PHYSICAL, BIOCHEMICAL AND PHYSIOLOGICAL EFFECTS OF ULTRAVIOLET RADIATION ON *BRASSICA NAPUS* AND *PHASEOLUS VULGARIS*

Yan-Ping Cen

SECTION OF PLANT PHYSIOLOGY
LUND UNIVERSITY, SWEDEN

LUND 1993

To my parents and my family

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故足之于地也践，虽践，恃其所不踩而后善博也；人之于知也少，虽少，恃其所不知而后知天之所谓也。

——《庄子》

Physical, Biochemical and Physiological Effects of Ultraviolet Radiation on *Brassica napus* and *Phaseolus vulgaris*

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Academic Dissertation

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Abstract

In order to follow some of the changes induced by ultraviolet-B (UV-B, 280-320 nm) radiation in *Phaseolus vulgaris* and *Brassica napus*, experiments were designed to localise sites of change in leaves and to correlate some of the physiological and biochemical changes with penetration of UV-B radiation. In addition, the influence of different levels of visible radiation on the UV response was studied. Action spectra were constructed on *B. napus* to determine the impact of UV radiation.

B. napus was exposed to 8.9 kJ m^{-2} day^{-1} biologically effective UV-B radiation ($UV\text{-}B_{BE}$). The penetration of UV-B radiation into the leaf was followed using a quartz fibre optic microprobe. Monochromatic radiation at 310 nm was decreased by ca 50 and 34% in the adaxial and abaxial epidermis, respectively, in plants not exposed to UV-B, whereas the radiation was decreased by ca 70 and 42%, respectively, in the same region in UV-treated plants. Polychromatic radiation showed a wavelength dependent change mainly for the collimated radiation. The results correlated with the distribution of phenolic compounds analysed from 40 µm paradermal leaf sections. The first adaxial section (40 µm) contained 35% of the whole leaf sample flavonoid glycosides in control plants, and 66% in UV-treated plants. Hydroxycinnamic acid derivatives increased by 26% in UV-treated plants relative to controls. The ratio of quercetin to kaempferol derivatives increased from 0.11 in controls to 0.91 in leaves of UV-treated plants. The leaf epidermis protected the inner leaf tissue where most of the photosynthetic apparatus is located.

P. vulgaris was subjected to 6.17 kJ m^{-2} day^{-1} $UV\text{-}B_{BE}$ with different levels of visible light. The largest UV-induced changes in photosynthesis, chlorophyll, carotenoids, UV-screening pigments, and surface leaf reflectance occurred under growth conditions of low levels of visible light together with UV radiation.

In general, under UV-B radiation, reductions in leaf dry weight and leaf area occurred, while leaf thickness and reflectance of visible light increased.

Action spectra for the effect of UV radiation on ultraweak luminescence (UL) from both leaf surfaces of *B. napus* showed a greater sensitivity of the abaxial surface towards UV radiation. Compared to the generalised plant action spectrum of Caldwell, these action spectra were flatter at wavelengths longer than 280 nm. The values of the UL action spectra give higher $UV\text{-}B_{BE}$ values, but the radiation amplification factor is lower. This means that there will be less impact from ozone depletion.

Key words: Action spectrum, Brassica napus, fibre optics, flavonoids, HPLC, hydroxycinnamic acids, ozone depletion, Phaseolus vulgaris, phenolics, photosynthesis, radiation penetration, reflectance, SEM, TLC, ultraviolet radiation, ultraweak luminescence, weighting function.

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This thesis is based on the following papers:

I. Cen, Y.-P. & Bornman, J.F. 1990. The response of bean plants to UV-B radiation under different irradiances of background visible light. - J. Exp. Bot. 41: 1489-1495.

II. Cen, Y.-P. & Bornman, J.F. 1993. The effect of exposure to enhanced UV-B radiation on the penetration of monochromatic and polychromatic UV-B radiation in leaves of *Brassica napus*. - Physiol. Plant. 87: 249-255.

III. Cen, Y.-P. & Björn, L.O. 1993. Action spectra for enhancement of ultraweak luminescence by ultraviolet radiation (270 - 340 nm) in leaves of *Brassica napus*. - J. Photochem. Photobiol. (in press).

IV. Cen, Y.-P., Weissenböck, G. & Bornman, J.F. 1993. The effects of UV-B radiation on phenolic compounds and photosynthesis in leaves of *Brassica napus*. - (Manuscript).

These papers will be referred to in the text by their Roman numbers.

Abbreviations

CFC, chlorofluorocarbon
DU, Dobson unit (atm. cm)
F, fluorescence induced by actinic light
FG, flavonoid glycosides
F_m', maximal fluorescence with actinic light on
F_m, maximal fluorescence
F_o', ground fluorescence with actinic light on
F_o, initial fluorescence
$F_v' = F_m' - F_o'$
$F_v = F_m - F_o$
HAD, hydroxycinnamic acid derivatives
PAR, photosynthetically active radiation (400 - 700 nm)
PS I, photosystem I
PS II, photosystem II
PSC, polar stratospheric cloud
Q_A, Q_B, functionally distinct quinone molecules
q_{NP}, non-photochemical quenching
q_P, photochemical quenching
RAF, radiation amplification factor
$t_{1/2}$, half-rise time from F_o to F_m
TOMS, total ozone mapping spectrometer
UL, ultraweak luminescence
UV, ultraviolet radiation
UV-A, ultraviolet-A radiation (320 - 400 nm)
UV-B, ultraviolet-B radiation (280 -320 nm)
UV-B_{BE}, biologically effective (or weighted) ultraviolet-B radiation
UV-C, ultraviolet-C radiation (200 - 280 nm)

1 Background

1.1 Ultraviolet radiation

In the late ninteenth century, J.W. Ritter discovered ultraviolet (UV) radiation, which is invisible to human eyes but can blacken silver chloride more effectively than the visible spectrum (400 - 700 nm) (Jagger 1967). The shorter the wavelength, and the higher the frequency of a quantum, the greater its energy. In biological research, the UV region can be designated as UV-A, UV-B, and UV-C. UV-C, 200 - 280 nm, is extremely harmful to organisms, but does not reach the Earth's surface. UV-B, 280 - 320 nm, is the most damaging part of the solar radiation reaching the surface of the Earth. UV-A, 320 - 400 nm, is the main component of solar UV but may be less biologically effective than UV-B depending on the biological process involved (Wellmann 1983). Important biological macromolecules, for example, proteins and nucleic acids, absorb effectively in the UV-B region, and thus any increase in this part of the solar radiation will constitute a potential threat to living organisms.

