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Effects of Ultraviolet-B Irradiance on Soybean[1]

V. THE DEPENDENCE OF PLANT SENSITIVITY ON THE PHOTOSYNTHETIC PHOTON FLUX DENSITY DURING AND AFTER LEAF EXPANSION

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ABSTRACT

Soybeans (*Glycine max* [L.] Merr. cv Essex) were grown in a greenhouse, and the first trifoliate leaf was either allowed to expand under a high photosynthetic photon flux density (PPFD) (1.4 millimoles per square meter per second) or a low PPFD (0.8 millimoles per square meter per second). After full leaf expansion, plants from each treatment were placed into a factorial design experiment with two levels of ultraviolet-B (UV-B) radiation (0 and 80 milliwatts per square meter biologically effective UV-B) and two levels of concomitant PPFD (0.8 and 1.4 millimoles per square meter per second) resulting in a total of eight treatments. Measurements of net photosynthesis and the associated diffusion conductances, ribulose-1,5-bisphosphate carboxylase activity, chlorophyll and flavonoid concentrations, and leaf anatomy were examined for all treatments. Leaves expanded in the high PPFD were unaffected by UV-B radiation while those expanded in the low PPFD were sensitive to UV-B-induced damage. Likewise, plants which were UV-B irradiated concomitantly with the high PPFD were resistant to UV-B damage, while plants irradiated under the low PPFD were sensitive. The results of this study indicate that both anatomical/morphological and physiological/biochemical factors contribute toward plant sensitivity to UV-B radiation.

In 1974, Molina and Rowland (17) warned of a partial depletion of the stratospheric ozone column by chlorofluorocarbons, primarily used as aerosol propellants and in refrigeration systems. Stratospheric ozone effectively determines the lower limit of short wavelength solar UV radiation penetrating the atmosphere and reaching the earth's surface, absorbing virtually all radiation below 290 nm. Therefore, the waveband affected by such atmospheric modifications is the UV-B (between 280 and 320 nm) portion of the electromagnetic spectrum. Even if stratospheric ozone concentrations were to be diminished by 90%, wavelengths below 280 nm would contain less energy than 10^{-3} w m^{-2} nm^{-1} at a solar zenith angle of 0° (7). There would, however, be a disproportionately large increase in the amount of biologically effective UV-B radiation received at the earth's surface. Caldwell (7) estimated that a 1% decrease in stratospheric ozone concentration would result in an approximate 2% increase in UV-B_{BE}[2] radiation at temperate latitudes. Therefore, the recently projected 5 to 9% stratospheric ozone reduction (18) would result in up to a 19% increase in UV-B_{BE} radiation.

An increase in UV-B irradiance is of particular concern since energy in this waveband is readily absorbed by proteins and it has been demonstrated that plant processes such as photosynthesis (25), transpiration (7, 25), leaf expansion (10, 23, 26, 27), dark respiration (22, 25), and biomass allocation (24) are affected. In the majority of UV-B studies, the UV-B dose utilized was 3- to 5-fold greater than the National Academy of Science's most recent estimates. Only a few studies have employed UV-B doses equivalent to less than a 20% reduction in the ozone layer. Nevertheless, these studies have also demonstrated the deleterious effects of UV-B irradiation upon plants (24–27).

In addition to unrealistically high UV-B irradiances, another criticism of many previous UV-B studies has been the low PPFD under which the plants were grown and irradiated. Only a few studies have examined the effects of UV-B radiation on plants grown under relatively high PPFDs, which more approximate natural conditions (24, 25). It was observed in a number of species that plants were less susceptible to UV-B-induced damage when grown under high PPFDs than under lower PPFDs, when all other conditions remained constant. This amelioration of UV-B-induced damage was attributed to photoprotection and photoreactivation.

High PPFDs might also affect plant sensitivity to UV-B radiation by eliciting plant responses which provide absorbing screens from UV-B radiation. Lautenschlager-Fleury (16) found that plants grown in the sun have greater concentrations of flavonoids in their leaves than shade plants. Several investigators (5, 20, 26, 32) have shown that the flavonoid content of leaves increases after UV-B irradiation, providing a protective mechanism for the plant. Sun plants also have smaller, thicker leaves compared to shade-adapted plants (2, 9). Since UV-B radiation must penetrate the leaf to produce any damage, a thicker leaf might be more protected from damage by UV-B radiation. This suggests that plants grown in the sun could be more resistant to UV-B-induced damage than plants grown in the shade, simply due to anatomical/morphological differences in response to visible radiation.

