamber under a 16-h photoperiod at an irradiance of 1200 μmol m^{-2} s^{-1} (cool white fluorescent tubes) and (light/dark) temperature of 28–30/20–25 °C (stock culture). Before being used the plants were surface-sterilized with 0.2% Clorox, then thoroughly washed with water.

Treatments

5 g of *Azolla* plants were transferred to 250 cm^3 vessels containing 2/5 modified Hoagland solution at laboratory conditions for 7 d, then exposed to UV-B radiation supplied by three UV-emitting tubes (TL12/100W/01; Philips, Holland) positioned 50 cm above leaf level. Rack height, lamp spacing and lamp power were adjusted as needed to maintain a total daily flux of biologically effective UV-B radiation, normalized to 1.6 W m^{-2} d^{-1} (Caldwell, 1971). This relatively high level of UV-B radiation, which corresponds to the maximum midsummer and clear sky irradiance, that would be anticipated, was selected to compare with other studies (*e.g.* Murali and Teramura, 1986) rather than to attempt a realistic simulation of field conditions. Control plants were illuminated by lamps wrapped with a polyester film (Folanrm 0.1 mm; Floex, Munich, Germany) which blocks both UV-B and UV-C radiation. UV-B radiation was routinely measured with a broad-band UV-B sensor (peak wavelength: 313 nm; Delta-T Devices Ltd., UK). Plants were rotated under the lamp banks in an attempt to minimize potential effects resulting from micro-environment variation for 0, 1, 8, 16, 24 and 48 h. Samples were taken for chemical analyses and ultrastructure examination.

Methods

The number of generations and doubling time (DT) were determined from the fresh mass and duration of experiment by employing the expression given by Peters *et al.* (1979): n (final mass) = $n_0 \cdot 2G$, where G is the number of generations and n_0 the initial mass of *Azolla* plants. Relative growth rate was calculated by using the formula of Subudhi and Watanabe (1981): RGR [kg kg^{-1} d^{-1}] = 0.693 DT^{-1}, where DT is the duration of experiment per one generation.

Photosynthetic pigments were extracted from fresh samples ground at a low light intensity in 10 ml 80% acetone at 4 °C. Absorbance of centrifuged extracts was measured with a spectrophotometer (Jenway 6305 UV/Vis, UK) at specified wavelengths required for computation of Chl a, b and total carotenoids from published formula (Lichtenthaler and Wellburn, 1983). Pellets remaining after centrifugations were dried at 60 °C and weighed. Photosynthetic pigments contents were expressed as g kg^{-1} dry mass (DM).

Absorption spectra of pigments at room temperature were measured using a UV-B-3000 double-beam spectrophotometer (LKB, UK). For monitoring emission spectra samples were excited

at 435 nm and the emission was measured between 550 and 800 nm.

Total phenolics were determined as reported by Sgherri *et al.* (2003), *i.e.* extracted from fresh fronds (2 g) for 1 h with 50% methanol containing 1% HCl under continuous stirring at room temperature. After centrifugation at $12000 \times g$ for 15 min, the supernatant was collected and the extraction was repeated three times on the pellet. Methanolic extracts were collected, dried and resuspended in 80% methanol. A measure of total phenolics was obtained by recording A_{280}. The calculations were performed using the absorbance and a calibration curve for total phenolics.

Flavonoids and anthocyanins pigments (UV-B screening pigments) were extracted from fresh samples ground in 10 ml acidified methanol (79:20:1, v/v methanol/water/HCl). Absorbencies of centrifuged extracts were measured between 300 and 700 nm after appropriate dilution (Mirecki and Teramura, 1984).

5-Aminolaevulinic acid (ALA) was extracted and estimated by the method of Stobart *et al.* (1985). The concentration of ALA was determined using the calculated extension coefficient $7.24 \cdot 10^4$.