1.2 Ultraviolet-B radiation and ozone

Most of the oxygen in the atmosphere originates from photosynthesis. As photosynthetic organisms evolved and oxygen started to accumulate, some of this oxygen was converted to ozone by the ultraviolet component of sunlight, forming a diffuse shell of ozone, which effectively absorbed wavelengths below 290 nm. This made it possible for life to emerge from the oceans. Most of the ozone is found in the lower stratosphere with the maximum concentration at about 22 km, depending on latitude (Jagger 1985, Green et al. 1980, Green 1983) (Fig. 1). In this lower stratosphere, a dynamic balance of ozone concentration is maintained by the UV radiation below 200 nm, which breaks down molecular oxygen, while chemical reactions reform the molecular oxygen. For example:

$O_2 + hv \longrightarrow O + O$ (1)

$O + O_2 \longrightarrow O_3$ (2)

$O_3 + hv \longrightarrow O_2 + O$ (3)

$O_3 + O \longrightarrow O_2 + O_2$ (4)

$Cl + O_3 \longrightarrow ClO + O_2$ (5)

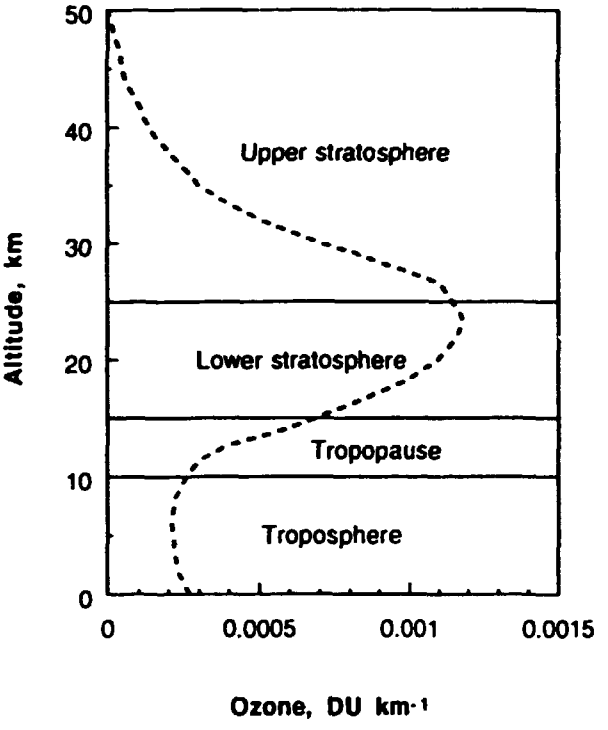

Fig. 1. Density distribution of atmospheric ozone. The dashed curve corresponds to 0.229 atm. cm of ozone. The ozone data were extracted from Green (1983).

$ClO + O \longrightarrow Cl + O_2$ (6)

The reactions 1 and 2 form ozone in the stratosphere, reactions 2 and 3 occur as ozone absorbs radiation, reaction 4 breaks down ozone, and reactions 5 and 6 are chlorine-promoted ozone breakdown. Ozone production takes place at a rate of about 300 million tons of ozone per day (Jagger 1985, Stolarski 1988, McFarland & Kaye 1992). Dobson units (DU) are used to describe the vertical atmospheric ozone thickness (atmospheric column ozone); 1 Dobson unit = 10^{-3} atm. cm (1 atm., atmospheric pressure at sea level = 101.325 kPa). One can also describe the ozone concentration in mm atm., whereby, for example, 300 DU would be equivalent to 3 mm at standard temperature and atmospheric pressure at sea level. The stratospheric ozone shell is not evenly distributed over the earth and also changes with season. The ozone levels are about 260 DU in the equatorial region where most ozone is produced. Ozone flows toward the poles by stratospheric circulation. At the north pole in late winter or early spring, the average is about 450 DU. At the south pole, ozone levels reach about 380 DU

(Stolarski 1988, McFarland & Kaye 1992). Only a few mm of ozone absorb practically all radiation below 290 nm and protect the biosphere on the earth from damaging, shortwave UV radiation.

1.3 Stratospheric ozone depletion

In 1974, Molina and Rowland reported that the inert gases of chlorofluorocarbons (CFCs) which have been widely used as coolants for refrigerators and air conditioners, propellants for aerosol sprays, agents for producing foam, and as cleansers for electronic parts, are not degraded in the troposphere (altitude 0 - 10 km), but are instead broken down by UV radiation in the stratosphere (altitude 20 - 50 km) into more reactive forms (such as chlorine atoms). This has disturbed the dynamic ozone balance, causing a decrease in stratospheric ozone.

Since November 1978, the Total Ozone Mapping Spectrometer (TOMS) on the Nimbus-7 satellite has monitored UV radiation entering the atmosphere and back scattering into space. TOMS has measured both the amount and distribution of the atmospheric ozone column. Analyses of the data from TOMS have shown a downward trend in column ozone outside the tropics during all seasons of the year (Bowman 1988, Frederick 1993, Gleason et al. 1993). Although up to a 40% ozone decrease has been observed in the springtime Antarctic ozone 'hole' region (Farman et al. 1985, Stolarski 1988, Roberts 1989, Moffat 1989, Hofmann et al. 1992), the trend for latitudes between 30 and 50 °N is -6.6 to -6.7 % per decade during winter and -2.6 to -3.0 % per decade during summer (Frederick 1993). Gleason et al. (1993) have published the data for annual average ozone over the last 13 years (Fig. 2).