The purpose of this study was to determine whether anatomical/morphological differences, such as leaf thickness and epidermal UV-B absorbance, are primarily responsible for a decrease in UV-B sensitivity in plants exposed to high PPFDs or whether

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[2] Abbreviations: UV-B_{BE}, biologically effective UV-B; PPFD, photosynthetic photon flux density; UV-B_{DNA}, DNA-effective UV-B; SLW, specific leaf weight; RuBPCase, ribulose-1,5-bisphosphate carboxylase.

physiological/biochemical processes, such as photoprotection and photoreactivation, are more important in regulating plant sensitivity to UV-B radiation.

MATERIALS AND METHODS

Plant Materials and Growth Conditions. Essex soybeans (*Glycine max* [L.] Merr.) were sown four seeds per 0.15-m pot filled with a standard greenhouse potting mixture. Seedlings were grown early in the summer under greenhouse conditions at the University of Maryland, College Park, MD. When the seedlings were approximately 50 mm tall, they were thinned to three plants per pot. The natural photoperiod was approximately 15 h, and midday irradiances under clear skies averaged 1.4 mmol m^{-2} s^{-1} (between 400 and 700 nm) at plant height for an average daily total of 1.8×10^4 mmol m^{-2}. Plants were watered every other day and were fertilized with half strength 20-20-20 (N,P,K) weekly.

Prior to expansion of the first trifoliate, half of the pots were randomly selected and placed under green neutral density Saran, providing 66% shade (low expansion PPFD) while the others remained under unshaded conditions (high expansion PPFD). The peak PPFD under the shade was 0.8 mmol m^{-2} s^{-1}. At this time, plants were treated with a systemic insecticide to prevent insect infestation. For the duration of the experiment, all leaves appearing above the first trifoliate, and all lateral leaves, were removed to minimize shading and to delay senescence of the first trifoliate. After the first trifoliate had become fully expanded (10 d after appearance), one-quarter of the plants from each of the two initial treatments were randomly placed into one of the four following irradiation treatments: high PPFD with UV-B, high PPFD with no UV-B, low PPFD with UV-B, and low PPFD with no UV-B. This resulted in a total of eight treatments, with four replicates in each. The treatments were: high PPFD expanded, concomitantly UV-B irradiated under either the high or low PPFD (H-H-UV and H-L-UV, respectively); high PPFD expanded, UV-B control under either the high or low PPFD (H-H-no UV and H-L-no UV, respectively); low PPFD expanded, simultaneously UV-B irradiated under either the high or low PPFD (L-H-UV and L-L-UV, respectively); and low PPFD expanded, UV-B control under either the high or low PPFD (L-H-no UV and L-L-no UV, respectively). Upon transfer, the plants expanded in the high PPFD treatment were elevated to bring them to the same height as the low PPFD plants which had longer internodes.

UV-B radiation was supplied by Westinghouse FS-40 sunlamps held in lamp frames suspended above the plants. The lamps had been preburnt for 100 h prior to use to minimize irradiation changes with time (25) and were filtered with either 0.076-mm cellulose acetate (transmission down to 292 nm) for the UV-B treatments or 0.13-mm Mylar Type S filters which absorb almost all radiation below 320 nm (controls). Cellulose acetate filters were changed every 5th d to insure uniform irradiation. Additionally, Mylar filters were also suspended between setups to absorb any scattered UV-B radiation. The plants were UV-B irradiated for 9 h daily, centered around solar noon, and the lamp frames were raised as the plants grew to provide a constant lamp-to-leaf distance of 0.20 m. The spectral irradiance at 0.20 m as measured with an Optronics Laboratories model 742 spectroradiometer is shown in Figure 1. The radiation filtered through cellulose acetate supplied a weighted irradiance of 80 effective mw m^{-2} UV-B_{BE} using the generalized plant response action spectrum (6). This is equivalent to 9.47 effective mw m^{-2} UV-B_{DNA} when weighted according to the DNA action spectrum (21). The weighted irradiance for the Mylar filtered lamps was 0 for both UV-B_{BE} and UV-B_{DNA}. The plants were irradiated for a total of 10 d and randomized within treatments daily.

