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THE RESPONSE OF FIVE TROPICAL PLANT SPECIES TO NATURAL SOLAR ULTRAVIOLET-B RADIATION

by

Peter S. Searles

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Range Ecology

Approved:

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Peter S. Searles

CONTENTS

LIST OF TABLES

LIST OF FIGURES

Page

Figure

ABSTRACT

The Response of Five Tropical Plant Species to Natural Solar Ultraviolet-B Radiation

by

Peter S. Searles, Master of Science
Utah State University, 1994

Major Professor: Martyn M. Caldwell
Program: Range Ecology

Tropical regions currently receive the highest global levels of solar ultraviolet-B radiation (UV-B, 280-320 nm) even without ozone depletion. Thus, the influence of natural, present-day UV-B irradiance in the tropics was examined for five tropical species, including three native rainforest tree species and two economically important species. Solar UV-B radiation conditions were obtained using either a UV-B excluding plastic film or a near-ambient UV-B transmitting film in a small clearing on Barro Colorado Island (BCI), Panama (9°N). Significant differences were often exhibited as increased foliar UV-B absorbing compounds, increased leaf mass per area, and reduced leaf blade length for plants receiving solar UV-B radiation. Plant height was typically reduced under solar UV-B, but some variation among species in response was seen. Biomass and photosystem II function using chlorophyll fluorescence were generally unaffected. The results of this study provide strong evidence that tropical vegetation, including native rainforest species, responds to the present level of natural solar UV-B. This suggests that even a small increase in UV-B radiation with ozone depletion may have biological implications.

(46 pages)

INTRODUCTION

A steep increase in UV-B radiation (UV-B, 280-320 nm) from the high to low latitudes accompanies the natural latitudinal gradient of decreasing ozone column thickness and shorter solar path length towards the equator (Caldwell Robberecht, and Billings, 1980). The increase is as much as seven-fold from the Arctic to high elevation, equatorial sites when weighted according to the generalized plant action spectra (Caldwell, 1971). Although terrestrial vegetation has previously been suggested to be somewhat more UV-B resistant with decreasing latitude (Caldwell et al., 1982; Teramura, Ziska, and Sztein, 1991), the high level of present-day UV-B flux in the tropical latitudes may have sizeable effects on plant life even without ozone depletion. Recently, the relatively low ambient UV-B in the temperate region has been shown to cause detrimental effects on the growth and photosynthesis of crop plants (Tevini, Mark, and Saile, 1989; Tevini et al., 1991).

If the current UV-B flux negatively affects tropical plants, small reductions in the stratospheric ozone layer resulting from increasing levels of chlorofluorocarbons (CFCs) may be crucial. The increase in absolute UV-B radiation even with marginal ozone depletion in the tropics will likely be greater than in the temperate region because of the already thin ozone layer and high solar elevation (Caldwell, 1991). Surprisingly, a strong trend for increased UV-B radiation from 1979 to 1992 has just been detected within fifteen degrees of the equator using the total ozone mapping system (TOMS) (Madronich and de Gruijl, 1993).

Very little emphasis has been placed on the study of species from natural ecosystems for UV-B sensitivity (Tevini and Teramura, 1989). Tropical rainforests, which supply 42% of net global primary productivity, have been neglected in UV-B studies (Teramura, 1990). As in the temperate zone, the UV-B sensitivity of crop species such as rice and cassava has primarily been examined for the tropical regions (Teramura, Ziska, and Sztein, 1991; Dai et al., 1992; Ziska et al., 1993). Based mostly on results from temperate agricultural systems, typical effects of UV-B radiation include growth reductions (Teramura, 1983), damage to photosystem II (PSII) reaction centers (Bornman, 1989), and augmentation of UV-B absorbing epidermal flavonoids (Caldwell, Robberecht, and Flint, 1983; Flint, Jordan, and Caldwell, 1985).

With respect to natural ecosystems, a wide variation in plant growth response was seen for species from a 3,000 meter Hawaiian elevational gradient (20°N) in a recent greenhouse study using artificial, supplemental UV-B radiation (Sullivan, Teramura, and Ziska, 1992). The ability of tropical, high elevational plants to increase epidermal flavonoid levels in response to supplemental UV-B has not been seen under greenhouse (Ziska, Teramura, and

Sullivan, 1992) or field conditions (Barnes, Flint, and Caldwell, 1987). The degree that lowland, equatorial tropical species respond to high UV-B flux by increasing UV-B absorbing compounds is not known. Increases in secondary compounds or leaf mass per area can be very important in reducing the penetration of UV-B to the mesophyll layer where photosytem II damage can occur (Tevini, Braun, and Fieser, 1991; Day, 1993).