Recently, measurements of the ozone destroyer, ClO, by the Microwave Limb Sounder on the Upper Atmosphere Research Satellite showed the winter increase in ClO in both polar regions, together with cold temperature which favour formation of Polar Stratospheric Clouds (PSCs) (Waters et. al. 1993). These PSCs, made up mainly of nitric acid trihydrate, serve as active surfaces for catalytic reactions involving chlorine and bromine, which are responsible for O_3 destruction upon spring time warming (Mahlman 1992). A downward trend in ozone has also been observed at all latitudes except the tropics in the lower

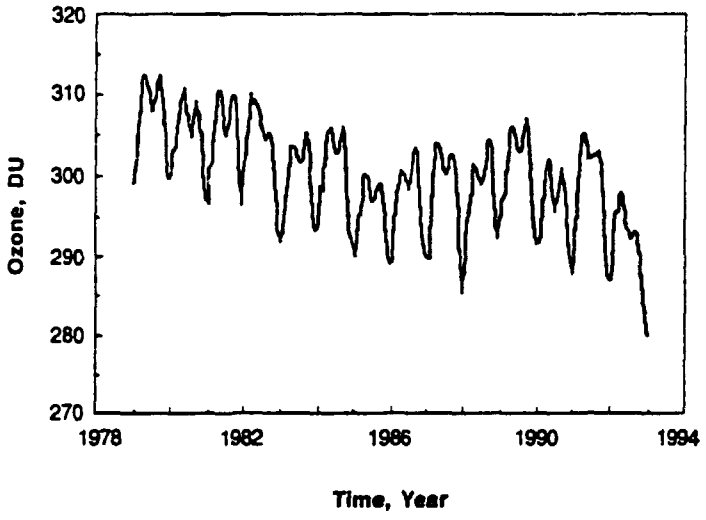

Fig. 2. The amount of annual average ozone for the latitude range 65°S to 65°N during 1 January 1979 and 31 December 1992. The curve was extracted from Gleason et al. (1993).

stratosphere (Waters et. al. 1993, Chipperfield 1993). Even an Arctic ozone hole may develop due to increases in atmospheric carbon dioxide concentration, causing cooling of the stratosphere (Austin et al. 1992, Mahlman 1992). The stratospheric ozone depletion and the UV-B radiation reaching the ground are related. As ozone depletion occurs, a selective increase in the amount of UV-B reaching the earth will result, with a greater increase at shorter wavelengths than longer wavelengths due to the absorption properties of ozone. However, the connection between ozone depleti and UV-B radiation increase is complicated. Aerosols, clouds, haze, ground reflectivity, zenith angle of the sun, and the proportion of UV-B that is scattered, all have an influence (Green 1983, Brasseur 1992, Kerr 1993).

It should also be noted that the daily variations in UV-B radiation and the day to day changes are dramatic as compared to the smaller UV-B radiation changes due to ozone depletion.

1.4 Biologically effective UV-B radiation

Most biological responses to UV radiation are highly wavelength dependent. In 1971, the term biologically effective UV-B radiation ($UV\text{-}B_{BE}$) was defined by

Caldwell (Caldwell 1971). In order to keep all terminology and abbreviations in this summary uniform, Caldwell's equation is rewritten as follows:

$$UV\text{-}B_{BE} = \int_{280}^{320} Q_\lambda E_\lambda \, d\lambda$$

Where λ is wavelength, Q_λ is the spectral irradiance at wavelength λ, and E_λ is the biological effectiveness at wavelength λ.

$UV\text{-}B_{BE}$ provides a way of expressing the amount of UV radiation as a function of the biological effectiveness of the radiation. Thus comparisons can more easily be made between results from various sources of UV-B irradiation applied in laboratories as well as from field studies using both artificial and solar UV-B sources. The so-called generalised plant UV-B action spectrum (Caldwell 1968, 1971) is generally used as a weighting function to simplify comparisons. It is also recommended that researchers normalise the action spectrum to 1.0 at 300 nm for comparison with other action spectra. However, no single weighting function is ideal for all situations, and therefore the few available action spectra will serve only as a guide for the estimation of biological effectiveness of UV-B radiation.

Changes in solar UV-B radiation reaching the ground due to atmospheric ozone depletion will increase the $UV\text{-}B_{BE}$. Other factors affecting daylight spectra, such as solar angle, atmospheric conditions, albedo, and elevation above sea level, will also influence the $UV\text{-}B_{BE}$ (Green et al. 1980, Green 1983, Caldwell et al. 1986). A computer program (Björn & Murphy 1985, Björn 1989) can be used to simulate daylight changes due to different ozone levels. When all the other conditions were the same, the $UV\text{-}B_{BE}$ showed a stronger dependence on ozone depletion than the unweighted solar UV-B radiation (Fig. 3).

Since the ozone absorption coefficient (Green et al. 1980, Green 1983) and the generalised plant action spectrum increase exponentially towards shorter wavelength in the UV-B region, a change in the ozone concentration will shift the wavelength composition of UV-B radiation, and an amplification of $UV\text{-}B_{BE}$ will occur (Fig. 4). The radiation amplification factor (RAF), which is defined as the ratio of percentage increase in $UV\text{-}B_{BE}$ to percentage decrease in ozone thickness (Rundel 1983, Caldwell 1981, Caldwell et al. 1986), is also used as a measure of a certain biological response due to ozone depletion.

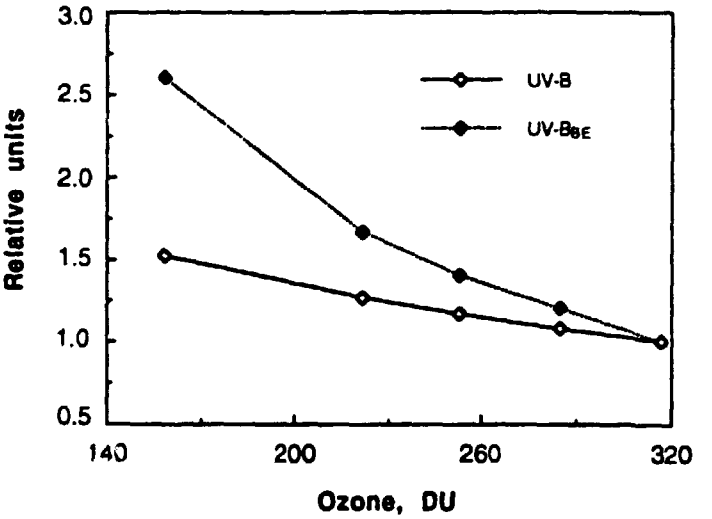

Fig. 3. The relative changes of solar ultraviolet-B radiation and biologically effective ultraviolet-B radiation (weighted by the generalised plant action spectrum of Caldwell) due to ozone depletion in Lund, Sweden (July 15). The data points were obtained by using a computer program (Björn & Murphy 1985, Björn 1989).