Gas Exchange Measurements. Net photosynthesis and transpiration were measured on the attached center leaflet. The fluxes of CO_2 and H_2O vapor were simultaneously measured in an open system using an Anarad AR-600R IR gas analyzer at a saturating leaf irradiance of 1.5 mmol m^{-2} s^{-1}, average leaf temperature of 34°C, and ambient CO_2 and H_2O vapor concentrations of 349 μl l^{-1} and 16.7 mg l^{-1}, respectively. Leaf temperatures were measured using a 0.13-mm copper-constantan thermocouple and PAR was measured using a LI-COR model LI-185 quantum sensor. Lighting was supplied by 300 w Westinghouse model 300 PAR 56/2WFL low temperature, wide floodlamps filtered through a 70-mm deep circulating water bath. Photosynthesis, transpiration, and the associated diffusive conductances were calculated similar to Gaastra (11) using 1.56 as the factor relating H_2O and CO_2 diffusivities. Nonstomatal conductance was calculated as a residual term.

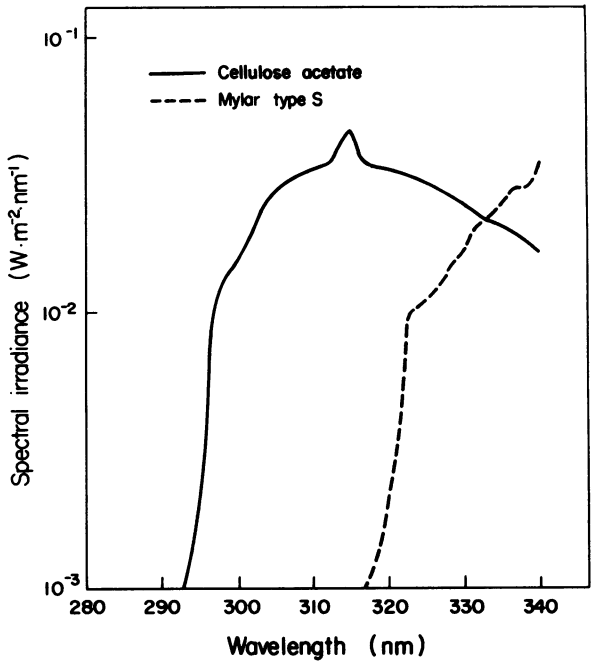

FIG. 1. Unweighted spectral irradiance of FS-40 sunlamps at 0.2 m filtered through either 0.076-mm cellulose acetate or 0.13-mm Mylar filters.

RuBPCase Assay. The center leaflet used for gas exchange measurements was ground in 5 ml of ice-cold buffer solution consisting of 0.12 M Tris-Cl (pH 8.0), 10 mM MgCl, 0.25 mM EDTA, and 7.5 mM reduced GSH. The assay was initiated by adding 100 μl of 0.1 mM RuBP to scintillation vials containing 500 μl reaction buffer (50 mM Tris-Cl [pH 8.0], 5 mM MgCl, 3 mM reduced GSH, and 20 mM $NaHCO_3$), 100 μl enzyme extract, and 2 μCi $NaH^{14}CO_3$ (9.5 μCi/μmol) which had been incubated for 10 min at 30°C. After 3 min, the reaction was stopped by adding 100 μl of 6 M acetic acid. The vials were dried at 90°C, 1 ml distilled H_2O was added, and then 10 ml scintillation cocktail (5.5 g PPO, 200 mg dimethyl POPOP, 666 ml Toluene, 333 ml Triton X-100) were added. Disintegrations per minute were counted with a Packard model 2425 Tri-Carb liquid scintillation spectrometer.

Leaf Anatomy, Chl, and Methanolic Extract. Leaf samples for anatomical studies were removed and stored in Sorensons buffer (0.2 M phosphate buffer [pH 7.0], 1.5 ml of 1.1% $CaCl_2$ [w/v] per 100 ml buffer) until analysis. Thickness of the upper and lower epidermis, the spongy layer, and the palisade layer were measured on five sections per leaflet. Chl was determined according to Arnon (1). Determination of the absorbance of methanolic extract was according to Caldwell (5) with the following modification: instead of using a scintered glass filter, the extract

was centrifuged for 3 min at 600 *g*. Absorbance scans were run using a Perkin-Elmer model 124 double beam grating spectrophotometer and the peaks at 270, 300, and 330 nm were arbitrarily used for analysis.

