

*Minireview*

## Is increased UV-B a threat to crop photosynthesis and productivity?\*

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### Abstract

It has been suggested that increases in ground-level UV-B, as a result of stratospheric ozone depletion, may have major deleterious effects on crop photosynthesis and productivity. The direct consequences of such effects have been projected by some as a world-wide decrease in crop yields of 20–25%. Further losses, or unrealized gains, have also been suggested as a result of increased UV-B counteracting the beneficial effects of elevated atmospheric CO<sub>2</sub>. Deleterious UV-B effects may be largely partitioned between damage to the plant genome and damage to the photosynthetic machinery. Direct damage to DNA is a common result of absorption of high energy UV-B photons. However, most plants possess repair mechanisms adequate to deal with the levels of damage expected from projected increases in ground-level UV-B. In addition, most plants have the ability to increase production of UV-absorbing compounds in their leaves as a result of exposure to UV-B, UV-A and visible radiation. These compounds contribute substantially to reducing UV-B damage in situ. It has also been shown that in some plants, under the proper conditions, almost every facet of the photosynthetic machinery can be damaged directly by very high UV-B exposures. However, electron transport, mediated by Photosystem II (PS II) appears to be the most sensitive part of the system. Various laboratories have reported damage to virtually all parts of the PS II complex from the Mn binding site to the plastoquinone acceptor sites on the opposite surface of the thylakoid membrane. However, a critical review of the literature with emphasis on exposure protocols and characterization of the radiation environment, revealed that most growth chamber and greenhouse experiments and very many field experiments have been conducted at unrealistic or indeterminate UV-B exposure levels, especially with regard to the spectral balance of their normal radiation environment. Thus, these experiments have led directly to large overestimates of the potential for damage to crop photosynthesis and yield within the context of 100 year projections for stratospheric ozone depletion. Indeed, given the massive UV-B exposures necessary to produce many of these effects, we suggest it is unlikely that they would occur in a natural setting and urge reconsideration of the purported impacts of projected increases of UV-B on crop productivity.

**Abbreviations:** C<sub>i</sub> – leaf internal CO<sub>2</sub> partial pressure; CPD – cyclobutane pyrimidine dimer; CVY – cultivar-year, one crop cultivar grown for one season; F<sub>v</sub>/F<sub>M</sub> – variable chlorophyll fluorescence ratio; kJ m<sup>-2</sup> d<sup>-1</sup> – daily radiation energy flux; PAR – photosynthetically active radiation; PAS300 – UV-B<sub>BE</sub> weighted by the generalized plant action spectrum normalized to 300 nm; TOMS – total ozone mapping spectrometer instrument mounted aboard the National Aeronautics and Space Administration's Nimbus-7 satellite; UV-A – ultraviolet-A radiation (400 nm ≥ λ > 320 nm); UV-B – ultraviolet-B radiation (320 nm ≥ λ ≥ 280 nm); UV-B<sub>BE</sub> – biologically effective UV-B (in this paper, irradiance weighted by the generalized plant action spectrum)

### Introduction

Projected decreases in the stratospheric ozone layer as a result of anthropogenic emissions of halogenated

carbon compounds such as chlorofluorocarbons may result in increased levels of ultraviolet-B (UV-B) irradiation at ground level. Recently Stolarski et al. (1992) published a statistical analysis of ground- and satellite-

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based ozone column measurements from the period 1958–1991. Their analysis of the more recent (1970–1991) data led them to conclude that the year-round rate of decline at mid-northern latitudes averaged about 1.8% per decade, although the rate of decline over most of the cropping season is closer to 1.0–1.3% per decade. These rates of decline may be moderated by implementation of the Montreal Protocols (United Nations Environment Program 1987), an international treaty providing for the phase out of production and use of the chlorinated fluorocarbons thought to catalyze destruction of stratospheric ozone. For purposes of this discussion however, we shall accept the interpretation of Stolarski et al. (1992) and further assume that the protocols will have no effect. Thus, we will take as a worst case scenario a year-round depletion of the column ozone of 18% for the next century. Naturally, there is a great deal of concern about the impact of potential UV-B increases on plant and animal life. With regard to agriculture there are concerns about direct effects on growth, development and yield of crops. Based on field studies in which soybean (*Glycine max* L.) had been subjected, throughout their growth, to levels of UV-B radiation equal to or greater than would occur in our worst case scenario, some workers reported substantial reductions (20–25%) in yields (Teramura and Murali 1986; Teramura et al. 1990a). Other studies, however, found no such effects (Sinclair et al. 1990; Sullivan and Teramura 1990; Sullivan et al. 1994; Miller et al. 1994). In fact, a critical review of the available field data revealed that 20 of the 50 cultivar years (CVY) reported at that time were from a putative sensitive cultivar Essex (Fiscus et al. 1994). This 'sensitive' cultivar exhibited yield decreases in only 25% of the years tested and only at UV-B exposures consistent with ozone depletions well in excess of our worst case scenario. In the remaining 75% of the 'sensitive' CVYs, yields either increased or showed no statistically significant change as a result of increased UV-B.

