Effects of prolonged UV-B exposure in plants

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Accepted 12 December, 2008

Over the past few decades, there has been a depletion of the stratospheric ozone layer due to emissions of halogen-containing compounds of anthropogenic origin. This has resulted in a concomitant increase in solar ultraviolet-B radiation. High levels of UV-B radiation are responsible for multiple biologically harmful effects in both plants and animals. In plants, these effects include DNA damage, which often causes heritable mutations affecting various physiological processes, including the photosynthetic apparatus, protein destruction and signal transduction via UV-B photoreceptors. High UV-B levels introduce a number of different lesions, predominantly cyclobutane pyrimidine dimmers (CPDs) and pyrimidine (6-4) pyrimidinone products [(6-4) PPs] in the genome. These could adversely affect plant growth, development and morphology, especially the productivity of sensitive crop species. This paper reviews the genetic effects of long-term UV-B exposure in plants.

Key words: UV-B radiation, ozone depletion, plant growth, morphology.

INTRODUCTION

The UV spectrum is generally divided into three regions: the UV-C region (220- 280 nm), the UV-B region (280 - 320 nm) and the UV-A region (320 - 400 nm). UV-B radiation is the most energetic component of sunlight reaching the earth's surface, and this has increased due to ozone depletion (Caldwell et al., 1989; Strid et al., 1994). All of the most damaging UV-C radiation and most of the UV-B are filtered out by atmospheric ozone before it reaches the earth's surface. However, UV influence rates at the earth's surface have been increasing recently as levels of atmospheric ozone have been decreasing. This progressively worsening situation has led to renewed impetus in efforts to understand the effects of UV radiation on plants and other organisms. The impact of increased UV-B radiation on the biosphere, as a result of stratospheric ozone depletion, has heightened awareness of the cytotoxic, mutagenic, and carcinogenic consequences of UV-irradiation (Longstreth et al., 1995), and increased interest in the mechanisms by which cells repair and/or tolerate UV-induced damage to DNA (Britt 1995, 1996; Cerutti et al, 1993; Sancar and Tang, 1993; Sancar and Sancar, 1988; Taylor, 1997; Taylor et al., 1997).

The increase in UV-B levels (Bais et al., 1996) is due to an accelerating depletion of the stratospheric ozone shield and is caused by man-made air-pollutants such as chlorofluorocarbons (CFCs) (Harm, 1980; Pang and Hays, 1991; Madronich, 1992; Stapleton, 1992; Stolarski et al., 1992). Despite its low concentration, ozone plays a critical role in chemical and biological processes by filtering ultraviolet radiation in the UV-B range.

Biological systems are vulnerable to wavelengths in the transitional range of 280 - 320 nm and are thus greatly affected by ozone losses (Caldwell et al., 1989; Rozema et al., 1997). Any perturbation that leads to an increase in UV-B radiation demands careful consideration of the possible consequences. As a result, the UV-B region of the ultraviolet spectrum has gained importance over other environmental factors (Madronich et al., 1999; Jordan 2002; Rozema et al., 2002).

The primary concern over ozone depletion is the potential impact on human health and ecosystems due to increased UV exposure. This enhanced exposure to UV-B is potentially detrimental to all living things but is particularly harmful to plants due to their obligatory requirement for sunlight for survival and their inability to move (Strid et al., 1994). Lower yields of certain cash crops and undesirable effects in agriculture may result due to increased UV-B stress (Caldwell et al., 1995); higher UV-B levels in the upper ocean layer may inhibit phytoplankton activities, which can have an impact on the entire marine ecosystem (Hader et al., 1995). Increases in skin cancer, skin ageing and cataracts in human populations are expected in a higher UV-environment

(Longstreth et al., 1995). In addition to direct biological consequences, indirect effects may arise through changes in atmospheric chemistry. Increased UV-B will alter photochemical reaction rates in the lower atmosphere that are important in the production of surface layer ozone which has detrimental effects on plants (Tang and Madronich, 1995).

A number of studies have been carried out to investigate whether increases in UV-B radiation resulting from ozone depletion would have a significant impact on plants, in particular on aspects of physiology and crop yield. Experimental work has been carried out in laboratory growth chambers, where plants are grown under artificial white light, to which is added different levels of UV-B, or outdoors, using artificial UV-B to supplement the UV-B in natural sunlight (Caldwell, 1977; Tevini and Teramura, 1989; Musil 1997). From these studies, it is now recognised that there are both direct and indirect effects of UV-B at the whole plant level, especially under natural conditions.

DIRECT EFFECTS OF UV-B ON PLANTS

Studies have shown that plants exhibit a tremendous variability in their sensitivity to UV-B radiation (Musil, 1995; Musil et al., 2002; Zuk-Golaszweska et al., 2003; Ryan et al., 1998). Responses that occur include changes in leaf secondary chemistry (flavonoid accumulation), alterations in leaf anatomy and morphology, reductions in net carbon assimilation capacity (photosynthesis) and changes in biomass allocation and growth (Musil, 1996). The direct UV-B action on plants that results in changes in form or function of plants appears to occur more often through altered gene activity rather than non-specific damage to DNA (Britt, 1997). These include both mechanistic damage to the photosynthetic apparatus and changes in growth and morphology which may reduce light interception and competitiveness. Direct effects include radiation-induced changes in photosynthesis, cell division and other life processes of direct importance to growth and development such as alterations in plant hormones or nucleic acids. These effects are observed after relatively short periods of irradiation, which could be hours or days (Bornman and Sundby-Emmanuelsson, 1995).

INDIRECT EFFECTS OF UV-B ON PLANTS

Indirect effects are those mediated by radiation-induced changes in the plant environment, or changes in the plant which are of importance mainly in relation to other organisms. Consequences of indirect solar UV-B radiation are quite important, yet less predictable, and act through changes in the chemical composition and growth form of plants through variations in the abiotic environment (Caldwell et al., 1998). Indirect effects include activities on other plants that compete with the plant under consideration, nutrient mobilisation, and on herbivores and micro-organisms of importance to the plant. Changes in the susceptibility of plants to attack by insects and pathogens in both agricultural and natural ecosystems could be some of the consequences. The direction of those changes can result in either a decrease or increase in susceptibility. Other indirect effects include changes in competitive balance of plants and nutrient cycling. To understand the effects of UV requires the study of several components of natural ecosystems.

