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Effects of ultraviolet radiation on plant cells

F. Hollósy*

Department of Medical Chemistry, Molecular Biology and Pathobiochemistry, Semmelweis University Budapest, Puskin u. 9., 1088 Budapest, Hungary

Abstract

Recent measurements of ozone levels have led to concern that the stratospheric ozone layer is being depleted as a result of contamination with man-made chlorofluorocarbons. Concomitantly, the amounts of solar UV-B radiation reaching the Earth's surface is increasing. UV-B radiation has been shown to be harmful to living organisms, damaging DNA, proteins, lipids and membranes. Plants, which use sunlight for photosynthesis and are unable to avoid exposure to enhanced levels of UV-B radiation, are at risk. Thus, mechanisms by which plants may protect themselves from UV radiation are of particular interest. This review will summarizes the main aspects of ultraviolet radiation on plants at physiological and biochemical level, with particular emphasis on protective structures and mechanisms. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Ultraviolet radiation; Plant cells; Target sites and damages; Cellular responses; Protective enzymatic and non-enzymatic mechanisms; Signal-transduction of UV radiation

1. Introduction

Ultraviolet radiation (UV) is a part of the non-ionizing region of the electromagnetic spectrum which comprises approximately 8–9% of the total solar radiation (Coohill, 1989; Frederick, 1993). UV is traditionally divided into three wavelength ranges: UV-C (200–280 nm) is extremely harmful to organisms, but not relevant under natural conditions of solar irradiation; UV-B (280–320 nm) is of particular interest because this wavelength represents only approximately 1.5% of the total spectrum, but can induce a variety of damaging effects in plants; UV-A (320–400 nm) represents approximately 6.3% of the incoming solar radiation and is the less hazardous part of UV radiation.

The stratospheric ozone layer efficiently filters out most of the detrimental, shortwave UV radiation, shorter than 280 nm. The absorption coefficient of ozone decreases rapidly at wavelengths longer than 280 nm and approaches zero at about 330 nm (Robberecht, 1989). Therefore, UV-A radiation is virtually unaffected by changes of ozone concentration. At the Earth's surface, radiation becomes significant between wavelengths of 290–315 nm, increasing rapidly within this range by about three orders of magnitude (Caldwell et al., 1989). A small decrease in ozone levels may cause a large relative increase in biologically effective UV radiation (Madronich, 1992, 1993). In general, each 1% reduction in ozone causes an increase of 1.3–1.8% in UV-B radiation reaching the biosphere. The decrease in ozone level originates from human activities such as the release of chlorofluorocarbons and nitrogen oxides, which act as ozone antagonists (Anderson et al., 1991; McFarland and Kaye, 1992). The relevant factors, such as atmospheric pathlength, elevation above sea level, cloud cover, ground reflectance and the natural latitudinal gradient of ozone, which are explanations for the great variability in the UV-B reaching the surface of the Earth, are discussed by Caldwell (1981). This paper will review the basic effects of UV radiation on higher plant cells.

2. Ultraviolet action spectra

The potential impacts of an increase in solar UV-B radiation reaching the Earth's surface have been investigated by numerous research groups during the past decades. Much of this research has focused on the effects of plant growth and physiology under artificial UV-B irradiation supplied to plants in growth chambers or greenhouses (Sinclair et al., 1990; Sullivan and Teramura, 1990; Teramura and Murali, 1986; Tevini and Teramura, 1989). Valid predictions of UV-B effects can only be made using conditions close to those of solar radiation. Since solar and artificial UV-B sources have different spectral energy distributions and due to the wavelength dependency of photobiological processes, this radiation must be weighted according to its

* Tel.: +36-1-266-2755, ext. 4092; fax: +36-1-266-7480.
E-mail address: fhollosy@puskin.sote.hu (F. Hollósy).

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biological effectiveness (Caldwell et al., 1986; Coohill, 1989; Teramura, 1986).

Among the methods of obtaining and analyzing photobiological data, action spectroscopy plays a central role in characterization of bioresponses (Coohill, 1992; Rundel, 1983). Action spectra serve as spectral weighting functions which can be used to determine whether a change in UV radiation quality, resulting from a given change in ozone, is biologically significant.

UV radiation can have damaging effects on DNA and/or physiological processes of the organisms as different mono- and polychromatic action spectra demonstrate (Setlow, 1974; Caldwell, 1971; Coohill, 1989, 1992; Caldwell et al., 1986).

Biological action spectra are normally developed with monochromatic radiation—often with tissue or organelle preparations. Monochromatic system is accurate, the preferred way for determining the target chromophores, and the classical method for generating an analytical action spectrum (Peak and Peak, 1982). In the case of only one chromophore, the action spectrum often corresponds to the absorption spectrum of this chromophore. The similarity of the absorption spectrum of DNA and the action spectra for cell inactivation, induction of chromosome aberrations, mutations and transformation within the wavelength region 220–300 nm indicated that DNA is the main chromophore for all these effects within this wavelength region. Action spectra were among the first assays to point to DNA as the genetic material (Gates, 1930) and to chlorophyll as the chromophore most responsible for plant growth.