Transverse sections of *Azolla* plants were examined by both light microscopy (LM) and transmission electron microscopy (TEM). Sectioning and staining were done as described in the pamphlet of EM unit, and then examined by a Jeol 100 CX electron microscope (Japan). Three digital TEM micrographs were taken from random cells; these magnification were chosen to discern clearly the structure of the chloroplasts. Numbers of chloroplasts, thickness of the cell wall and areas of chloroplasts were measured. The number of plastoglobuli was counted and the total area of the plastoglobuli was measured; the area of one plastoglobuli was obtained by dividing the total area with the number of plastoglobuli. All measurements were done using Adobe Photoshop 7.0 (Adobe Systems, San Jose, CA, USA).

Statistics

One-tailed t-test of mean differences was obtained with a stat view SE+ graphics computer application (Abacus Concepts, Berkeley, CA, USA) for size and number of chloroplasts. Photosynthetic data were analyzed using analysis of variance (ANOVA). Comparison for UV-B levels were made by least significant difference (LSD).

Results

In a preliminary experiment, exposure of *Azolla caroliniana* to UV-B radiation for 72 h induced their complete death. However, exposure of plants to UV-B radiation for 1, 8, 16, 24, and 48 h exhibited a significant decrease in biomass and this reduction was severe at a prolonged period of exposure. Consequently, the doubling time increased (decrease in relative growth rate) which was approx. 4-fold in comparison to the control value at the end of experiment (Table I).

The photosynthetic performance and pigment contents of *A. caroliniana* were markedly changed in response to UV-B treatment. Chl a and b contents were significantly decreased especially after 16 h of exposure. The decreases in Chl a and b after this period were 73% and 53% compared to the control. Also 5-aminolaevulinic acid (precursor of chlorophyll) was decreased in a similar manner, reaching a 85% reduction at the end of the experiment (Table II). On the other hand, carotenoids increased significantly up to 16 h by 48% and then decreased till the end of the experimental period. The carotenoids/total chlorophyll ratio

Treatment [h]		Biomass [g culture^{-1}]	*DT* [d]	*RGR* [kg kg^{-1} d^{-1}]
1	Control	10.03 ± 1.01	7.02 ± 1.47	0.10 ± 0.002
	+ UV	8.97 ± 0.82	7.85 ± 0.98	0.09 ± 0.001
8	Control	10.98 ± 2.08	6.67 ± 1.08	0.1 ± 0.010
	+ UV	8.65 ± 1.03	8.47 ± 1.4	0.08 ± 0.002
16	Control	12.33 ± 1.25	6.22 ± 1.28	0.11 ± 0.01
	+ UV	8.0 ± 0.98	9.58 ± 0.98	0.07 ± 0.003
24	Control	13.54 ± 2.07	5.91 ± 1.2	0.12 ± 0.012
	+ UV	6.27 ± 0.98	12.76 ± 1.98	0.05 ± 0.01
48	Control	15.88 ± 2.52	5.67 ± 0.85	0.12 ± 0.025
	+ UV	4.43 ± 1.78	20.32 ± 3.21	0.03 ± 0.001

Table I. Growth characterized by biomass, doubling time (DT) and relative growth rate (RGR) of *Azolla* plants exposed to UV-B radiation for 0 (control), 1, 8, 16, 24 and 48 h (values are means ± SE of 3 samples).

Table II. Changes in chlorophylls (Chl), carotenoids and 5-aminolaevulinic acid (ALA) content of *Azolla* plants exposed to UV-B radiation for 0 (control), 1, 8, 16, 24 and 48 h (values are means ± SE of 3 samples).

Treatment [h]		Chl a [g kg^{-1} DM]	Chl b [g kg^{-1} DM]	Carotenoids [g kg^{-1} DM]	Carotenoids/ Chl a+b	ALA [μmol g^{-1} FM]
1	Control	2.81 ± 0.41	0.95 ± 0.06	0.81 ± 0.39	0.22	150.5 ± 5.26
	+ UV	2.28 ± 0.25	0.86 ± 0.07	0.96 ± 0.11	0.31	140.6 ± 6.01
8	Control	2.90 ± 0.39	0.93 ± 0.08	0.90 ± 0.103	0.23	153.2 ± 4.58
	+ UV	1.61 ± 0.21	0.69 ± 0.069	1.18 ± 0.21	0.51	110.3 ± 6.25
16	Control	3.00 ± 0.65	1.00 ± 0.012	0.96 ± 0.09	0.24	168.6 ± 7.1
	+ UV	0.81 ± 0.04	0.47 ± 0.03	1.42 ± 0.13	1.11	71.3 ± 0.25
24	Control	3.11 ± 1.04	1.13 ± 0.074	1.01 ± 0.10	0.24	185.2 ± 5.2
	+ UV	0.40 ± 0.01	0.31 ± 0.001	1.28 ± 0.098	1.80	40.3 ± 3.21
48	Control	3.20 ± 1.08	1.20 ± 0.01	1.10 ± 0.087	0.25	186.6 ± 6.52
	+ UV	0.20 ± 0.03	0.18 ± 0.003	0.87 ± 0.018	2.29	28.9 ± 2.65

