Ambient levels of UV-B in Hawaii combined with nutrient deficiency decrease photosynthesis in near-isogenic maize lines varying in leaf flavonoids: Flavonoids decrease photoinhibition in plants exposed to UV-B

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Abstract

Near-isogenic lines of maize varying in their genes for flavonoid biosynthesis were utilized to examine the effects of foliar flavonoids and nutrient deficiency on maximum net photosynthetic rate (P_N) and chlorophyll (Chl) fluorescence (F_v/F_m) in response to ultraviolet-B (UV-B) radiation. Plants with deficient (30 to 70 % lower N, K, Mn, Fe, and Zn) and sufficient nutrients were exposed to four irradiation regimes: (*1*) no UV-B with solar photosynthetically active radiation (PAR), (*2*) two day shift to ambient artificial UV-B, 8.2–9.5 kJ m^{-2} d^{-1} (21–25 mmol m^{-2} d^{-1}); (*3*) continuous ambient artificial UV-B; (*4*) continuous solar UV-B in Hawaii 12–18 kJ m^{-2} d^{-1} (32–47 mmol m^{-2} d^{-1}). The natural ratio of UV-B : PAR (0.25–0.40) was maintained in the UV-B treatments. In the adequately fertilized plants, lines b and lc had higher contents of flavonoids and anthocyanins than did lines hi27 and dta. UV-B induced the accumulation of foliar flavonoids in lines hi27 and b, but not in the low flavonoid line dta or in the high flavonoid line lc. In plants grown on deficient relative to adequate nutrients, flavonoid and anthocyanin contents decreased by 30–40 and 40–50 %, respectively, and Chl *a* and Chl *b* contents decreased by 30 and 70 %, respectively. The UV-B treatments did not significantly affect P_N and F_v/F_m in plants grown on sufficient nutrients, except in the low flavonoid lines dta and hi27 in which P_N and F_v/F_m decreased by ~15 %. P_N, F_v/F_m, and stomatal conductance decreased markedly (20–30 %) in all lines exposed to UV-B when grown on low nutrients. The decrease in F_v/F_m was 10 % less in higher flavonoid lines b and lc. The photosynthetic apparatus of maize readily tolerated ambient UV-B in the tropics when plants were adequately fertilized. In contrast, ambient UV-B combined with nutrient deficiency significantly reduced photosynthesis in this C_4 plant. Nutrient deficiency increased the susceptibility of maize to UV-B-induced photoinhibition in part by decreasing the contents of photoprotective compounds.

Additional key words: anthocyanins; chlorophyll fluorescence; CO_2-assimilation; stomatal conductance; *Zea*.

Introduction

Ultraviolet-B radiation (UV-B; 280–320 nm) inhibits photosynthesis at several levels, including photosystem 2 (PS2) photochemistry, maximum net photosynthetic rate (P_N), the activity of carbon fixing enzymes such as ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) and phospho*enol*pyruvate carboxylase, and electron transport (*e.g*. Bennett 1981, Vu *et al.* 1982, 1984, Strid *et al.* 1990, Kulandaivelu and Nedunchezhian 1993, Baker *et al.* 1997, Nogués *et al.* 1998, Teramura and Ziska 1996, Ghetti *et al.* 1999, Kolb *et al.* 2001, Rousseaux *et al.* 2004). The inhibition of PS2 is thought to occur *via* UV-B-induced damage to D1 and D2 subunits of PS2 and *via* photooxidation of associated chlorophyll *a* (Chl *a*) and quinone (Greenberg *et al.* 1989, Christopher and Mullet 1994, Jansen *et al.* 1996, Barbato *et al.* 1999, Melis 1999). UV-B can affect PS2 and RuBPCO activity by decreasing the abundance of *psbA* and *rbcL* mRNAs encoding the D1 protein and the large

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subunit of RuBPCO (Jordan *et al.* 1992), decreasing RuBPCO contents (Mackerness *et al.* 1997, Keiller *et al.* 2003) and *via* photomodification of tryptophan residues in RuBPCO (Wilson *et al.* 1995, Greenberg *et al.* 1996). Decreases in the activities of PS2 and electron transport in response to UV-B could be responsible for decreases in activities of CO_2-fixing enzymes. However, UV-B inhibits P_N in a variety of plants without direct correlation with Chl fluorescence or PS2 activity, suggesting that photodamage of PS2 is not the primary reason for reduced rates of P_N (Allen *et al.* 1997, 1998). In some cases, alterations in stomatal function by UV-B have lead to decreases in P_N (Deckmyn *et al.* 1994, Day and Vogelmann 1995, Kolb *et al.* 2001). These two processes are not always correlated, in particular, under conditions of drought with high levels of UV-B (Nogués *et al.* 1998). Finally, some effects of UV-B on photosynthesis are actually caused by UV-B-induced reactive oxygen species, low levels of PAR, or by contaminating UV-C from lamps (Allen *et al.* 1998, Gerhardt *et al.* 2005).

