Plant Physiol. (1993) 103: 741–752

The Role of Flavonol Glycosides and Carotenoids in Protecting Soybean from Ultraviolet-B Damage[1]

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The increase in ultraviolet-B (UV-B; 0.290–0.320 μm) radiation received by plants due to stratospheric ozone depletion heightens the importance of understanding UV-B tolerance. Photosynthetic tissue is believed to be protected from UV-B radiation by UV-B-absorbing compounds (e.g. flavonoids). Although synthesis of flavonoids is induced by UV-B radiation, its protective role on photosynthetic pigments has not been clearly demonstrated. This results in part from the design of UV-B experiments in which experimental UV-A irradiance has not been carefully controlled, since blue/UV-A radiation is involved in the biosynthesis of the photosynthetic pigments. The relationship of flavonoids to photosynthetic performance, photosynthetic pigments, and growth measures was examined in an experiment where UV-A control groups were included at two biologically effective daily UV-B irradiances, 14.1 and 10.7 kJ m^{-2}. Normal, chlorophyll-deficient, and flavonoid-deficient pigment isolines of two soybean (*Glycine max*) cultivars that produced different flavonol glycosides (Harosoy produced kaempferol, Clark produced quercetin and kaempferol) were examined. Plants with higher levels of total flavonoids, not specific flavonol glycosides, were more UV-B tolerant as determined by growth, pigment, and gas-exchange variables. Regression analyses indicated no direct relationship between photosynthesis and leaf levels of UV-B-absorbing compounds. UV-B radiation increased photosynthetic pigment content, along with UV-B-absorbing compounds, but only the former (especially carotenoids) was related to total biomass ($r^2 = 0.61$, linear) and to photosynthetic efficiency (negative, exponential relationship, $r^2 = 0.82$). A reduction in photosynthesis was associated primarily with a stomatal limitation rather than photosystem II damage. This study suggests that both carotenoids and flavonoids may be involved in plant UV-B photoprotection, but only carotenoids are directly linked to photoprotection of photosynthetic function. These results additionally show the importance of UV-A control in UV-B experiments conducted using artificial lamps and filters.

Anthropogenic chemical depletion of the stratospheric ozone layer has resulted in an increase in UV-B radiation (0.290–0.320 μm) reaching terrestrial organisms (Blumthaler and Ambach, 1990). Common plant responses to UV-B irradiation include inhibition or impairment of plant function, subsequently resulting in a decline in productivity (Tevini and Teramura, 1989). Reduction in plant function is primarily associated with damage to DNA, proteins, and plant membranes. DNA damage occurs when cyclobutane-type pyrimidine dimers are formed by cross-linking of strands; these dimers can subsequently be monomerized, a process known as photoreactivation or photorepair, by the blue/UV-A-activated enzyme photolyase (Sutherland, 1981; Langer and Wellman, 1990; Pang and Hays, 1991). Photosynthetic damage is associated primarily with PSII (Tevini et al., 1991), as well as disruption of the chloroplast membrane (Bornman, 1989).

The effect of UV-B irradiation on the associated light-harvesting complexes is equivocal, since inconsistent results have been reported on the relative change in the constituent photosynthetic pigments, the Chl's, and carotenoids. However, the greatest effects of UV-B radiation on photosynthetic pigments and other plant responses have been observed when UV-B exposure is provided concomitant with low PPF, whereas plants grown under high PPF conditions (>1000 μmol m^{-2} s^{-1}) are less affected by UV-B radiation, a result that has been attributed to photoprotection or photorepair induced by visible light (Warner and Caldwell, 1983; Mirecki and Teramura, 1984; Cen and Bornman, 1990).

Protective responses are also stimulated by UV-B radiation, including increased production of UV-B-absorbing compounds (e.g. flavonoids), secondary compounds ubiquitous in higher plants (Wellmann, 1971; Flint et al., 1985; Beggs et al., 1986a, 1986b; Murali et al., 1988; Tevini et al., 1991). Flavonoids are thought to protect photosynthetic tissues by acting as screening pigments, absorbing UV-B radiation. The evidence for this comes from several sources: UV-B-induced flavonoid production is generally localized in the leaf upper epidermis and has a maximal absorption around 0.300 μm

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[1] This research was supported in part by U.S. Department of Agriculture Competitive Grants Program (No. 89-37280-4744).

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Abbreviations: E, transpiration; *g*, conductance to water vapor; HC, high control; HT, high treatment; LC, low control; LT, low treatment; P_{CO2}, net photosynthetic capacity based on carbon dioxide exchange; P_{CO2} g^{-1} Chl, photosynthetic efficiency expressed as carbon dioxide exchange per unit Chl; P_{O2}, maximum net photosynthetic capacity based on oxygen evolution; P_{O2} g^{-1} Chl, photosynthetic efficiency expressed as oxygen evolution per unit Chl; PPF, photosynthetic photon flux, 0.400–0.700 μm; *SLW*, specific leaf weight (leaf mass to area ratio); UV, ultraviolet radiation between 0.200 and 0.400 μm; UV-A, ultraviolet radiation between 0.320 and 0.400 μm; UV-B, ultraviolet radiation, 0.290–0.320 μm; UV-B_{BE}, biologically effective ultraviolet radiation, weighted with a generalized action spectrum.

Table I. *Characteristics of select isolines for soybean cultivars Clark and Harosoy*

Cultivar and Isoline [Strain Designation]	Symbol	Characteristics	Flavonol Glycoside Type and Amount
Clark			
Normal [Clark]	Clk	Normal Chl and flavonol glycoside levels	Moderate kaempferol and quercetin levels
Magenta [L72-2181]	Clk-wm	Normal Chl levels, reduced flavonol glycosides	Low kaempferol and quercetin levels
Chl mutant [L69-4755]	Clk-y_9	Deficient in photosynthetic pigments, especially in Chl *b*	Low-moderate kaempferol and quercetin levels
Harosoy			
Normal [Harosoy]	Hsy	Normal Chl and flavonol glycoside levels	Moderate kaempferol levels
Magenta [T235]	Hsy-wm	Normal Chl levels, reduced flavonol glycosides	Low kaempferol levels
Chl mutant [L69-4318]	Hsy-y_9	Deficient in photosynthetic pigments, especially in Chl *b*	Moderate kaempferol levels

(Beggs et al., 1985, 1986a, 1986b; Bornman et al., 1986; Teramura, 1986). Increased flavonoid production in leaves exposed to UV-B irradiation has been reported consistently. Also, flavonoid accumulation in parsley and soybean cell-suspension cultures is linked to UV-B-induced activation of chalcone synthase and "group I" enzymes in the flavonoid synthesis pathway (Wellmann, 1971; Ragg et al., 1981; Chappell and Hahlbrock, 1984; Stafford, 1990), although red and blue light and many other environmental factors can modify the induction response. Additionally, plants collected in naturally high UV-B radiation environments tend to be less affected by experimental exposure to UV-B radiation (Robberecht and Caldwell, 1986; Sullivan et al., 1992; Ziska et al., 1992), possibly due to adaptive accumulation of high levels of leaf flavonoids.