Statistical Analysis. The data were analyzed using the Statistical Analysis Systems (SAS) statistical package, version 79.5. ANOVA was used to detect treatment differences and means were separated at the 5% level using Duncan's multiple range comparison test. CORR was used to obtain correlation coefficients. Unless otherwise specified, all significant differences are at the 5% level.

RESULTS

Gas Exchange. Overall, UV-B irradiation significantly reduced ($P < 0.01$) net photosynthesis, expressed either on a leaf area or a leaf weight basis (Table I). On an area basis, the average photosynthetic rate of the controls was 13% greater than that of UV-B irradiated plants, while on a weight basis the controls were 11% greater. UV-B-irradiated leaves also exhibited a 29% ($P < 0.01$) decrease in nonstomatal conductance and a 27% decrease ($P < 0.001$) in stomatal conductance compared to control leaves. These reductions were significantly correlated ($r = 0.80$, $P < 0.0001$ and $r = 0.78$, $P < 0.0001$, respectively) to the reduction observed in photosynthetic rate. UV-B radiation also significantly reduced transpiration rates.

Regardless of the concomitant PPFD during irradiation, low PPFD-expanded leaves were sensitive to UV-B radiation, indicated by a 20% reduction in net photosynthesis when both irradiation PPFDs were averaged over each expansion PPFD (Fig. 2; Table I). However, net photosynthesis was unaffected by UV-B radiation in leaves which expanded in the high PPFD. The high PPFD-expanded leaves which were UV-B irradiated had 30% lower nonstomatal conductances than their controls; however, the low PPFD-expanded leaves were nearly twice as sensitive to UV-B radiation as their high PPFD counterparts, exhibiting a 56% decrease in nonstomatal conductance (Table I). There was no significant difference in stomatal conductance between the high PPFD-expanded, UV-B-irradiated leaves and their controls; however, low PPFD-expanded leaves which were UV-B irradiated had a 42% decrease in stomatal conductance relative to their controls. When both irradiation PPFDs were averaged in each expansion PPFD, transpiration was not significantly affected by UV-B radiation.

Despite different expansion PPFDs, leaves were not sensitive to UV-B radiation if simultaneously irradiated with the high PPFD (mean of H-H and L-H) and, therefore, photosynthetic rates were unaffected; however, UV-B reduced photosynthetic rates by 20% in leaves which concomitantly received the low PPFD (Fig. 2; Table I). For nonstomatal conductance, stomatal conductance, and transpiration, leaves UV-B irradiated under the low PPFD had smaller values than their controls, while leaves UV-B irradiated under high PPFD were not significantly different from their controls (Table I).

The only treatment combinations to exhibit significant differences relative to their controls were the low expansion, low irradiation PPFD (L-L) and the high expansion, low irradiation PPFD (H-L). In the L-L-UV, photosynthesis was reduced 25%, nonstomatal conductance 48%, stomatal conductance 55%, and transpiration 28%, relative to the L-L-no UV treatment. In the H-L-UV treatment, nonstomatal conductance was reduced 42% relative to the H-L-no UV treatment.

RuBPCase Assay. There was no significant difference in RuBPCase activity between any treatment and its control, either on a leaf area or a leaf fresh weight basis (Table II).

Chlorophyll. UV-B radiation had significant effects on Chl concentration only in leaves expanding in the low PPFD (L-L and L-H treatments) (Table II). In the L-L-UV treatment, the concentrations of Chl *a* and *b*, and the total Chl concentration were significantly greater than those of the L-L-no UV treatment. In the L-H-UV treatment, only the concentration of Chl *b* was significantly reduced relative to the L-H-no UV treatment.

Anatomy. All leaf anatomical parameters showed significant differences due to expansion PPFD (Table III). The irradiation PPFD and UV-B irradiation had no effect upon any of the examined morphological parameters (data not shown). The thickness of the upper epidermis, palisade layer, spongy mesophyll, and lower epidermis, and SLW were all significantly greater in leaves expanded in the high PPFD than in those expanded in the low PPFD, irrespective of UV treatment or irradiation PPFD.