In addition to the direct effects listed above, DNA damage reactions and also adaptive responses, including the switching on of a range of defence mechanisms to afford protection against UV radiation, can be included. Damage to DNA has long been recognised as an important consequence of exposure to UV and the products formed as a result of damage to DNA, and the activity of the photolyase enzyme(s) involved in repair have been described (McLennan, 1987; Pang and Hays, 1991; Quaite et al., 1992; Stapleton, 1992; Taylor et al., 1997). Some of the products are UV radiation-induced pyrimidine dimers, of which there are two major classes: the pyrimidine [6-4] pyrimidone photoproduct (6-4 product), and the cyclobutane pyrimidine dimer (CPD). Clearly, some of the UV-B induced down-regulation of photosynthetic genes (Jordan et al., 1992; Baker et al., 1997; Britt, 1997; Mpoloka, 2001) is not simply a consequence of non-specific damage to DNA, since other defence-related genes are up-regulated, and there is developmental variation in response to UV-B. Furthermore, protection under high light appears to be due to some component of photosynthetic activity rather than to photolyase activity.

As already stated, plants exhibit a wide range of responses to UV-B, including physiological responses which help to protect them from damaging UV-B wavelengths (Tevini and Teramura, 1989; Stapleton, 1992). The best studied direct UV protection mechanism is the differential production of pigments, especially flavonoids (Fohnmeyer et al., 1997; Musil, 1996). This type of response involves the stimulation of expression of particular genes by UV-B, implying specific UV-B light detection systems and signal transduction processes, which lead to the regulation of transcription. For example, the synthesis of UV-absorbing protective molecules such as flavonoids, hydroxycinnamic acids and related compounds is not a damage response and involves the stimulation of expression of particular genes (Jenkins et al., 1997a, b). The largest concentration of these pigments is located in the epidermis, effectively reducing the penetration of UV-B deeper into the mesophyll cells of the leaf (Bornman et al., 1997), thus acting to screen out the UV-B. It is evident that not all the effects of UV-B on plants involve macromolecular damage. Understanding the molecular basis of perception and signal transduction is therefore likely to be of direct relevance to a scenario of global climatic change.

Unlike studies of direct effects, where responses occur quite rapidly, studies of indirect effects need to be carried out over a long period of time since they are likely to be of a slow, cumulative nature. To date, more effort has been given to understanding the direct effects, and indeed these are probably the most important ones in crops, growing as they do in a partly human-controlled environment, and mostly in monoculture. Indirect effects, however, may be more important for wild populations in a natural environment.

The potential impacts of an increase in the solar UV-B radiation reaching the earth's surface have been investigated by several research groups during the past two decades (Caldwell et al., 1998; Grammatikopoulos et al., 1998; Strid et al., 1994; Tevini and Teramura, 1989). In these studies, UV-B radiation was found to be particularly detrimental to plants and this could possibly be due to the fact that the studies were performed with non-realistic doses and spectral distributions of UV-B radiation, usually in growth chambers or glasshouses where, in addition, the natural balance between UV-B, UV-A and visible radiation was considerably altered. Possible anomalies in assessing the biological effectiveness of the irradiation sources and of predicted ozone depletion could occur because of the wavelength dependency of photobiological processes and the fact that the artificial sources do not precisely match the solar spectrum. To interpret results from such investigations, weighting functions based on action spectra for specific responses, have been developed (Quaite et al., 1992; Tevini and Teramura, 1989; Caldwell et al., 1998).

The process by which light regulates aspects of plant growth is termed photomorphogenesis (Kendrick et al., 1997). Some of the sub-processes that are regulated by light signals include changes in structure and form, such as seed germination, leaf expansion, stem elongation, flower initiation and pigment synthesis. These photomorphogenic responses confer an enormous survival advantage on organisms. However, relatively little is known about the types of photomorphogenic responses and signal transduction pathways that plants employ in response to UV-B radiation. What is known is that competitive interactions may also be altered indirectly by differential growth responses, while photosynthetic activity may be reduced by direct effects on photosynthetic enzymes, metabolic pathways or indirectly through effects on photosynthetic pigments or stomatal function (Baker et al., 1997).

Overall, the effect of UV-B varies both among species and among cultivars of a given species. Sensitive plants often exhibit reduced growth (plant height, dry weight, leaf area, *etc.*,), photosynthetic activity and reduced flowering. Most of these responses are mediated by a number of light-absorbing molecules that enable organisms to respond to changes in the natural light environment. Because UV-B has been implicated in the inhibition of plant growth and possibly mutations, studies have been conducted to screen for mutants with enhanced sensitivity to either of these effects (Jenkins, 1997). It should be noted, however, that mutants expressing either of these UV-sensitive phenotypes might be defective in processes other than DNA repair, including defects in the production of UV-B absorbing pigments, defects in the ability to cope with a variety of stresses, or defects in other UV-sensitive processes such as photosynthesis. Notwithstanding, useful insights into the biochemical basis of UV-induced growth inhibition are likely to be gained through this approach.

The impact of an increase in UV-B on the physiological parameters and morphological features of plants has been studied extensively (Bornman and Teramura, 1993). However, the knowledge of the effects of UV-B at the biochemical and molecular levels is limited. It is well established that a major site of damage by UV-B is the chloroplast, leading to impairment of photosynthetic function (Bornman, 1989). In the chloroplast, the integrity of the thylakoid membrane seems to be much more sensitive than the activities of the photosynthetic apparatus bound within. A few studies have focused on the molecular mechanisms underlying UV-B sensitivity of photosynthesis (Strid et al., 1994; Baker et al., 1997; A-H Mackerness et al., 1999). Changes in gene expression reported in response to supplemental UV-B include reduction in expression and synthesis of key photosynthetic genes including Rubisco (*rbc*S and *rbc*L), D1 polypeptide of photosystem II (*psb*A), chlorophyll *a/b*-binding protein (*Lhcb* or *cab*), a decline in total RNA enzyme activity and the ATPase complex. The ATPase complex is involved in the hydrolysis of ATP to ADP and orthophosphate, as well as functioning as an exchanger or transporter for NA^+, K^+ or Ca^{2+} (Jordan, 1996; A-H Mackerness et al., 1997b; Baker et al., 1997). Decreases of mRNA transcripts for photosynthetic complexes and other chloroplast proteins have been reported as being among the very early events of UV-B damage, pointing to the effect of UV-B on photosynthesis as well as protein synthesis (Strid et al., 1994; Mpoloka, 2001). Other genes encoding defence-related enzymes e.g. of the flavonoid biosynthesis pathway, are rapidly up-regulated following commencement of UV-B exposure.