Since photosynthesis is the engine driving overall growth, development and yield, physiological studies of UV-B effects have tended to concentrate in this area. The effects of UV-B on photosynthetic processes seem to be less ambiguous than those on growth and yield, and it has been demonstrated in publications too numerous to list that UV-B exposures of sufficient magnitude can have deleterious effects on almost every aspect of photosynthesis, both in vivo and in vitro. There is, however, some question whether these effects are relevant to the crop production environment expected to occur with projected stratospheric

ozone depletions. In previous work it was suggested that methodological difficulties leading to understatement of the actual exposure levels, in terms of ozone depletion, are inherent in most of the reports of decreased productivity as a result of UV-B supplementation (Fiscus et al. 1994). Understatement of exposures leads naturally to overestimates of the potential for damage. The same difficulties apply to many in situ photosynthetic studies. Our main objective here is to examine current evidence for UV-B induced damage to photosynthetic machinery from the perspective of projections of real world changes in the UV-B environment. Because of the objective, this review will be less thorough in a physiological sense and tend to concentrate on a few examples drawn to illustrate particular points and their relationship to the environment. Because exposure levels are often difficult to interpret from the literature, we shall spend rather more time discussing exposure calculations than might seem warranted, but we believe that understanding these methodologies provides the key to arriving at more realistic assessments of the potential impact of increased UV-B. Unfortunately, estimating actual exposure levels, in terms of ozone depletion, is usually a complex and difficult exercise since sufficient information frequently is lacking, especially in reports of greenhouse and field studies.

To set the stage for much of the following discussion, it will be useful to discuss briefly several significant points relating to plant responses to UV-B. These points are: 1) many plants synthesize UV-absorbing compounds that may protect them; 2) in many instances, UV damage can be repaired; and 3) there are interactions between UV-B and UV-A and/or visible radiation that can induce or enhance these protective and repair processes. Neither the UV-A nor visible radiation bands are much affected by the stratospheric ozone layer.

#### *Protective mechanisms*

A general consensus has emerged, based on several critical experiments (Teramura 1980; Teramura et al. 1980; Bennett 1981; Warner and Caldwell 1983; Mirecki and Teramura 1984; Teramura 1986; Latimer and Mitchell 1987; Cen and Bornman 1990; Caldwell et al. 1994) and numerous observations, that UV-B-induced damage can be moderated or eliminated when plants are grown under levels of PAR approaching normal outdoor conditions. In a recent experiment Caldwell et al. (1994) demonstrated that there is a threshold

level for PAR, below which UV-B damage is manifest. They also showed that if PAR is below that threshold value, UV-A is effective in moderating UV-B damage. Precise values for that PAR threshold have not been established, but it is clear that most growth chamber and very many greenhouse studies have been conducted below that threshold. While the mechanisms of protection are not entirely clear, damage moderation may result from increases in protective pigments and/or repair of the UV damage. Production of UV-absorbing compounds such as flavonoids, which can be stimulated by exposure to UV-B radiation (Cen and Bornman 1990, for example), in itself could provide a protective mechanism. Very high levels of UV-B, however, may inhibit flavonoid synthesis (Flint et al. 1985). Flavonoid synthesis also can be stimulated by higher levels of visible radiation, as well as the UV-A that generally accompanies it (Beggs et al. 1986; Cen and Bornman 1990); thus, UV-B photoprotection will be much more effective at higher PAR levels. Even moderate levels of PAR ( $750 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) resulted not only in higher leaf flavonoid content but also in thicker leaves (Warner and Caldwell 1983; Cen and Bornman 1990), which may provide additional photoprotection (Flint et al. 1985; Cen and Bornman 1990).

Flavonoids may be induced through the phytochrome system and by high intensity blue, green or red light (McClure 1975). Red, blue and UV-B irradiation also have been shown to induce flavonoid synthesis (Bruns et al. 1986) in cultured parsley cells. In addition, accumulation of the mRNA for chalcone synthase, a key enzyme in flavonoid biosynthesis, is induced in parsley cell cultures by either red or blue light alone, but the induction is considerably enhanced by combination with UV-B irradiation (Ohl et al. 1989). Early work by Lautenschlager-Fleury (1955) suggests that the pool of UV-absorbing compounds in *Vicia faba* is dynamic and capable of fairly rapid decreases and increases in response to the visible radiation environment.