Table III. Changes in phenolics, flavonoids, and anthocyanins content in *Azolla* plants exposed to UV-B radiation for 0 (control), 1, 8, 16, 24 and 48 h (values are means ± SE of 3 samples).

Treatment [h]		Phenolics [mg kg^{-1} FM]	Flavonoids [mg kg^{-1} FM]	Anthocyanins [mg kg^{-1} FM]
1	Control	131.4 ± 3.98	52.7 ± 3.26	16.3 ± 1.68
	+ UV	152.9 ± 4.25	57.9 ± 4.12	20.1 ± 1.98
8	Control	131.8 ± 6.25	51.4 ± 6.58	17.2 ± 2.1
	+ UV	251.3 ± 4.15	70.9 ± 6.03	28.3 ± 3.65
16	Control	140.7 ± 6.98	52.9 ± 4.25	19.2 ± 2.9
	+ UV	389.5 ± 8.65	91.6 ± 5.98	44.7 ± 4.1
24	Control	142.3 ± 9.25	60.3 ± 4.10	21.4 ± 1.32
	+ UV	451.3 ± 12.8	191.3 ± 6.98	89.6 ± 3.8
48	Control	141.13 ± 1.65	69.0 ± 4.25	22.1 ± 1.25
	+ UV	298.6 ± 9.58	86.2 ± 4.6	69.2 ± 2.98

showed a significant increase during all experimental periods, reaching an approx. 5-fold increase after 16 h. Absorption spectra of the extract showed the presence of major absorption peaks at 330 and 663 nm. Prolonged exposure to UV-B radiation showed a marked decrease of these absorption peaks.

Total phenolics were markedly increased from the first hour of exposure to UV-B radiation; their values were approx. 1.2- and 3.2-fold after 1 and 24 h, respectively, in comparison to the control values (Table III). Flavonoids and anthocyanins contents were also increased in response to UV-B treatment. After 24 h, the corresponding increases were 3- and 4-fold compared to the control, respectively.

Examination of plants by LM and TEM showed that there were two types of changes that could be distinguished in chloroplasts:

(1) Chloroplasts became rounded instead of lobate and thylakoids in a slightly and distinctly wave-like oriented from were a little swollen.

(2) Chloroplasts had a well preserved internal lamellar system but separated from a limiting membrane.

The lowest number of chloroplasts (48%) with changed structure was observed after 48 h UV-B radiation treatment. Consequently, there was an increment in the number of damaged chloroplasts to almost 90% (data not shown). The average chloroplast cross sectional area and the number of plastoglobuli were significantly altered by UV-B radiation and depending on the duration of exposure their responses differed greatly. After 24 h of UV-B exposure, the average cross sectional area increased significantly, and thereafter it increased linearly with increasing UV-B exposure period. The most striking effect of the enhanced UV-B treatment on the ultrastructure of *A. caroliniana* fronds was that the chloroplast area was significantly occupied by a large amount of plastoglobuli in fronds stressed by UV-B radiation, while plastoglobuli were occasionally detected in the chloroplasts of control (chloroplasts under elevated UV-

Table IV. Changes in ultrastructure of *Azolla* plants exposed to UV-B radiation for 0 (control), 24 and 48 h. The cell wall thickness, chloroplast area and number of plastoglobuli per μm^2 chloroplast stroma are shown (values are means ± SE, $n = 5$). Values carrying different letters are significantly different at $P \leq 0.05$.