UV-B-absorbing flavonoids accumulate in the vacuoles of epidermal cells (Markham 1982, Robinson 1991) and can protect plants from the damaging effects of UV-B (Beggs and Wellmann 1985, Murali and Teramura 1986, Tevini *et al.* 1991, Li *et al.* 1993, Lois 1994, Krause *et al.* 1995, Ryan *et al.* 2002, Warren *et al.* 2003). Damage to DNA caused by UV-B (Mazza *et al.* 1999) and UV-C is lowered in maize lines that accumulate flavonoids (Stapleton and Walbot 1994). The synthesis of flavonoids, including the anthocyanins, is induced by UV-B (Strid and Porra 1992, Chaves *et al*. 1997, Wilson *et al.* 1998, Warren *et al.* 2003), nutrient deficiency (Epstein 1972, Pinto *et al*. 1999), low temperature (Rabino and Mancinelli 1986, Kulandaivelu and Nedunchezhian 1993), and water deficit (Chaves *et al.* 1997, Nogués *et al.* 1998). Anthocyanins are formed by the glycosylation of the anthocyanidins and absorb UV less efficiently compared to other flavonoids. Anthocyanins may reduce visible radiation that causes photoinhibition (Tevini *et al.* 1991) and protect flavoenzymes from photodamage (Beggs and Wellmann 1994, Ryan *et al.* 2002). Enhancing flavonoid contents through genetic manipulation is one strategy to reduce the adverse effects of UV-B on plants (Li *et al.* 1993, Stapleton and Walbot 1994, Wilson *et al.* 1998).

The ability of flavonoids to protect plants from the inhibitory effects of UV-B on photosynthesis is of particular interest in the tropics. Tropical latitudes receive high levels of UV-B and ozone reductions have occurred there (Caldwell *et al.* 1986, Madronich and de Gruijl 1993, Madronich *et al.* 1995, Searles *et al.* 1995). Minor reductions in the ozone layer will result in greater UV-B levels in the tropics than in temperate latitudes (Madronich and de Gruijl 1993, Searles *et al.* 1995). Small increases in UV-B reduce the height and increase foliar flavonoid contents of native tropical trees (Krause *et al.* 1995, Searles *et al.* 1995). However, very little information is available on the effects of flavonoids in protecting photosynthesis from ambient levels of UV-B in any tropical locale.

When analyzing the effects of UV-B on plants, the source of UV-B and photosynthetically active radiation (PAR), whether natural or artificial, need to be considered. In addition, the UV-B : PAR ratios are critical. PAR in growth chambers, combined with narrow-spectrum artificial sources of UV-B, increase the sensitivity of photosynthesis to UV-B (Biggs *et al.* 1981, Deckmyn *et al.* 1994, Teramura and Ziska 1996, Allen *et al.* 1998). However, many photoprotective processes require high ambient levels of PAR. Therefore, UV-B : PAR ratios appear more important in determining the sensitivity of plants to UV-B than is the total level of UV-B (Deckmyn *et al.* 1994, Allen *et al.* 1998).

We examined the role of foliar UV-B absorbing compounds in protecting photosynthesis in plants exposed to ambient levels of artificial and natural UV-B in Hawaii. We used near-isogenic lines of maize that vary in the genes for flavonoid biosynthesis (Brewbaker 1996). Because plants commonly grow in nutrient poor soils, the effects of UV-B on plants were also tested under nutrient-deficient and nutrient-sufficient regimes. In nutrient sufficient conditions, UV-B absorbing compounds protected photosynthesis from photoinhibition. However, nutrient deficiency increased plant susceptibility to photoinhibition by ambient UV-B.

Materials and methods

Plants, growth conditions, and nutrient analyses: All experiments were conducted at the University of Hawaii, Manoa campus, at 21°19'N and 157°50'W. Three isogenic lines of *Zea mays* L., which are designated dta, b, and lc, were derived from the Hawaii 27 (hi27) parental inbred (Table 1). These three lines and hi27 were obtained from the University of Hawaii Foundation Seed Facility. All of the lines are near-isogenic, and only genetically differ in the genes for flavonoid biosynthesis (Table 1). Nutrient-deficient plants were grown in a 1 : 1 mixture of vermiculite and *Sunshine* potting media and

Table 1. Description of genotypes responsible for altered flavonoid levels in the near isogenic maize lines used in this study. Genes are described in Cone (1994).

Maize line	Symbol	Genotype
Dotted aleurone	dta	*a b Pl C r dt*
Hawaii 27, inbred	hi27	*A b Pl C r Dt*
Booster color	b	*A B Pl C R Dt*
Red leaf color	lc	*A b Pl C r Lc*

watered with 0.1×Hoagland's solution for the first two weeks, followed by 10 mM NaH_2PO_4 (pH 7.0) for the remainder of the experiment to prevent phosphorus deficiency, which can induce flavonoid accumulation. Nutrient-sufficient plants were grown similarly except they were fertilized (*Bandini 7 Iron*, 19-19-19, N-P-K with micronutrients). Plant pots were randomly assigned weekly to provide uniform lighting. Plants were maintained either in the greenhouse for the no-UV-B and artificial UV-B treatments or grown outside for exposure to solar UV-B (described below) at 26–35 °C for four weeks prior to photosynthetic measurements and pigment analyses. Plants grown outdoors were protected from the wind using vertical barriers of cellulose diacetate. Plantings were done every two weeks from April to August in 1996, 1997, 1998, 2002, and 2003. All measurements were taken on plants that were six weeks post-germination.