Most of the inducible flavonoids are concentrated in the vacuoles of epidermal cells, although inducible quantities also are found within the mesophyll and chloroplast (McClure 1975; Robberecht and Caldwell 1983). Using epidermal peels, Robberecht and Caldwell (1978) found that epidermal transmittance of UV-B under normal solar radiation generally was less than 10% and epidermal attenuation was 95–99% for more than half the 25 species examined. These data led Caldwell et al. (1983b) to state that, 'The existence of selective filtration in plants makes it unlikely

that most plants in their native habitats are suffering reduced photosynthetic capacity or significant nucleic acid damage due to solar UV-B radiation.' Direct micro-probe measurements of UV-B penetration into intact leaves (De Lucia et al. 1991, 1992) indicated similar degrees of attenuation of incident UV-B radiation by the epidermis of mature leaves. More recently, however, Day et al. (1993) have confirmed the prediction of Caldwell et al. (1983b) that UV-B might easily penetrate the anticlinal cell walls (those perpendicular to the plane of the epidermis) so that screening may be incomplete. In fact, Day et al. (1993) showed that epidermal transmittance in two herbaceous species averaged about 40% and 55%, although transmittance  $100 \mu\text{m}$  below the adaxial leaf surface was about 10% or less for both species. One striking feature of this work was the very high degree of effectiveness of the epidermal protoplasts in attenuating UV. Further analyses of this sort at radiation angles of incidence other than normal to the leaf surface might show whether the anticlinal walls effectively channel the UV to the leaf interior or scatter it to be absorbed by adjacent protoplasts. The key role of UV-absorbing compounds as an effective protective mechanism, however, still seems beyond refute since: 1) it has long been known that plants grown under conditions that suppress flavonoid production are extremely sensitive to damage on subsequent exposure to UV-B (Bennett 1981; Bogenrieder and Klein 1977; Wellmann 1983); and 2) mutant plants that lack the ability to synthesize flavonoids are also extremely sensitive to UV-B radiation (Li et al. 1993; Britt et al. 1993).

The absence of flavonoid increases on exposure to UV-B is, however, not necessarily indicative of UV-B susceptibility. Some plants have a normally high flavonoid content, show very little increase with UV-B treatment, and remain undamaged (Barnes et al. 1987). Others, especially a few alpine species, may possess photosynthetic machinery that is, for unknown reasons, more tolerant of UV-B (Caldwell et al. 1982; Barnes et al. 1987).

#### *DNA damage*

Another way that visible light might moderate UV-B damage is through its role in DNA repair. Cyclobutane pyrimidine dimers (CPD) and pyrimidine (6–4) pyrimidone photoproducts are the most common lesions induced by UV-B and UV-C radiation and the cause of most DNA-mediated damage (Beggs et al. 1986; Britt et al. 1993). Photoreactivation (photorepair) is

a process whereby pyrimidine dimers are enzymatically repaired in situ during a light-requiring process (UV-A or visible). However, in another common non light-requiring mechanism, called excision repair, a damaged DNA sequence is physically removed and replaced by a sequence newly synthesized from the complementary strand. Although both photoreactivation and excision repair are widespread in the plant kingdom, Beggs et al. (1986) concluded that photoreactivation is the predominant repair mechanism operating in higher plants and that the capacity for photoreactivation is more than sufficient to cope with damage caused by projected increases in ambient UV-B. Also, Hays and Pang (1994) found that excision repair was only about 5% as efficient in removing CPDs as photoreactivation in *Arabidopsis thaliana*. They suggested, based on the developmental pattern of photolyase expression (the enzyme effecting photoreactivation), that younger plants depended more on protection by flavonoids but photoreactivation became more important with age. However, Sutherland et al. (1994) found that the relative importance of the photoreactivation and excision repair paths in alfalfa seedlings depended largely on the initial level of DNA damage. They also found that UV-A can induce dimer production and, given the much higher fluences of UV-A, the damage inflicted from that waveband is a significant portion of the total. Incidentally, the recent in situ DNA damage action spectrum for alfalfa developed by Quate et al. (1992) suggests that the basal level of DNA damage may be much higher than indicated by Setlow's DNA action spectrum, bacteriophage DNA in solution, or the generalized plant action spectrum (Caldwell 1971, to be discussed later). The new damage spectrum indicated considerable effect into the UV-A region, which is relatively unaffected by the ozone layer, resulting in higher basal levels of DNA damage than would be indicated by the other action spectra. These other spectra therefore lead to overestimates of the potential for proportionally increased damage by UV-B as a result of stratospheric ozone depletion.

The capacity to repair pyrimidine (6–4) pyrimidone photoproducts has been less well characterized than photoreactivation in higher plants. Recently, however, based on work with *Arabidopsis*, Britt et al. (1993) suggested that the 6–4 photoproducts rather than CPDs may be the critical cytotoxic lesion and that rapid removal of these photoproducts by excision was necessary for UV tolerance. They reported rapid and efficient dark repair of the 6–4 photoproducts and

that growth of an *Arabidopsis* mutant deficient in this ability was inhibited by very low levels of UV-B.

## The radiation environment and exposure levels

### *The importance of the overall radiation environment*

It is important that the radiation environment be characterized as completely as possible in photobiological effects research. Yet, the knowledge that low PAR levels during UV-B experiments constitutes a substantial confounding factor seems to have had little effect on experimental procedures, methodologies, or reporting. It is still rare in greenhouse and field experiments for any but the most cursory PAR values to be reported and these occasional reports frequently include only maximum irradiances at canopy height for solar noon on an exceptionally clear day during the course of the experiment. Average daily PAR integrals at canopy height, which slowly are finding their way into research papers, are of considerably more use in evaluating an experiment. The most informative approach would be to present plots or tables of daily integrals of UV-B and PAR for ambient conditions and at canopy height for the controls and treatments. Also, in view of Caldwell et al. (1994) it may be desirable to provide similar information for UV-A, especially in greenhouse and growth chamber studies where PAR and UV-A can be extraordinarily low compared to the UV-B supplements provided by lamp banks. Clearly, there is a need to provide more information than is now typical in order to properly evaluate the results of an experiment.