Since all of the above tropical studies have been done under artificial UV-B light sources, the comparison of these studies to the effects of solar UV-B radiation can be difficult (Caldwell et al., 1986; Tevini, Mark, and Saile, 1989). Artificial UV-B light sources have considerably different spectral distributions from natural sunlight. Thus, individual wavelengths in the UV-B region must be weighted with respect to their ability to cause damage (i.e., biological effectiveness) for comparisons between artificial and solar radiation. Previous field experiments are further complicated by having been done in the temperate and not the tropical latitudes. Additionally, greenhouse studies often show plants to be more sensitve than in the field (Teramura, 1983).

Another important potential factor in determining UV-B sensitivity is the background visible light level (photosynthetic photon flux density, PFD, 400-700 nm waveband) because of its ability to protect plants by increasing flavonoids and leaf thickness and to repair UV-B-induced pyrimidine dimers (Pang and Hays, 1991). However, the role of excess, photoinhibitory visible light has been little explored in ultraviolet radiation studies (Lovelock, Clough, and Woodrow, 1992; Ziska et al., 1993). Following treefall gap openings in tropical forests, understory shade-tolerant species and early successional, invasive species may experience excess PFD around midday.

The primary objective was to determine if growth, photosystem II, and the level of UV-B absorbing compounds are affected by the UV-B radiation in natural sunlight. Five tropical species were examined in a small clearing in Panama (9°N), including three rainforest tree species, a timber tree (Swietenia macrophylla), and a crop species (Manihot esculenta). The relative importance of UV-B-induced changes in plant morphology and in UV-B absorbing compounds versus damaging photosynthetic and growth reductions was of particular interest. Although large decreases in plant biomass may not occur, subtle changes in plant morphology and secondary chemistry may also be of tremendous ecological importance. Secondarily, the importance of excess visible radiation on the level of UV-B sensitivity was examined.

MATERIALS AND METHODS

Site Description

A field experiment under natural sunlight was conducted in a small clearing on Barro Colorado Island (BCI). BCI, administered by the Smithsonian Tropical Research Institute, is located in Lake Gatun in the Panama Canal Area at 9°N latitude. Detailed descriptions of the island's biology and climate are provided by Leigh, Rand, and Windsor (1982) and Leigh and Wright (1990). The experimental work was done from January through mid-April, 1993, which overlapped with the pronounced dry season from mid-December through March. Plants were grown in 3-m long x 1-m wide, A-shaped aluminum frames and received full solar radiation for most of the day as both the eastern and southern ends of the clearing were open. A building to the west partially attenuated diffuse radiation in the lower third of the sky, but did not block any direct radiation until approximately 30 min before the sun set behind the uphill forest canopy.

Plant Culture

Cecropia obtusifolia, *Tetragastris panamensis*, and *Calophyllum longifolium* were all grown from seed collected on BCI. Seeds of the shade-intolerant (gap pioneering) tree, *Cecropia obtusifolia*, were collected in late September 1992 near the lakeshore from a separate, single catkin for each of two experimental trials. Numerous seeds of Cecropia were germinated together in large pots 2 mo before the start of each trial. The two shade tolerant tree species, Tetragastris and Calophyllum, were germinated similarly in April and May, 1992, and grown for 7 to 8 mo as suppressed seedlings under shade cloth. Tetragastris and Calophyllum received less than 100 μmol m^{-2} s^{-1} PFD (PFD, photosynthetic flux density, 400-700 nm) for the initial 4 mo and at a midday, peak visible irradiance of 500 μmol m^{-2} s^{-1} PFD thereafter. Growth under such low PFD is typical for seedlings germinating in the tropical forest understory. At the start of the experimental period, Tetragastris and Calophyllum received a large increase in irradiance as would simulate a forest gap opening. Seeds of the timber tree, Swietenia macrophylla (mahogany), were collected from the Panama Canal area and germinated in early January, 1993. Manihot esculenta (cassava), an agricultural tuber crop, was grown from stem cuttings of many parent plants.

Seedlings were transferred to 15-l or 30-l (cassava only) pots containing Pro-Mix soil (Premier Brands) shortly before the start of the experiment. Tetragastris, Calophyllum, and the first trial of Cecropia were placed in the plant frames in early to mid-January 1993. Swietenia, Manihot, and the second Cecropia trial were started on February 2, February 11, and March 1 respectively. Five frames were exposed to ambient UV-B (+UV-B) and five frames received no UV-B (-UV-B). One pair of plants from each of five species was placed on each frame with a pair of plants forming a row in a frame. Each row was moved one pot length per frame every other day to spread any within-frame variability among the pairs. Additionally, each plant pair was randomly assigned to a different frame every 2 to 3 wk to spread between-frame variability among the pairs. A pair of plants was the experimental unit, and five replicate pairs were employed for each UV-B level per species. The plants were watered each morning with lake water and fertilized once per week with standard Johnson's solution the first month and half-strength 20N-20P-20K commercial fertilizer thereafter.