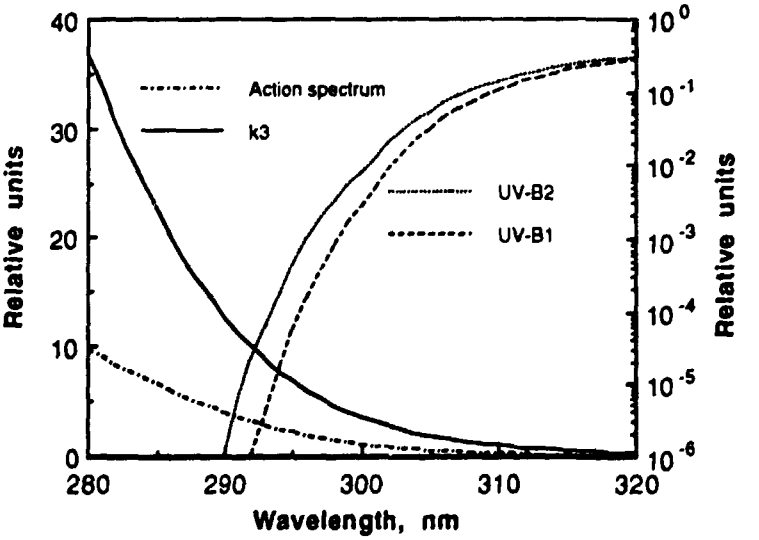

Fig. 4. The spectra of solar ultraviolet-B radiation with normal ozone concentration, UV-B1; or 25% ozone depletion, UV-B2 in Lund, Sweden (July 15, logarithmic scale; right ordinate). The ozone absorption coefficient, k3 (Green 1983) and the generalised plant action spectrum of Caldwell, normalised to 300 nm (left ordinate). Solar ultraviolet-B radiation was obtained by a computer program (Björn & Murphy 1985, Björn, L.O. 1989).

2 Some approaches for investigating the influence of UV-B radiation on higher plants

2.1 UV-B penetration within leaves

A direct way to measure radiation within plant organs is by the use of glass or quartz optical fibres. This has been done both for visible and UV radiation (Vogelmann & Björn 1984, Bornman & Vogelmann, 1988, 1991, Day et al. 1992, II). The tapered, metal-coated end of a fibre is driven into a leaf using an automated stepper motor. The opposite end of the fibre is positioned at the entrance port of a spectroradiometer which detects the light which is guided through the fibre. Data acquisition is computer controlled. In this way it is possible to measure gradients of light as the fibre is driven towards a light source striking the opposite leaf surface. Alternatively, a defined region of the radiation spectrum can be measured by positioning the fibre probe at a pre-determined depth and scanning the region of interest. In this way one can obtain information about the internal spectral regime, internal fluence rate, and light gradients within, e. g. a leaf. As the optical fibre is only sensitive to light incident within a certain acceptance angle, separate measurements of the light fluxes at different angles within the sample are needed to estimate the internal fluence rates (Vogelmann & Björn 1984, Vogelmann et al. 1991).

When UV radiation strikes a leaf surface, some will be reflected in the cuticle-air interface without entering the leaf (Gausman et al. 1975). Rayleigh scattering within the epicuticular wax deposits is stronger at shorter wavelengths, and this will reduce the relative penetration of UV in comparison with longer wavelengths (Clark & Lister 1975). As radiation is propagated within the interior of a plant sample, it is attenuated by absorption of pigments as well as the reflectance and scattering properties of the tissues. Thus the presence of UV-screening pigments and changes in structure due to growth under enhanced levels of UV radiation, e. g. increases in leaf thickness, will decrease penetration of UV radiation. Mainly because of the presence of the UV-screening pigments, the gradients of UV radiation are steeper than those for visible radiation.

The leaf epidermis is the first layer to be directly exposed to solar UV radiation. The penetration of UV-B radiation within plant leaves is largely

attenuated by the epidermal layer. However, this attenuation ability differs among plant species and the developmental stage of the leaf (DeLucia et al.1992, Day et al.1992). Day et al. (1992) found that in mature conifer needles almost all UV-B radiation is attenuated in the epidermis. In woody dicotyledons 3-12% of the UV-B radiation reached the mesophyll, while in herbaceous dicotyledons 18 - 41 % reached the mesophyll. In paper II not only the penetration of monochromatic UV-B radiation, but also that of polychromatic radiation was determined within leaves of *Brassica napus*. These results correlated well with the internal distribution of UV-screening pigments.

2.2 UV-screening pigments - Flavonoids

The flavonoids are a group of water-soluble phenolic derivatives, mainly found in the vacuoles of plant epidermal cells, although some are found in chromoplasts and chloroplasts, as well as in cell walls. Anthocyanins, flavones and flavonols are three groups of flavonoids which are of particular interest. Anthocyanins contribute to the colour of the plant, although they have weak absorption in the UV-B region. Flavones and flavonols absorb in the UV region of the daylight. The flavonoids in plants serve not only to attract insects to flowers, and protect against microbial infection but they also protect plants from damaging UV radiation (Wellmann 1983, Salisbury & Ross 1985, Hedin & Waage 1986, Tevini et al. 1991b, IV).

The flavonoids reduce the penetration of UV-B radiation into inner leaf tissues (Robberecht & Caldwell 1980, 1986, Tevini et al. 1991a, Caldwell et al. 1983, DeLucia et al. 1992, II). Flavonoids are thus related to plant sensitivity to UV-B radiation. UV-B sensitive plants have generally less UV absorption in the epidermis than more tolerant plants due to the flavonoid content (Robberecht & Caldwell 1978, 1986). Both visible light and UV radiation can induce flavonoid accumulation in leaves of higher plants (Wong 1976, Wellmann 1976, 1983, 1985, Tevini et al. 1983, 1991b, Beggs et al. 1986, Schmelzer et al. 1988, I). The UV-induced flavonoid production is one example by which the plant can reduce the penetration of damaging UV-B radiation into plant tissues. Parallel measurements of flavonoid content and UV-B penetration have also illustrated this (II).

2.3 UV-B radiation and photosynthesis

Photosynthesis is the process whereby light energy is converted and stored as chemical energy within green plants. It is the main mechanism of energy input into the living world by plants and bacteria. Photosynthesis can be measured in different ways, and certain parameters are used as indicators of changes in the photosynthetic system. For example, the partial reactions of photosynthesis are often measured by using different chlorophyll fluorescence parameters. Changes in net photosynthesis, measured by determining the assimilation of CO_2 , are not always as large as those determined from the partial reactions. This is particularly true of short-term irradiation studies.