The plasma membrane of plant cells undergoes a number of changes in response to UV-B exposure. These changes include an efflux of K^+ ions, depolarisation of the cell's electrical potential, synthesis of H_2O_2, and oxidation of reduced glutathione (GSH) to oxidised glutathione (GSSG) (Strid et al., 1990). The changes to the plasma membrane are considered to be induced in response to UV-B and not a result of direct photochemical damage and subsequent loss of membrane integrity. Many different plant responses to supplemental UV-B radiation have been observed. These include biomass reduction, decreases in the percentage of pollen germination, changes in the ability of crop plants to compete with weeds, epidermal deformation, and changes in

cuticular wax and increased flavonoid levels. These changes could result from any number of primary UV-B events, DNA damage, direct photosynthetic damage, membrane changes, protein destruction, hormone inactivation, signal transduction through phytochrome (which photo-converts in response to UV-B), or signal transduction via an UV-B photoreceptor.

Nuclear encoded genes are reportedly more sensitive than genes encoded by the chloroplast (Jordan et al., 1992). The relative sensitivity of transcripts to UV-B radiation is also dependent on the developmental stages of the tissue studied. In contrast to the down-regulation of genes encoding photosynthetic proteins, UV-B irradiation results in the up-regulation of some defence genes such as chalcone synthase and glutathione reductase. Chalcone synthase (*chs*) is a key enzyme in the synthesis of flavonoids, which are produced under a variety of conditions, including UV-B exposure, and are thought to act as UV-B screening pigments. Glutathione reductase on the other hand, is thought to be an important part of a system designed to scavenge active oxygen produced in response to oxidative damage caused by exposure of plants to a multitude of stresses. This is essential for survival and for prevention of genetic diseases since unrepaired or improperly repaired lesions can be cytotoxic and mutagenic. Damage is initiated by a wide variety of environmental and endogenous agents, particularly reactive oxygen species (ROS) that are generated spontaneously during respiration.

An increase in the level of mRNA for these genes indicates that repression of gene expression for chloroplast proteins is a specific response to UV-B treatment and not a result of non-specific damage to DNA. Studies of the effects of UV-B on mRNA levels indicate that UV-B mediated inhibition of photosynthetic performance can, to some extent, be related to decreases in levels of photosynthetic proteins. However, they do not show whether the effect of UV-B arises through changes in transcription, translation or post-translational events (Mackerness et al., 1997a, b). The accumulation of flavonoid compounds in response to UV-B has been shown to be due to an increase in the rate of transcription of the chalcone synthase gene. However, the level at which UV-B down-regulates the genes encoding for the chloroplast proteins has not been studied.

PERCEPTION AND SIGNAL TRANSDUCTION OF UV-B RADIATION

Because plants are constantly exposed to solar radiation, the consequences of any increase in UV-B radiation are likely to be most obvious in their effect upon plant growth and development (Caldwell, 1981; Teramura, 1983; Tevini and Teramura, 1989). Individual plant cells have the capacity to respond to a wide range of signals that regulate their growth and differentiation and affect their survival. A key point is that the cells must have mechanisms that allow signals to be detected and acted upon to give rise to particular responses. For instance, to enable the UV-B radiation to have an effect, the plant must first absorb it. The plant may perceive the UV-B radiation by a specific mechanism involving photoreceptor molecules or by non-specific absorption by other cellular constituents (Frohnmeyer et al., 1997; Jenkins et al., 1997a; Jenkins, 1997). The detection of signals in many cases is likely to involve specific cellular components termed receptors, and reception is coupled to the terminal response by signal transduction mechanisms. Once the plant perceives the UV-B radiation, the information must be transmitted through cells or tissues to target sites where it may elicit a response. This transmission is frequently referred to as the signal transduction pathway and is composed of the secondary messenger(s), an amplification mechanism and the responsive component within the cell.

Biochemical approaches have mainly been used to study UV-B and blue/UV-A signal transduction pathways (Lois and Buchanan, 1994). A major advance was the cloning of the *Arabidopsis* CRY1 (cryptochrome) photoreceptor encoded by the *hy*4 gene (Ahmad and Cashmore, 1993, 1996). CRY1 mediates some responses to UV-A, blue and green light. Mutants in the *hy*4 gene are impaired in the suppression of hypocotyl extension by these wavelengths, and several other extension growth responses. In addition, *hy*4 mutants have reduced induction of flavonoid biosynthesis gene expression and anthocyanin synthesis in blue light (Jenkins, 1997). CRY1 is postulated to regulate the extension growth and gene expression responses through separate or branching signal transduction pathways. Studies of the CRY1 protein indicate that it binds flavin and pterin chromophores, pointing to the possibility of electron transport being an initial event in CRY1 signal transduction. Evidence that membrane processes are involved in signal transduction is also available. For instance, calcium fluxes appear to be part of the signalling events which couple CRY1 to the regulation of transcription (Christie and Jenkins, 1996). In addition, membrane potential changes and ion fluxes are associated with blue light–induced extension growth responses. Furthermore, these blue light induced membrane potential changes and H^+ fluxes are associated with stomatal openings in several species.

FLAVONOIDS BIOSYNTHESIS AND THEIR ROLE IN PLANT DNA PROTECTION

Rapid protective response to the damaging effects of UV irradiation is paradigmatic of active defence mechanisms in plants. In many cases, protection is thought to derive from the induced accumulation of strongly UV-absorbing flavonoid compounds in the outer tissue layers, preferentially in epidermal layers, which presumably protect sensitive targets from UV-damage (Schulze-Lefert et al.,

1989). Most higher plants accumulate UV-B absorbing pigments in their leaves particularly phenylpropanoids such as cinnamoyl esters, flavones, flavonols, and anthocyanins esterified with cinnamic acids after irradiation with UV-B (Wellmann, 1983). In addition to phenyl propanoids, other important products of the shikimic acid pathway such as furanocoumarins, and polyketides and terpenoids such as canabinoids, also accumulate under increased UV-B radiation. Several researchers have found key enzymes in the biosynthetic pathways of these compounds to be specifically induced by UV-B irradiation (Schulze-Lefert et al., 1989; Stapleton, 1992; Middleton and Teramura, 1993; Kootstra, 1994).

These phenolic compounds attenuate the damaging UV-B radiation but transmit photosynthetically active radiation (PAR) to the underlying palisade and mesophyll tissue where the bulk of photosynthetic reactions take place. For example, in maize UV-B absorbing flavonoid pigments have been implicated in UV-protection (Stapleton and Walbot, 1994), whereas sinapic esters which are biosynthetic precursors of lignin may be particularly important as sunscreens in *Arabidopsis* (Landry et al., 1995). However, sunscreens are not completely effective and plants must also have mechanisms that enable them to cope with the cellular damage caused by the UV radiation that is able to penetrate the cells. To mitigate the effects of UV-induced DNA damage on transcription and replication, all organisms exhibit a range of DNA damage control strategies, and many of these repair mechanisms appear to be broadly conserved.