Despite these documented associations between UV-B irradiation and leaf flavonoid content, a direct dependence of photosynthetic response on flavonoid content has not been consistently observed (Tevini et al., 1991). Moreover, if flavonoids protect photosynthetic cells by absorbing UV-B radiation, the photosynthetic pigments should be maintained at normal levels as flavonoids increase in response to UV-B irradiation; if flavonoids fail to increase, as is often observed in UV-sensitive plants, losses in photosynthetic pigments would be expected. However, an increase in photosynthetic pigments would imply that their synthesis is also induced, directly or indirectly, by UV-B radiation.

To determine the effect of UV-B radiation on photosynthetic pigments, and whether flavonoids are involved in protecting them, it is necessary to control UV-A radiation simultaneously provided by the UV-B light source. This is especially important in glasshouse and growth chamber studies where the solar UV-A irradiance is either restricted or lacking altogether. Middleton and Teramura (1993) found that filtered UV-B lamps produced substantially different UV-A irradiances for two contrasting UV-B irradiances (e.g. biologically effective UV-B, or UV-B_{BE}, daily irradiances of 14.1 and 10.7 kJ m^{-2}). Besides the photorepair of UV-B-induced DNA damage, UV-A/blue radiation is involved in several steps in the synthesis of both carotenoids and Chl's (Rau and Schrott, 1987; Senger, 1987) and is positively linked to photosynthetic performance (Kawallik, 1987). Consequently, the UV-A irradiances associated with UV-B treatments may require greater attention, analogous to that of visible light.

Soybean (*Glycine max* [L.] Merr.) genotypes exhibit a wide range in sensitivity to UV-B radiation, attributable in part to differences in flavonoid content (Sullivan and Teramura, 1990; Reed et al., 1992). Flavonol glycosides are the major type of flavonoids produced in soybean, dominated by kaempferol and quercetin (Stafford, 1990). To determine whether flavonoids protect photosynthetic pigments, two soybean cultivars, Clark and Harosoy, with analogous isolines for normal, flavonoid-deficient, and Chl-deficient traits, were utilized in this study. In addition to the pigment concentration differences among these isolines, the type of flavonol glycosides differ between the two cultivars: the three Harosoy isolines produce only kaempferol; the three Clark isolines produce both kaempferol and quercetin (Buzzell et al., 1980). Neither cultivar had been examined previously for sensitivity to UV-B radiation.

This is the first of several studies to evaluate whether UV-A radiation that was received concomitantly with UV-B radiation contributed to differences in plant response, especially for photosynthetic pigment-related variables and processes, in glasshouse studies where the solar UV-A irradiance (especially $\lambda < 0.350$ μm) was restricted by the glass. Additionally, the present study examined whether flavonoids conferred protection to the photosynthetic apparatus, and whether there were differences in effectiveness for different flavonoids under high visible irradiances (PPF > 1200 μmol m^{-2} s^{-1} at midday).

MATERIALS AND METHODS

Plant Material

The study included three pigment isolines of two soybean (*Glycine max*) cultivars (Table I), Clark and Harosoy. The wild-type, or normal, isolines of each cultivar (Clk; Hsy) produced moderate flavonol glycoside levels; the flavonoid mutant, or magenta, isolines of each cultivar (Clk-wm; Hsy-wm) had reduced flavonol glycoside levels; the Chl *b* mutant, or Chl-deficient lines of each cultivar (Clk-y_9; Hsy-y_9), had low to moderate flavonol glycoside levels, in combination with reduced Chl content. Plants were grown individually in 2.5-L pots from seed (provided by R.L. Nelson, U.S. Department of Agriculture, University of Illinois, Urbana) in standard potting mixture (ProGro 300S), inoculated at planting with *Bradyrhizobium japonicum*, watered twice daily, and fertilized biweekly (20–20–20, N-P_2O_5-K_2O); all plants were nodulated at harvest.

UV Radiation Treatments

The experiment was conducted during April and May of 1990 in an unshaded glasshouse at the University of Maryland, College Park, MD, which was equipped with fans and water-evaporative cooling pads. Ambient daytime temperature was 29 ± 6°C and clear-sky midday PPF averaged 1300 to 1600 μmol m^{-2} s^{-1}.

UV radiation was provided by fluorescent sun lamps (UVB-313; Q-Panel Co., Cleveland, OH). Lamps were placed above benches in hanging horizontal racks, each holding 12 parallel lamps spaced 0.3 m apart. Racks were equally subdivided, with half assigned to a UV-B treatment and half assigned to the corresponding control. The lamps were wrapped with different types of presolarized plastic cut-off filters: for the UV-B treatments, cellulose diacetate (0.08 mm) was used to eliminate UV-C radiation ($\lambda < 0.290$ μm), thereby transmitting UV-B and longer wavelengths, including UV-A radiation; for controls, polyester film (Mylar Type S, 0.13 mm) was used to eliminate UV-B and UV-C radiation ($\lambda < 0.320$ μm), transmitting UV-A and longer wavelength radiation. Consequently, UV-B treatments are actually "UV-B + UV-A," whereas controls are "UV-A," with all plants receiving the same level of ambient visible irradiance. Typically, the effect of UV-B radiation is determined as the difference between the plant response to the UV-B treatment and its control, which assumes that the UV-A components of both are equivalent.

Differences in UV irradiance between the LC and HC groups were achieved by using lamps with different UV outputs and by utilizing different rack heights. Within the LC and HC groups, the treatment irradiances were maintained with a constant rack height above the plants. Measurements of UV-B radiation were made at the beginning of the experiment for all treatment and control groups; all of the plants were wrapped with presolarized cellulose diacetate. After matching controls with UV-B treatments on the basis of equivalent UV-B radiation, the cellulose diacetate filters on lamps assigned to the controls were replaced with presolarized polyester filters. UV-A irradiances were estimated by regression from UV-B and UV-A measurements made on the lamps at a later date.

Two UV-B irradiances (HT and LT) and two controls (HC and LC) were established, based on 9 h of irradiation centered at solar noon. The UV-B radiation was spectrally weighted for biological effectiveness (Caldwell, 1971), whereas the UV-A was unweighted. The mean HT daily irradiance was 14.1 ± 0.4 kJ m^{-2} UV-B$_{BE}$ and 8.9 ± 0.5 kJ m^{-2} UV-A. The mean LT daily irradiance was 10.7 ± 0.4 kJ m^{-2} UV-B$_{BE}$ and 5.2 ± 0.5 kJ m^{-2} UV-A. The mean daily irradiances for HC and LC were 8.2 ± 0.8 and 4.9 ± 0.5 kJ m^{-2} UV-A, respectively. The variabilities given include the filter degradation effects. The UV-B$_{BE}$ irradiances corresponded to those anticipated with a 30% and 15% stratospheric ozone depletion at the summer solstice over College Park, MD (39°N), as determined by an empirical model (Green et al., 1980). Approximately 80 ± 5% of the total UV-A radiation from the lamps (with new, presolarized filters) was in the shortwave region (0.320–0.350 μm); the daily ambient clear-sky UV-A radiation inside the glasshouse was approximately 20 kJ m^{-2}, but only 15% of this (approximately 3 kJ m^{-2}) was in the shortwave UV-A region (Middleton and Teramura, 1993).