Solar PAR reached a maximum fluence of 2.2±0.2 mmol m^{-2} s^{-1} at noon during June. PAR was measured with a cosine corrected *LI-190SA* quantum sensor (*LI-COR*, Lincoln, NE, USA) attached to a *21X* data logger (*Campbell Scientific*, Logan, UT, USA). Nutrient contents of leaves were determined at the University of Hawaii Agriculture and Diagnostic Service Center using the combustion method for N (*LECO CN-2000*) and the inductively coupled plasma method for the other elements (K, Zn, Fe, Mn), as described in Kalra (1998).

UV-B irradiation: Maize plants with sufficient and deficient-nutrients were exposed to the following four irradiation regimes: (*1*) No UV-B consisting of solar PAR in the greenhouse under UV-B lamps (see below) covered with 0.13 mm-thick *Mylar D* fluoropolymer film (*Cadillac Plastic and Chemical Co.*, N. Hollywood, CA, USA) that filtered out UV-B (<312 nm). (*2*) Artificial UV-B consisting of solar PAR in the greenhouse under UV-B lamps covered with 0.13 mm-thick cellulose diacetate film (*Cadillac Plastic and Chemical Co.*, N. Hollywood, CA, USA) that filtered out the UV-C (<288 nm). (*3*) Solar UV-B consisting of full spectrum solar radiation outdoors. (*4*) UV-B shift consisting of no UV-B for 40 d as in (*1*) followed by a shift for two days to artificial UV-B as in (*2*). Ambient solar and artificial UV levels were recorded throughout the day using a digital spectroradiometer with a *UVX-31* sensor for detecting UV-B (*UVP*, Upland, CA, USA) attached to a *21X* data logger (*Campbell Scientific*, Logan, UT, USA) and spectrally weighted for biological effectiveness (Caldwell *et al.* 1986). Artificial UV-B radiation was provided by four lamps (*Spectroline*), each fitted with two *UVB-313* bulbs (*Q-Panel Lab Products*, Cleveland, OH, USA) in the greenhouse. The *UVB-313* lamps emit 95 % radiation from 273 to 399 nm with a peak at 313 nm. Lamps were hung 15.2 cm above the plants and the lamp height was adjusted to maintain a consistent distance from the apex. The cellulose diacetate and *Mylar* films were changed weekly to maintain transparency and consistent UV-B doses. There were 18 plant pots below each lamp, with nine pots per row in two rows, and one plant per pot.

Greenhouse glass reduced UV-B by 90 % for wavelengths >315 nm and completely eliminated UV-B <310 nm. Total biologically weighted ambient UV-B outdoors in June ranged 12–18 kJ m^{-2} d^{-1} (32–47 mmol m^{-2} d^{-1}). The natural photon ratios of UV-B/PAR ranged from 0.25 from 09:00 h to 0.40 at noon, and from 0.30 at 13:00 to 0.25 at 16:00 h, respectively. The UV-B : PAR ratios in the artificial UV-B were 0.25–0.30 to ensure that plants were treated with ecologically relevant ratios of UV-B : PAR and to avoid overexposure to UV-B. The level of artificial UV-B radiation was 8.2–9.5 kJ m^{-2} d^{-1} (21–25 mmol m^{-2} d^{-1}). An automatic timer turned lamps on and off each day from 08:30 to 16:30. On overcast days, when PAR dropped below a level to maintain the UV-B : PAR ratio, the height of the lamps was raised to restore the ratio. After the UV-treatments, non-invasive measurements of Chl fluorescence and CO_2 assimilation were done first, and then pigments were extracted. Measurements were made on the same area of the first fully expanded leaf mid-length from the leaf base.

CO_2 assimilation, stomatal conductance (g_s), and Chl fluorescence: P_N portable system (model *LI-6200*, *LI-COR*, Lincoln, NE, USA) was used to determine P_N and g_s under each treatment. Three to five replicate measurements were taken on the same leaf between set time periods and then averaged. Chl *a* fluorescence was measured at room temperature (23 °C) with a *PAM 101* Chl fluorometer (*H. Walz*, Effeltrich, Germany) according to Schreiber *et al.* (1986). Separate *KL1500* electronic cold light sources (*Schott*, Weisbaden, Germany) supplied actinic radiation of 0.4 mmol m^{-2} s^{-1}, and a saturating pulse of 4.6 mmol m^{-2} s^{-1}. Fluorometric readings were taken on leaves dark-adapted for 15 min. One-second saturating pulses were given every ten sec for a total of 20 min until a steady-state fluorescence level was achieved. F_v/F_m was calculated according to Schreiber *et al.* (1986).

Pigment analyses: Total Chl was extracted from fresh leaf tissue in 80 % acetone by homogenizing with a mortar and pestle. After centrifugation, Chl extracts were diluted and scanned from 600 to 750 nm with a diode array spectrophotometer, model *8452A* (*Hewlet-Packard*, Waldbronn, Germany), in 1.0 cm quartz cuvettes after baseline corrections for turbidity. Chl content was calculated according to Wellburn and Lichtenthaler (1984). Absorbance by ethanol extracts at 300 nm is characteristic of flavonoids (Markham 1982). Flavonoids were extracted in acidified 95 % ethanol and 1 % HCl [v/v] from fresh leaf tissue by homogenizing with a mortar and pistil (Rabino and Mancinelli 1986). After clarifying by centrifugation, the extract was diluted with

solvent to obtain uniform readings and then scanned from 280 to 550 nm with a diode array spectrophotometer in 1.0 cm quartz cuvettes as above. Anthocyanin content was calculated as absorbance at 530 nm per gram of fresh tissue. Conversion factors for each maize line (dta, 0.0147; hi27, 0.0162; b, 0.0154; lc, 0.0145) were used to convert Chl and anthocyanin absorbances to leaf area basis.