It has been shown that flavonol accumulation is specifically UV-induced and is linearly dependent on UV-B fluence (Wellman, 1983; Schemelzer et al., 1988). This increase in flavonoid concentration is due to a higher activity of the key enzyme PAL (phenylalanine ammonium lysase) and/or to higher rates of biosynthesis of this enzyme (Tevini and Teramura, 1989). Biosynthesis of various flavonoids from phenylalanine is brought about via transcriptional activation of genes of phenylpropanoid metabolism. The biosynthetic pathway of all classes of flavonoids (Grisebach, 1979; Halbrock and Grisebach 1979; Wong 1976) is initiated by the enzyme chalcone synthase (CHS) (Heller and Forkmann, 1988; Mol et al., 1996). CHS and other enzymes involved in biosynthesis serve as model systems for a better understanding of molecular aspects of gene regulation by light as these pigments have an important function against damaging effects from shorter wavelength solar radiation.

EFFECTS OF UV-B ON DNA

Radiation and some chemical agents frequently produce modified bases within DNA that prevent accurate replication or transcription (D'mitry et al., 1995). For example, ultraviolet radiation UV-B and or UV-C gives rise to a multitude of DNA photoproducts (Sancar and Sancar, 1988; Taylor et al., 1997). Biological effects of these lesions have been studied extensively in mammalian and microbial systems where the UV-B induced damage to DNA has been shown to lead to either mutagenesis or toxicity (Britt, 1997). These damaged DNA molecules may cause mutations if replicated (Jiang and Taylor, 1993), thus repair of UV-B radiation-induced damage to DNA is important for all living organisms, especially plants. However, very few studies with plants have been directed toward the identification of DNA photoproducts, subsequent mutations and repair mechanisms (Stapleton, 1992; Strid et al., 1994; Kootstra, 1994; Britt, 1995; Buchholz et al., 1995). At the molecular level, pyrimidine dimers are known to inhibit the progress of microbial and mammalian DNA polymerases. The formation of these DNA photoproducts gives rise to changes in the base-pairing properties between two DNA strands at the site of the lesion. The change in base-pairing properties is most notable for the cytosine (C)-derived pyrimidine adducts and results in a high degree of adenosine (A) incorporation into the new complementary DNA strand during replication. With TT photoproducts, such misincorporation occurs only rarely. Because pyrimidine dimers cannot effectively base pair with other nucleotides, they are not directly mutagenic, but instead act as blocks to DNA replication (Britt, 1997). Thus a single pyrimidine dimer, if left unrepaired, is sufficient to completely eliminate expression of a transcriptional unit. In addition to the two most common UV-induced photoproducts already mentioned, other types of DNA photoproducts exist, such as purine containing photoproducts, hydration of pyrimidines, insertion or deletion of base pairs resulting in frame-shifts, DNA strand breaks and cross-linking of DNA to proteins.

Although the detailed mechanism by which biological effects of UV are produced and the results of such increased UV-B exposure at the molecular level are largely unknown, the evidence is overwhelming that changes in DNA such as formation of pyrimidine dimers and other photochemical products, have important biological consequences (Stapleton, 1992; Kootstra, 1994; Strid et al., 1994; Britt, 1995). Despite the lack of thorough characterisation of the biological effects of these lesions, they are generally assumed to be similar to those observed in other living organisms. In many cases, for microbial systems, it has been possible to correlate the production of specific photoproducts in DNA with biological changes such as the inactivation of biologically active DNAs, the killing of cells and the induction of mutations (Setlow and Setlow, 1972; Setlow, 1974).

The formation of different photoproducts upon UV-B radiation exposure damages DNA molecules and eventually blocks DNA replication and transcription in plants cells. Even a single persisting UV-induced lesion can be a potentially lethal event, particularly in haploid tissues such as pollen grains (Britt, 1997). This is because DNA is a highly reactive molecule that is sensitive to damage from a wide range of both physical and chemical

agents (making it an especially vulnerable target for UV-induced damage), as well as being considered to be the primary absorbing chromophore in the cell in the UV-B region of the spectrum. If every pyrimidine dimer acts as a block to transcription and replication, while only a small fraction of dimers result in a mutation, the inhibitory effects of UV on transcription and DNA replication are probably more significant in terms of plant growth than its mutagenic effects.

DNA double-strand breaks (DSBs) on chromosomes may arise during DNA replication, cleavage by site-specific endonucleases, or due to exogenous factors such as ionising radiation or chemical DNA-damaging agents (Michel et al., 1997; Haber, 1999). DSBs are key intermediates in recombination reactions of living organisms and play an important role in homologous recombination in eukaryotes (Puchta et al., 1996). Induction of DSBs has been shown to increase the frequency of homologous recombination. Since DSBs represent potential lethal lesions, all organisms have developed repair pathways to correct such errors.

Since the UV component of sunlight produces cytotoxic, mutagenic and carcinogenic lesions in DNA, a variety of mechanisms for repairing or circumventing DNA damage have been reported in both prokaryotes and eukaryotes, and the genes that control these mechanisms are regulated by DNA damage. In order to prevent alterations in their genetic information due to DNA damage, organisms have developed efficient mechanisms of DNA repair and recombination (Britt, 1995, 1996; Cerutti et al., 1993). A number of studies aimed at elucidating these repair processes that counteract the deleterious effects of UV radiation on cellular DNA have been conducted. The most important discovery in this area of research in recent years is that all cells have a remarkable capacity to repair damage that is produced by UV in their DNA, and the most detailed studies have been carried out on *Escherichia coli*, yeast and mammalian cells (Sancar and Tang, 1993).

Based on these studies, cells appear to have evolved a variety of biochemical mechanisms to restore the integrity and retain the stability of the genetic material after DNA damage. These processes, defined as DNA repair, are organised into a number of pathways that are functionally distinguished by the type of chemical modification which they modulate (Taylor et al., 1997). The systems of repair responsible for the removal of damage by UV-B radiation from cellular DNA include photoreactivation (PHR), excision repair, including nucleotide excision repair (NER) and base excision repair (BER), recombinational repair and post replication repair (Sancar and Sancar, 1988; Sakamoto et al., 1998; Caldwell, 1981; Vonarx et al., 1998; Van Dyck et al., 1999).

RECOMBINATIONAL REPAIR

Double strand breaks in DNA can be repaired in several ways and the most economical is ligation with another available DNA end by a process called non-homologous end-joining (also called illegitimate recombination) or the break may be repaired through genetic exchange with a homologous chromosome (homologous recombination). This dark repair system, also termed post-replication or recombinational repair, takes place after normal DNA replication (Caldwell, 1981; Vonarx et al., 1998; Van Dyck et al., 1999). It is of great importance that cells recognise the DNA DSBs and act upon them rapidly and efficiently, because major deleterious consequences can result if these are left unrepaired or are repaired inaccurately.