At room temperature, the chlorophyll fluorescence emission from leaves is mainly emitted by photosystem II (PS II) and the attached light harvesting chlorophyll complex (Clayton 1980, Krause & Weis 1984). A fluorescence induction curve or Kautsky curve (Govindjee and Papageorgiou 1971), is obtained upon illumination of a dark adapted leaf. Different phases of the resultant curve have been defined. The fast phase is related to primary processes of (PS II). The initial fluorescence, F_o, is thought to represent emission by excited antenna chlorophyll molecules when all the reaction centres are open. The variable fluorescence, F_v, reflects the reduction state of a primary electron acceptor, Q_A. The maximum fluorescence, F_m is reached with saturating irradiance, and the reaction centres are closed. The ratio of F_v/F_m has a range of 0.75 - 0.85 in intact photosystems (Björkman & Demmig 1987, Bolhar-Nordenkampf & Öquist 1993). This ratio can be shown to be proportional to the quantum yield of photochemistry (Björkman & Demmig 1987, Clayton 1980, Demmig-Adams & Adams 1992). The half-rise time ($t_{1/2}$) from F_o to F_m can also be used as an estimate for the size of the plastoquinone pool (Anderson et al. 1988), and has been shown to be a particularly sensitive indicator of changes induced by UV radiation (Bornman et al. 1984, I)

The slow kinetics of fluorescence starts after the P peak, and is related to interactions between the thylakoid membranes and stroma. Photochemical quenching (q_P) causes a decrease in fluorescence as Q_A is partially re-oxidised by photosystem I (PS I). Non-photochemical quenching (q_{NP}) also contributes to the decline in fluorescence. This quenching is mainly due to de-excitation through

heat generation. The energy dependent quenching is thought to result from the formation of a proton gradient across the thylakoid membrane and is probably the main contributor to q_{NP} (Horton & Bowyer 1990). Although few UV-B studies have been carried out with these parameters, an increase in non-photochemical quenching and a decrease in photochemical quenching have been documented (Tevini et al. 1988) These effects suggested changes in the oxidation state of the electron acceptor Q_A and a decreased turnover of reduction and energy equivalents in the Calvin cycle (Tevini et al. 1988).

A pulse amplified modulation (PAM, Walz, Effeltrich, Germany) fluorimeter is generally used to determine these and other fluorescence parameters. According to Schreiber & Bilger (1987) and Horton & Bowyer (1990) the quenching coefficients can be derived as follows:

$q_P = (F_m' - F) / (F_m' - F_o')$

$q_{NP} = (F_v - F_v') / F_v$

for the definition of F_m', F_o', F_v', see Fig. 5.

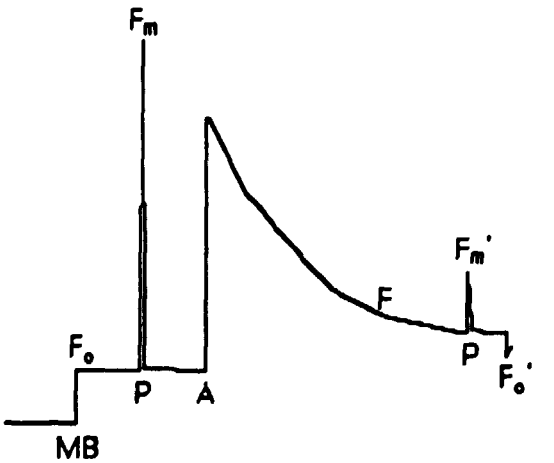

Fig. 5. Definition and parameters of quenching analysis. MB, measuring beam; P, saturation pulse; A, actinic light. F_o, initial fluorescence with weak modulated measuring beam; F_m, maximal fluorescence of a dark-adapted sample; F_m', maximal fluorescence with actinic light on; F, fluorescence induced by actinic light; F_o', ground fluorescence with actinic light on. Other fluorescence parameters are obtained from the above, $F_v = F_m - F_o$, $F_v' = F_m' - F_o'$.

Generally UV-B radiation mainly influences PS II, with only slight inhibition of photosystem I (PS I) when high irradiance UV-B is used (Bornman 1989). The influence of UV-B radiation on reaction centres of PS II will be reflected in the fluorescence kinetics. F_o, F_v, F_v/F_m, and F_v/F_o are often used to indicate changes in the fast phase of the fluorescence induction curve. Both the oxidizing and reducing sides of PS II appear to be sensitive to UV-B radiation (Tevini 1985, Renger et al. 1986, 1989, Bornman 1989, He et al. 1993). UV-B radiation may thus modify the binding protein of the primary and secondary plastoquinones of the PSII acceptor side (Q_A-Q_B apoprotein), simultaneously leading to a functional blocking of the primary acceptor. Using isolated PS II membrane fragments of spinach, Renger et al. (1989) further suggested that UV-B radiation also disrupts the integrity of the water oxidation complex.

Studies on the $t_{1/2}$ together with above parameters of fluorescence showed that impairment of PS II activity by UV-B radiation was due to blockage of PS II reaction centers (Noorudeen et al. 1982). A slower recovery from UV-B irradiation compared to high visible light, i. e. photoinihibition, could mean that UV-B photons are more likely to damage the cellular components such as nucleic acids and proteins (Chow et al. 1993).

2.4 Ultraweak luminescence and UV-B radiation

Luminescence is the emission of electromagnetic radiation from atoms or molecules in the transition of (both singlet and triplet) electronically excited states to lower energy states. The emission spectrum can be in the UV, visible and infrared regions, and chemical reactions are the source of energy (Campbell 1988). This differs from fluorescence which is defined as the light emitted from a singlet electronically excited state and is of very short duration after removal of the source of excitation. When the intensity of luminescence is less than 10 000 photons cm^{-2} s^{-1}, it is called ultraweak luminescence (UL; Campbell 1988). The quantum efficiency of UL is 10^{-3} to 10^{-14} photons per activated molecule (Abeles 1986). The emission spectra can be used to aid in identifying the possible light emitters (Campbell 1988). This UL is invisible to the human eye, but can be detected using sensitive photomultipliers (Inaba et al. 1982).

In higher plants and in other living cells, the emission of UL is associated

with certain important cellular functions, such as mitochondrial respiration, photosynthesis, cell division, eicosanoid production and detoxification. The main metabolites thought to be responsible for UL are:

1) Excited carbonyls; >C=O*

2) Singlet oxygen; $^1O_2$ (dimol, $^1O_2$ - $^1O_2$)

3) Energy transfer from an excited state molecule (X*) to an endogenous fluorochrome (F);

Reaction ---> X* +F ---> X + F* ---> F + light

Details of UL from dark adapted green leaves, chloroplasts, peroxisomes, and mitochondria have been documented (Abeles et al. 1978, Abeles 1986, Hideg et. al. 1990, 1991, Hideg 1993).