The spectral irradiance at plant height below the racks was measured with a spectroradiometer (Optronics model 742, Optronics Laboratory, Inc., Orlando, FL) equipped with a double monochromator with dual holographic grating, interfaced with a printing calculator (Hewlett-Packard 85, Cupertino, CA). The spectroradiometer was calibrated separately for UV-B and UV-A wavebands while fitted with Teflon and quartz filters, respectively, using a National Institute of Standards and Technology traceable 1000-W tungsten halogen lamp. Wavelength alignment was checked using mercury vapor emission lines from a mercury arc lamp. With the Teflon filter, the UV-B$_{BE}$ was determined from spectral measurements made between 0.260 and 0.350 μm at 0.001-μm intervals, weighted with the generalized plant response action spectrum (Caldwell, 1971) and normalized to 0.300 μm. With the quartz filter, the total unweighted UV-A irradiance was calculated from spectral measurements made between 0.260 and 0.400 μm at 0.001-μm intervals.

Experimental Design

Two racks/benches were assigned to the HT and HC and two to the LT and LC. The UV-B treatment and control in each rack were separated by a hanging sheet of polyester. Each treatment group was assigned 18 pots with 2-week-old seedlings (three replicates per isoline per treatment group). Pot positions were randomized within each group twice weekly to minimize position effects, and lamp rack heights were adjusted at least weekly to maintain the plant canopy-to-lamp rack distance, and cellulose diacetate filters were changed weekly and polyester was changed biweekly.

A randomized complete block design was used, replicated twice, with treatments arranged as a split-plot. Here, the traditional comparison between "UV-B" and "no UV-B" was made to evaluate UV-B radiation effects on each variable. This comparison is actually a contrast of spectral irradiance quality (UV-B + UV-A versus UV-A only); treatments were also compared to evaluate the effect of relative irradiance (high or low). These two radiation factors served as the whole-plot experimental factors, UV spectral irradiation (H_O: UV-B + UV-A = UV-A) and irradiance (H_O: high = low). The two subplot factors were cultivar (H_O: Clark = Harosoy) and isoline (H_O: normal = magenta = Chl deficient). The experiment was conducted as a full factorial [(2 UV spectral

Table II. *Leaf-level pigment and gas-exchange variables exhibiting UV-B effects: statistical significance*[a] *for experiment-wide sources of variance*

Source of Variation	df	Growth			Pigments			Gas Exchange			
		Leaf area	Root dry weight	Plant biomass	Chl *a*	Total carotenoids	UV-B-absorbing compounds	Photosynthesis	*g*	*E*	Photosynthetic efficiency
		cm^{-2}	*g*	*g*	$\mu g\ cm^{-2}$	$\mu g\ cm^{-2}$	$A_{300}\ cm^{-2}$	$\mu mol\ CO_2\ m^{-2}\ s^{-1}$	$mol\ H_2O\ m^{-2}\ s^{-1}$	$mmol\ H_2O\ m^{-2}$	$\mu mol\ CO_2\ g^{-1}\ Chl$
Whole plot factors											
Block	1	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Irradiance	1	ns	*	ns	*	ns	*	ns	*	*	*
UV spectral irradiation	1	**	ns	ns	**	**	**	*	**	*	**
Irradiance × UV spectral irradiation	1	*	*	*	***	**	ns	ns	ns	ns	ns
Error (a)	3										
Sub plot factors											
Cultivar	1	***	***	ns	***	***	***	***	***	***	***
Isoline	2	***	***	***	***	***	***	ns	ns	ns	***
Cultivar × isoline	2	*	**	***	***	***	***	ns	ns	ns	***
Cultivar × irradiance	1	ns	ns	ns	ns	ns	ns	ns	ns	ns	*
Cultivar × UV spectral irradiation	1	*	ns	ns	ns	ns	**	*	**	*	ns
Isoline × irradiance	2	ns	ns	ns	ns	ns	ns	ns	ns	ns	**
Isoline × UV spectral irradiation	2	ns	ns	ns	ns	ns	ns	ns	ns	ns	*
All three-factor interactions	9	ns	ns	ns	ns	ns	ns	ns	ns	ns	*[b]
Error (b)	115										

[a] Significance levels are denoted: significant, * $P \le 0.05$; highly significant, ** $P \le 0.01$; and very highly significant, *** $P \le 0.001$. [b] The significant three-factor interaction is cultivar × isoline × irradiance.

irradiations) × (2 irradiances) × (2 cultivars) × (3 isolines)]. Statistical analyses were performed using SYSTAT V. 5.0 (1990, SYSTAT, Inc., Evanston, IL).

Measurements

At flowering (after 8 weeks of growth), gas-exchange variables (photosynthesis, P_{CO2}, measured as carbon dioxide exchange, μmol m^{-2} s^{-1}; *g*; and *E*) were measured using a portable photosynthesis system (ADC LCA-3, Analytical Development Co., Ltd., Hoddesdon, England) on the youngest fully expanded terminal leaflet. These laboratory measurements were made at light saturation (PPF = 1800 μmol m^{-2} s^{-1}), ambient CO_2 (350 ± 20 ppm), and leaf temperatures of 32 ± 1°C. Maximum photosynthesis (light-saturated, 5% CO_2 in air), P_{O2}, was also determined by an oxygen evolution system (Hansatech Corp., Kingslynn, England) on leaf discs (chamber approximately 31°C) taken from the youngest, fully expanded terminal leaflet of all plants in the Clark isolines. Photosynthetic efficiency (photosynthesis per unit Chl) was calculated and expressed as either P_{CO2} g^{-1} Chl or P_{O2} g^{-1} Chl).

Coincident to the first set of photosynthetic measurements, several leaf discs were taken from the same trifoliate leaf for determination of photosynthetic pigments (Chl *a*, Chl *b*, and carotenoids) and bulk UV-B-absorbing compounds. Photosynthetic pigments were extracted in 5 mL of DMSO for 12 h in the dark (Chappelle and Kim, 1992). The absorption spectra between 0.300 and 0.750 μm were determined using a computerized dual beam spectrophotometer (Perkin-Elmer Lambda 3, Perkin-Elmer, Norwalk, CT) with a resolution of 0.001 μm. Photosynthetic pigment concentrations were calculated from absorbances at 0.664, 0.648, and 0.470 μm according to equations given by Lichtenthaler (1987) and Chappelle and Kim (1992). UV-B-absorbing compounds were extracted from dry leaf discs after 72 h in the dark in 10 mL of acidified methanol (79:20:1 by volume, methanol:water:HCl), according to standard procedures (Mirecki and Teramura, 1984), and absorbance was arbitrarily measured with the spectrophotometer at 0.300 μm. All absorbances were determined on 10-mL samples in quartz cuvettes.

Plants were harvested after 8 weeks of UV irradiation. The one-sided leaf area for the plant and for a single leaflet of the youngest fully expanded leaf was determined by an area meter (Li-Cor model 3100, Li-Cor, Inc., Lincoln, NE). Roots were harvested and dry biomass for plant organs were obtained after oven-drying for 1 week at 60°C. *SLW* was computed on dried material.

RESULTS

General Experiment-Wide Treatment Effects and Interactions

The experiment-wide analysis of variance, summarized in Table II, examined the overall average response for each experimental factor. The plant response to the whole-plot radiation factors was of particular interest. All pigment, gas-exchange, and some growth variables showed significant ($P \leq 0.05$) UV-B radiation effects. However, P_{CO_2} ($P < 0.05$) was the only variable that was influenced by UV-B irradiation only. The three other gas-exchange variables and the UV-B-absorbing compounds exhibited significant irradiance effects in response to either UV-B or UV-A radiation. Several growth variables (total biomass, root mass, and individual leaf area) and the photosynthetic pigments exhibited a significant ($P \leq 0.05$) UV spectral irradiation × irradiance interaction, indicating that the pattern of growth or pigment response for UV-B treatments and controls was different at high and low irradiances.