However, in some cases the action spectra are slightly red shifted compared with the DNA absorption spectrum. This suggests that light absorption by proteins also plays some role. Action spectra of UV damage to functions directly related to proteins closely resemble the absorption spectra of proteins.

The spectral weighting functions need to be representative of responses of intact organisms exposed to polychromatic radiation as occurs in sunlight (Caldwell et al., 1986). A major advantage of using polychromatic system in the development of polychromatic action spectrum is that interactions of biological responses to different wavelengths can be empirically incorporated into the composite spectrum (Coohill, 1989). Polychromatic action spectra include the beneficial effects of background white light on photorepair of DNA damage, or on accumulation of filter pigments and are thus closer to the natural conditions.

Recently, a new action spectrum for pyrimidine dimer induction in intact alfalfa leaves indicated that the slope in the short wavelength band is less steep when DNA is shielded, which lowers the predicted impact of ozone depletion, when only DNA-related responses are considered (Quaite et al., 1992). This impact could even be absent where DNA dimers are repaired by photoreactivation repair. Therefore, spectral weighting functions may be developed with monochromatic radiation, but should be tested with polychromatic radiation in realistic combinations using intact organisms to ensure that the weighting functions are realistic. In addition, the degree to which these functions may be modified by other environmental factors, such as temperature, needs to be assessed. The usefulness of these spectra are limited and they will be replaced in the future by more accurate and common spectra as they are generated.

With a slight shift of the spectrum towards shorter wavelengths, the biologically effective radiation increases steeply or is amplified. This effect is expressed as a radiation amplification factor (RAF), which is the percentage increase of biologically effective radiation caused by a given percentage of total column ozone (Caldwell et al., 1986). RAF values are determined both by the action spectra used and by other factors, which determine irradiation received. Thus, for a given action spectrum, a RAF value of, e.g. 2, indicates that a 1% decrease in total column ozone would lead to an increase in biologically effective irradiance of 2%. These calculations are important in predicting likely biological damage, such as reduction in plant growth.

3. Identification of target sites of UV radiation

3.1. Nucleic acids

DNA is one of the most notable targets of ultraviolet radiation. Irradiation in both the UV-B and UV-C regions results in a multitude of DNA photoproducts (Sancar and Sancar, 1988), which may cause mutations during replication (Jiang and Taylor, 1993). The most common DNA photoproducts are cyclobutane-type pyrimidine dimers and the pyrimidine(6,4)pyrimidone dimer (Hutchinson, 1987). DNA protein cross-links, and DNA strand breaks and deletion or insertion of base pairs can also be induced by UV exposure (Smith, 1989). Cyclobutane-type pyrimidine dimers (CPDs), produced by the dimerisation of adjacent pyrimidines (TT, TC, CT or CC) on the same strand of DNA and the pyrimidine(6,4)pyrimidone (the 6-4 photoproduct) are the most important UV-induced base pair changes (Cadet et al., 1992). CPD formation and removal were investigated both in intact plants and in plant cell cultures (McLennan 1987a,b; McLennan and Eastwood, 1986; Pang and Hays, 1991) and it was found that CPDs represent approximately 75% of the total DNA damage. CPD-formation was induced by all wavelengths of UV radiation in alfalfa cotyledons (Quaite et al., 1992). UV-induced damages to DNA have been studied in detail in humans, mammals, fungi and bacteria (see reviews Stapleton, 1992; Strid et al., 1994).

3.2. Amino acids

Proteins have strong absorption at about 280 nm as well as at higher wavelengths of the UV-B region due to absorption by aromatic amino acids phenylalanine, tryptophane and tyrosine, as well as histidine, cysteine and cystine

and, therefore, can be direct targets of UV-radiation. UV-induced destruction of tyrosine and tryptophane has been observed both in the form of free amino acids and proteins (Khoroshilova et al., 1990). UV-B can induce photooxidation of tyrosine to 4,4-dihidroxyphenylalanine and the formation of dityrosine (Creed, 1984). Tryptophane can be excited either directly by UV or by energy transfer from neighboring amino acids such as tyrosine or phenylalanine. Photochemical changes initiated by tryptophane are often attributed to formation of N-formyl kinurenine (Kochevar, 1990). After absorption of UV-A radiation this photoproduct can react with nucleic acids.

Cysteine is a relative poor absorber in the UV-B region, but undergoes UV-induced photolysis at a high quantum efficiency (Creed, 1984). The disulfide group of cystine can be split by UV radiation into reactive sulfhydryl groups. The disulfide bridges are important for the tertiary structure of many proteins, and the effects of UV-B on these bonds can strongly influence protein structure and function (Creed, 1984).