Statistical analyses: The *Minitab 11.21* statistical package (*Minitab*, State College, PA, USA) was used to perform statistical analyses. Responses to radiation treatment and nutrition were analyzed using one-way ANOVA or two sample *t*-tests. When necessary, the data was either square-root or logarithmically transformed to correct for non-normally distributed data. *DeltagraphPro 3.0* software (*Deltapoint*, Monterey, CA, USA) was used for graphing.

Results

Flavonoid, anthocyanin, and Chl contents in response to UV-B and nutrient status: Leaf tissues of nutrient-deficient maize were low in two major and three minor nutrients compared to those grown with sufficient nutrients (Table 2). Nutrient concentrations fell by at least 60 % for N, 33 % for K, 70 % for Mn, 57 % for Fe, and 33 % for Zn in the low nutrient-treated plants (Table 2). P contents did not decrease due to P supplementation.

Table 2. Foliar nutrient contents of maize lines grown with low and ample nutrients and exposed to the non UV-B-regime. The percent decrease in each nutrient in low relative to ample nutrients is given.

Nutrient	Maize line	Ample nutrients	Low nutrients	Decrease [%]
N [%]	dta	2.27	0.90	60.4
	hi27	2.19	0.86	60.7
	b	2.21	0.82	62.9
	lc	2.39	0.91	61.9
K [%]	dta	3.61	2.36	53.0
	hi27	2.97	1.96	34.0
	b	3.33	1.43	57.1
	lc	3.06	2.06	32.7
P [%]	dta	1.71	1.68	1.8
	hi27	1.89	1.77	6.3
	b	1.82	1.72	5.5
	lc	1.74	1.79	0
Mn [g m^{-3}]	dta	98.5	24.0	75.6
	hi27	119.0	36.2	69.6
	b	92.5	22.5	75.7
	lc	94.0	20.0	78.7
Fe [g m^{-3}]	dta	95.5	36.5	61.8
	hi27	108.0	40.5	62.5
	b	94.0	32.0	66.0
	lc	90.0	38.5	57.2
Zn [g m^{-3}]	dta	18.0	12.0	33.3
	hi27	18.5	12.1	34.5
	b	14.5	9.0	37.9
	lc	15.0	10.0	33.3

In the adequately fertilized plants in the no-UV-B treatment, lines b and lc had higher contents of flavonoids (Fig. 1*A*) and anthocyanins (Fig. 1*B*) than did lines hi27 and dta (Fig. 1*A*). Lines b and lc maintained the contents of flavonoids and anthocyanins in the artificial and solar UV-B treatments. Line hi27 exposed to 4 weeks of artificial and solar UV-B accumulated higher amounts of UV-B absorbing flavonoids (Fig. 1*A*) and anthocyanins (Fig. 1*B*) than hi27 not exposed to UV-B. Further, the contents of these compounds in line hi27 exposed to artificial and solar UV-B were similar to the contents in lines b and lc with UV-B. In the two-day UV-B shift treatment, flavonoid contents in lines hi27, b, and lc did not change relative to controls, and anthocyanin contents did not change in lines dta, b, and lc. However, anthocyanin contents did increase by 20 % in hi27 in the UV-B shift treatment. Line dta consistently had low contents of flavonoids and anthocyanins in all treatments of plants grown with sufficient nutrients.

The contents of flavonoids and anthocyanins were also determined in each of the lines when grown on nutrient deficiency and exposed to the four irradiation regimes. Flavonoid contents decreased by 30–40% in plants grown on deficient (Fig. 1*C*) relative to adequate (Fig. 1*A*) nutrient contents. Lines b and lc had 30 % higher average contents of flavonoids than line dta (Fig. 1*C*). Flavonoid contents increased in line hi27 by 25 % in the artificial and solar UV-B treatments. However, in lines dta, b, and lc, there were no significant effects of the UV-B treatments on flavonoid contents.

Anthocyanin contents were 40–50 % lower under nutrient deficiency (Fig. 1*D*) relative to adequately fertilized plants (Fig. 1*B*). The low contents of anthocyanins in line dta did not decrease much further upon withholding nutrients. Line lc had higher contents of anthocyanins than lines dta, hi27, and b at nutrient deficiency (Fig. 1*D*). There were no significantly different effects of the UV-B treatments on the anthocyanin contents within a particular line except for line dta, in which content of anthocyanins decreased under the artificial UV-B and solar UV-B treatments.