UV-B radiation caused an increase in the levels of UL in leaves, which was interpreted as an indirect indication of the formation of free radical species and their products (Panagopoulos et al. 1989, 1990, 1992, Levall & Bornman 1993). In paper III an action spectrum was determined to obtain more detailed information on the effect of UV radiation on the processes of UL.

2.5 Action spectrum in UV research

An action spectrum is the specific radiation response of a system as a function of wavelength (Jagger 1985, Caldwell et al. 1986, Caldwell 1971). It gives information about the biomolecule responsible for the photon absorption and a direct relationship between the chromophores and the respective responses (Peak & Peak 1983, Ensminger 1993). Biological responses to UV are wavelength dependent, and therefore an action spectrum can be used as a weighting function for evaluating the biologically effective UV-B radiation (Caldwell 1968, 1971). The UV-B_{BE} gives a biologically meaningful expression of the UV-B radiation either from the sun or from lamps. A proper UV action spectrum not only serves to define the chromophore but also may provide a true measure of the biological UV-B effect. However, different action spectra used as weighting functions result in different values of UV-B_{BE} for the same daylight UV-B radiation (Fig. 6). This may lead to different conclusions about the significance of solar UV-B radiation increase due to stratospheric ozone depletion (Caldwell et al. 1989, Rundel 1983). The sensitivity of UV-B_{BE} due to ozone depletion can be described by RAF. This

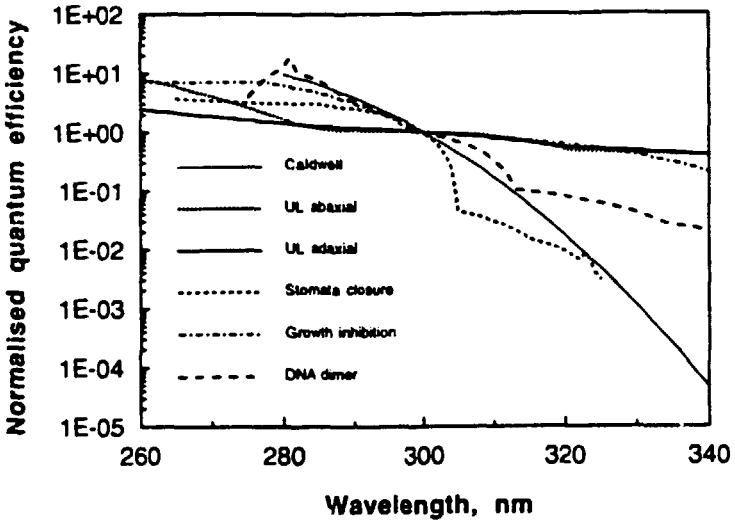

Fig. 6. Several action spectra for UV effects on higher plants. Caldwell, generalised plant action spectrum (Caldwell 1971); UL abaxial, UL enhancement from the abaxial leaf surface (Cen & Björn 1993); UL adaxial, UL enhancement from the adaxial leaf surface (Cen & Björn 1993); Stomatal closure, (Negash & Björn 1986); Growth inhibition, hypocotyl inhibition (Steinmetz & Wellmann 1986); DNA dimers (Quaite et al. 1992).

Tab. 1. Biological effective UV-B radiation weighted by several action spectra with daylight* of 5% ozone depletion in Lund, Sweden (July 15), and the radiation amplification factor (RAF) according to different action spectra.

Weighting function**	UV-B_{BE} (kJ m^{-2} day^{-1})	RAF
Caldwell	6.4	1.59
UL abaxial	50.1	0.94
UL adaxial	49.3	0.91
Stomata closure	1.8	1.99
Growth inhibition	52.9	0.90
DNA dimers	12.0	1.39

*Daylight values were calculated using a computer program (Björn & Murphy 1985, Björn 1989).

**See legend on Fig. 6.

RAF may also vary as different action spectrum was used (Tab. 1)

There are many action spectra for different UV effects on higher plants. A single action spectrum is not proper for evaluating different higher plant responses to UV-B radiation. Therefore, a separate action spectrum should be determined for a special aspect or a certain plant species (Coohill 1989, 1991). In paper III, action spectra for the enhancement of UL by UV radiation have been constructed from both leaf surfaces of *B. napus*. Several different action spectra on higher plants including our own are presented in Fig. 6. (Caldwell 1971, Negash & Björn 1986, Steinmetz & Wellmann 1986, Quaite et al. 1992). These spectra were used to calculate UV-B_{BE} for simulated daylight in Lund (July 15, with or without 5% ozone depletion). Our action spectra gave higher UV-B_{BE} than others except for growth inhibition (Tab. 1). However, the RAF values were smaller using our spectra and the one for growth inhibition (Tab. 1), corresponding to a smaller impact by ozone depletion.

2.6 Plant growth and morphology

UV-B radiation can cause growth and morphological changes in plants. However, these changes may differ according to plant species and growth conditions (Tezuka et al. 1993, Dumpert & Knacker 1985, Basiouny et al. 1978, Vu et al. 1982). The levels of the PAR are important for the plant response to UV-B radiation (Warner & Caldwell 1983, I, Adamse & Britz 1992). The UV-B damage is magnified under low PAR, since plants lack fully developed protective mechanisms (Tevini & Teramura 1989). In many cases, UV-B radiation inhibits plant elongation, so that decreases in height occur (Biggs et al. 1981, Teramura 1983, Murali & Teramura 1986, Kulandaivelu et al. 1989, Tevini & Teramura 1989, Barnes et al. 1990, I, Tevini et al. 1991b). The growth inhibition may be due to destruction of the growth regulator indole-3-acetic acid (IAA) and the formation of growth inhibiting IAA photoproducts (Ros 1990).

Plant leaf area and total dry weight may also be reduced by UV-B radiation (Bennet 1981, Vu et al. 1981, Murali et al. 1988, I, II), with increases in leaf thickness sometimes also occurring (Murali et al. 1988, Bornman and Vogelmann, 1991, I, II). Increased number of leaves, increased branching and decreased leaf length were also found after UV-B radiation (Barnes et al. 1990).

UV-B radiation can also alter the leaf epidermis (Basiouny 1986, Santos et al. 1993, I). However, severe damage is only found when UV-B is applied together with low visible light (I). In greenhouse and growth chamber studies, higher levels of visible light generally reduce plant response to UV radiation (Teramura 1980, I). In the field, changes in morphology and plant growth may influence the competitive balance of the plant in ecological systems (Caldwell 1981).