It should be noted that very little is known about the genes required for illegitimate recombination in plants, but some *Arabidopsis* mutants displaying sensitivity to ionising radiation have been isolated (Britt, 1996). In *E. coli*, UV-photoproducts that are not repaired or removed prior to DNA replication may be "tolerated" by *recA*-dependent translession synthesis or post-replication mechanisms, and similar tolerance mechanisms have been reported in yeast involving members of the *RAD6* epistasis group for repair of UV-damage (Vonarx et al., 1998). Genes encoding these post-replication homologues have now been isolated from wheat and *Arabidopsis*. For example, two cDNAs (*DRT111* and *DRT112)* for genes potentially involved in homologous recombination were isolated and they are postulated to play a role in DNA repair and /or recombination in *Arabidopsis*.

Ries et al. (2000a) reported a reduction in genome stability in plants due to elevated UV-B radiation. Elevated solar UV-B doses increased the frequency of somatic homologous DNA rearrangements in *Arabidopsis* and tobacco plants. The authors analysed the recombination frequency in somatic (non-reproductive) cells in response to natural spectral tolerance and also when UV-B was raised to higher levels. The progeny of plants exposed to high UV-B levels had a higher somatic mutation rate than their predecessors, and elevated UV-B was found to reduce genome stability in *A. thaliana*, suggesting that plants were undergoing heritable and cumulative changes in the expression of genes involved in DNA metabolism.

Even though UV-B has been found to cause changes mainly in somatic tissue that is not going to transmit genes to the next generation, Ries et al. (2000a) reported UV-B induced recombination in the reproductive (germ) cells, pointing to the likelihood of permanent changes in plant populations as a result of increased UV-B radiation levels. Plants showed somatic recombination in response to ecologically relevant increases in UV-B radiation, and large elevations in UV-B radiation caused further genomic instability. In the same study, increases in recombination were accompanied by strong induction of expression of genes putatively involved in major DNA repair pathways (photoreactivation and recombination repair), namely photolyase and Rad51. This study presented intriguing data suggesting that elevated UV-B exposure

over several generations may lead to progressive increases in somatic recombination rates as well as to higher numbers of permanently altered plants. An interesting observation was that effects of UV-B on genomic instability increased with each generation, suggesting that plants are undergoing heritable and cumulative changes in the expression of genes involved in DNA metabolism.

The strategies employed for the removal of DNA lesions show a high degree of evolutionary conservation between micro-organisms and humans, and CPD product repair in most organisms is due in large part to different combinations of the repair processes already described. Only recently have investigations focused upon DNA repair mechanisms in plants where it is assumed that similar mechanisms to those in other organisms operate. The precise mechanism of the DNA excision repair, which is prevalent from prokaryotes to eukaryotes, remains unknown, although a full understanding of its molecular basis is crucial for cell biology and the medical sciences.

PHYSIOLOGICAL AND BIOCHEMICAL EFFECTS OF CUMULATIVE UV-B EXPOSURE

In vitro physiological and biochemical studies on effects of exposure of a desert annual, D*imorphotheca sinuata* to UV-B (Musil and Wand, 1993; Musil, 1995, 1996; Midgley et al., 1998) point to the possibility that UV-B effects could be cumulative. The studies also showed that accumulated UV-B effects had a greater impact on plant performance than immediate UV-B effects (Musil, 1994, 1995). Changes brought about by accumulated UV-B included earlier reproductive effort, substantial reductions in dry mass, decreased stem and inflorescence production, diminished steady-state fluorescence yields, chlorophyll-*a* concentrations, pollen tube growth and germination of seeds set. On the other hand, immediate UV-B effects caused only diminished non-photochemical quenching, reduced concentrations of chlorophyll-*a*, soluble sugar and starch, decreased pollen germination and increased carotenoid contents (Musil, 1996). The effects of UV-B irradiation on growth and allocation of biomass appeared to accumulate as subsequent generations were exposed to UV-B irradiation. Furthermore, after four generations of UV-B irradiation, the effects persisted in a fifth generation that was not exposed to UV-B treatment, implying that the effects of UV-B irradiation changes could be amplified (Musil, 1996). This phenomenon could also be apparent in long-lived woody plants (Xiong and Day, 2001; Day et al., 1999; 2001).

Damage to DNA caused by UV-B exposure during plant development may not be fully repaired, and thus could be inherited by offspring and accumulated over successive generations of different plant species (Musil, 1996). Since the genomes of plant reproductive organs are generally well screened from UV-B during both developmental and mature phases (by thick ovary walls and thick anther sacs for male gametes and perianth tissue that has been reported to transmit low UV-B levels), it has been postulated that pollen grains are the most likely candidates for damage by UV-B radiation. Pollen grains have indeed been reported to be particularly susceptible to UV-B radiation during the short period between anther dehiscence and pollen tube penetration into stigmatic tissue (Musil and Wand, 1994; Midgley et al., 1998). Findings of UV-B-induced reductions in pollen viability have also been reported in several South African annual species grown under enhanced UV-B (Musil, 1995). From these findings it has been postulated that pollen grains form an ecologically critical developmental stage of the plant, and in its natural state, *D. sinuata* pollen could be exposed to UV-B during the period between anther dehiscence pollination, and therefore is potentially vulnerable to genetic damage by UV-B.

Diminished pollen quality could imply that UV-B radiation interferes with pollen development or that there has been DNA damage in the pollen grains, which could cause mutations during replication of damaged DNA after fertilisation. This would, in turn, alter DNA integrity sufficiently to affect subsequent plant performance (Jiang and Taylor, 1993). McLennan (1987) reported the partial purification of the enzyme photolyase from maize pollen and several types of bean, implying that repair of DNA damage to pollen is essential for survival. UV-B irradiation of maize pollen has been reported to activate cryptic transposable elements and under such conditions, recombination processes may also be induced, thus affecting the genome stability of future plant generations (Walbot, 1999).

Even though UV-B has been found to cause changes mainly in somatic tissue that is not going to transmit genes to the next generation, UV-B-induced recombination has been reported in the reproductive (germ) cells, pointing to the likelihood of permanent changes in plant populations as a result of increased UV-B radiation levels (Ries et al., 2000a,b). The progeny of plants exposed to high UV-B levels had a higher somatic mutation rate than their predecessors, suggesting that plants were undergoing heritable and cumulative changes in the expression of genes involved in DNA metabolism. In one study, elevated UV-B was found to reduce genome stability in *A. thaliana*, and the results suggested that plants were undergoing heritable and cumulative changes in the expression of genes involved in DNA metabolism (Ries et al., 2000a). Another study involving chloroplast DNA analysis showed polymorphisms that could be attributed to evolutionary processes acting on natural populations exposed to elevated UV-B levels that may result in rearrangements of the genome (Mpoloka et al., 2007).