The contents of Chl *a* and *b* were measured in lines grown on low relative to adequate nutrients exposed to the four irradiation regimes (Table 3). Under sufficient nutrients, Chl *a* and *b* contents did not change significantly due to genotype or irradiation. In the nutrient deficient plants, average Chl *a* and *b* contents were 30 and 70 % lower, respectively, relative to Chl contents in plants grown on sufficient nutrients (Table 3). In

addition, Chl *a*:*b* ratios were two- to three-fold greater in the nutrient-deficient plants relative to those with sufficient nutrients. The increase in Chl *a*:*b* ratios was primarily due to the markedly lower Chl *b* contents in all lines grown low nutrients.

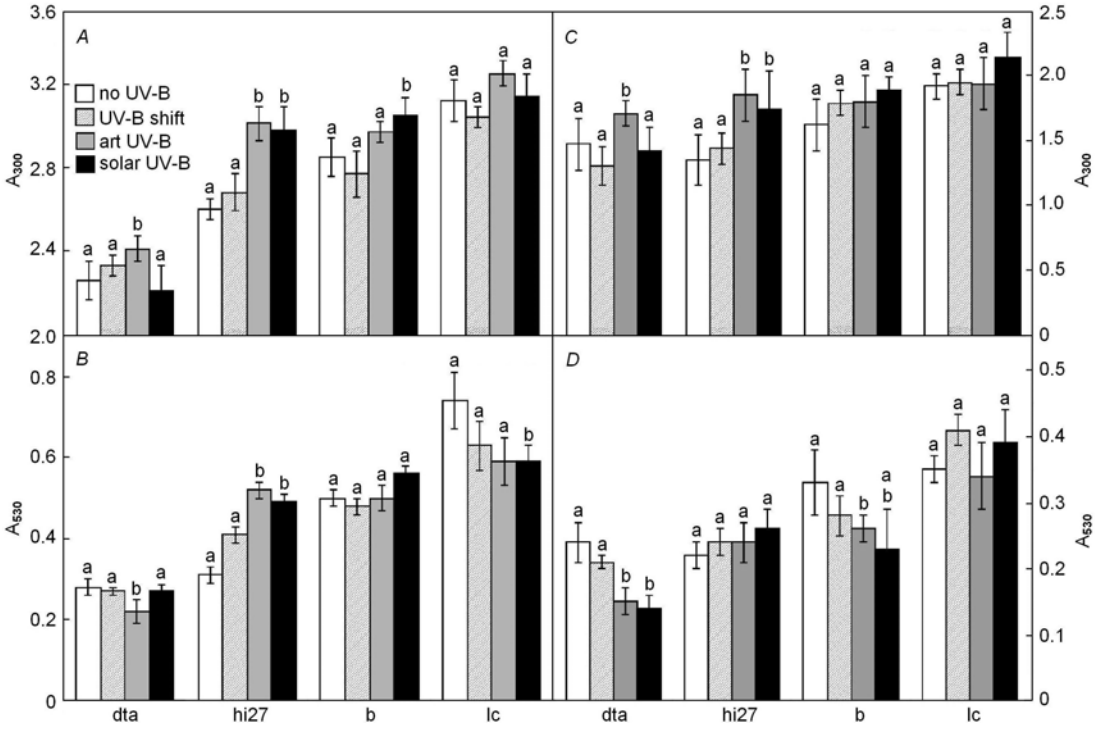

Fig. 1. Effects of UV-B treatments on flavonoid and anthocyanin contents in four near-isogenic maize lines dta, hi27, b, and lc differing in genes for flavonoid biosynthesis. Plants were grown with sufficient (*A, B*) or deficient (*C, D*) nutrients under the four radiation regimes (Art. = artificial UV-B) as described in the Materials and methods. Means ± SE (n = 8 plants per treatment) given on leaf surface area basis. The contents in ethanol extracts of leaves were determined by measuring the absorbance for flavonoids, *A* at 300 nm [A_{300} cm^{-2}] and anthocyanins, *B* at 530 nm [A_{530} cm^{-2}]. Values in each group with the same letter (a or b) are not statistically different according to an ANOVA test (p<0.05).

Table 3. Chlorophyll *a* and *b* contents [g m^{-2}(leaf)] were measured in the four maize lines grown on sufficient and deficient nutrient conditions and exposed to the four radiation regimes.