3.3. Proteins

UV irradiation causes not only the modification or destruction of amino acid residues, but leads to inactivation of whole proteins and enzymes (Grossweiner, 1984; Prinsze et al., 1990). Inactivation of proteins and enzymes can be caused directly by UV photolysis of aromatic amino acids or disulfide groups if affected residues are included in the active site. It is also important to note that there exists the possibility that UV absorption within the protein matrix can cause damage far from the actual absorption site via energy migration to functionally important amino acids of an active center, as suggested for the sensitization of cysteine destruction by aromatic residues (Jordan, 1993). Characteristic examples of this effect include ribulose 1,5-bisphosphate carboxylase (Rubisco) (Vu et al., 1984; Strid et al., 1990), ATP-ase, violaxanthin de-epoxidase (Pfündel et al., 1992), and protein subunits of the photosystem I and II as well as insulin, pepsin, trypsin, myosine (Jordan, 1993).

Protein components of the plant cytoskeleton may be possible targets for UV radiation. Subjecting *Petunia hybrida* protoplasts to UV radiation (UV-A and UV-B) shortened cortical microtubule organization (Staxén et al., 1993). Tubulin may be a particularly sensitive target, since it has a high content of amino acids with aromatic side chains (Zaremba et al., 1984).

UV radiation delayed progression through the cell cycle, with the phases S, G1 and G2, being affected. There may be a correlation between cortical microtubule disruption and the delay in the onset of DNA synthesis (Staxén et al., 1993).

3.4. Lipids

Lipids with isolated or conjugated double bonds can also be photochemically modified by UV absorbance. Phospho- and glycolipids, which are the main components of plant cell membranes, contain unsaturated fatty acids, which are destroyed by UV-B radiation in the presence of oxygen (Kramer et al., 1991; Panagopoulos et al., 1990). Oxidative damage can be detected by lipid peroxidation. Hydroxyl radicals and singlet oxygen can react with the methylene groups forming conjugated dienes, lipid peroxy radicals and hydroperoxides (Smirnoff, 1995). Either radicals or singlet oxygen can be produced by photosensitization with dyes, aromatic carbohydrates or porphyrins (Smirnoff, 1995). The peroxy radicals can abstract hydrogen from other polyunsaturated fatty acids, leading to a chain reaction of peroxidation. The peroxidation of membrane lipids leads to breakdown of their structure and function (Mishra and Singhal, 1992; Murphy and Vu, 1996). One of the products of lipidperoxidation is malondialdehyde which is often used as a measure of peroxidation. Hydrocarbons such as ethylene, ethane and pentane can also be released and they can be measured (Halliwell, 1987). Hydrogen peroxide can inactivate enzymes, particularly some of the light-activated Calvin-cycle enzymes (Charles and Halliwell, 1980). Hydroxyl radicals can denature proteins and react with bases in DNA causing mutations and the aldehydes formed by lipid peroxidation can conjugate and inactivate proteins (Wolff et al., 1986).

The composition of membrane lipids in chloroplasts such as mono- and digalactosyldiglicerides (MGDG, DGDG), may change due to the UV-B radiation. Since a high degree of unsaturation of MGDG is necessary for stability of chloroplast membrane structure (Hugly et al., 1989), the decreases in the MGDG/DGDG ratio with UV-B radiation may influence this stability (Predieri et al., 1995).

3.5. Plant growth regulators

UV-induced changes in DNA and/or plant growth regulators are the probable molecular reasons for changes in growth, general development, and flowering.

Altering the concentrations of plant growth regulators influences processes which are dependent on them (Yang et al., 1993). Photolytic degradation of indole-3-acetic acid (IAA) has been demonstrated in sunflower seedling (Ros and Tevini, 1995). The reduced IAA concentration and the growth inhibitor IAA photoproduct, 3-methyleneoxindole, may be responsible for the inhibition of hypocotyl growth. Furthermore, the action of peroxidases, functioning as IAA-oxidase, may also inhibit the elongation since the cell wall extensibility is reduced.

Early experiments demonstrated that 2,4-dichlorophenoxyacetic acid (2,4-D), an often used synthetic auxin, is destroyed with radiation between 340 and 390 nm and 2,4-D is converted to 2,4-dichlorophenol by light which is a cell poison (Bell, 1956). The phytohormone abcisic acid (ABA) also absorbs strongly in the UV-B region, and it can be inactivated by photolysis (Lindo et al., 1979).

Growth can be stimulated by gibberellins in UV-irradiated seedlings (Ballaré et al., 1991). Another phytohormone, ethylene, which changes elongation to radial

growth, is produced to a greater extent in UV-B irradiated plants (Ros and Tevini, 1995).

3.6. Pigments

Pigments of the photosynthetic apparatus can be destroyed by UV radiation, with concomitant loss of photosynthetic capacity (Strid et al., 1990; Jordan et al., 1994). Chlorophylls (*Chl*) and carotenoids may be adversely affected by relatively large amounts of UV-B radiation, with carotenoids generally being less affected than the chlorophylls (Pfündel et al., 1992). It has been reported that UV-B radiation resulted in greater reduction in the amount of *Chl b* as opposed to *Chl a* and may point to a more selective destruction of *Chl b* biosynthesis or degradation of precursors (Marwood and Greenberg, 1996). However, the effect of UV-B radiation on *Chl a/b* ratios varies among growth conditions and species.

3.7. Membranes

UV-induced damage to membranes is mainly focused on transport phenomena (Murphy, 1990; Murphy et al., 1985; Murphy and Wilson, 1982). High fluences accelerate molecule flow by producing large breaks. Low fluences inactivate membrane function like transport systems, electrogenic pumps, ATP-ase and photosynthetic activity (Wright et al., 1981).