Cultivar differences were highly significant ($P \leq 0.01$) for all variables except total biomass. A significant cultivar × UV spectral irradiation interaction, indicating that the two cultivars responded differently to UV-B and/or UV-A irradiation, was associated with several variables (leaf area, UV-B-absorbing compounds, P_{CO_2}, g, and E). Isoline differences were also highly significant for all growth and pigment variables, but not for the primary gas-exchange variables (P_{CO_2}, g, and E); the cultivar × isoline interaction was also significant for many of these variables, displaying a different response by the analogous isoline per cultivar. Other two- and three-factor interactions were unimportant for most variables, except photosynthetic efficiency, which exhibited isoline × (UV spectral irradiation and irradiance) interactions.

Specific Responses per Plant Group and Radiation Treatment

Generalizations in plant response were not possible for many plant variables because of the statistical interactions among experimental factors. Therefore, the actual mean values for those variables exhibiting UV-B effects are given in Tables III through V. Comparisons were made between the UV-B treatment and its corresponding control at each irradiance (e.g. HT versus HC). The two magenta isolines were extremely sensitive to UV-B irradiation as evidenced by the number of traits demonstrating significant effects: reductions in individual leaf area, root mass, biomass, and photosynthetic efficiency, coupled with increased photosynthetic pigments, and UV-B-absorbing compounds (Harosoy only), at one or both UV-B treatments. The two Chl-deficient isolines were moderately sensitive to UV-B irradiation, demonstrating significant reductions in leaf area and photosynthetic efficiency, coupled with increases in pigment content (Chl, UV-B-absorbing compounds, or carotenoids), at the LT.

Cultivar and isoline differences were strongly expressed. On average, Harosoy had higher pigment levels (photosynthetic and UV-B-absorbing compounds), higher gas-exchange rates, greater *SLW*, greater pod mass, and greater total biomass than did Clark. Conversely, Clark produced larger individual leaves, greater root mass, and higher photosynthetic efficiency than Harosoy. Normal isolines had larger plant organs and higher pigment concentrations than the magenta or Chl-deficient lines. The magenta isolines had greater *SLW* than the other two isolines, whereas the photosynthetic efficiency of the Chl-deficient isolines exceeded that of the normal and magenta lines.

The Harosoy normal line showed no UV-B effects on growth variables, but all pigments increased significantly at one or both UV-B treatment levels; conversely, the Clark normal isoline showed no UV-B effect on photosynthetic pigments, but increases in UV-B-absorbing compounds (at

Table III. *Isoline-specific UV-B effects for growth variables, determined by comparison of the treatment and control at each irradiance*

Variable[a]	UV-B Treatment	Treatment Means[b]						SE[c]
		Clark isolines			Harosoy isolines			
		Clk	Clk-y$_9$	Clk-wm	Hsy	Hsy-y$_9$	Hsy-wm	
Leaf area, upper leaf (cm^2)	LT	44.92*	31.49	34.11	31.98	27.02	29.15*	(a) 2.43 (2.67)
	LC	52.99	35.67	33.06	32.90	24.60	22.85	(b) 2.50 (2.75)
	HT	43.96*	33.03	31.14**	29.48	19.43*	23.11	
	HC	50.59	39.03	43.51	31.90	26.63	27.99	
Dry weight, roots (g)	LT	2.67	0.90	1.75	1.50	0.95	1.34	(a) 0.15 (0.17)
	LC	1.97	1.01	1.95	1.47	0.90	1.10	(b) 0.17 (0.18)
	HT	1.48	1.06	1.18***	1.47	1.05	0.86	
	HC	1.76	1.08	2.02	1.46	0.87	1.17	
Total biomass (g)	LT	11.49	5.33	8.95	10.64	7.10	9.06**	(a) 0.92 (1.01)
	LC	11.08	5.19	9.36	11.03	7.25	6.02	(b) 0.97 (1.07)
	HT	10.13	6.24	7.43**	11.83	7.98	5.99	
	HC	10.43	5.73	10.89	11.94	8.53	8.28	

[a] Total sample size was 143 for most variables. The sample size per group was 6, except for (a) Clk LC, where $n = 5$, all variables; and (b) for biomass, $n = 5$ in these groups: Hsy (HC), Hsy-y$_9$ (LT), and Hsy-wm (LC). [b] Differences between pairs of means (LT versus LC; HT versus HC) are statistically significant at * $P \leq 0.05$, ** $P \leq 0.01$, and *** $P \leq 0.001$. All variables are normally distributed. [c] The SE is given: (a) for comparison of means within treatment groups (for Clk LC); (b) for comparison of means among isolines (for Clk LC).

Table IV. *Isoline-specific UV-B effects for pigment and gas-exchange variables, determined by comparison of the treatment and control at each irradiance*

Variable[a]	UV-B Treatment	Treatment Means[b]						SE[c]
		Clark isolines			Harosoy isolines			
		Clk	Clk-y_9	Clk-wm	Hsy	Hsy-y_9	Hsy-wm	
Chl a ($\mu g\ cm^{-2}$)	LT	20.65	7.70	19.83**	25.53**	16.22**	23.88***	(a) 0.90 (0.98)
	LC	20.31	6.45	16.45	22.20	13.34	19.30	(b) 1.08 (1.19)
	HT	21.62	9.98	17.52	23.99	15.39	20.07	
	HC	21.01	7.74	18.49	23.69	14.12	21.78	
Total Chl ($\mu g\ cm^{-2}$)	LT	24.83	8.57	24.44**	30.75*	17.70	29.16***	(a) 1.13 (1.45)
	LC	24.71	7.31	20.00	26.82	14.53	23.53	(b) 1.44 (1.58)
	HT	26.66	11.63	20.77	29.02	16.84	24.26	
	HC	25.94	8.62	22.53	28.03	15.74	26.28	
Total carotenoids ($\mu g\ cm^{-2}$)	LT	5.29	2.47	4.91*	6.31**	4.46*	5.73*	(a) 0.22 (0.24)
	LC	5.14	2.35	4.29	5.31	3.87	4.73	(b) 0.25 (0.28)
	HT	5.41	2.77	4.64	5.82	4.12	4.91	
	HC	5.07	2.40	4.62	5.61	3.78	5.19	
UV-B-absorbing compounds ($A_{300}\ cm^{-2}$)	LT	3.46	2.59*	2.51	4.88**	4.23***	2.86**	(a) 0.34 (0.37)
	LC	3.12	1.51	2.80	3.50	2.85	1.61	(b) 0.36 (0.40)
	HT	3.40**	2.57	2.80	4.29**	4.02***	3.01**	
	HC	2.31	1.79	2.41	3.16	1.84	1.68	
Photosynthetic efficiency ($\mu mol\ CO_2\ g^{-1}$ Chl)	LT	0.77	2.08**	0.66**	0.60	1.16**	0.69*	(a) 0.08 (0.09)
	LC	0.73	2.54	1.10	0.84	1.55	1.03	(b) 0.12 (0.13)
	HT	0.66	1.33***	0.59*	0.61	1.10**	0.70	
	HC	0.76	1.94	0.89	0.82	1.84	0.87	
Photosynthetic capacity ($\mu mol\ O_2\ m^{-2}\ s^{-1}$)	LT	22.2	20.6	28.9*				(a) 1.03 (1.08)
	LC	23.3	20.9	24.3				(b) 1.70 (1.70)
	HT	15.9**	25.2	25.0				
	HC	22.7	20.2	25.5				

[a] See footnote a, Table III. [b] See footnote b, Table III. [c] See footnote c, Table III.