Maize line	UV treatment	Low nutrients			Sufficient nutrients		
		Chl *a*	Chl *b*	Chl *a/b*	Chl *a*	Chl *b*	Chl *a/b*
dta	No UV-B	11.6 ± 1.1	1.20 ± 0.12	9.67	15.0 ± 1.1	3.82 ± 0.30	3.93
	UV-B shift	9.5 ± 0.9	1.19 ± 0.10	7.98	14.8 ± 0.6	3.57 ± 0.30	4.14
	Artificial UV-B	10.1 ± 2.7	1.22 ± 0.17	8.28	15.2 ± 2.0	4.11 ± 0.31	3.70
	Solar UV-B	9.7 ± 1.3	1.06 ± 0.10	9.15	15.8 ± 1.4	4.17 ± 0.44	3.79
hi27	No UV-B	10.4 ± 0.3	0.99 ± 0.09	10.50	14.4 ± 1.5	4.22 ± 0.47	3.41
	UV-B shift	9.6 ± 1.2	0.87 ± 0.23	11.03	15.3 ± 0.9	4.51 ± 0.52	3.39
	Artificial UV-B	10.6 ± 1.4	1.08 ± 0.30	9.81	16.7 ± 1.7	4.86 ± 0.50	3.44
	Solar UV-B	11.4 ± 0.8	1.16 ± 0.49	9.83	15.0 ± 2.3	4.26 ± 0.41	3.52
b	No UV-B	11.8 ± 0.9	1.40 ± 0.27	8.43	15.1 ± 1.9	4.62 ± 0.53	3.27
	UV-B shift	10.9 ± 1.1	1.33 ± 0.42	8.20	14.3 ± 1.1	4.10 ± 0.17	3.49
	Artificial UV-B	12.0 ± 1.2	1.41 ± 0.31	8.51	15.6 ± 0.8	4.31 ± 0.22	3.62
	Solar UV-B	12.5 ± 1.7	1.32 ± 0.44	9.47	14.3 ± 1.3	3.94 ± 0.40	3.63
lc	No UV-B	11.3 ± 0.8	1.52 ± 0.25	7.43	15.7 ± 1.2	4.94 ± 0.31	3.18
	UV-B shift	12.2 ± 1.0	1.54 ± 0.20	7.92	16.2 ± 1.5	4.20 ± 0.25	3.86
	Artificial UV-B	12.3 ± 1.4	1.70 ± 0.19	7.22	17.3 ± 0.9	5.41 ± 0.27	3.20
	Solar UV-B	13.1 ± 2.2	1.67 ± 0.24	7.84	16.4 ± 1.1	4.67 ± 0.41	3.51

Gas assimilation and exchange: The effects of UV-B on gas exchange were examined in each isogenic line (Fig. 2). In plants with adequate nutrients, P_N in dta plants exposed to the UV-B shift, artificial UV-B, or solar UV-B treatments decreased by 10–20 % relative to non-UV-B-treated controls (Fig. 2*A*). In line hi27 exposed to the UV-B shift, P_N decreased by ~10 %. No differences in P_N were observed in lines hi27, b, and lc exposed to artificial and solar UV-B. In contrast, g_s decreased in each line exposed to UV-B (Fig. 2*B*). The decrease in g_s (Fig. 2*B*) did not always correlate with P_N (Fig. 2*A*).

In nutrient-deficient plants (Fig. 2*C*), P_N was generally 15 % lower in the non UV-B-treated controls compared to plants grown with adequate nutrients (Fig. 2*A*) except for line lc in which P_N did not decrease significantly in the no UV-B controls grown on low nutrients. The three UV-B treatments markedly decreased P_N in all lines when grown on low nutrients (Fig. 2*C*). In the UV-B treatments of nutrient deficient plants, the decrease in P_N was greater than 50 % in line dta exposed to solar UV-B relative to the no UV-B controls (Fig. 2*C*). The decrease in P_N was slightly less, 30–40 %, in lines hi27, b, and lc. The g_s decreased in each line for each UV-B treatment relative to non-UV-B-treated controls (Fig. 2*D*).

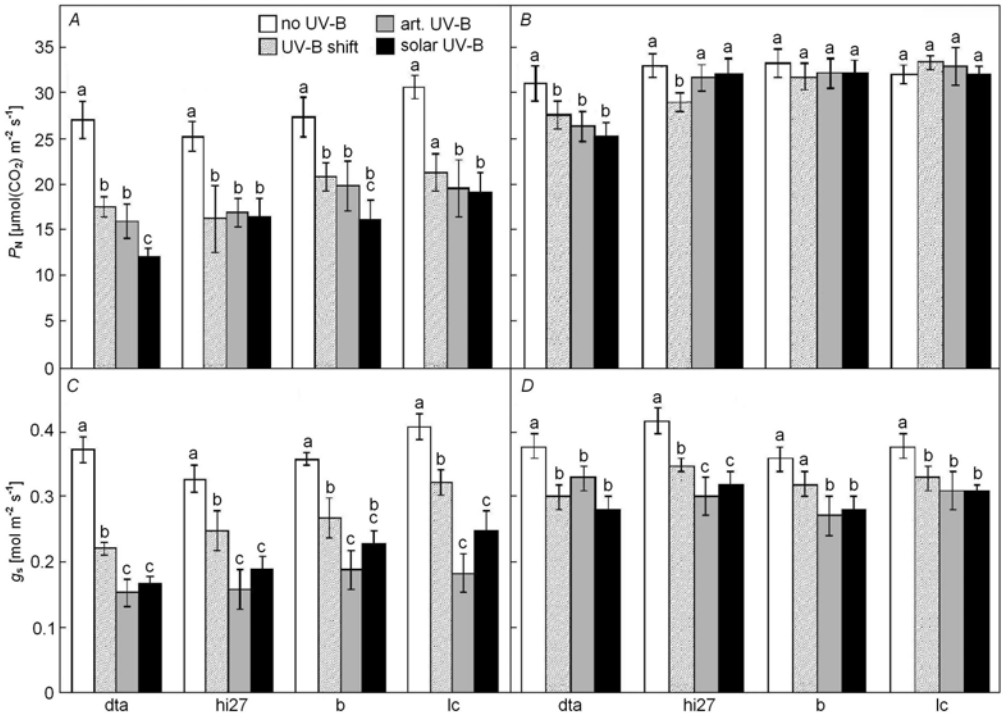

Fig. 2. Influence of UV-B radiation and adequate (*A*, *B*) or deficient nutrients (*C*, *D*) on maximum net CO_2 assimilation, P_N (*A*, *C*) and stomatal conductance, g_s (*B*, *D*) in four maize isogenic lines exposed to four radiation regimes. Means of 3–5 measurements per leaf ± SE (n = 5–7 plants). Column colors and statistical ANOVA tests are described in the legend for Fig. 1.