Calculating and reporting UV-B exposures is another substantial problem for two major reasons: 1) the spectral distribution of the lamps used to provide UV-B supplements is different from that of the sun, and 2) for satisfactory performance, the models used to predict ground-level UV-B and to calculate supplements require inputs that frequently are not available (e.g. aerosol coefficients and ozone column thicknesses). The former problem has been ameliorated somewhat by the adoption of weighting functions to compensate for the facts that the shorter UV-B wavelengths tend to be more biologically effective than the longer ones and that UV-B lamps contribute more energy at the lower end of the UV-B range than the normal solar spectrum at ground level. Although there are many action spectra for specific physiological processes, because

of numerous technical and theoretical difficulties, it has not been possible thus far to construct one that describes the effects of UV-B on overall growth, development and yield. Indeed, it is not clear that such a spectrum can exist in any meaningful sense. However, by combining several specific action spectra Caldwell (1971) formulated a generalized plant action spectrum. When normalized to 300 nm this spectrum is the one in most widespread use today in whole higher-plant studies and we shall refer to UV-B radiation weighted according to this scheme as biologically effective UV-B (UV-B<sub>BE</sub>) or as PAS300 throughout the remainder of this paper. Even though this spectrum does not accurately describe specific processes and may even introduce an additional important degree of uncertainty, it has provided a simple basis for comparison of UV-B irradiances under a wide range of circumstances, and because of this we urge its continued use. However, in the event that the generalized plant action spectrum is superseded, it is important for comparisons to historical data that appropriate procedures be developed for comparing PAS300 irradiances to the new standard.

#### *Predicting ground-level UV-B*

In an attempt to establish comparability among studies and relevance to stratospheric ozone depletion, it has become customary to express UV-B treatments in terms of a percentage column ozone depletion. The difficulty with this approach is that in many instances neither the column nor ground level UV-B have been measured throughout the experiment; rather, both have been predicted from models. The problem of predicting ground level UV-B and calculating exposures on the basis of that prediction has led to another major difficulty in UV-B research. In the past, partly due to historical necessities, it was often, and still is more often than not, the practice to calculate UV-B supplements for clear sky conditions for the summer solstice rather than the actual experimental conditions or period. Unfortunately, inadequate information usually was available to make accurate calculations and the models most widely used (Green et al. 1974 and later Green et al. 1980) tended to overestimate ground level UV-B, especially at lower elevations and in areas containing significant levels of atmospheric aerosols. Further algorithmic revisions were published (Green 1983) in an attempt to firm up that aspect of the predictive model. However, these latest revisions have been somewhat slow to be adopted and still require good aerosol and ozone column inputs to provide reasonable

accuracy. Even though there can be reasonable agreement between these models under some circumstances, ground level measurements are still required for confirmation and should be considered an experimental necessity. Because of the tendency of the earlier models to overestimate ground level UV-B, supplemental treatments were also overestimated so that, in terms of ozone column depletion, the supplements were often higher than reported (Fiscus et al. 1994). The potential discrepancies between the earlier model (Green et al. 1980), which is currently the most widely used, the later revisions (Green 1983) encoded by Björn and Murphy (1985), and ground measurements made in Raleigh, North Carolina in 1992 are illustrated in Fig. 1. The lines were calculated from the models using Total Ozone Mapping Spectrometer (TOMS) satellite data for ozone column inputs and assuming clear sky conditions (zero aerosols). Model calculations under these conditions should adequately predict the boundary line for the actual data. It can be seen that in the one case the agreement appears reasonable, while with the earlier model (Green et al. 1980) the overestimates are substantial, especially during the growing season. Further discussion of this point may be found in Fiscus et al. (1994).

Other factors that usually have not been taken into account are seasonal changes and cloud cover. Seasonal considerations are illustrated by Fig. 1. For example, it is clear that the supplement calculated to simulate a column ozone depletion of 20% on day 200 will represent a much larger depletion on day 250.

The potential effects of cloud cover on the target ozone depletion simulation in a system using a fixed daily supplement are illustrated in Fig. 2. For clear sky conditions and TOMS ozone data we can calculate the fixed daily supplement for the summer solstice from the Green et al. (1980) model. This calculation shows that to simulate a 20% decrease in column ozone, we would need to increase PAS300 by 55% over ambient or in this case  $3.8 \text{ kJ m}^{-2} \text{ d}^{-1}$ . However, ground-based measurements and the Björn and Murphy (1985) model indicate that this supplement could actually represent a 23% ozone depletion and a 63% increase in PAS300, not a terribly large discrepancy. However, on a day with average cloud cover or radiation attenuation ( $\text{PAR}/\text{PAR}_{\text{MAX}} = 0.69$ ) that same supplement would be an increase of 88% over ambient and represent an ozone depletion in excess of 30%. Every tenth day, when  $\text{PAR}/\text{PAR}_{\text{MAX}} = 0.25$ , the supplement would be 220% over ambient or 4 times the target level.