Methanolic Extract Absorbance. At all three wavelengths, there were highly significant differences ($P < 0.001$) on a leaf area basis due to expansion PPFD, irradiation PPFD, and UV-B irradiation level. The greater the total irradiation dose, either UV or visible, the higher the methanolic extract absorbance. H-H-UV leaves consistently had the highest absorbances, while L-L-no UV leaves consistently had the lowest absorbance values. Leaves irradiated with UV-B (Fig. 3) averaged a 36% higher absorbance than controls, leaves expanded in the high PPFD averaged a 27% higher absorbance than those expanded in the low PPFD, and fully expanded leaves irradiated with the high PPFD averaged an 18% higher absorbance than those under low PPFD irradiation.

DISCUSSION

When weighted for biological effectiveness (using Caldwell's [6] generalized plant weighting function), the daily UV-B dose

Table I. *Means ± SE for Net Photosynthesis, Transpiration, and Associated Conductances as Affected by Expansion PPFD, Irradiation PPFD, and UV-B Irradiation Flux*

Gas exchange measurements were conducted at a saturating irradiance of 1.4 mmol s^{-1} m^{-2}, an average leaf temperature of 34°C, and ambient CO_2 and H_2O vapor concentrations of 349 μl l^{-1} and 16.7 mg l^{-1}, respectively. Means in the same column followed by a different letter are significantly different at the 5% level.

Treatment Code	Expansion PPFD	Irradiation PPFD	UV-B Flux Level	Photosynthesis	Nonstomatal Conductance	Stomatal Conductance	Transpiration
	mmol s^{-1} m^{-2}		*mw m^{-2}*	*μmol CO_2 s^{-1} m^{-2}*	*cm s^{-1}*		*μg H_2O m^{-2} s^{-1} × 10^{-4}*
H-H-noUV	1.4	1.4	0	22.0 ± 1.75 ab	0.371 ± 0.077 bcd	0.784 ± 0.013 ab	6.37 ± 0.22 ab
H-H-UV	1.4	1.4	80	23.2 ± 1.14 a	0.395 ± 0.038 bc	0.757 ± 0.009 ab	6.04 ± 0.35 bc
H-L-noUV	1.4	0.8	0	24.8 ± 0.81 a	0.612 ± 0.125 a	0.769 ± 0.021 ab	6.01 ± 0.43 bc
H-L-UV	1.4	0.8	80	21.6 ± 1.14 ab	0.357 ± 0.063 bcd	0.633 ± 0.006 bc	6.13 ± 0.11 abc
L-H-noUV	0.8	1.4	0	19.0 ± 1.61 bc	0.229 ± 0.016 bcd	0.597 ± 0.024 bcd	5.27 ± 0.20 c
L-H-UV	0.8	1.4	80	16.5 ± 0.84 c	0.198 ± 0.013 d	0.479 ± 0.014 cd	5.56 ± 0.21 bc
L-L-noUV	0.8	0.8	0	22.5 ± 0.45 ab	0.418 ± 0.026 b	0.990 ± 0.009 a	7.11 ± 0.21 a
L-L-UV	0.8	0.8	80	16.9 ± 1.04 c	0.216 ± 0.021 cd	0.444 ± 0.022 d	5.09 ± 0.59 c

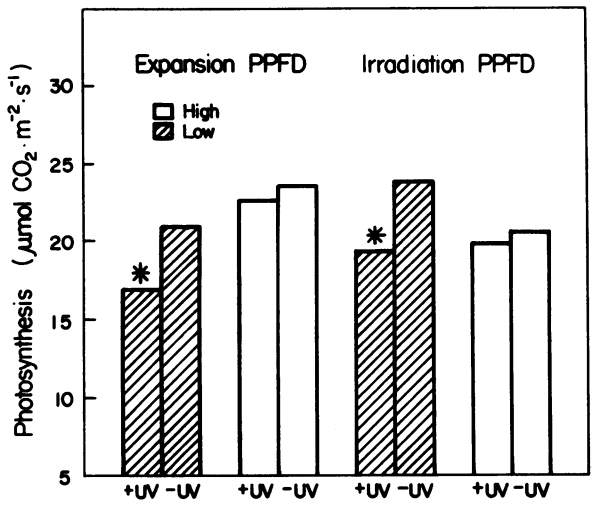

FIG. 2. Photosynthetic rate based on interactions between two levels of expansion PPFD and two levels of UV-B or two levels of irradiation PPFD and two levels of UV-B. An asterisk denotes a significant difference from the control at the 5% level.