HT) and reductions in leaf area were significant (at LT and HT). Both cultivars showed significant reductions in P_{CO_2}, g, and E for one or both UV-B treatments (Table V), but did not show evidence of any isoline-specific responses. P_{O_2} rates (Table IV) were isoline-specific, but were significantly reduced in only one group—Clk at HT—whereas either an increase or no change was observed in the other Clark groups. The overall average maximum photosynthetic capacity was 22.9 μmol O_2 m^{-2} s^{-1}.

Plant Responses to UV-B or UV-A Radiation

The significant UV spectral irradiation × irradiance interaction observed in the analysis of variance reveals that some variables performed differently at the high and low irradiances in response to either the UV-B or UV-A irradiance. This is examined further in Table VI, where means in the low versus high groups are compared (LT versus HT, LC versus HC); only significant ($P \leq 0.05$) differences are listed, expressed as a relative percent difference. This analysis shows that the relative irradiance (high or low) was important in plant response for both the UV-B and UV-A radiation in some isoline/variable combinations. Most variables, except UV-B-absorbing compounds, showed evidence of irradiance responses to UV-B irradiation in at least one isoline, and significant UV-A irradiance effects were observed in at least one isoline for most variables. However, although experiment-wide average effects were observed for P_{CO_2}, E, and root/total biomass, isoline-specific differences for the two UV-A doses were not observed for these variables (Table VI).

The higher UV-B irradiance resulted in some positive responses in the magenta and Chl-deficient isolines (e.g. Chl for Clk-y_9). More typically, the higher UV-B irradiance (compared with LT) reduced photosynthetic pigments in the magenta lines, shoot biomass in Hsy-wm, P_{CO_2} and root mass in the Clark lines, photosynthetic efficiency (P_{O_2} g^{-1} Chl) for Clk, and g in all lines. Most importantly, these reductions in response to the UV-B treatments (LT versus HT) are contrasted with increases in response to UV-A (LC versus HC) for the photosynthetic pigments and shoot mass in both magenta lines. However, photosynthetic efficiency (P_{CO_2} g^{-1} Chl) was reduced in the Chl-deficient lines by both high UV-B and UV-A radiation. UV-A irradiance also influenced UV-B-absorbing compounds in Clk.

Table V. *Cultivar-specific[a] UV-B effects on gas-exchange variables, determined by comparison of the treatment and control at each irradiance*

Variable[b]	UV-B Treatment	Treatment Means[c]		SE[d]
		Clark	Harosoy	
Photosynthesis (μmol CO_2 m^{-2} s^{-1})	LT	17.38	19.28*	0.70 (0.72)
	LC	18.50	22.57	
	HT	14.94**	17.57**	
	HC	18.51	22.30	
Conductance (mol H_2O m^{-2} s^{-1})	LT	0.329	0.374***	0.043 (0.045)
	LC	0.356	0.642	
	HT	0.209*	0.259***	
	HC	0.324	0.508	
Transpiration (mol H_2O m^{-2}) × 10^{-2}	LT	4.18	4.96**	0.233 (0.241)
	LC	4.15	5.93	
	HT	3.03*	4.16**	
	HC	3.93	5.57	

[a] Cultivar-wide responses (no isoline differences) are indicated here and in Table VI as Clark or Harosoy. [b] Total sample size was 144 (or 143). The sample size per group was 18, except for Clk LC, where $n = 17$. [c] See footnote b, Table III. [d] The SE is given for comparison of means within treatment groups (for Clk LC).

Regression Analyses

Various experiment-wide multivariate models were examined for relationships among pigment and growth variables. Most notably, UV-B-absorbing compound content was not related to photosynthesis or photosynthetic efficiency. Although *SLW* did not respond to UV-B radiation, it was positively, although weakly, related to UV-B-absorbing compound levels (A_{300} g^{-1} or A_{300} cm^{-2} basis; $r^2 = 0.25$, $P \leq 0.001$) over all isolines. Carotenoid content was the only variable well correlated with UV-B-absorbing compounds (although only under limited conditions), photosynthesis, and productivity (Figs. 1-3).

Understanding the relationship of leaf carotenoid content to UV-B-absorbing compounds required a separate analysis of UV-B treatments and UV-A controls. In the UV-A control groups, carotenoids showed no relationship to the UV-B-absorbing compound content for any isoline. But for the UV-B-irradiated plants of the normal isolines only (Fig. 1), an overall positive correlation was revealed between leaf carotenoid concentration and UV-B-absorbing compounds ($r = 0.66$, $P \leq 0.001$), with cultivar and UV-B treatment differences providing an additional 15% of the experimental variance. This represented a 0.42-μg increase in carotenoid content per unit increase in A_{300} cm^{-1}. The carotenoid content also increased for UV-B-irradiated plants in the two mutant lines (Table IV), but this increase could not be related to UV-B-absorbing compound level.

The carotenoid content, whether inherent or altered in response to UV treatments, was strongly correlated with leaf Chl content (Fig. 2A) across all cultivars, isolines, and treatment groups ($r = 0.97$, $P \leq 0.01$); the average Chl:carotenoid ratio was 6:1. The close quantitative association of these two pigments provides strong evidence that the increase in carotenoids occurred in the photosynthetic antennae complexes rather than in other leaf structures. The model that best predicted total biomass was a multivariate linear model that included carotenoids, UV-B-absorbing compounds, and several co-variates ($P \leq 0.01$, all factors: cultivar, isoline, UV group; $r^2 = 0.61$); this is notable because the pigments were determined from a single leaf several weeks before harvest. The relationship of biomass to carotenoids, the primary variable, is shown in Figure 2B. Similar but weaker relationships to Chl's were also demonstrated (data not shown).

Photosynthetic efficiency was negatively and exponentially related to leaf carotenoid concentration. The general nonlinear model ($r^2 = 0.72$, $P \leq 0.01$) for P_{CO_2} g^{-1} Chl is given in Figure 3A. For P_{O_2} g^{-1} Chl (Fig. 3B), available for Clark only, a similar although stronger relationship ($r^2 = 0.81$, $P \leq 0.001$) was obtained, with the curve shifted upward to higher photosynthetic efficiency values per unit carotenoid.

DISCUSSION

Was UV-A Radiation Important?

UV-A radiation directly influenced the response of several variables, including stomatal conductance (*g*). This is consistent with studies showing UV-A/blue radiation influence on stomatal behavior (Assman and Grantz, 1990; Grantz and Assman, 1991). Additionally, the inclusion of two UV-A controls revealed significant UV-B effects at high irradiances that otherwise would have been obscured. For example, the following variables would have been judged nonsignificant at the HT (by inappropriate comparison with LC), assuming the same standard errors: leaf area reductions for Clk-wm, increased UV-B-absorbing compounds for Clk, and biomass reduction for Clk-wm. Moreover, a few responses would have been incorrectly judged significant (e.g. decrease in P_{O_2} g^{-1} Chl in Clk-y_9, not shown).