CONCLUSION

From the literature (Caldwell, 1977; Caldwell et al., 1989,

1995, 1998; Madronich 1992; Madronich et al., 1998; Rozema et al., 1997; Teramura 1983; Tevini and Teramura, 1989) it is apparent that there has been a reduction in the amount of ozone in the stratosphere due to man-made CFCs resulting in an increase in UV-B radiation reaching the earth's surface. This increase in UV-B has been found to cause both photomorphogenic as well as genetic changes in plants. Photoreceptors acting through signal transduction pathways are responsible for sensing this ultraviolet radiation. Several components of the photosynthetic apparatus have been found to be affected by UV-B, with nuclear encoded genes being more sensitive to UV-B than chloroplast encoded genes. However, long-term effects of UV-B radiation in plants are still not well understood, therefore, more research need to be carried out over longer time periods to provide definitive answers to questions such as cumulative effects of UV-B, effects of UV-B at ecosystem level, and interactions of elevated UV-B with other stress factors. Currently not enough is known about the reasons for the large UV-B response differences among cultivars observed by a number of researchers (Musil, 1994; Mpoloka, 2001; Chimphango et al., 2007). Ideally it is necessary to understand the genetic basis (heritability) of UV-tolerance and sensitivity. Once this is known, an estimate of the possibility of using conventional breeding practices to minimise the potential effects of UV-damage could be made. At present plant breeders have not yet considered UV-sensitivity as a selective factor.

REFERENCES

Ahmad M, Cashmore AR (1993). *HY4* gene of *A. thaliana* encodes a protein with characteristics of a blue-light photoreceptor. Nature 366: 162-166.

Ahmad M, Cashmore AR (1996). Seeing blue: The discovery of cryptochrome. Plant Mole. Biol. 30: 851-861.

Bais AF, Zerefos CS, McElroy CT (1996), Solar UVB Measurements with the Double- and Single-monochromator Brewer Ozone Spectrophotometers, Geophy. Res. Lett. 23(8): 833-836.

Baker NP, Nogues S, Allen DJ (1997). Photosynthesis and photoinhibition. *In* Lumsden P (ed.). Plants and UV-B : Responses to Environmental Change, Cambridge University Press pp 95-111.

Bornman JF (1989). Target sites of UV-B radiation in photosynthesis of higher plants. J. Photochem. Photobiol. 4: 145-158.

Bornman JF, Sundby-Emmanuelson C (1995). Response of plants to UV-B radiation:some biochemical and physiological effects. *In* N. Smirnoff (ed), Environment and Plant Metabolism, Bioscientific, Oxford pp 245-262.

Bornman JF, Teramura AH (1993). Effects of UV-B radiation on terrestrial plants. In Young AR, Bjorn LO, Moan J and Nultsch W, eds. Environmental UV Photobiology. Plenum Publishers Co, New York. pp 427-471

Bornman JF, Reuber S, Cen YP, Weissenbock G (1997). Ultraviolet radiation as a stress factor and the role of protective pigments. *In* Lumsden P (ed.). Plants and UV-B : Responses to Environmental Change, Cambridge University Press pp 156-168.

Britt AB (1995). Repair of DNA damage induced by ultraviolet radiation. Plant Physiol. 108: 891-896.

Britt AB (1996). DNA damage and repair in plants. Ann. Rev. Plant Physiol. Plant Mole. Biol. 47: 75-100.

Britt AB (1997). Genetic analysis of DNA repair in plants. In Lumsden P (ed.). Plants and UV-B : Responses to environmental change, Cambridge University Press. pp 77-93.

Buchhols G, Ehmann B, Wellmann E (1995). Ultraviolet light inhibition of phytochrome-induced flavonoid biosynthesis and DNA photolyase formation in mustard cotyledons (*Sinapsis alba* L.). Plant Physiol. 108: 227-234.

Caldwell MM (1977). The effects of solar UV-B radiation (280-315nm) on higher plants : implications of stratospheric ozone reduction. In Castellani A. ed., Research in Photobiology, Academic Press, New York pp 597-607.

Caldwell MM (1981). Plant response to solar ultraviolet radiation. Encyclopaedia Plant Physiol. 12A: 169-197.

Caldwell MM, Björn LO, Bornman JF, Flint SD, Kulandaivelu G, Teramura AH, Tevini M (1998). Effects of increased solar ultraviolet radiation on terrestrial ecosystems. J. Photochem. Photobiol. B: Biol. 46: 40-52.

Caldwell MM, Teramura AH, Tevini M, Bornman JF, Bjorn LO, Kulandaivelu G (1995). Effects of increased solar ultraviolet radiation on terrestrial plants. Ambio 24: 166-173.

Caldwell MM, Teramura AH, Tevini M (1989). The changing solar ultraviolet climate and the ecological consequences for higher plants. TREE 4: 363-367.

Cerutti H, Ibrahim HZ, Jagendorf AT (1993). Treatment of pea (*Pisum sativum* L.) protoplasts with DNA-damaging agents induces a 39-kilodalton chloroplast protein immunologically relateed to *Escherichia coli* RecA. Plant Physiol. 102: 155-163.

Chimphango SBM, Brown CF, Musil CF, Dakora FD (2007). Effects of UV-B radiation on seed yield of *Glycine max* and an assessment of F_1 generation progeny for carryover effects. *Physilogia Plantarum* 131: 378-386.

Christie JM, Jenkins GI (1996). Distinct UV-B and UV-A/blue light signal transduction pathways induce chalcone synthase gene expression in Arabidopsis cells. Plant Cell 8: 1555-1567.

Day TA, Ruhland CT, Xiong F (2001). Influence of solar ultraviolet-B radiation on Antarctic terrestrial plants: results from a four year field study. J. Photochem. Photobiol. 62: 78-87.

Day TA, Ruhland CT, Grobe CW, Xiong F (1999). Growth and reproduction of Antarctic vascular plants in response to warming and UV radiation reductions in the field. Oecologia. 119: 24-35.

Dmitry GV, Kashiwagi T, Mikami Y, Ariyoshi M, Iwai S, Ohtsuka E, Morikawa K (1995). Atomic model of a pyrimidine dimer excision repair enzyme complexed with a DNA substrate: structural basis for damaged DNA recognition. Cell *83*: 773-782.

Frohnmeyer H, Bowler C, Schafer E (1997). Evidence of some signal transduction elements involved in UV-light-dependent responses in parsley protoplasts. J. Exp. Bot 48: 739-750.