UV-B Filters and Measurement

The experimental treatments were obtained using a 0.13-mm thick polyester plastic film (optically equivalent to Mylar-D, DuPont Co., Wilmington, DE, USA) for the no UV-B treatment and 0.038-mm thick Aclar plastic (Allied Signal, Pottsville, PA, USA) for the ambient UV-B treatment. Polyester has a sharp transmittance cut-off at 320 nm, while Aclar allows full transmittance above 220 nm. The plastic film-covered frames were open on the north and south ends with one 15-cm fan per frame used to enhance ventilation. Air temperature did not increase by more than 1°C above ambient inside the frames. The UV-B level under the Aclar covered frames was 90-93% of full ambient UV-B flux and no biologically effective UV-B was measured under the polyester-covered frames using an Optronics 742 double-monochromator spectroradiometer (Orlando, FL, USA) modified with a Peltier heat exchange unit. Calibration for wavelength accuracy and absolute intensity of the spectroradiometer was performed in the laboratory using a low-pressure mercury lamp with distinct emission lines and a 1000-W tungsten-halogen standard lamp from the National Institute of Standards and Technology, respectively. Additionally, wavelength accuracy was rechecked in the field. The PFD transmittance levels under the plastic filters were 89% for polyester and 92% for Aclar using a LiCor quantum sensor (Lincoln, NE, USA). The plastic filters were cleaned daily and replaced once per month. Only minimal photodegradation for the polyester filter in the shortwave UV-A (320-360 nm) occurred in comparison to greenhouse experiments where UV fluorescent lamps have been shown to cause significant photodegradation of plastic filters. No photodegradation occurred for the Aclar plastic.

Since the biological effectiveness of UV-B increases with decreasing wavelength and solar spectral irradiance increases steeply with increasing wavelength, UV-B radiation is typically weighted using the generalized plant action spectra (Caldwell, 1971) and normalized to 300 nm. The weighting of individual wavelengths is critical in studies with artificial lamps because their spectral distributions differ from solar radiation. In the present study, the measured biologically effective UV-B under the plastic filters was approx. 5.5 kJ m^{-2} day^{-1} in January and increased to 8.5 kJ m^{-2} day^{-1} in March under cloudless conditions. The percentage of clear days was 12, 68, 61, and 79% in January, February, March, and April based on PFD data from the Lutz canopy tower on BCI.

Plant Measurements

Plant growth parameters (see Figs. 1 and 2, Tables 1 and 2) were measured 3 or 4 times over the course of the experimental treatment for each species including the day before harvest. Plant harvest occurred on day 61 and day 50 of plant growth for the first and second Cecropia trials, respectively. Tetragastris, Calophyllum, Swietenia, and Manihot were harvested after 71, 76, 76, and 56 d. Each plant was divided into leaves, petioles, stem, and roots for the determination of dry weight after 72 hr at 65°C.

Chlorophyll fluorescence was measured to determine if either predawn, midday (solar noon), or late afternoon (17:00 hr. solar) UV-B inhibition of PSII occurred. The leaf ages for the two trials of Cecropia and for Manihot were 20-25 d, while leaf ages for Swietenia were approximately 35 d. Leaves of Tetragastris and Calophyllum that developed under low-PFD and UV-B prior to the experiment and subsequently exposed to full sunlight were measured 3 wk after the start of the experiment. Measurements of high-PFD, high-UV-B leaves (leaf age=60 d) that developed during the experiment were performed before the harvest of each shade-tolerant species. Measurements of light-adapted midday fluorescence were not taken for Tetragastris and Calophyllum because strong visible light photoinhibition made repeatable measurements difficult.

Predawn ratios of variable-to-maximal fluorescence (Fv/Fm) were determined for each species using a Walz PAM-2000 (PAM, Pulse amplitude Modulation) portable fluorometer or Walz PAM-101 system (H. Walz Co. Effeltrich, Germany). The Fv/Fm is calculated as (Fm-Fo)/Fm with Fo and Fm being the minimal and maximal fluorescence levels of dark adapted leaves. For midday and late afternoon measurements with the portable instrument, the quantum yield of PSII photochemistry under the given PFD conditions was calculated as (Fm'-Ft)/Fm' (Genty, Briantais, and Baker, 1989). Ft represents the steady-state fluorescence and Fm' is the maximal fluorescence under the light-adapted conditions. Leaf temperature and PFD at the site of fluorescence measurement were determined with a thermocouple and microquantum sensor as part of a leaf positioning clip (Model 2030-B, H. Walz Co.). All measurements were nondestructive except for predawn measurements using the Walz PAM 101 system where leaf discs were punched for measurement in the laboratory.