used in this study was equivalent to that which would be received at College Park, MD (39° N) under clear sky conditions on June 21 with an anticipated 19% ozone depletion (12). This UV-B irradiance produced significant changes in net photosynthesis, diffusive conductances, transpiration, and pigment concentrations of irradiated soybean plants without having any visible effect upon them. UV-B radiation resulted in a reduction in net photosynthesis which was highly correlated ($P < 0.0001$) with a decrease in nonstomatal conductance. Several other investigators (3, 22, 25) have also reported large UV-B-induced decreases in nonstomatal conductance, leading them to the conclusion that this was a major factor in the decrease of photosynthetic rates in UV-B irradiated plants.

The reduction in nonstomatal conductance in the current study could be due to several factors, among them a decrease in carboxylation activity and/or a decrease in electron transport activity (3, 29–31). RuBPCase activity was unaffected by the UV-B dose employed in this study in general agreement with the results of Thai (28). Other investigators (29, 31) have shown that UV-B radiation significantly reduced RuBPCase activity in a variety of crops. However, in all cases, the UV-B doses employed were much greater than that supplied in the current study, and the PPFD under which the plants were grown was much lower.

Since no differences in RuBPCase activity were found in the current study, one likely explanation for the reductions in net photosynthesis might be a UV-B effect on PSII. It has been demonstrated that UV-B radiation can decrease Hill reaction activity (3, 29, 30) and variable fluorescence (13, 19). Adding dichlorophenolindophenol (DCPIP) which has been reduced by ascorbic acid as an artificial electron donor to PSI results in Hill reaction activities in UV-B irradiated plants which are greater than the UV-B irradiated plants which had no DCPIP added (3). This disruption of electron transport coincided with UV-B-induced structural damage to chloroplast membranes. Brandle *et al.* (3) found 26.2% of UV-B-irradiated cells exhibited damage to organelles as compared to less than 1% for control cells. While photosynthetic rates of the L-L-UV leaves in the present study were significantly less than those of the L-L-no UV leaves, the Chl content of the L-L-UV leaves was significantly greater. This, combined with the nonstomal conductance decrease and the lack of any effect on RuBPCase activity levels due to UV-B irradiation of the L-L plants implies that Chl concentration was not a limiting factor and that UV-B radiation decreases photosynthesis by interrupting electron transport. More recently, studies by Noorudeen and Kulandaivelu (19) and Iwanzik *et al.* (13) have concluded that UV-B radiation inactivates the reaction centers of PSII, transforming them into dissipative sinks for excitation energy.

In addition to nonstomatal conductance, a significant decrease in stomatal conductance followed UV-B irradiation. Although stomatal conductance accounted for only one-third of the total leaf diffusive conductance, it was highly correlated with the decrease in photosynthesis ($r = 0.78$, $P < 0.0001$). Earlier studies with soybean (25) have also shown that stomatal conductances can be affected by ambient levels of UV-B radiation when grown under light-limited conditions. In *Cucumis sativus* L., a very UV-B sensitive species, Teramura *et al.* (27) found that a low UV-B irradiance (11.7 mw m^{-2} UV-B_{BE}) dramatically altered the diurnal pattern of stomatal conductance. The mechanism of the effects of UV-B radiation on stomatal conductance is presently unknown.

In the present study, UV-B radiation had no effect on the gas exchange characteristics of leaves which expanded in the high PPFD. The decreased UV-B sensitivity resultant from the high PPFD growth environment was correlated with increases in methanolic extract absorbance and differences in anatomy between leaves expanded in the high PPFD compared with the low PPFD. This increase in absorbance may have been due to an increase in the biosynthesis of flavonoids and related phenolics (32). Leaves which expanded and remained in the high PPFD had greater absorbances than leaves from other treatments in the absence of UV-B radiation. UV-B radiation additionally produced a substantial increase in extract absorbance, a phenomenon that has been observed in many plant species (5, 26) and has been proposed as an adaptive mechanism, screening plant tissues from the deleterious effects of UV-B radiation. These absorbance changes primarily occur in cells of the upper epider-

Table II. *Means ± SE for RuBPCase Activity, Total Chl, Chl a, Chl b, and the Chl a/b Ratio as Affected by Expansion PPFD, Irradiation PPFD, and UV-B Irradiation Flux*

Means in the same column followed by a different letter are significantly different at the 5% level.