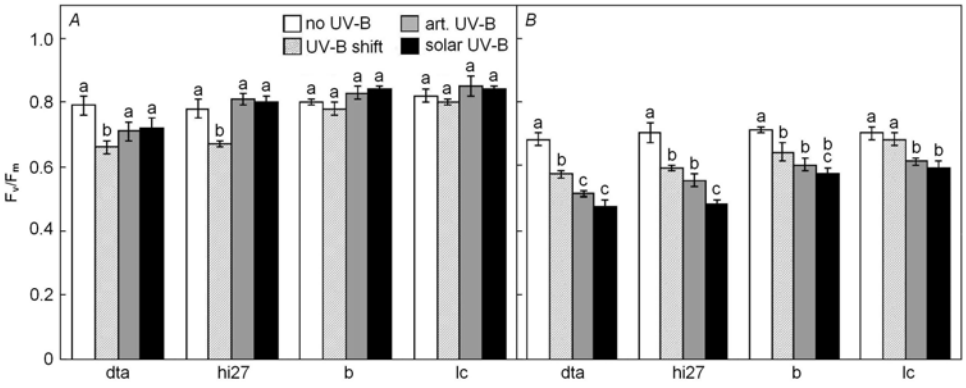

Fig. 3. Measurements of F_v/F_m for the four isogenic maize grown with sufficient (*A*) or deficient (*B*) nutrients, and exposed to four radiation regimes. Means ± SE (n = 4–7 plants per treatment) are shown. Column colors and statistical ANOVA test are described in the legend for Fig. 2.

Modulated Chl fluorescence: F_v/F_m was not significantly affected by the UV-B treatments in plants grown on sufficient nutrients (Fig. 3*A*), except in the low flavonoid lines dta and hi27 exposed to the UV-B shift, in which F_v/F_m decreased by 15 %. In contrast, F_v/F_m decreased markedly (20–30 %) in all lines exposed to UV-B when grown on low nutrients (Fig. 3*B*). The decrease in F_v/F_m was 10 % less in lines b and lc. In general, F_v/F_m was 12 % lower in the no UV-B treatments in plants that had deficient (Fig. 3*B*) compared to sufficient (Fig. 3*A*) nutrients.

Discussion

Tropical regions such as Hawaii have some of the highest levels of solar UV-B in the world, even in the absence of ozone depletion (Searles *et al.* 1995). We employed natural ratios of UV-B : PAR in the artificial UV-B treatments. The photosynthetic apparatus of maize adapts and readily tolerates ambient UV-B when adequately fertilized. However, we found that nutrient-deficiency, combined with exposure to ambient UV-B, inhibited photosynthesis in this C_4 plant. In addition, UV-B absorbing compounds in lines hi27, b, and lc (when grown on adequate nutrients) protected leaves from photoinhibition when the plants were abruptly shifted from conditions lacking UV-B into UV-B. Nutrient deficiency increased the susceptibility of maize to UV-B-induced photoinhibition in part by decreasing the contents of photoprotective compounds. These results have implications for tropical agriculture and ecosystems that do not have access to adequate fertilization and/or lack available nutrients. Furthermore, this experimental system will also be useful to determine the effects of UV-B and flavonoids on photosynthesis in combination with individual nutrient-deficiencies, drought and temperature stresses.

Differential accumulation of flavonoids among isogenic lines in response to UV-B: The increase in A_{300} values in response to both artificial and natural UV-B (Fig. 1) is interpreted to result from stimulation of flavonoid biosynthesis in the lines hi27 and b that occurs in response to blue radiation, UV-A, and UV-B (Beggs and Wellmann 1985, 1994, Strid and Porra 1992, Chaves *et al*. 1997, Wilson *et al.* 1998, Ryan *et al.* 2002, Warren *et al.* 2003). UV-B did not stimulate the accumulation of flavonoids in line dta, which lacks UV-B-responsive genes for flavonoid biosynthesis (Table 1). Line lc had high flavonoid contents without UV-B and further accumulation did not occur in response to UV-B. Line lc may have a feedback mechanism that reduces flavonoid production in flavonoid-rich tissue. Nutrient-deficiency resulted in lower flavonoid contents in lines dta, hi27, b, and lc in all irradiation regimes. This is in contrast to the report that N deficiency leads to flavonoid accumulation (Pinto *et al.* 1999). We believe the multiple nutrient deficiencies used here are rather severe and limit flavonoid biosynthesis. This prevented determining the possible photoprotective effects of high flavonoid contents on photosynthesis in response to UV-B in nutrient-deficient plants. The precise molecular identification of the flavonoids in these lines requires further quantitative and qualitative analyses.