Treatment [h]	Cell wall thickness [μm]	Chloroplast area [μm^2]	Chloroplast area (% of cell)	Plastoglobuli number/μm^2
0	0.42 ± 0.103[a]	2.6 ± 0.85[a]	16.0 ± 1.03[a]	4.5 ± 1.09[a]
24	0.65 ± 0.069[b]	3.6 ± 0.99[b]	22.6 ± 2.11[a]	7.1 ± 1.28[b]
48	0.29 ± 0.084[c]	4.4 ± 1.02[c]	36.2 ± 2.85[b]	11.2 ± 1.22[c]

B radiation, after 24 and 48 h, were filled with plastoglobuli, taking up 58 and 148% of the chloroplast profile). Therefore, chloroplasts were significantly widened at elevated UV-B radiation (Table IV). The cell cross sectional area occupied by chloroplasts was significantly increased by an elevated UV-B exposure period in a linear manner with increasing UV-B radiation. Cell wall got significantly altered across a prolonged exposure period to UV-B radiation relative to the control. The cell wall increased in thickness under elevated UV-B exposure for 24 h, then decreased after 48 h.

Discussion

Biomass and relative growth rate of UV-B-treated *Azolla* plants decreased significantly resulting in an increase in doubling time over the control (Table I). Our results are consistent with Wilhelm *et al.* (1997) who found that UV-B radiation affects growth and several other physiological processes including photosynthesis in algae. The physiological basis for growth reductions under UV-B radiation may involve changes in membrane integrity due to lipid peroxidation caused by free radicals and/or to a disruption in the synthesis or transport of plant hormones (Kramer *et al.*, 1991; Ros and Tevini, 1995). Also, UV-B exposure resulted in a marked decrease in photosynthetic pigment contents and altered thylakoid integrity, suggesting the photosynthetic machinery as the primary target of UV-B stress. Therefore, enhanced UV-B radiation causes damage to Chl a and b due to the inhibition in Chl biosynthesis (El-Mansy and Salisbury, 1971). As *Azolla* fronds possess only two or three layers of mesophyll tissue, the photosynthetic pigments are bleached upon UV-B treatment. Carotenoids act as photoprotective pigments avoiding the generation of singlet oxygen by quenching the triplet state in chlorophyll molecules and by scavenging any singlet oxygen produced avoiding the chlorophyll photooxidation (Young, 1991). In this study UV-B radiation showed a great increase in carotenoids content as well as the carotenoids/total chlorophyll ratio increased and maintained almost always a higher ratio throughout the UV-B exposure period (Table II). This means that there was a strongly change in favour of carotenoids and, concomitantly, chlorophyll was degraded. Therefore, UV-B-treated *Azolla* fronds appeared to be more capable of avoiding the production of singlet oxygen and to scavenge it better than the control ones. In contrast, the correlation between the UV-B-induced degradation of chlorophyll and carotenoids in *Azolla microphylla* was reported by Jayakumar *et al. (*2002).

To cope with UV-B radiation damage, plants have evolved a variety of mechanisms including: screening out UV-B radiation by accumulating UV-absorbing phenolic compounds such as flavonoids and anthocyanins in the leaf epidermis, repairing UV-induced DNA damage, and formation of antioxidants to scavenge peroxides and oxygen radicals (Jordan, 1996). In our study, a likely protective mechanism to minimize damage caused by UV-B radiation is the highly significant increase in UV-B-absorbing pigments such as phenolics as well as flavonoids and anthocyanins in UV-B-treated *Azolla* fronds (Table III). The relative increase in absorption under the effect of UV-B radiation was greatest between 330 and 360 nm, which is in the range of flavonol absorption especially after 8 and 16 h. Low level of cell damage can be explained by the progressive accumulation of these pigments in response to UV-B radiation, probably reflecting that phenolics may act synergistically with flavonoids to stabilize macromolecules, thereby stabilizing the protoplasm (after exposure for 24 h to UV-B radiation flavonoids concentration increased by 311% and for 48 h by 124% relative to control). This assumption is supported by the observations of Larkin *et al.* (2003).