The factorial design of the experiment also revealed a statistically significant interaction between the UV spectral irradiation (i.e. UV-B treatment, UV-A control) and irradiance (high or low), demonstrating that for several variables the UV-B and UV-A wavebands produced opposite responses at the two irradiances (e.g. leaf area, biomass accumulation per organ and/or plant, and photosynthetic pigment content). In most of these cases, UV-B radiation was deleterious to plant function, whereas the UV-A radiation produced positive effects (Table VI), most likely through photorepair or photoprotection (Sutherland, 1981; Beggs et al., 1985). On the other hand, it is important to note that this UV spectral irradiation × irradiance interaction was not significant for P_{CO_2}, *g*, *E*, or increases in UV-B-absorbing compounds. These are among the suite of characters often found to be significantly affected by UV-B radiation. Although the effect of UV-B radiation on these variables is often strongly expressed, the lack of an interaction between the two radiation factors (UV spectral irradiation and irradiance) has surely contributed to the common finding of significant effect on these plant attributes. Moreover, some of these, as noted above, showed a direct response to UV-A radiation that was similar to that

Table VI. *Significantly different irradiance responses for HT and LT groups*[a]

Variable	LT versus HT		LC versus HC	
	Isoline	Percent relative difference	Isoline	Percent relative difference
Leaf area, upper leaf (cm^2)	Hsy-y_9	39.1**	Clk-wm	−24.0***
Dry biomass, pods (g)	Hsy	−27.0**		
	Hsy-y_9	−22.6	Hsy-y_9	−29.0**
	Hsy-wm	85.8	Hsy-wm	−35.3**
Dry biomass, roots (g)	Clk	80.4***		
	Clk-wm	48.3**		
	Hsy-wm	−55.8*		
Total biomass (g)	Hsy-wm	51.3**		
Chl *a* ($\mu g\ cm^{-2}$)	Hsy-wm	19.0**	Hsy-wm	−11.4*
Total Chl ($\mu g\ cm^{-2}$)	Clk-wm	17.7*		
	Hsy-wm	20.2**		
Total carotenoid ($\mu g\ cm^{-2}$)	Hsy-wm	16.7**		
UV-B-absorbing compounds ($A_{300}\ cm^{-2}$)			Hsy-y_9	54.9**
Photosynthetic efficiency ($\mu mol\ CO_2\ g^{-1}$ Chl)	Clk-y_9	56.4**	Clk-y_9	30.9**
			Hsy-y_9	−15.8*
Photosynthetic capacity ($\mu mol\ O_2\ m^{-2}\ s^{-1}$)	Clk	39.6***		
	Clk-y_9	−18.2*		
	Clk-wm	15.6*		
Photosynthesis ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)	Clark[b]	16.3**		
Conductance ($mol\ H_2O\ m^{-2}\ s^{-1}$)	Clark	57.4*	Harosoy	26.4*
Transpiration ($mmol\ H_2O\ m^{-2}$)	Clark	38.0**		

[a] The percent relative difference = [(low − high)/high × 100, computed separately for treatments and for controls. Statistical significance was determined for the actual difference of means (LT versus HT; LC versus HC). Significance levels (*, **, ***) are described in footnote b, Table III. [b] See footnote a, in Table V.

produced by UV-B radiation, thus enhancing the UV-B effects.

This study demonstrates that UV-A radiation modifies UV-B radiation effectiveness for many plant characteristics. For these variables, UV-A radiation may represent a confounding factor, especially since the variability among plant isolines was at least as great as the UV-B radiation-induced change, as can be seen by the overlapping ranges of these groups in Figures 2 and 3. These results should have an impact on the future design of UV-B experiments, especially those conducted in glasshouses and growth chambers where UV-A radiation is supplemented substantially by the fluorescent lamps, as discussed by Middleton and Teramura (1993). Control of UV-A radiation in these settings is essential due to the basic assumption of the experimental design, UV-$A_{TREATMENT}$ = UV-$A_{CONTROL}$. But only one UV-A control, functionally equivalent to the LC in this experiment, is typically utilized in the standard UV experimental protocol, even when multiple UV-B irradiances (e.g. LT and HT) are included. Without proper UV-A controls, the determination of statistical significance for UV-B effects on plant responses is more difficult to demonstrate.

Did UV-B-Absorbing Compounds Protect the Photosynthetic Apparatus?

In this experiment, the general trends found in plant response to UV-B radiation were in agreement with those found in other UV-B studies, including reduced P_{CO_2}, *g*, and leaf area and increased levels of UV-B-absorbing compounds. The magenta (flavonoid-deficient) lines were most affected by the UV-B treatments, in terms of the number of traits showing significant responses. But no gas-exchange parameter was correlated with UV-B-absorbing compound content, indicating that UV-B-absorbing compounds (e.g. flavonoids) did not directly affect photosynthetic function.

Although both UV-B-absorbing compounds and photosynthetic pigments increased in response to UV-B irradiation, and a positive association existed between these two classes of pigments under specific conditions, only the photosynthetic pigments (especially carotenoids), and not the UV-B-absorbing compound levels, could be related to photosynthesis and overall productivity. This relationship was best seen in the normal isolines; the increase in carotenoids was weaker in the flavonoid-deficient lines and unrelated to increases in UV-B-absorbing compounds in the Chl-deficient isolines, perhaps revealing an uncoupling of separate processes. This suggests that both pigments perform a photopro-

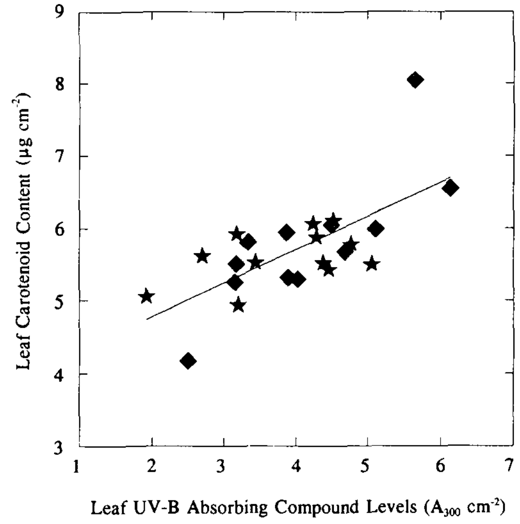

Figure 1. Relationship of leaf carotenoid concentration ($\mu g\ cm^{-2}$) to UV-B leaf-absorbing compound levels ($A_{300}\ cm^{-2}$) for the normal isolines of both cultivars ($r = 0.66$, $P \leq 0.001$). The mutant plants are not shown since no relationship with UV-B-absorbing compounds was observed. Only the UV-B-irradiated plants are shown since carotenoid content was not associated with UV-B-absorbing compound content in controls of any isoline. Symbols indicate UV LT (◆) and UV HT (★).