Ambient UV-B combined with nutrient-deficiency inhibits photosynthesis: The adverse effects of UV-B were more pronounced for nutrient-deficiency, as significant reductions in P_N and F_v/F_m occurred. The decrease in photosynthesis in nutrient-deficient plants could be linked to the reduced contents of Chl *a* + *b* and flavonoids. Chl contents, especially Chl *b*, were markedly reduced in all lines under nutrient-deficiency. This indicates that the light-harvesting antennae for both PS1 and PS2 were smaller (Ghirardi and Melis 1988). The decreased light-harvesting capacity would limit Chl *a* fluorescence and carbon assimilation through reductions in resonance transfer, electron transport, and ATP and NADPH production. Carbon allocation to the synthesis of Chl may be reduced, Chl photodegraded (Biggs *et al.* 1981, Vu *et al.* 1984, Tevini *et al.* 1991, He *et al.* 1994, Day and Vogelmann 1995), antenna size (Melis 1999), and the abundance of mRNAs that encode enzymes for Chl biosynthesis decreased (Strid *et al.* 1990, Jordan *et al.* 1991). Three of the elements that were deficient in the maize leaves (N, Fe, and Mn) play major roles in the photosynthetic complexes and their deficiencies decrease the contents of these complexes (Stocking 1975, Lawlor 1993). Nutrient-deficiency diminishes the amount of carbon and cofactors allocated to the photosynthetic subunits and pigments, thus limiting the ability to replace the photodamaged D1 and D2 subunits (Greenberg *et al.* 1989, Christopher and Mullet 1994, Jansen *et al.* 1996, Melis 1999).

The low F_v/F_m values indicated moderate to severe photoinhibition in nutrient-deficient plants exposed to solar UV-B and correlate with our observations of reduced P_N in these plants. Using mutants deficient in the C_4 and C_3 cycles showed that CO_2 assimilation correlates with PS2 activity (Maroco *et al.* 1998). In the solar UV-B treatments, the lower Chl fluorescence was correlated with lower P_N. In contrast, the degree of P_N decrease was not always correlated with a proportional decrease in Chl fluorescence as, for example, in nutrient-deficient plants exposed to artificial and solar UV-B (Figs. 2 and 3), which is consistent with observations of Allen *et al.* (1997). It is possible that P_N is also affected by UV-B independently of electron transport, as in the case of the photo-modification of RuBPCO by UV-B (Wilson *et al.* 1995, Allen *et al.* 1998). We observed that lower g_s did

not always correlate with proportionally lower P_N, as observed in pea by Nogués *et al.* (1998).

Flavonoids ameliorate the degree of photoinhibition in plants exposed to UV-B: Foliar flavonoids lowered the degree of photoinhibition in plants grown in UV-B, but photoinhibition still occurred in nutrient-deficient plants that also had low flavonoid contents. Lines with higher flavonoid contents, such as lc and b in all irradiation regimes, and hi27 in artificial and solar UV-B regimes, had higher F_v/F_m and P_N relative to line dta, which had low flavonoid contents. When shifted from a regime lacking UV-B into artificial UV-B, lines b and lc grown on sufficient nutrients were less photoinhibited than lines dta and hi27 (Figs. 2*A* and 3*A*), and the lower photoinhibition was correlated with the higher contents of flavonoids in line lc, which protected PS2 and RuBPCO from photodamage (Wilson and Greenberg 1993, Wilson *et al.* 1995).

Comparing natural and artificial UV-B: Radiation from UV lamps filtered through *Mylar* served as a no UV-B treatment in this study. However, neither *Mylar* nor cellulose acetate remove the UV-A and blue radiation emitted by the lamps (Teramura and Ziska 1996). Exposure to a UV lamp produces higher proportions of UV-A than occur in the solar spectrum. A biological effect of the UV-A in the lamps is likely because plants have UV-A/blue radiation receptors involved in stomatal opening (Zeiger 1994), plant development, and photosynthetic processes (Vu *et al.* 1984, Gorton *et al.* 1993, Christopher and Mullet 1994, Senger and Schmidt 1994, Short and Briggs 1994, Allen *et al.* 1998, Mazza *et al.* 1999) and UV-A can decrease the inhibitory effect of UV-B on photosynthesis (Gartia *et al.* 2003). The system used in our study carefully supplied UV-B : PAR levels that did not exceed the ratios in the natural environment, unlike other studies that used higher ratios (reviewed in Teramura and Ziska 1996, Allen *et al*. 1998). In a greenhouse, PAR is more comparable to natural radiation because glass attenuates only about 10 % of natural PAR. Higher UV-B dosages can be given to the plants while still maintaining a more realistic ratio of radiation types than growth chambers. Therefore, we believe this study realistically determined the effects of solar UV-B levels on a C_4 plant species.

The maize lines with higher flavonoid contents are relatively tolerant to UV-B when grown under nutrient-sufficiency. They were developed in a breeding program that used the inbred parent line (hi27) which is more tolerant of tropical irradiance than maize bred for temperate regimes (Jong *et al.* 1982, Brewbaker 1996). However, many crop and native plant communities undergo short- and long-term shifts in nutrient availability and farmers in third world countries have limited access to adequate fertilizer. The ability of plants to acclimate to increases in UV-B is lessened under nutrient-limited environments. The findings indicate that sufficient nutrients are needed to maintain flavonoid and Chl biosyntheses and the repair processes by which the photosynthetic apparatus adapts to UV-B.

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