UV-B radiation seems to exert adverse effects not only on various protein and pigment-protein complexes of the photosynthetic apparatus, but also on the structure of the thylakoid membrane that contains these complexes.

An early consequence of UV-B radiation is an increase in ion-permeability of the thylakoid membrane (Chow et al., 1992). This phenomenon is apparent as an increase in the rate of relaxation of the flash-induced absorption change of chloroplast pigments at 515 nm, resulting in accelerated electric potential difference. UV-induced ion permeability of the membranes has also been observed with plasma membranes (Doughty and Hope, 1973) and cultured cells of higher plants (Murphy and Wilson, 1982) and explained by association with specific ion channels. UV-B induced loss of K^+ from guard cells may be responsible for the observed loss of stomatal conductance in irradiated plants (Negash and Björn, 1986).

3.8. Photosynthesis

Studies of more than 300 plant species and cultivars have been carried out, and about 50% have been considered sensitive, 20–30% moderately sensitive, and the rest insensitive to UV-B radiation (Teramura et al., 1990; Teramura and Sullivan, 1994). In many sensitive plant species (e.g. wheat, rice, maize, rye, sunflower, and cucumber), reduced leaf area and/or stem growth has been found (Teramura and Sullivan, 1994).

Photosynthesis is one of the most studied processes under UV-B accompanied mainly by growth experiments. Despite the diversity of UV targets in plants, it seems that the photosynthetic apparatus is among the main action sites of UV-B, and its damage contributes significantly to the overall UV-B damage (Kulandaivelu et al., 1993).

The *direct effect* of enhanced UV-B radiation in sensitive plants are the following:

impairment of photosystem II (PSII) and to lesser extent photosystem I (PSI);
decrease in Rubisco activity;
decreased carbon dioxide fixation and oxygen evolution;
reduction in dry weight, starch and chlorophyll content.

In addition to the direct effect of UV-B radiation, photosynthesis may also be *indirectly effected*:

induction of stomatal closure may reduce the efficiency of gas exchange;
changes in leaf thickness and anatomy may alter light environment within the leaf;
changes in canopy morphology may also indirectly affect whole plant photosynthesis.

3.8.1. Photosystems I and II

The effects of UV-B radiation do not seem to be evenly distributed between the two photosystems. Based on a variety of experiments, a general consensus has emerged that UV-B has has little or no effect on PSI as compared to PSII (Renger et al., 1989; Bornman, 1989). The lack of UV-effect on PSI can be demonstrated by adding an artificial donor couple to UV-B-treated chloroplasts, whereby nearly all function is restored (Kulandaivelu and Noorudeen, 1983). UV-B radiation may inhibit PSI-mediated cyclic photophosphorylation (Pang and Hays, 1991). Inhibition of PSI by UV-C irradiation has also been observed, but its impairment was much less pronounced than that of PSII. The possible targets within PSI have not been studied in detail.

While there is general consensus that UV-B radiation influences primarily PSII, there are many different reports on possible targets (Bornman, 1989; Renger et al., 1989; Noorudeen and Kulandaivelu, 1982). Different techniques were used to reveal the possible target sites of UV radiation such as fluorescence induction, flash-induced absorption changes, and measurement of oxygen evolution (Fiscus and Booker, 1995; Cen and Björn, 1994; Bornman, 1989). Critical comparison of the published data is often complicated by the essentially different experimental conditions: spectral composition of the applied UV source (presence of UV-C component besides UV-B or UV-A radiation), the presence or absence of visible light, and different light intensities. However, it seems to be well established that the redox components of PSII are effected by UV-B to some degree.

From previous experiments it has been assumed that

UV-B acts on either the reaction center itself, producing dissipative sinks for excitation energy, which quench the variable fluorescence and/or the reducing site of PSII (Iwanzik et al., 1983). Other works, however, suggested the quinone electron acceptors (Melis et al., 1992; Trebst and Depka, 1990), the redox active tyrosines (Tyr-Z, Tyr-D), or the primary step of PSII electron transport (Iwanzik et al., 1983; Kulandaivelu and Noorudeen, 1983) as the primary targets of UV-B action in PSII electron transport. Recent comparative studies confirm earlier suggestions that the water-oxidizing complex seems to be the most UV-sensitive part of PSII (Bornman and Sundby-Emanuelsson, 1995). Since the Mn cluster of water oxidation seems to be the most fragile component of the electron transport chain, UV-B absorption by the protein matrix or other redox components may lead to conformational change and inactivation of the Mn cluster. Q_A and Q_B acceptors have been frequently suggested as sensitizers of D1 and D2 protein damage due to the similarity of the action spectrum of D1 protein degradation and the absorption spectrum of plastosemiquinones (Greenberg et al., 1989a,b; Jansen et al., 1996). However, recent data point to the importance of the donor side components of PSII, primarily that of the water-oxidizing system, in sensitizing D1 protein cleavage via hydroxyl radical formation.