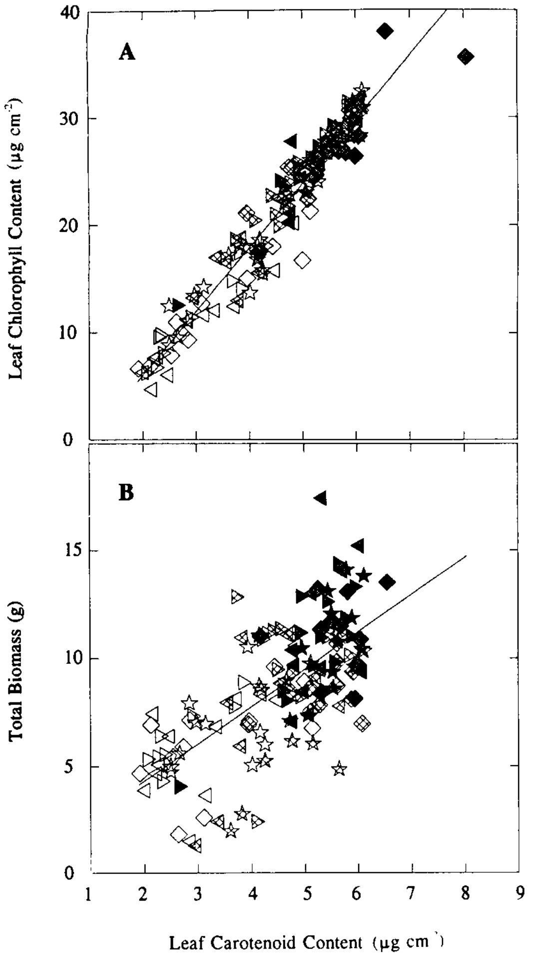

Figure 2. Linear relationships of two variables to leaf carotenoid content (μg cm^{-2}). A, Leaf total Chl content (μg cm^{-2}) measured at the same time ($r = 0.97$, $P \leq 0.001$); B, total biomass (g dry weight) determined 2 weeks later at harvest, where $r^2 = 0.61$ for the full model including co-variates isoline ($P = 0.005$), cultivar ($P \leq 0.001$), UV treatment group ($P = 0.001$), and UV-B-absorbing compounds ($P = 0.014$). In this and the following figures, symbols indicate UV treatment group: ◇, UV-B LT; ☆, UV-B HT; ◁, LC; and ▷, HC. Shaded symbols, Normal isolines; hatched symbols, magenta (flavonoid-deficient) isolines; open symbols, Chl-deficient isolines.

tective function, but only the carotenoids are implicated in photoprotection of the photosystems. Recent studies have shown that carotenoids serve a protective function against UV-B (Rau et al., 1991) and UV-C (Campos et al., 1991) radiation.

The efficacy of carotenoids in protecting the photosystems is likely due to their function as efficient quenchers of high-energy shortwave radiation. The mechanism by which this is accomplished was first proposed to involve a photochemical state change of singlet oxygen to triplet form by interaction with carotenoids, removing the potentially dangerous oxygen radicals produced in photooxidative processes (Krinsky, 1979). Functionally, the carotenoids, especially xanthophylls, absorb the shortest wavelength radiation within the light-harvesting complexes. More recently, a radiationless dissipation process (i.e. heat) involving the xanthophyll cycle in the presence of excessive light has been proposed (Demmig-Adams, 1990; Demmig-Adams and Adams, 1990). Zeaxanthin, produced by conversion from violaxanthin (or β-carotene) in excessively high radiation, lowers the excited singlet state of Chl in the photosynthetic pigment antennae complexes, diverting excitation energy away from the reaction centers. Larger pool sizes of the the photosynthetic carotenoids, especially the xanthophylls, result under long-term exposure to high radiation (Demmig-Adams, 1990).

The increase in both classes of pigments (photosynthetic and UV-B-absorbing compounds) with UV-B irradiation might indicate a similar UV-B induction stimulus for their biosynthesis. Campos et al. (1991) suggest that UV-B and UV-C irradiation increases the levels of 3-hydroxy-3-methylglutaryl CoA reductase mRNA, inducing additional synthesis of carotenoids for protection of Chl against UV damage,

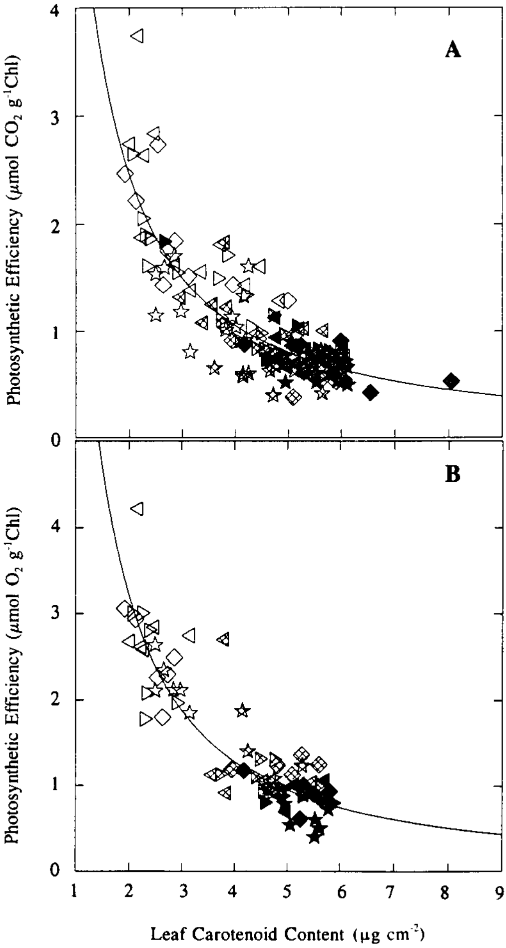

Figure 3. Photosynthetic efficiency as a function of leaf carotenoid content. A, Determined from CO_2 exchange (P_{CO_2} g^{-1} Chl), which includes the stomatal influence, where $r^2 = 0.72$ ($P \leq 0.001$) and photosynthetic efficiency = exp[1.557 − 0.352 × (carotenoid content)]. B, Determined from O_2 evolution (P_{O_2} g^{-1} Chl) for Clark only ($r^2 = 0.81$, $P \leq 0.001$), where the maximum photosynthetic efficiency = exp[(1.846 − 0.375 × (carotenoid content)], with the stomatal limitation removed. Symbols are as described in Figure 2.