Treatment Code	Expansion PPFD	Irradiation PPFD	UV-B Flux Level	RuBPCase Activity	Total Chl	Chl *a*	Chl *b*	Chl *a/b*
	mmol s^{-1} m^{-2}		*mw* m^{-2}	*dpm* $m^{-2} \times 10^{-8}$		*g* $m^{-2} \times 10^{-2}$		
H-H-noUV	1.4	1.4	0	2.72 ± 0.22 ab	3.59 ± 0.14 c	2.74 ± 0.10 bc	0.85 ± 0.03 cd	3.24 ± 0.05 a
H-H-UV	1.4	1.4	80	2.79 ± 0.33 a	3.91 ± 0.17 abc	2.97 ± 0.13 abc	0.94 ± 0.04 abc	3.15 ± 0.09 a
H-L-noUV	1.4	0.8	0	1.82 ± 0.27 c	4.17 ± 0.12 a	3.15 ± 0.11 a	1.01 ± 0.02 a	3.11 ± 0.07 a
H-L-UV	1.4	0.8	80	2.00 ± 0.19 bc	4.06 ± 0.14 ab	3.09 ± 0.10 ab	0.97 ± 0.04 ab	3.19 ± 0.03 a
L-H-noUV	0.8	1.4	0	2.91 ± 0.21 a	3.11 ± 0.07 d	2.36 ± 0.05 d	0.75 ± 0.02 de	3.15 ± 0.05 a
L-H-UV	0.8	1.4	80	2.90 ± 0.24 a	2.93 ± 0.04 d	2.24 ± 0.03 d	0.70 ± 0.02 c	3.22 ± 0.06 a
L-L-noUV	0.8	0.8	0	2.67 ± 0.20 ab	3.14 ± 0.06 d	2.37 ± 0.06 d	0.77 ± 0.01 de	3.06 ± 0.06 a
L-L-UV	0.8	0.8	80	2.41 ± 0.12 abc	3.62 ± 0.29 bc	2.73 ± 0.22 c	0.89 ± 0.07 bc	3.05 ± 0.03 a

Table III. *Thickness of the Upper Epidermis, Palisade Layer, Spongy Mesophyll, and Lower Epidermis, and Specific Leaf Weight (SLW) as Affected by Expansion PPFD*

Means in the same column followed by a different letter are significantly different at the 5% level.

Expansion PPFD	Upper Epidermis Thickness	Palisade Layer Thickness	Spongy Mesophyll Thickness	Lower Epidermis Thickness	SLW
mmol m^{-2} s^{-1}		*μm*			*g m^{-2}*
1.4	13.86 ± 0.34 a	51.65 ± 0.92 a	92.29 ± 1.96 a	13.28 ± 0.22 a	39.09 ± 0.58 a
0.8	12.36 ± 0.37 b	48.78 ± 0.64 b	82.28 ± 1.32 b	12.59 ± 0.23 b	34.46 ± 0.46 b

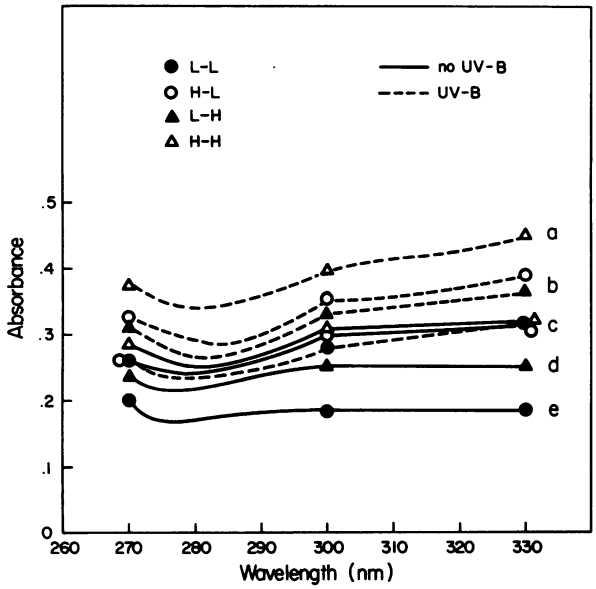

FIG. 3. Absorbance of methanolic extract (on a leaf area basis) as determined by interactions among expansion PPFD, irradiation PPFD, and UV-B irradiation level. Absorbance values at 330 nm followed by the same letter are not significantly different at the 5% level.