3.8.2. The cytochrome b/f complex

Cytochrome *b/f* mediates electron transport between the two photosystems: it oxidizes plastoquinol produced by PSII and reduces plastocyanin, which serves as electron donor to PSI. Cytochrome *b/f* complex together with PSI seems to be the least affected part of the thylakoid membrane by UV-B radiation (Cen and Bornman, 1990).

3.8.3. Light harvesting complex

Since the light harvesting complex of PSII (LHCII) plays an important role in light absorption, and energy transfer to the reaction center as well as thylakoid stacking, any damage to these systems results in multiple effects on the photosynthetic apparatus. UV-B radiation decreases the transcription of the *cab* gene responsible for the synthesis of the chlorophyll *a/b*-binding proteins of LHCII and may lead to the functional disconnection of LHCII from PSII (Jordan et al., 1994).

3.8.4. Rubisco and ATP-ase

The main CO_2-fixing enzyme in C3 plants is Rubisco, and comprises it about 50% of the total soluble plant protein and consists of a large (55 kDa) and small (14 kDa) subunit. Rubisco activity may decline with enhanced levels of UV-B radiation (Vu et al., 1984; Strid et al., 1990). High UV-B irradiance in combination with low PAR levels produce significant reduction in the concentration of carboxylating enzymes (Barbato et al., 1995). When high PAR (>1000 $\mathrm{mol}^{-2}\,\mathrm{s}^{-1}$) was applied in conjuction with low UV-B levels the Rubisco was unaffected. The high UV-B levels were reported to decrease the amount of carboxylating enzyme and the levels of both subunits are decreased (Strid et al., 1990; Jordan, 1993). UV-B-induced inactivation of Rubisco could be due to modification of the peptide chain, degradation of the protein, and/or diminished transcription of the gene (Jordan et al., 1992, 1994; Caldwell, 1993). Recently, in vivo photomodification of Rubisco holoenzyme was observed in UV-B-treated plants (Wilson et al., 1995). In UV-B-irradiated plants, both the amount and the activity of ATP-ase of thylakoids decreased. There is also a marked decline in the amount and activity of ATP-synthase (Zhang et al., 1994).

3.9. Structural modifications

UV radiation has been shown to cause alterations in physiological and biochemical processes as well as in plant morphology. Plants exposed to UV radiation commonly respond by reducing the penetration of the radiation. The attenuation of UV radiation with depth in a leaf has been demonstrated using quartz fiber optic microprobes to measure directly the gradients of radiation within leaves (Cen and Bornmann, 1993). Depending on a number of factors, such as species, cultivar and growth conditions, plant growth may be inhibited or stimulated by different levels of UV radiation (Staxén and Bornman, 1994). The seemingly different mechanisms operative in inhibition and stimulation are not well understood.

Given the lack of uniformity and range of exposures used, it is difficult to generalize about the exact sites of UV-B damage. However, morphological alterations in leaf anatomy have been well-documented in numerous plants (Tevini et al., 1981; Tevini, 1993). These changes include an increase in leaf thickness concomitant with a decrease in leaf area, and decrease in stomatal frequencies (Barnes et al., 1990). The increase in leaf thickness has been interpreted as a protective mechanism against damage caused by UV-B radiation. Contrary to reports on other species (Murali et al., 1988; Mirecki and Teramura, 1984; Cen and Bornman, 1993), the thickness of the leaves in *Zea mays* was decreased (Santos et al., 1993). This reduction was the result of the collapse of the adaxial epidermal cells and also was due to the decrease in water content (Teramura et al., 1983). An ultrastructural investigation on sugar beet leaves showed the collapse of these cells were caused by UV-C, and not by UV-B and also showed that with UV-C not only epidermal cells were damaged but mesophyll cells as well (Bornman et al., 1983).

3.9.1. In vivo ultrastructural investigations

In order to characterize the exact sites of UV damage at cellular level we have investigated the effects of UV-A and UV-B irradiation resulted alterations on ultrastructure of the shoot apex covering very young leaves of *Lysimachia nummularia* L. plant. For these experiments plants were cultivated in a growth chamber and illuminated by warm