3 Goals of the present investigations and the measures taken to achieve them

Recent reports from satellite measurements document a decrease in total stratospheric ozone (Frederick 1993). Since the effects of many of the compounds, e. g., CFCs and bromine, are long-lived in the stratosphere, it is likely that ozone depletion will continue for some time to come. It is therefore important to attempt to analyse the potential impact on plants by an enhanced UV-B environment.

To understand some of the effects of UV-B radiation on higher plants, structural, physiological, biochemical and physical studies were done using *Phaseolus vulgaris* and *Brassica napus*, two important crop plants. Structural investigations included anatomical changes of the leaf using light and scanning electron microscopes, while physiological studies dealt mainly with measurements of some of the partial reactions of photosynthesis, as well as plant growth. The biochemical response of the plants was investigated by determining pigment changes, specifically for some of the different phenolic compounds using chromatographic techniques. Also the ultraweak light emission from biochemical reactions involving free radical formation was measured as a stress indicator. Physical measurements involved measuring some of the changes in radiation both externally from leaf surfaces, i.e. reflectivity, as well as the internal spectral regime of leaves using fibre optic microprobes. These different aspects were investigated in order to correlate the physiological and biochemical changes with the actual distribution of UV-B radiation within plant tissues. Action spectra were constructed for investigating the biological importance of UV-B radiation at individual wavelengths, as well as the possible biomolecules responsible for the response. It is hoped that this work will have furthered our understanding of some of the effects of an enhanced level of UV-B radiation.

4 Summary of papers I - IV

UV-B irradiance applied and the simulation of ozone depletion

In the growth chamber used for the experiments, UV-B radiation was supplied by Westinghouse FS 20, Westinghouse FS 40 sunlamps (Westinghouse, U.S.A.) (I), Philips Ultraviolet-B sunlamps (TL 40 W/12, The Netherlands) (II, IV), and UVB-313 (Q-PANEL Co. U.S.A.) tubes (IV). The latter two types of tubes generated the same spectral energy distribution after filtering through a single layer of cellulose acetate (0.8 mm thickness) together with a Plexiglas sheet (FBL.2458, Röhm GMBH, Chemische Fabrik, Germany; 3.0 mm thickness), which removed all radiation below 280 nm (Fig. 7). In the earlier experiment with *Phaseolus vulgaris (P. vulgaris)* only the Plexiglas was placed between the Westinghouse FS sunlamps and plants, giving a small amount of radiation below 280 nm, which corresponded to 0.3% of the radiation from 260 to 320 nm (I). The spectrum of this 0.3% radiation has been published by Panagopoulos et al. (1990). This 0.3% of UV-C radiation may have contributed to plant damage. The applied UV-B radiation varied from 3.5 to 5 h daily during the middle of the photoperiod to achieve 6.17 kJ m^{-2} day^{-1} (I), or 8.9 kJ m^{-2} day^{-1} (II, IV) biologically effective UV-B radiation ($UV\text{-}B_{BE}$). The calculations were done with a daylight model computer program (Björn & Murphy 1985, Björn 1989). The weighting spectrum used was the generalised plant action spectrum (Caldwell 1971) normalised to 1.0 at 300 nm. The $UV\text{-}B_{BE}$ applied in the experiments was intended to simulate 5% or 25% ozone depletion, respectively, on a cloudless summer day at Lund, 15 July (Fig. 8). The UV-B radiation applied in the growth chamber gave a spectrum of $UV\text{-}B_{BE}$ similar to that which would (or will) occur under natural conditions with an increase in UV-B levels in the future (Fig. 8).

General plant appearance, growth, and leaf structural changes

After UV-B irradiation plants exhibited a shiny leaf surface compared to the control ones. These shiny surfaces were also indicated in the leaf reflectivity measurements in both *B. napus* and *P. vulgaris* (I, IV). A higher leaf reflectivity in the PAR region of the spectrum was monitored in UV-B irradiated plants compared to the controls. Inhibition of growth was seen in plants after UV-B

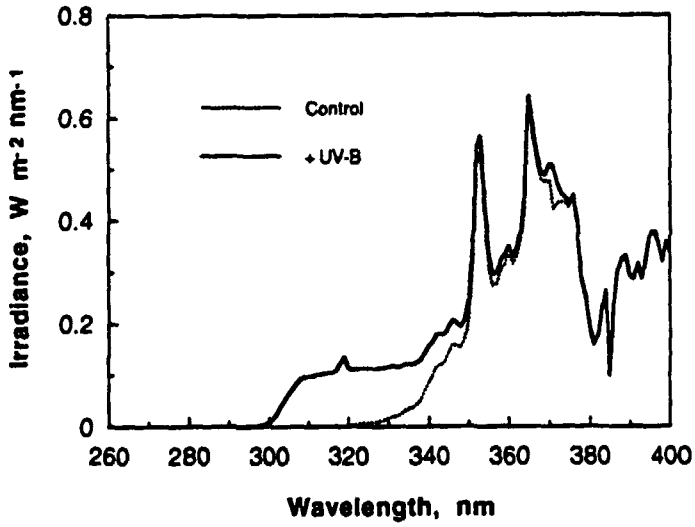

Fig. 7. The spectral energy distribution of UV radiation received by the UV-treated plants in a growth chamber simulating a 25% stratospheric ozone depletion in Lund, Sweden. The UV radiation was from Philips ultraviolet-B sun lamps (TL 40 W/12). Control plants received irradiation only from Osram Power Star (AWAKE 400 W/D, Germany) dysprosium lamps.

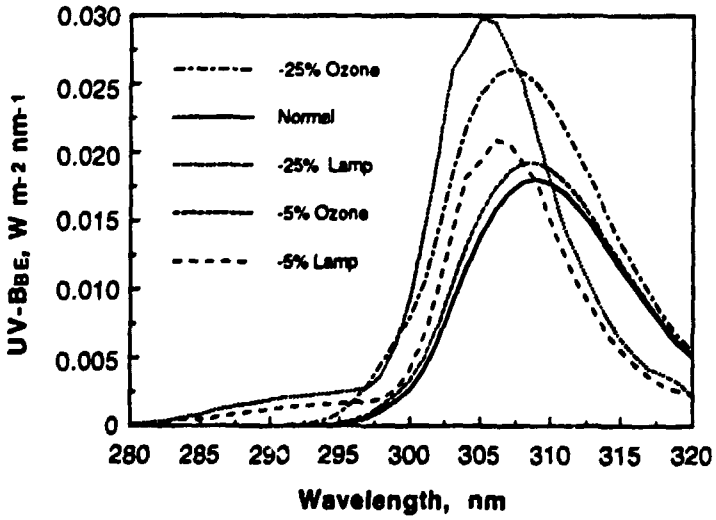

Fig. 8. The spectral distribution of biologically effective UV-B radiation (weighted by the generalised plant action spectrum of Caldwell) for the artificial broadband UV-B radiation used in our experiments (papers I, II, IV), as well as for simulated daylight in Lund (middle day, July 15, with or without 5% or 25% ozone depletion). Daylight simulations were obtained by using a computer program (Björn & Murphy 1985, Björn, L.O. 1989).