This study shows that exposure of *Azolla caroliniana* to UV-B radiation in a growth chamber obviously changed the ultrastructure of the cells, therefore measurable changes in the chloroplast ultrastructure were observed. Forty-eight hours after application of UV-B radiation, the content of deformed chloroplasts increased up to 95%. The chloroplast size was significantly increased in comparison to the cell and follows a linearly progressive response to the duration of UV-B exposure (Table IV). Similar observations in many earlier studies with other plant species were discussed by Kivimaenpaa *et al.* (2003). Similarly, the change in chloroplast size followed changes in the area of starch; also one notable difference in this study is that starch was readily detected in controlled-grown *Azolla caroliniana*, while it was rarely observed in the UV-B-treated *Azolla*. Lack of starch in the chloroplasts in response to UV-B exposure suggests little photosynthetic activity. Plastoglobuli (carotenoid-bearing structure), which consist mainly of triacylglycerols, plastohydroquinone and α-tocopherol, and may function as storage pools of thylakoid constituents (Murphy, 2001), are markedly affected by UV-B radiation. In *Azolla*, UV-B radiation increased the size and the number of plastoglobuli linearly with the dose. These findings are in agreement with many observations on the increase in the number and/or size of plastoglobuli in response to abiotic stresses (Britvec *et al*., 2001). Thinner cell walls under UV-B exposure may result from a delay in cell wall differentiation. Cell wall measurements are also prone to inaccuracies because the cell wall thickness varies considerably between and within cells.

This study reports, for the first time, ultrastructural responses of *Azolla caroliniana* to prolonged UV-B exposure. The observed responses in all the above experiments indicate that low dose of UV-B radiation stimulates progressively the production of carotenoids, carotenoid-bearing structures, as well as phytochemicals such as UV-B-absorbing phenolics, whereas prolonged exposure to UV-B radiation results in metabolic changes and inhibits the growth of *Azolla*; this may be due to the destruction of photosynthetic activity and plant growth hormones through direct absorption by the fronds.

Britvec M., Reichenauer T., Soja G., Ljubesic N., Eid M., and Pecina M. (2001), Ultrastructure changes in grapevine chloroplasts caused by increased tropospheric ozone concentrations. Biologia Bratislava **56**, 417–424.

Caldwell M. M. (1971), Solar UV radiation and the growth and development of higher plants. In: Photophysiology, Vol. 6 (Giese A. C., ed.). Academic Press, New York, pp. 137–171.

Chaves M. M., Maroco J. P., and Pereira J. S. (2003), Understanding plant responses to drought from genes to the whole plant. Functional Plant Biol. **30**, 239–264.

Chinnici F., Bendini A., Gaiani A., and Riponi C. (2004), Radical scavenging activities of peels and pulps from cv Golden Delicious apples as related to their phenolic composition. J. Agric. Food Chem. **52**, 4684–4689.

Coleman R. S. and Day T. A. (2004), Response of cotton and sorghum to several levels of subambient solar UV-B radiation: a test of the saturation hypothesis. Physiol. Plant. **122**, 362–372.

El-Mansy H. I. and Salisbury F. B. (1971), Biochemical responses of *Xanthium* leaves to ultraviolet radiation. Radiat. Bot. **11**, 325–328.

Foyer C. H., Descourvieres P., and Kunert K. J. (1994), Protection against oxygen radicals: an important defense mechanism studied in transgenic plants. Plant Cell Environ. **17**, 507–523.

Gould K. S. (2004), Nature's Swiss army knife: The diverse protective roles of anthocyanins in leaves. J. Biomed. Biotech. **5**, 314–320.

Haupt W. (1990), Chloroplast movement. Plant Cell Environ. **13**, 595–614.

Holopainen T., Anttonen S., Wulff A., Palomaki V., and Karenlampi L. (1992), Comparative evaluation of the effects of gaseous pollutants, acidic deposition and mineral deficiencies: structural changes in the cells of forest plants. Agric. Eco. Environ. **42**, 365–398.