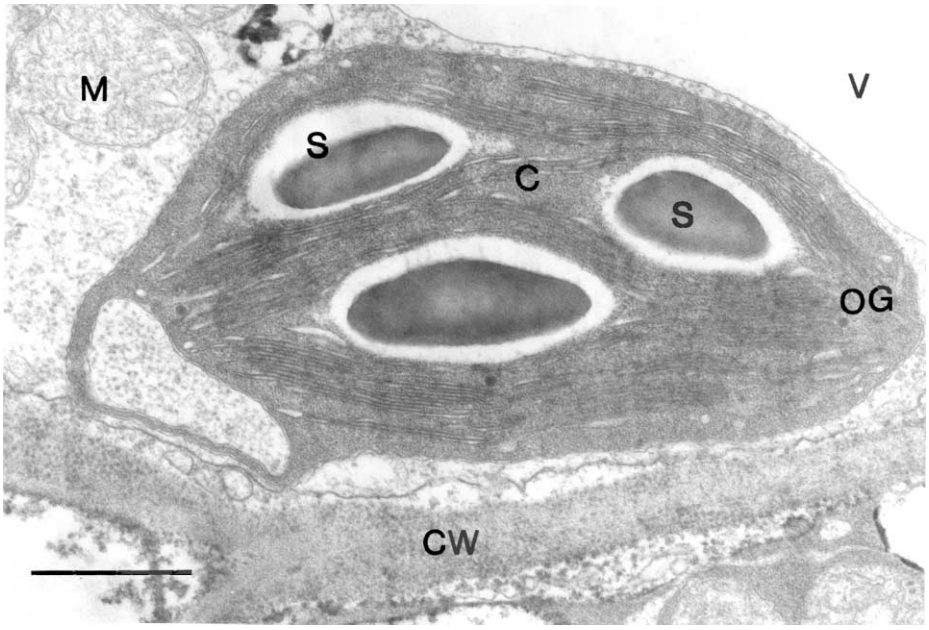

Fig. 1. Portion of mesophyll cell from control tissue fixed with osmium tetroxide and picric acide. Large starch grains (S) and small osmiophilic globules (OG) are present. CW, cell wall; C, chloroplast; M, mitochondrion; V, vacuole. Bar 1 m.

white tubes (F29, Tungsram 36 W, 17 W/m^2) with a 16/8 h day/night regime. During the 7 days of experiments, plant were illuminated with F29 tubes (controls), or supplemented with UV-A radiation from Philips TL40 tubes (max. 370 nm, 0.5 W/m^2) or with UV-B from FS40 sunlamps (Westinghouse, 9 10^{-2} W/m^2) which were filtered through aged 0.1-mm thick cellulose acetate, effectively cutting off radiation below 290 nm. Samples were harvested daily for both ultrastructural investigations and for flavonoid determination as well.

As illustrated in Fig. 1, leaf material from control plant was characterized by well-defined cell structure. The highly vacuolate mesophyll cells contained numerous chloroplasts with well-developed grana and stroma thylakoids. Plastoglobuli of variable size were also present in the finely granular stroma. The nucleate mesophyll cells contained large number of starch grains, large population of mitochondria and microbodies. Some dictyosomes, variable numbers of plasmalemmosomes and abundant tubular endoplasmic reticulum were present.

Effect of UV-A irradiation is shown in Fig. 2. All of the cellular components seen here are normal in appearance. However, the great majority of the cells contained lesser amount of starch grains than that could be counted in control cells. In many chloroplasts starch grains seemed to be absent which may be a reflection of the increased ability of such chloroplasts to mobilize starch. Another symptom of UV-A radiation is the appearance of electron dense globuli in the stroma of chloroplasts. In many sections, the most typical phenomena is the increased accumulation of electron dense precipitate in vacuoles bordering the tonoplast membranes. In the presence of phenolic compounds within the vacuoles such kind of precipitate always could be formed when picric acid was used during the fixation process. The increased formation of precipitate may have a possible indicator of increased production of phenolic filter pigments.

The most notable effects caused by UV-B radiation is illustrated by in Fig. 3, which shows parts of two mesophyll cells. Among the various organelles the chloroplasts appear to be the most sensitive to UV-B. The damaged cell on the left contains a chloroplast, which had begun to lose its structural integrity. The orderly pattern of grana and stroma thylakoids has been lost, and some of the thylakoids appear slightly dilated. Most of the stroma has a quasi-crystalline appearance which may have the result of water stress imparted by the UV-B treatment. Tevini et al. (1981) reported that UV-B irradiated plants suffered water stress. However, no evidence of incipient plasmolysis could be detected in any of the cells. Some places osmiophylic precipitate can be found near the membranes of vacuoles. Other types of discernible damage to cellular organelles seemed to be absent. This means that the mesophyll cells in leaves covering the shoot apex are not only well shielded against the harmful effect of UV radiation but represent effective protective multilayer for the apical meristems.

UV radiation may cause more serious ultrastructural alterations as were found in other plant species. In a study of *Pisum sativum*, UV-B-induced ultrastructural changes included dilation of the nuclear membrane, chloroplast swelling, thylakoid dilation, rupture of chloroplast outer membrane, swollen cisternae in the endoplasmic reticulum, and vesiculation of the plasmalemma and tonoplast (Brandle et al., 1977). Damage to ultrastructure accumulated with exposure time and led to both vesiculation in the chloroplast stroma and endoplasmic reticulum, and rupture of the plasmalemma and tonoplast in about 26%