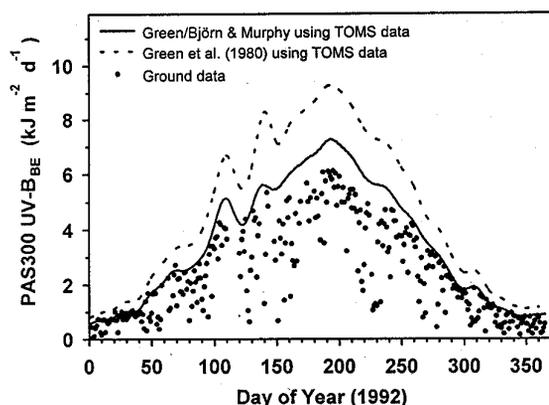


Fig. 1. Comparison of PAS300 UV-B<sub>BE</sub> data for the field site at Raleigh NC (35.75° N, 78.67° W) for 1992 with two predictive models. Lines were calculated using the specified model, aerosol coefficients = 0, and ozone column data from NASA's Total Ozone Mapping Spectrometer (TOMS) on the Nimbus-7 satellite (For the TOMS data we would like to acknowledge Drs Richard D. McPeters and Arlin J. Krueger of NASA GSFC, members of the TOMS Nimbus Experiment and Ozone Processing Teams, and the National Space Science Data Center/World Data Center-A for Rockets and Satellites.). The TOMS data were subjected to a 5% FFT smoothing procedure using TableCurve software (Jandel Scientific, San Rafael, CA) before the curves were calculated from the models so the lines appear much smoother than normal. (Mention of a product or company name does not constitute an endorsement by the United States Department of Agriculture.)

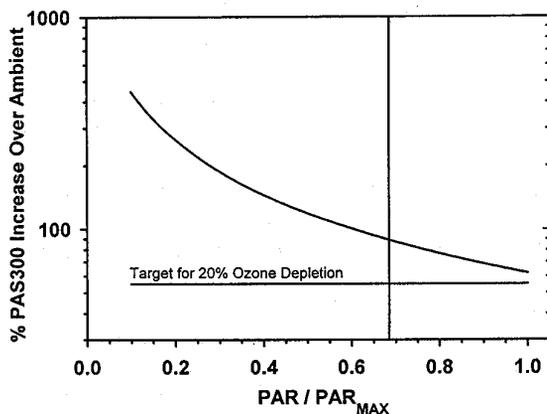


Fig. 2. Potential impact of cloud cover and choice of model on ozone column depletion simulations for Raleigh NC, 21 June 1992. The target simulation, indicated by the horizontal line on the graph, is for a fixed daily supplement calculated from the Green et al. (1980) model using TOMS ozone and clear sky conditions. The curve expresses the fixed supplement as a percentage of ambient UV-B<sub>BE</sub>, which was calculated from ground based measurements of the relationship between PAR and UV-B<sub>BE</sub> during June 1992. The vertical line is the arithmetic mean of PAR/PAR<sub>MAX</sub> for 1992.

The problems of exposure levels can be further compounded in greenhouse experiments. Most greenhouse glass transmits virtually no UV-B and the entire treatment must be supplied from artificial sources; thus, greenhouse treatment levels are especially subject to understatement because of model-based overestimates of ground-level UV-B and should be scrutinized very carefully. For example, at Raleigh, NC the Green et al. (1980) model might predict normal ground level PAS300 of 8.5 kJ m<sup>-2</sup> d<sup>-1</sup>, indicating a supplement of 3.4 kJ m<sup>-2</sup> d<sup>-1</sup> would be necessary to simulate a 20% column ozone reduction on the summer solstice. Therefore, the lamps would be set to deliver total PAS300 of 8.5 and 11.9 kJ m<sup>-2</sup> d<sup>-1</sup> for the control and treatment respectively. Ground-level measurements allow estimates of the UV-B<sub>BE</sub> daily irradiance envelope (Fig. 1) which indicate that the 'control' already represented an 18% column reduction and the 'treatment' is really simulating a 32% depletion if the plants are grown during mid summer. Average cloud conditions, as illustrated in Figs. 1 and 2, could make the 'control' level and the 3.4 kJ m<sup>-2</sup> d<sup>-1</sup> supplement look like 31% and 42% ozone column reductions, respectively. In addition, PAR and UV-A will be decreased, both in absolute terms and relative to UV-B, by average weather conditions, thus further aggravating the potential for damage. Moreover, the 15–20% shading effect in even a well-constructed greenhouse will contribute to the problem of adequately assessing exposure levels.