Holopainen T., Anttonen S., Palomaki V., Kainulainen P., and Holopainen J. K. (1996), Needle ultrastructure and starch content in Scots pine and Norway spruce after ozone fumigation. Can. J. Bot. **74**, 67–76.

Jayakumar M., Eyini M., Lingakumar K., and Kulandaivelu G. (2002), Effect of enhanced ultraviolet-B (280–320 nm) radiation on growth and photosynthetic activities in aquatic fern *Azolla microphylla* kaulf. Photosynthetica **40**, 85–89.

Jordan B. R. (1996), The effect of ultraviolet B radiation on plants: a molecular perspective. Adv. Bot. Res. **22**, 97–162.

Kivimaenpaa M., Sutinen S., Karlsson P. E., and Sellden G. (2003), Cell structural changes in the needles of Norway spruce exposed to long term ozone and drought. Ann. Bot. **92**, 779–793.

Kramer G. F., Norman H. A., Krizek D. T., and Mirecki R. M. (1991), Influence of UV-B radiation on polyamines, lipid peroxidation and membrane lipids in cucumber. Phytochemistry **30**, 2101–2108.

Larkin J. C., Brown M. L., and Schiefelbein J. (2003), How do cells know what they want to be when grow-

ing up? Lessons from epidermal pattering in *Arabidopsis*. Annu. Rev. Plant Biol. **54**, 403–430.

Lichtenthaler H. K. and Wellburn A. R. (1983), Determination of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Biochem. Soc. Trans. **603**, 591–592.

Mirecki R. M. and Teramura A. H. (1984), Effects of ultraviolet B-irradiance on soybean V.: The dependence of plant sensitivity on photosynthetic photon flux density during and after leaf expansion. Plant Physiol. **74**, 475–480.

Murali N. S. and Teramura A. H. (1986), Effectiveness of UV-B radiation on the growth and physiology of field grown soybean modified by water stress. Photochem. Photobiol. **44**, 215–219.

Murchie E. H. and Horton P. (1997), Acclimation of photosynthesis to irradiance and spectral quality in British plant species: chlorophyll content, photosynthetic capacity and habitat preference. Plant Cell Environ. **20**, 438–448.

Murphy D. J. (2001), The biogenesis and functions of lipid bodies in animals, plants and microorganisms. Prog. Lipid Res. **40**, 325–438.

Nogues S. and Baker N. R. (1995), Evaluation of the role of damage to photosystem II in the inhibition of CO_2 assimilation in pea leaves on exposure to UV-B radiation. Plant Cell Environ. **18**, 781–787.

Peters G. A., Mayhe B. C., Ray T. B., and Toia R. E. (1979), Physiology and biochemistry of the *Azolla-Anabaena* symbiosis. In: Nitrogen and Rice. International Rice Research Institute, Los Banosa Laguna, Philippines, pp. 325–344.

Ros J. and Tevini M. (1995), Interaction of UV-radiation and IAA during growth of seedlings and hypocotyl segments of sunflower. J. Plant Physiol. **146**, 295–302.

Sgherri C., Cois E., and Navari-Izzo F. (2003), Phenol and antioxidative status of *Raphanus sativus* grown in copper excess. Physiol. Plant. **118**, 21–28.

Stobart A. K., Griffiths W. T., Ameen-Bukhari I., and Sherwood R. P. (1985), The effect of Cd^{2+} on the biosynthesis of chlorophyll in leaves of barley. Physiol. Plant. **63**, 293–298.

Subudhi B. P. R. and Watanabe I. (1981), Differential phosphorus requirements of *Azolla* species and strains in phosphorus limited continuous culture. Soil Sci. Plant Nutr. **27**, 237–247.

Wetzel R. G. (2001), Limnology: Lake and River Ecosystems, 3rd ed. Academic Press, San Diego.

Wilhelm C., Bida J., Domin A., Hilse C., Kaiser B., Kesselmeier B., Lohr M., and Muller A. M. (1997), Interaction between global climate change and the physiological responses of algae. Photosynthetica **33**, 491–503.

Young A. J. (1991), The photoprotective role of carotenoids in higher plants. Physiol. Plant. **83**, 702–708.