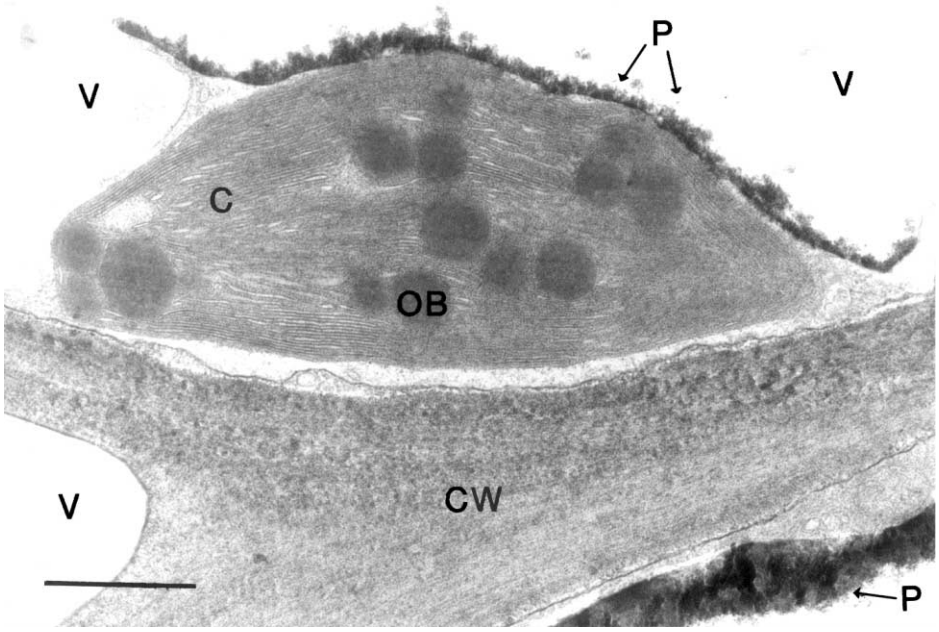

Fig. 2. Chloroplast from leaf tissue irradiated with low UV-A dose; fixed with osmium tetroxide and picric acide.The starch grains have completely disappeared. The number and size of osmiophylic bodies (OB) have increased. Arrows point to precipitate (P) in vacuoles bordering the membranes apparently resulting from the UV-A treatment. CW, cell wall; C, chloroplast; V, vacuole. Bar 1 m.

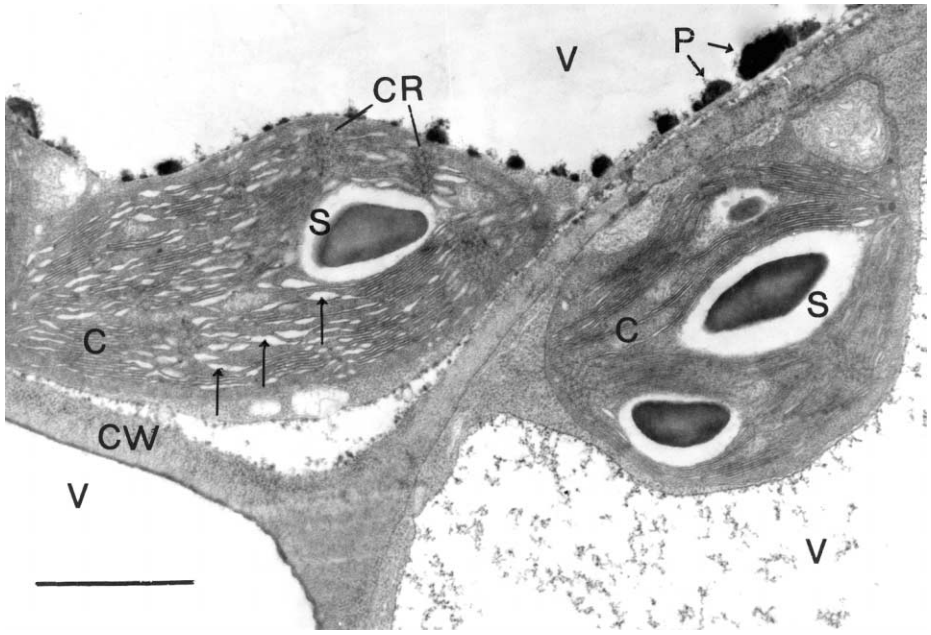

Fig. 3. Portion of mesophyll cells from leaf exposed to UV-B radiation. Unlabeled arrows point to disruptions of thylakoid membranes of the chloroplast (C) on the left; chloroplast on the right was normal in appearance. The stroma in both chloroplasts contain quasi-crystalline (CR) areas in appearance. Arrows point to osmiophilic precipitate (P) in vacuoles bordering the membranes. CW, cell wall; C, chloroplast; M, mitochondrion; V, vacuole. Bar 2 m.

of the cells during the second day of exposure. Studies with *Zea mays* showed a different situation for cellular compartment in UV-B irradiated material. In mesophyll cells only mitochondria appeared damaged and some degree of alteration was observed in dictyosomes and endoplasmic reticulum. This sensitivity of mitochondria was found neither in *Pisum sativum* (Brandle et al., 1977) nor in *Beta vulgaris* (Bornman et al., 1983), in which the most sensitive organelle was the chloroplast. Morphometric analysis of *Zea* chloroplasts showed that their fractional volume was reduced by the UV-B irradiation. The reduction in the fractional volume of chloroplasts can be partly due to an osmotic adjustment of the cells under water stress, as admitted by Lesser and Shich (1990) for the genus *Symbiodinum*.