Grammatikopoulos G, Karousou R, Kokkini S, Manetas Y (1998). Differential effects of enhanced UV-B radiation on reproductive effort in two chemotypes of *Mentha spicata* under field conditions. Austr. J. Plant Physiol. 25: 345-351.

Grisebach H (1979). Selected Topics in flavonoid biosynthesis. In Swain T et al (eds) Recent Advances in Phytochemistry Plenum Press, NY, London. 12: 221-248.

Haber JE (1999). Gate keepers of recombination. Nature 398: 665-667.

Hader DP, Worrest RC, Kumar HD, Smith RC (1995). Effects of increased solar ultraviolet radiation on acquatic ecosystems. Ambio 24: 174-180.

Halbrock K, Grisebach H (1979). Enzymic control in the biosynthesis of lignin and flavonoids. Ann. Rev. Plant Physiol. 30: 105-130.

Harlow GR, Jenkins ME, Pittalwala TS, Mount DW (1994). Isolation of *uvh1*, an *Arabidopsis* mutant hypersensitive to ultraviolet light and ionizing radiation. Plant Cell 6: 227-235.

Harm W (1980). Biological effects of ultraviolet radiation (Cambridge University Press).

Heller W, Forkmann G (1988). Biosynthesis. In: Harborne JB (ed). The flavonoids. Advances in research since 1980 Chapman Hall, London. pp 399- 425.

Jenkins GI (1997). UV and blue light signal transduction in *Arabidopsis*. Plant, Cell Environ. 20: 773-778.

Jenkins GI, Christie JM, Fuglevand G, Long JC, Jackson JA (1997b). Plant responses to UV and blue light: biochemical and genetic approaches. Plants Sci. 112: 117-138.

Jenkins GI, Fuglevand G, Christie JM (1997a). UV-B perception and signal transduction. In Plants and UV-B: Responses to environmental change. Lumsden P (ed.) (Cambridge University Press) pp. 135-156.
Jiang N, Taylor JS (1993). *In vivo* evidence that UV-induced C->T mutations at dipyrimidine sites could result from the replicative bypass of cis-syn cyclobutane dimers on their deamination products. Biochemistry 32: 472-481.
Jordan BR (1996). The effects of ultraviolet radiation on plants: a molecular perspective. Adv. Bot. Res. 22: 97-162.
Jordan BR (2002). Molecular response of plant cells to UV-B stress. Functional Plant Biol. 29: 909-916.
Jordan BR, He J, Chow WS, Anderson JM (1992). Changes in mRNA levels and polypeptide subunits of ribulose 1,5-bisphosphate carboxylase in response to supplementary ultraviolet radiation. Plant Cell Environ. 15: 91-98.
Kendrick RE, Kerckhoffs LHJ, Tuinen AV, Koornneef M (1997). Photomorophogenic mutants of tomato. Plant Cell Environ. 20: 746-751.
Kootstra A (1994). Protection from UV-B induced DNA damage by flavonoids. Plant Mol. Biol. 26: 771-774.
Landry LG, Chapple CCS, Last RL (1995). Arabidopsis mutants lacking phenolic sunscreens exhibit enhanced ultraviolet-B injury and oxidative damage. Plant Physiol. 109: 1159-1166.
Lois R, Buchanan BB (1994). Severe sensitivity to ultraviolet radiation in an *Arabidopsis* mutant deficient in flavonoid accumulation II. Mechanisms of UV-resistance in *Arabidopsis*. Planta 194: 504-509.
Longstreth JD, de Gruijl FR, Kripke ML, Takizawa Y, van der Leun JC (1995). Effects of increased solar ultraviolet radiation on human health. Ambio 24: 153-165.
Mackerness AHS, Jordan BR, Thomas B (1997a). UV-B effects on the expression of genes encoding proteins involved in photosynthesis. In Lumsden P (ed.). Plants and UV-B: Responses to Environmental Change, Cambridge University Press. (pp 113-134).
Mackerness AHS, Jordan BR, Thomas B (1997b). The effects of supplementary ultraviolet-B radiation on mRNA transcripts, translation and stability of chloroplast proteins and pigment formation in *Pisum sativum* L. J. Exp. Bot. 48: 729-736.
Mackerness AHS, Surplus SL, Blake P, John CF, Buchana-Wollaston V, Jordan BR, Thomas B (1999). Ultraviolet-B-induced stress and changes in gene expression in *Arabidopsis thaliana*: role of signalling pathways controlled by jasmonic acid, ethylene and reactive oxygen species. Plant Cell Environ. 22: 1413-1423.
Madronich S (1992). Implications of recent total atmospheric ozone measurements for biologically active ultraviolet radiation reaching the earth's surface. Geophy. Res. Lett. 19: 337-40.
Madronich S, McKenzie RL, Bjorn LO, Caldwell MM (1998). Changes in biologically active ultraviolet radiation reaching the earth surface. In Environmental Effects of Ozone Depletion:1998 Assesment, United Nations Environment Programme, Ozone Secretariat, Nairobi, Kenya. pp1-27.
McLennan AG (1987). The repair of ultraviolet light-induced DNA-damage in plant cells. Mutation Res. 181: 1-7.
Michel B, Ehrlich SD, Uzzest M (1997). DNA double-strand breaks caused by replication arrest. EMBO J. 16: 430-438.
Middleton EM, Teramura AH (1993). The role of flavonol glycosides and carotenoids in protecting soybean from UV-B damage. Plant Physiol. 103: 741-752.
Midgley GF, Wand SJE, Musil CF (1998). Repeated exposure to enhanced UV-B radiation in successive generations increases developmental instability (fluctuating asymmetry) in a desert annual. Plant Cell Environ. 21: 437-442.
Mol J, Jenkins G, Schafer E, Weiss D (1996). Signal perception, transduction, and gene expression involved in anthocyanin biosynthesis. Crit. Rev. Plant Sci. 15: 525-557.
Mpoloka SW (2001). Genetic effects of prolonged UV-B exposure in a Namaqualand daisy – *Dimorphotheca sinuata*. PhD Thesis, University of Cape Town, South Africa.
Mpoloka SW, Abratt VA, Mundree SG, Thomson JA, Musil CF (2007). Potential effects of prolonged ultraviolet radiation exposure in plants: chloroplast DNA analysis. Am.-Eurasian J. Agric. Environ. Sci. 2(4): 437-441.
Musil CF (1994). Ultraviolet-B irradiation of seeds affects photochemical and reproductive performance of the arid-environment ephemeral *Dimorphotheca pluvialis*. Environ. Exp. Bot. 34: 371-378.
Musil CF (1995). Differential effects of elevated ultraviolet-B radiation on the photochemical and reproductive performances of dicotyledonous and monocotyledonous arid-environment ephemerals. Plant Cell Environ. 18: 844-854.
Musil CF (1996). Cumulative effect of elevated ultraviolet-B radiation over three generations of the arid environment ephemeral *Dimorphotheca sinuata* DC (Asteraceae). Plant Cell Environ. 19: 1017-1027.
Musil CF, Wand SJE (1993). Responses of *Sclerophyllous* Ericaceae to enhanced levels of ultraviolet-B radiation. Environ. Exp. Bot. 33: 233-242.
Musil CF, Wand SJE (1994). Differential stimulation of an arid-environment winter ephemeral *Dimorphotheca pluvialis* (L.) Moench by ultraviolet-B radiation under nutrient limitation. Plant Cell Environ. 17: 245-255.
Musil CF, Chimphango SBM, Dakora FD (2002). Effects of elevated ultraviolet-B radiation on native and cultivated plants of southern Africa. Anna. Bot. 90: 127-137.
Pang Q, Hays JB (1991). UV-B-inducible and temperature-sensitive photoreactivation of cyclobutane pyrimidine dimers in *Arabidopsis thaliana*. Plant Physiol. 95: 536-543.
Puchta H, Dujon B, Hohn B (1996). Two different bu related mechanisms are used in plants for the repair of genomic double-strand breaks by homologous recombination. Proceed. Nat. Acad. Sci. 93: 5055-5060.
Quaite FE, Sutherland BM, and Sutherland JC, (1992). Action spectrum for DNA damage in alfalfa lowers predicted impact of ozone depletion. Nature 358: 576-578.
Ries G, Buchholz G, Frohnmeyer H and Hohn B (2000b). UV-B-mediated induction of homologous recombination in *Arabidopsis* is dependent on photosynthetically active radiation. Proc. Natl. Acad. Sci. 97: 13425-13429.
Ries G, Heller W, Puchta H, Sandermann H, Seidlitz HK and Hohn B (2000a). Elevated UV-B radiation reduces genome stability in plants. Nature 406: 98-101.
Rozema J, Bjorn OL, Bornman JF (2002). The role of UV-B radiation in aquatic and terrestrial ecosystems – an experimental and functional analysis of the evolution of UV-B absorbing compounds. J. Photochem. Photobiol. B: Biol. 66: 2-12.
Rozema J, Staalj van de J, Bjorn OL, Caldwell M (1997). UV-B as an environmental factor in plant life: stress and regulation. Trends Ecol. Evol. 12(1): 22-28.
Sakamoto A, Tanaka A, Watanabe H and Tano S (1998). Molecular cloning of *Arabidopsis* photolyase gene (*PHR1*) and characterization of its promoter region. DNA Sequence- J. Sequencing Mapping 9: 335-340.
Sancar A, Sancar GB (1988). DNA repair enzymes. Annu. Rev. Biochem. 57: 29-67.
Sancar A and Tang MS (1993). Photobiology school:nucleotide excision repair. Photochem. Photobiol. 57: 905-921.
Schmelzer E, Jahnen W, Halbrock K (1988). *In situ* localization of light-induced chalcone synthase mRNA and flavanoid end products in epidermal cells of parsley leaves. Proceed. Nat. Acad. Sci. USA.85: 2989-2993.
Schulze-Lefert P, Becker-Andre M, Schulz W, Hahlbrock K, Dangl JL (1989). Functional Architecture of the light-responsive chalcone synthase, and flavonoid end products in epidermal cells of parsley leaves. Proceed. Nat. Acad. Sci. USA. 85: 2989-2993.
Setlow RB (1974). The wavelengths in sunlight effective in producing skin cancer: A theoretical analysis. Proceed. Natl. Acad. Sci USA 71: 3363-3366.
Setlow RB, Setlow JK (1972). Effects of radiation on polynucleotides. Ann. Rev. Biophy. Bioeng. 1: 293-346.
Stapleton AE (1992). Ultraviolet Radiation and Plants: Burning Questions. Plant Cell 4: 1353-1358.
Stapleton AE, Walbot V (1994). Flavonoids protect maize DNA from the induction of ultraviolet radiation damage. Plant Physiol. 105: 881-889.
Stolarski R, Bojkov R, Bishop L, Zerefos C, Staehelin J, Zawodny J (1992). Measured trends in stratospheric ozone. Science 256: 342-349.