mis (20) and are therefore relatively independent of anatomical changes, such as increases in leaf thickness. Since high PPFDs also increase methanolic extract absorbances in the UV region, a plant growing naturally in a high insolation environment may be inherently less sensitive to UV-B radiation.

Generally speaking, plants grown in the sun tend to have thicker leaves than those grown in the shade (2, 4, 9). Although neither UV-B radiation itself nor the concomitant PPFD had any effect upon leaf anatomical characteristics, leaves which expanded in the high PPFD possessed significantly thicker upper epidermal, palisade, spongy mesophyll, and lower epidermal layers than leaves expanded in the low PPFD. Since UV-B radiation must penetrate into the leaf to produce any damage, a thicker leaf which has a greater proportion of sensitive organelles, such as chloroplasts, located in deeper, more protected tissue layers would be more resistant to UV-B radiation damage than would a thinner leaf without the benefit of these anatomical screens.

Increased flavonoid biosynthesis and changes in anatomy do not, however, explain why plants irradiated with UV-B concomitantly with a high PPFD were less sensitive to UV-B-induced damage than those irradiated in the low PPFD. Several investigators (5, 22, 24, 25) have demonstrated that longer wavelength (315–550 nm) radiation, supplied either immediately prior to, during, or after UV radiation can minimize or completely eliminate any deleterious effects that the UV radiation might otherwise have had upon the plant. It has been demonstrated (8, 14, 15) that supplying plants with longer wavelength radiation before UV-B irradiation results in decreased respiration and cell division which allows a cell to undergo more dark repair of UV-B-induced damage. This decreases plant sensitivity to UV-B radiation and is termed photoprotection. A reduction of UV-B-induced damage by simultaneous or subsequent exposure to longer wavelength (315–550 nm) radiation has also been observed. This reduction is thought to be attributed to photoreactivation, which involves the activation of specific enzymes which repair UV-B-induced damage.

In the current study, plant sensitivity to UV-B radiation was determined at least in part by the PPFD concomitant with UV-B irradiation. Plants UV-B irradiated concomitantly with the low PPFD were more sensitive to UV-B-induced damage than those irradiated with UV-B in the high PPFD. This supports the possible role of photoreactivation and photoprotection since the UV-B irradiation was bracketed for several hours by and was also accompanied by the high PPFD. Since photosynthesis was affected by UV-B only in leaves both expanded and irradiated under the low PPFD, this implies that a high PPFD, either at the time of expansion or at the time of UV-B irradiation, can be effective in minimizing the detrimental effects of the UV-B radiation.

This study suggests that some plant adaptations to high insolation environments, such as leaf thickening, reduction in leaf area, and increased leaf pigment production, additionally contribute toward protection from the deleterious effects of UV-B radiation. The ecological implications of this conclusion are that leaves expanding under unshaded field conditions would consequently be less sensitive to UV-B radiation than those expanding in the shade. Leaves which had developed in the shade, but were subsequently exposed to higher levels of UV-B radiation such as might occur due to sunflecks or canopy removal, would have some of the effect of UV-B radiation ameliorated by the simultaneous increase in visible radiation which would accompany the UV-B. Nevertheless, the effects of UV-B radiation may not entirely be mitigated and therefore may ultimately have serious adverse effects.

In summary, the results of this study confirm that the sensitivity of soybean to UV-B irradiation is affected by longer wavelength radiation. A high PPFD, provided either during leaf expansion prior to UV-B irradiation (preconditioning PPFD), or concomitantly with UV-B irradiation decreases soybean sensitivity to UV-B radiation. This protective effect appears to involve both anatomical/morphological changes and biochemical/physiological process.

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