That these exposure calculations and methodologies can result in substantial experimental and interpretational ambiguities can be illustrated by discussion of one recent greenhouse study on the interaction of UV-B and elevated CO<sub>2</sub> on photosynthesis and productivity (Teramura et al. 1990b). In this study, wheat, rice and soybean were grown in a greenhouse at mid latitudes (36° N) where the annual maximum ambient PAS300 may have been about 6 kJ m<sup>-2</sup> d<sup>-1</sup>. Exposure levels of 8.8 and 15.7 kJ m<sup>-2</sup> d<sup>-1</sup> were calculated as representing ambient conditions at the growth site and a 10% column ozone depletion for Singapore on the maximum annual UV-B day. Ground based measurements less than 30 km from the greenhouse indicated that, in terms of the actual growth site, the 'control' level already represented a column ozone depletion of at least 18% and the enhancement level was in the 45% range (see also Figs. 1 and 2). The highest PAR/UV-B<sub>BE</sub> ratio achieved during these experiments was only slightly over 600, which is only about half the summer average. In addition, these ozone deple-

tion levels were calculated for clear skies and were not discounted for cloud cover or greenhouse shading which would push them even higher and further reduce the PAR/UV-B<sub>BE</sub> ratio. The experimental data from this study indicated that elevated CO<sub>2</sub> significantly increased light-saturated photosynthesis in all three species, but the increase in rice was cut in half by the high UV-B treatment; high CO<sub>2</sub> increased apparent quantum efficiency but high UV-B counteracted that increase in wheat and rice; apparent carboxylation efficiency was decreased by combined high CO<sub>2</sub> and UV-B in wheat and rice; CO<sub>2</sub>-induced water use efficiency increases were unaffected by UV-B in wheat and soybean but halved in rice; and UV-B tended to reduce CO<sub>2</sub>-induced increases in seed yield and total biomass in wheat and rice while soybean was unaffected. The authors interpreted these data as indicating that increased levels of UV-B could negate any beneficial effects on growth and yield that might accrue from increased atmospheric CO<sub>2</sub> levels. In this particular case, use of alternative exposure calculations and ground level monitoring might have completely changed the interpretation of the data. Since their 'controls' already represented an 18% ozone depletion at the growth site, the data might be interpreted to mean that CO<sub>2</sub> elevation will result in increased photosynthesis and yield *despite* enhanced UV-B levels consistent with the worst case scenario for the next century.

Generally speaking, most of the experimental problems and ambiguities concerning exposure levels just discussed could be avoided in field and greenhouse experiments by continuous ambient and canopy level monitoring of UV-B and PAR at the experimental site. The best solution is to use a modulated UV-B dispensing system (Caldwell et al. 1983a; Sullivan et al. 1994) which requires continuous monitoring for proper operation. Interpretation of the exposures in terms of ozone column depletion still depends on the use of accurate UV-B atmospheric transmission models and knowledge of the actual ozone column.

### **Is there evidence that future UV-B levels will damage crop photosynthesis?**

Earlier reviews (Teramura 1983; Sisson 1986; Bornman 1989; Teramura and Sullivan 1994) generally can be summarized as indicating that PS II is the most sensitive component of the photosynthetic system to UV-B and there is little effect of UV-B on stomatal response. With the exception of a few species, UV-B

has little effect on net photosynthesis at field levels of PAR unless exposure levels substantially exceed our worst case scenario. For example, in a 2-year study in the field and greenhouse, in which greenhouse PAR was supplemented to 1600  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , Beyschlag et al. (1988) could find no difference in light- or CO<sub>2</sub>-saturated photosynthesis, stomatal conductance, leaf internal CO<sub>2</sub> partial pressure (C<sub>i</sub>), quantum efficiency or carboxylation efficiency in wheat and wild oats for ozone column depletion simulations of 30–45%. In other studies with these species, Barnes et al. (1987) could find no UV inhibition of photosynthesis. In field-grown *Vicia faba*, Flint et al. (1985) could find no effects of depletion simulations of up to 32% on light-saturated photosynthesis, stomatal conductance or C<sub>i</sub>. And, Miller et al. (1994) could find no differences in net photosynthesis in soybean (cv. Essex) grown in open-top field chambers during depletion simulations of 35%.

Given the lack of uniformity and range of exposures used, it is difficult to generalize about the exact sites of UV-B damage except to say that at low PAR levels non-DNA damage to photosynthetic systems tends to be in the nature of widespread structural disruption. For example, in a study of *Pisum sativum* in greenhouse and growth chamber, Brandle et al. (1977) reported decreases in net photosynthesis after exposures for as little as 15 min. For this same time ultrastructural changes included dilation of the nuclear membrane, chloroplast swelling, thylakoid dilation, rupture of the chloroplast outer membrane, swollen cisternae in the endoplasmic reticulum, and vesiculation of the plasmalemma and tonoplast. Ultrastructural damage accumulated with exposure time and led to both vesiculation in the chloroplast stroma and endoplasmic reticulum and rupture of the plasmalemma and tonoplast in about 26% of the cells during the second day of exposure. As nearly as we can estimate from the paper, the exposure levels were probably simulating ozone column depletions in the range of 50 to 60%.