Strid A, Chow WS, Anderson JM (1990). Effects of supplementary ultraviolet-B radiation on photosynthesis in *Pisum sativum*. Biochem. Biophys. Acta 1020: 260-268.
Strid A, Chow WS, Anderson JM (1994). UV-B damage and protection at the molecular level in plants. Photosynth. Res. 39: 475-489.
Tang X, Madronich S (1995). Effects of increased solar ultraviolet radiation on trophospheric composition and air quality. Ambio 24: 188-190.
Taylor CB (1997). Damage control. The Plant Cell 9: 111-114.
Taylor RM, Tobin AK, Bray CM (1997). DNA damage and repair in plants. Plants and UV-B: Responses to environmental change. Edited by Lumsden P, Cambridge University Press.
Teramura AH (1983). Effects of UV-B radiation on the growth and yield of crops. Physiol. Plant. 58: 415-427.
Tevini M, Teramura AH (1989). UV-B effects on terrestrial plants. Photochem. Photobiol. 50: 479-487.
Van Dyck E, Stasiak AZ, Stasiak A, West SC (1999). Binding of double-strand breaks in DNA by human Rad52 protein. Nature 398: 728-731.
Vonarx EJ, Mitchell HL, Karthikeyan R, Chatterjee I, Kunz BA (1998). DNA repair in higher plants. Mutation Res. 400: 187-200.
Walbot V (1999). UV-B damage amplified by transposons in maize. Nature 397: 398-399.
Wellman E (1983). UV radiation : definitions, characteristics and general effects. In Encyclopedia of Plant Physiology, New Series. Photomorphogenesis (Edited by Shroshirre W, Mohr H) 16B: 745-756, Springer, Berlin.
Wong E (1976). Biosynthesis of flavonoids. In : Goodwin TW (ed). Chemistry and biochemistry of plant pigments Academic Press, NY, London 1: 464-526.
Xiong F, Day TA (2001). Effects of solar ultraviolet-B radiation during spring time ozone depletion on photosynthesis and biomass production of Antarctic vascular plants. Plant Physiol. 125: 738-751.
Zuk-Golaszewska K, Upadhyaya MK, Golaswewski J (2003). The effect of UV-B radiation on plant growth and development. Plant Soil Environ. 49: 135-140.