irradiation. A decreased whole leaf dry weight was found in both *B. napus* and *P. vulgaris* (I, II). Also reduced plant height occurred in *P. vulgaris* (I). Leaf structural changes included an increased leaf thickness and a decreased leaf area in *B. napus* and *P. vulgaris* (I, II), while little difference was seen for leaf fresh weight (I). The thicker leaves after UV-B irradiation were due to elongated palisade cells in *P. vulgaris* (I), while both palisade and sponge cells contributed to a thicker leaf in *B. napus* (II). UV-B radiation can also cause partial collapse of the epidermis, followed by extensive internal damage to leaf tissues as in *P. vulgaris* (I). However, this epidermal damage was only found under growth conditions of low background visible light (I).

Photosynthetic capacity

Photosynthetic capacity was monitored by measuring the chlorophyll fluorescence induction mainly of PS II at room temperature in intact plant leaves. UV-B irradiated plants showed a decreased F_v/F_o ratio, and an increased half-rise time in *P. vulgaris* (I). Trends towards a slightly decreased F_v/F_m ratio, together with a lower photochemical quenching, and a slightly higher non-photochemical quenching was measured in *B. napus* (IV) compared to control plants. These changes in the fluorescence induction parameters reflected a decreased photosynthetic capacity as discussed previously. In paper I, the inhibition of photosynthesis was statistically significant only under low PAR conditions. While in all the other cases the UV-B radiation-induced changes in photosynthetic capacity were not statistically significant (I, IV), which may imply an effective protective system functioning under higher PAR conditions.

The photosynthetic pigments, chlorophylls and carotenoids, which are responsible for light harvesting and photosynthesis were also affected by UV-B radiation. UV-B radiation resulted in a decreased total leaf chlorophyll content in both *B. napus* and *P. vulgaris* (I, IV). These changes were mainly due to a decreased leaf area. On a unit leaf area basis, a decreased chlorophyll content was only found in *P. vulgaris* grown under low PAR (I). Carotenoid content showed a similar response as chlorophylls in *P. vulgaris*. However, a slight increase in carotenoids was found in *B. napus* (IV).

Phenolic compounds as UV-screening pigments

Flavonoids are believed to be the main UV-screening pigments. These UV-screening pigments accumulated in leaves of both *B. napus* and *P. vulgaris* after UV-B irradiation (I, II). Using paradermal sections (40 µm) of *B. napus* leaves, it was seen that the first adaxial epidermal section contained over 30% of the whole leaf UV-screening pigments, while the lowest abaxial epidermal section contained over 12% of the UV-screening pigments in the leaf (II). This distribution is strong evidence for the importance of these tissue layers in attenuating UV-B radiation. In further experiments, the UV-screening pigments were separated chromatographically into flavonoid glycosides (FG) and hydroxycinnamic acid derivatives (HAD). The first adaxial epidermal section contained 35% and 66% of the whole leaf sample of FG in control and UV-treated plants, respectively (IV). After UV treatment, this upper layer showed an increase in FG and HAD of 134% and 26%, respectively. The increase in phenolic compounds probably afford good protection from UV-B damage (IV). A shift in the composition of the phenolic compounds from kaempferol to quercetin derivatives in leaves of UV-treated plants was found. The ratio of quercetin to kaempferol derivatives increased from 0.11 in control plants to 0.91 in UV-treated plants. This shift in the ratio may also provide plants with more effective antioxidant properties, and under natural conditions may also be an added defence against microbial attack.

Penetration of monochromatic and polychromatic UV-B radiation

Control and UV-B irradiated plants of *B. napus* were used in this experiment (II). The penetration of 310 nm radiation in leaves of *B. napus* was attenuated strongly by the epidermal and mesophyll tissues (II). The adaxial leaf surface had a higher attenuation compared to the abaxial surface. Total radiation (collimated, forward and back scattered) was decreased from 50% to 62% in the adaxial epidermis of control plants, while 34% to 46% of the total radiation was attenuated in the abaxial epidermis. UV-B radiation enhanced the attenuation ability of the plants. In plants grown under UV-B radiation, the attenuation to radiation at 310 nm of the adaxial epidermis was more than 70% and for the abaxial epidermis, over 42%.

Steric energy flux of polychromatic UV-B radiation inside the leaf showed a wavelength dependent change mainly for the collimated radiation (II). This may have been due to the UV-screening pigments as shown in the pattern of their absorption spectra (I, II). The effective decrease in UV-B in the adaxial epidermis and the nearby tissue was mainly due to a higher content of FG and HAD in these tissues (IV).

UV action spectra for enhancement of ultraweak luminescence

Ultraweak luminescence emitted from living organisms is positively correlated to the levels of free radicals within these organisms, and therefore the enhancement of ultraweak luminescence by UV radiation in the plant tissues is also a measure of potential UV damage (Abeles et al. 1978, Abeles 1986, Campbell 1988, Hideg 1993). Two UV action spectra for the enhancement of ultraweak luminescence were constructed from both the adaxial and adaxial leaf surfaces of *B. napus* (III). A similar spectral pattern was found for these two spectra except for wavelengths less than 290 nm. Fairly steep slopes with increasing wavelength were seen in the short UV region (<290 nm), compared to those for longer wavelengths (III). The higher sensitivity of the abaxial leaf surface further indicates the importance of the protective role of the adaxial epidermis from UV radiation. The flatter slopes in our action spectra make them different from the generalised plant action spectrum (Caldwell 1971). The latter has been used in our laboratory and in many others as a weighting function for calculating UV-B_{BE}. Using our action spectra as the weighting function (normalised to unity at 300 nm), much higher (increased by a factor of 4 to 7) UV-B_{BE} values will result. However, the RAF values due to stratosphere ozone depletion are reduced by a factor of 2. This would mean less impact of ozone depletion.

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Lund 1993

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