In cases where it is possible to determine the ozone column depletion simulation, UV-B exposure levels simulating column decreases of 50 to 60% were very common, especially in the earlier literature during a period when projections for stratospheric ozone depletion were also much higher than today. Unfortunately, similar and higher exposure levels are frequently used to the present. Simulations of this magnitude certainly exceed the threshold for damage in many species, especially at low concurrent levels of PAR and UV-A. In an early series of papers, Van and several coworkers

(Van and Garrard 1976; Van et al. 1976) found that all of the six  $C_4$  species tested, but only peanut among the seven  $C_3$  species, were unaffected even at these very high UV-B levels in the greenhouse. The other six  $C_3$  species showed decreased net photosynthesis. The same UV-B exposure level in growth chambers significantly reduced net photosynthesis in all the species tested. Later, using various species under similar growth chamber conditions ( $PAR < 400 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), Garrard et al. (1977) demonstrated decreases in reducing sugars, sucrose, starch, total non-structural carbohydrates, chlorophyll concentrations and Hill activity. Decreases in Hill activity, cyclic photophosphorylation,  $\text{CO}_2$  uptake in leaf disks and Rubisco activity were confirmed in greenhouse experiments but it should be noted that in this study approximately 23% of the annual maximum total daily UV-B irradiance was administered in a period of 10 min (Van et al. 1977). Vu and coworkers also published a series of papers reporting many of the same effects (Vu et al. 1981, 1982a, b, 1984) in addition to changes in Rubisco kinetic parameters, carotenoids and soluble proteins. From these experiments, which were conducted in the greenhouse under more moderate levels of UV-B (maximum column ozone depletion simulation was about 36%) they also reported visual symptoms such as dwarfism, shoot distortion, chlorosis and bronzing. Their reported changes in Hill activity and net photosynthesis did not become apparent until exposure levels reached a simulated 21% ozone depletion, even though reported midday clear sky PAR at canopy height in these experiments was only about  $250 \mu\text{mol m}^{-2} \text{s}^{-1}$ . The culmination of this type of experiment was reported by Strid and co-workers (Strid et al. 1990; Strid and Porra 1992) who irradiated peas under growth chamber conditions of about  $150 \mu\text{mol m}^{-2} \text{s}^{-1}$  of PAR and, as nearly as we can determine from the data supplied, about  $55 \text{ kJ m}^{-2} \text{d}^{-1}$  of PAS300 UV-B<sub>BE</sub> (about 7–9 times the clear sky daily exposure or a column ozone depletion in excess of 70% for summer at mid ( $35^\circ$ ) latitudes). After 8 days of exposure they reported 60% decreases in both total chlorophyll and carotenoids. On a unit chlorophyll basis, PS I and cytochrome f were stable but PS II activity decreased by 55%, ATP hydrolysis by 47%, and maximum Rubisco activity by 80%. However, on the basis of leaf area, PS I and cytochrome-f each were reduced by 58% and the other three parameters by 80%, 80%, and 90%, respectively. Quantum yields, net  $\text{O}_2$  evolution, maximum photosynthesis and  $F_v/F_m$  also were decreased substantially by the treatments. Rubisco activation state was the

only thing to markedly increase. They concluded that UV-B radiation at low PAR levels could be extremely detrimental to mature pea leaves. They also concluded that the UV-B damage to PS II might have been effected indirectly through the D1 subunit by virtue of its high turnover and the effects of UV-B on protein synthesis, which might also account for the declines in Rubisco activity. This latter speculation was confirmed by Jordan et al. (1992) who found, using the same exposure regime, substantial decreases in both the large and small Rubisco polypeptide subunits and their corresponding mRNAs on exposure to supplemental UV-B.

PS II is implicated in many of the studies showing photosynthetic effects and encompassing studies with both intact leaf tissue and isolated chloroplasts or thylakoid membranes. The most susceptible parts of PS II are reported to be either the water splitting enzyme complex (Bornman et al. 1984; Kulandaivelu et al. 1991; Renger and Eckert 1991) or the D1 peptide of the reaction center (Strid et al. 1990; Strid and Porra 1992), or both (Kulandaivelu and Annamalaiathan 1991). In fact, Renger et al. (1989) concluded that UV-B alters the structure of the D1/D2 complex thereby destroying the function of the catalytic site for water oxidation through distortion of the binding sites of the Mn cluster. The distortion of the complex also decreased the number of Atrazine-binding sites located at the opposite membrane surface from the Mn binding locale. Consistent with this latter effect, an earlier report by Renger et al. (1986), along with others (Iwanzik et al. 1983; Bornman et al. 1984; Renger and Eckert 1991), also implicated the plastoquinone binding sites and the formation of additional unspecified dissipative energy sinks. The results of any particular experiment may depend on the exact circumstances of the exposure and perhaps species (Kulandaivelu et al. 1991), and it is very difficult at this time to critically compare work from different laboratories. The major obstacle is a lack of standards for UV-B exposure procedures and reporting which makes it very difficult to determine if the damage reported is generalized in nature or if there is some rate- or cumulative exposure-dependent progression of dysfunction. In particular, exposures generally have been massive without any attempts to relate *in vivo* or *in vitro* treatment levels to conditions in the outside world. Nevertheless, projections connecting the levels of PS II damage to stratospheric ozone losses and reduced crop production have not been lacking.