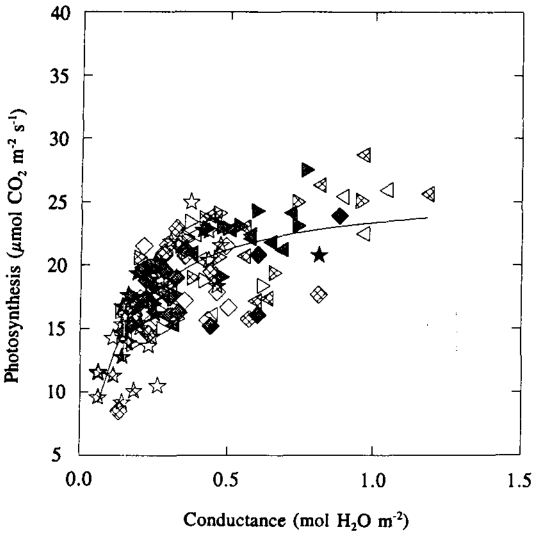

Figure 4. Light-saturated photosynthesis as a function of leaf conductance (mol H_2O m^{-2}), $r^2 = 0.54$ ($P \leq 0.001$). $P_{CO2} = [(26\ \mu mol\ m^{-2}\ s^{-1}) \times g]/(g + 0.112)$. Symbols are as described in Figure 2.

which could be used as a marker of UV-induced stress. They also observed an enhancement of Phe ammonia lyase mRNA levels, a group I enzyme in flavonoid synthesis. DNA itself may act as a UV-B photoreceptor, stimulating synthesis of both pigments, a mechanism already proposed for flavonoids (Beggs et al., 1985, 1986b) and stilbenes (Fritzemeier and Kindl, 1981). By whatever means these two pigments are biochemically associated, increased flavonoid levels may serve as an "indicator" or a "correlate" to the actual, physiologically relevant photoprotector of the photosynthetic apparatus, the carotenoids. Under UV-B irradiation, increases in flavonoid content have been far easier to recognize than have increases in photosynthetic pigments, probably due to lack of control for UV-A radiation. Consequently, flavonoids have been assumed to protect the photosystems, based largely on circumstantial evidence.

The reduction in photosynthetic efficiency as a function of carotenoid content implies that there may have been a "cost" to the production of carotenoids. Approximately one molecule of CO_2 per unit of Chl was fixed in leaves with normal leaf levels of carotenoids (5-6 $\mu g\ cm^{-2}$) under optimal conditions (Fig. 3B). At low carotenoid levels, the mutant pigment lines achieved higher P_{CO2} efficiencies than the normal isolines because they maintained gas-exchange rates equivalent to those of the normal isolines. Overall, UV-B irradiation was associated with both reductions in carbon fixation and, simultaneously, increased production of all pigments. There is little evidence that this photosynthetic reduction was due to PSII damage, since the available P_{O2} values for Clark are higher than the corresponding P_{CO2} values. A stomatal limitation to the measured P_{CO2} data was implied in the relationship between P_{CO2} and g (Fig. 4), since the majority of UV-B-irradiated plant values were located at the lower end of the linear portion of the response curve. This relationship demonstrates that similar gas-exchange kinetics were responsible for the responses elicited from individuals of all isolines, although the values characteristic of some isolines cluster at different segments of the curve. Furthermore, when the stomatal limitation calculated as $[(P_{O2} - P_{CO2})/P_{O2}]$ using the Farquhar and Sharkey (1982) method is plotted relative to P_{CO2}, a negative linear relationship is revealed for the UV-B-irradiated plants (Fig. 5). This calculation is approximate because leaf temperatures were not strictly controlled, but they averaged approximately 31°C in both data sets. These data show that a stomatal limitation was operating that limited carbon uptake in UV-B-irradiated plants. It is possible that stomata responded directly to UV-B (and UV-A) radiation, in addition to indirect responses mediated through photosynthetic dysfunction.

Was Kaempferol More Effective than Quercetin?

Differences in response to UV-B radiation among other soybean cultivars have been observed in other studies for leaf area, UV-B-absorbing compounds, P_{CO2}, g, and E (Murali et al., 1988; Sullivan and Teramura, 1990; Reed et al., 1992). Whatever the protective function of the UV-B-absorbing compounds, some forms may be more effective than others. Because Harosoy outperformed Clark, it is tempting to conclude that kaempferol is a more effective compound. However, Harosoy apparently fared better because it inherently produced a greater *SLW* and more photosynthetic and UV-B-absorbing pigments rather than because it produced kaempferol. Although the relative amounts of quercetin and kaempferol in Clark were not determined, the greater total amount of kaempferol in Harosoy must have exceeded the kaempferol produced in Clark. If kaempferol, directly or indirectly, was more effective in photoprotection, we would expect

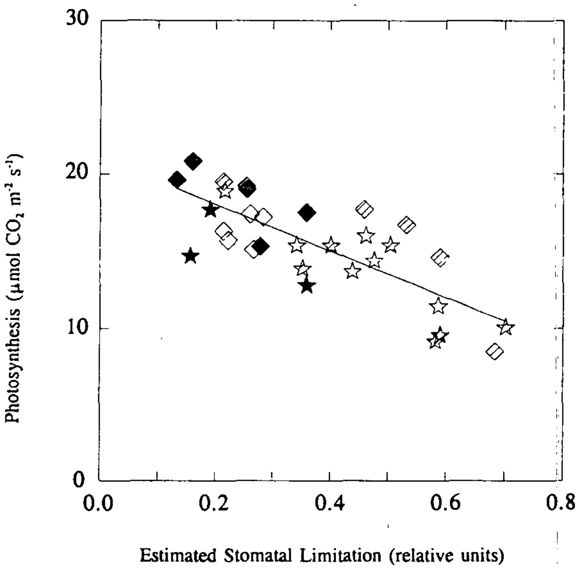

Figure 5. The estimated relative stomatal limitation to photosynthesis is given as a function of P_{CO2}. An inverse linear relationship ($r^2 = 0.66$, $P \leq 0.001$, with UV treatment as a co-variate, $P = 0.036$) is shown for the UV-B-irradiated plants. A small positive offset was applied so initial $X = 0$. There was no stomatal limitation evident in control plants (not shown). Symbols are as described in Figure 2.

separate curves for Harosoy and Clark relating photosynthetic efficiency to the UV-B-absorbing compound levels. Instead, we find that they fall on different ranges of a continuum that is explained by the total amount of UV-B-absorbing compounds present; quantitative rather than qualitative differences associated with these two flavonol glycosides were most important. This is supported by the overall performance of the normal, wild-type lines, which were less affected by UV-B and UV-A irradiation, in general, than were the pigment mutants. Therefore, there is no evidence that kaempferol is more effective than quercetin. UV-B tolerance may be linked to inherently higher levels of flavonoids, as shown by enhanced UV-B tolerance and higher leaf flavonoids in plants from high UV-B environments (Teramura, 1986; Sullivan et al., 1992; Ziska et al., 1992).

CONCLUSIONS

The factorial design of this experiment revealed the complexity of interactions among treatment factors for plant growth, pigment, and gas-exchange variables in UV-B experiments. The importance of including a UV-A control to complement each UV-B treatment was clearly demonstrated, as was the benefit of including specific genetic lines for evaluation of UV radiation responsiveness. Furthermore, these data suggest that UV-B photoprotection is more complex than is usually assumed and involves the increased synthesis of photosynthetic pigments, especially carotenoids, in the chloroplasts for direct protection of the photosystems. These results were obtained with a relatively high background PPF (midday average $\geq 1200\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$), making them relevant to levels of visible solar radiation naturally found outdoors.

ACKNOWLEDGMENTS

The authors wish to thank the following individuals for assistance: Larry Douglass (Animal Sciences Department, University of Maryland), experimental design; Edward L. Middleton, III, plant harvest; Emmett W. Chappelle (NASA Goddard Space Flight Center, Greenbelt, MD), laboratory facilities for pigment extractions; Irwin N. Forseth (Botany Department, University of Maryland), use of laboratory facilities and the oxygen electrode system; and Lewis L. Ziska (Climate Stress Laboratory, USDA, Beltsville, MD), manuscript revisions.

Received December 4, 1992; accepted June 25, 1993.
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