Exposure of cells to UV radiation has been shown to lead to a delay in the onset of mitosis. Using *Petunia hybrida*

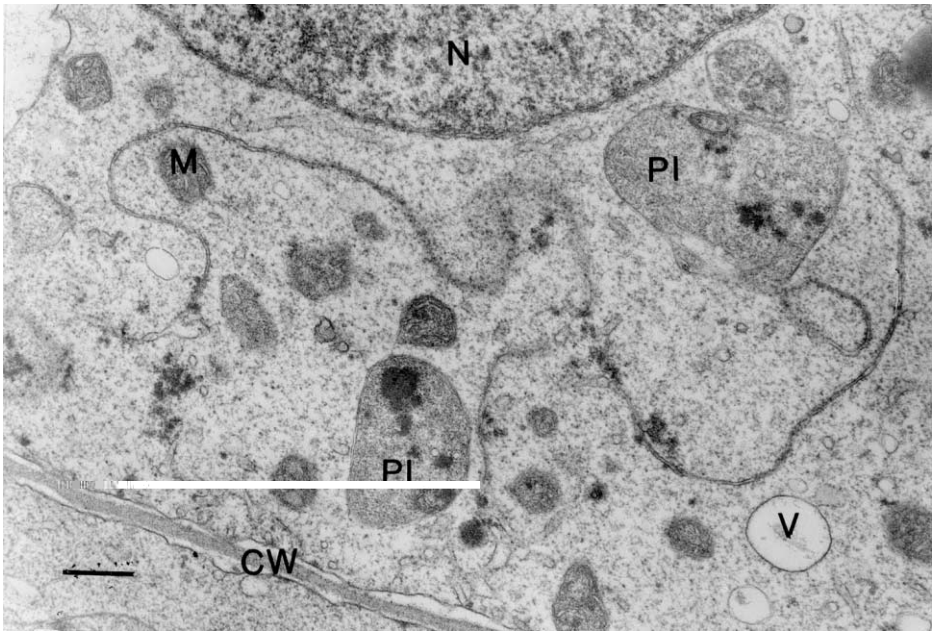

Fig. 4. Portion of callus cells from control culture of *Lysimachia nummularia*. CW, cell wall; Pl, plastid; M, mitochondrion; V, vacuole; N, nucleus. Bar 2 m.

protoplasts, Staxén et al. (1993) found that the protoplasts were delayed in their progression through the cell cycle, with G1 and G2 phases being affected as well as the S phase. Also, UV radiation induced breaks in cortical microtubules resulting in shorter fragments with increasing dose. After UV-treatment re-establishment of a normal organization of the cortical microtubule network and the resumption of cell division could also be detected (Staxén et al., 1993). The question arises as to the mechanisms underlying the changes in morphology. According to Staxén and Bornman (1994) two mechanisms could explain a direct action of UV-B on leaf morphology:

1. Alteration of the deposition of cell wall microfibrils. Microtubules, which have been implicated in the deposition of cell wall microfibrils depolimerized when irradiated by UV-B and the disruption of the cortical microtubule network of epidermal cells could result in altered cell shape, consequently altered leaf morphology.
2. Changes in cell division rates in leaf meristems. Divisions that occur during leaf expansion would be more susceptible to inhibition by UV-B. After UV-treatment, fewer cells will be formed on a whole leaf area without an alteration in cell size.

Cell size and shape would be affected in the case of both mechanisms.

3.9.2. In vitro ultrastructural investigations

Using the same experimental conditions for *Lysimachia* cultures mentioned in Section 3.9.1., we have investigated the effects of UV-A and UV-B radiation on ultrastructure of *Lysimachia nummularia* L. in vitro culture. In these experiments, calli of *Lysimachia* plant were cultivated on Murashige-Skoog synthetic medium supplemented with 1 mg/l of 2,4-dichlorophenoxyacetic acid (2,4-D) as plant growth regulator. Calli were grown under sterile conditions in plastic plant boxes which were transparent for both UV-A and UV-B radiation. Calli were subjected to UV-A and UV-B irradiation for 7 days. Samples were harvested daily for both ultrastructural investigations and for flavonoid determination as well.

3.9.2.1. Control callus Callus cells of *Lysimachia* were consisted of a wide variety of cell types. Most of the cells seemed to be young, highly vacuolated and parenchymatous, as presented in Fig. 4. The cells were rich either in cytoplasm or organelles. Most of the cells contained large and diffusively stained nuclei, each with a strongly stained nucleolus. Callus cells formed plastids with profound invaginations and sometimes the whole plastids was filled with starch grains (amyloplast). In certain plastids had small osmiophilic globules and also deposits of phytoferritin. As illustrated in Fig. 4, the endoplasmic reticulum had shorter-longer swollen cisternae. Matrix of mitochondria was dense. Only few dictyosomes could be observed, however, they seemed to have normal structure. The presence of large number of free ribosomes and lomasomes were also characteristic in the control callus.

3.9.2.2. UV-A and UV-B treated callus cells The most conspicious alteration in the UV-A and UV-B irradiated cells is shown in Figs. 5 and 6 by most of their endoplasmic reticulum, mitochondria and dictyosomes. A common deviation from the control was the development of abundant RER and numerous membrane delimited apparatus that