While the studies of isolated organelles reported here can be very revealing from the point of view of delimiting the tolerance properties of those organelles, it may not be useful to extrapolate these effects to *in vivo* conditions and further to crop growth and yield effects. We must realize that the exposure levels frequently used on isolated chloroplasts or thylakoid membranes in the lab represent several times the annual maximum UV-B irradiance, and that these treatments are more valuable in testing the limits of plant stability than in determining how they normally cope with environmental stresses. For example, in a recent study of UV-B effects, Kulandaivelu et al. (1991) reported that in isolated chloroplasts for 5 out of 6 species examined, the main site of damage was the water oxidizing side of PSII, between the donor sites for Mn or NH<sub>2</sub>OH, while for the other species the PSII reaction center was inhibited directly. In this experiment isolated chloroplasts were exposed to UV-B levels that we estimate as equivalent to about 3× the annual ambient peak. An earlier study from the same lab (Kulandaivelu and Noorudeen 1983) also showed inactivation of the PSII reaction center in isolated chloroplasts when subjected to what we estimate to be about 7× the annual maximum daily exposure. Plants in both of these studies were grown in environmental chambers where PAR levels are unknown. However, based on values from previously published work, PAR was probably no greater than about 100 μmol m<sup>-2</sup> s<sup>-1</sup>. Further, in the field or other high radiation environments, 95% or more of ambient UV-B radiation is filtered out by the epidermis before it can reach a chloroplast. As a result, the exposures used often represent fluence levels at least 100 times the maximum levels of UV-B radiation that an organelle might see in the field where high ambient PAR levels would facilitate photoreactivation and photoprotective processes. Even with the 50% epidermal transmittance values reported by Day et al. (1993), the exposures would still be at about 50 times maximum field levels.

Satisfactory resolution of the question of the primary target in the PSII complex, in a way that is meaningful at the organ or plant level, will require repeating many of the experiments discussed. These experiments need to be conducted under conditions of high PAR, which should be closely monitored, and over a range of well characterized UV-B exposures encompassing natural radiation levels. The progression of damage and susceptibility could be elucidated by exceeding this range by whatever magnitude is necessary to demonstrate the desired effects. However, if the results are to

be interpreted in relation to stratospheric ozone depletion and if that is to be used as a rationale for the research, the experiments need, at a minimum, to span a realistic range of irradiances.

## Conclusions

It is often stated that plant responses to UV-B exposure are quite variable but the rarely recognized corollary of this statement is that the exposure methodologies are as varied as the results. In fact the methodologies may be the main source of variability in the results. Even so, we think it is possible from the available literature and analysis of exposure procedures to draw some generalizations: (1) under extremely high UV-B exposures, far in excess of the worst case for the next century, or very low PAR levels, it is possible to detect damage in virtually any part of the photosynthetic apparatus; (2) it is difficult to find decreases in net photosynthesis or damage to the photosynthetic apparatus in leaves grown under levels of PAR and UV-A typically found in the field, even for levels of ozone depletion at or far exceeding the worst case; and (3) although there appear to be a few species or cultivars susceptible to damage by current UV-B levels (*Rumex patientia* (Sisson and Caldwell 1977), *Cucumis sativa* L. cv Poinsett (Bennett 1981), *Oenothera stricta* (Robberecht and Caldwell 1983) and a few Arctic and northern Alpine species (Caldwell et al. 1982)) it appears that the levels of damage reported under the usual experimental circumstances reflect a much hardier nature for most of the plants studied than previously believed.

One sees in the literature the generalization that one-third to two-thirds of all plants tested are susceptible to damage by UV-B (e.g. Teramura 1990; Teramura and Sullivan 1991a, b, c, 1994; Chow et al. 1992). This generalization is based primarily on growth chamber, greenhouse and field experiments in which the exposure levels were frequently very high, especially with reference to PAR, and just as frequently underestimated. Also, a cultivar or species was considered sensitive if it exhibited a response in a growth chamber or greenhouse regardless of UV-B exposure levels, PAR levels or whether or not that response was also observed in the field. Because the generalization lumps data from a very wide range of experimental conditions, mostly so extreme as to have little environmental relevance, the impression created is one of widespread susceptibility to even modest increases in ground-level UV-B. Contrariwise, during the course of surveying the liter-

ature for this review, one of the truly surprising facts that emerged about these past studies was the extreme measures which had to be employed to produce UV-B effects and how truly insensitive were most of the plants tested, especially when viewed in the context of their normal radiation environment. Insensitivity to UV-B may be due to damage repair, protective mechanisms or some heretofore unrecognized ability to withstand environmental stress. Finally, while acknowledging the utility of using very high UV-B exposures as a probe for physiological responses and functioning, it is our present view that currently projected declines in stratospheric ozone and the associated increases in UV-B that may result from these declines will have no substantial or enduring effect on in situ photosynthetic processes or productivity in the vast majority of crop species.

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