A determination of leaf UV-B absorbing compounds was done using fresh leaf discs (1.13 cm^2) sampled at the time of final harvest and stored in individual vials containing 99:1 ethanol/glacial acetic acid solution for later analysis. Absorbance at 305 nm was measured in a spectrophotometer (Shimadzu, Japan) after refluxing for 10 min in a hot water bath (Flint, Jordan, and Caldwell, 1985; Barnes, Flint, and Caldwell, 1987). The extracted leaf disc and sample solution were then dried and weighed to express absorbance on a dry mass basis.

Samples for chlorophyll and carotenoid determination including xanthophylls were collected at noon on March 6 and before dawn on March 7 from the first trial of Cecropia obtusifolia. Leaf temperature and PFD were measured for the midday samples using a thermocouple unit (Wescor, Logan, UT, USA) and quantum sensor, respectively. Leaf discs of 3.38 cm^2 were punched from an approximately 20-day-old leaf and immediately frozen in liquid nitrogen.

The pigments were separated and quantified using high-pressure liquid chromatography (Waters Milipore HPLC system, Milford, MA, USA). Pigments were extracted from mortar-ground frozen samples with 100% acetone and microcentrifuged for 5 min. The supernatant was collected and the pellet resuspended in 100% acetone for recentrifugation. This procedure was repeated until all of the pigments were extracted. The collected supernatant of each sample was then filtered through a 0.22-μm PTFE filter (Alltech, Deerfield, IL, USA) and analyzed using a 20-μl injection volume into the HPLC system. A spherisorb ODS-1 column (5-μ particle size, 250 mm x 4.6 mm I.D., Alltech, Deerfield, IL, USA) and a C_{18} guard-pak pre-column (Waters Milipore, Milford, MA) were used for separation. Pigments were eluted at a flow rate of 2 ml min^{-1} using solvent A (acetonitrile-methanol-Tris HCl; 72:12:7) for 6 min, a 10 min linear gradient from solvent A to solvent B (methanol-hexane; 7:1), and solvent B for 4 min. The detector wavelength was set at 440 nm for integration. Quantified pigments included chl a, chl b, α-carotene, ß-carotene, lutein, neoxanthin, violaxanthin, antheraxanthin, and zeaxanthin.

Statistical Analysis

A General Linear Model procedure (SAS PC version 6.04, SAS Institute, USA) was used for all analyses. The effect of UV-B radiation on final dry weight harvest, chlorophyll fluorescence, and UV-B absorbing compounds was evaluated using one-way ANOVA. Plant growth measurements over time were examined for the effect of UV-B level using one-way ANOVA with repeated measures. For *Cecropia* (day 23) pre-dawn, chlorophyll fluorescence in which two leaf ages were measured, a two-way ANOVA was used for the analysis of UV-B effect. A two-way ANOVA was also used for the xanthophyll measurements where time was a main effect.

RESULTS

Plant Growth

Of the morphological parameters investigated, plant height was the most affected by UV-B radiation (Fig. 1). The two shade-tolerant species, Tetragastris panamensis ($P<0.05$) and Calophyllum longifolium ($P<0.01$), showed that UV-B-induced reductions for change in plant height (i.e., plant height increment) using repeated measures ANOVA over time. A decrease ($P<0.10$) in absolute plant height for Cecropia trial 1 was seen with a strong UV-B*time interaction ($P<0.01$) occurring between days 38 and 61. This interaction reflects an amelioration of UV-B-reduced plant height over time. In the second Cecropia trial, a small UV-B stimulation in plant height was evident ($P<0.10$) with a strong UV-B*time interaction ($P<0.05$) as well. However, the growth rate of this second trial was unusually low for Cecropia in general and not as representative of the species as the first trial. Mahogany showed a small increase in plant height at final harvest ($P<0.05$), while no differences were seen in cassava. Internode measurements taken for Cecropia trial 1, mahogany, and cassava paralleled plant height differences (Table 1).

Blade length was significantly reduced for Cecropia trial 1 ($P<0.10$) for an approx. 25 day-old-leaf measured during each time period and for cumulative blade length of all leaves produced in Calophyllum ($P<0.05$) (Fig. 2). Leaf

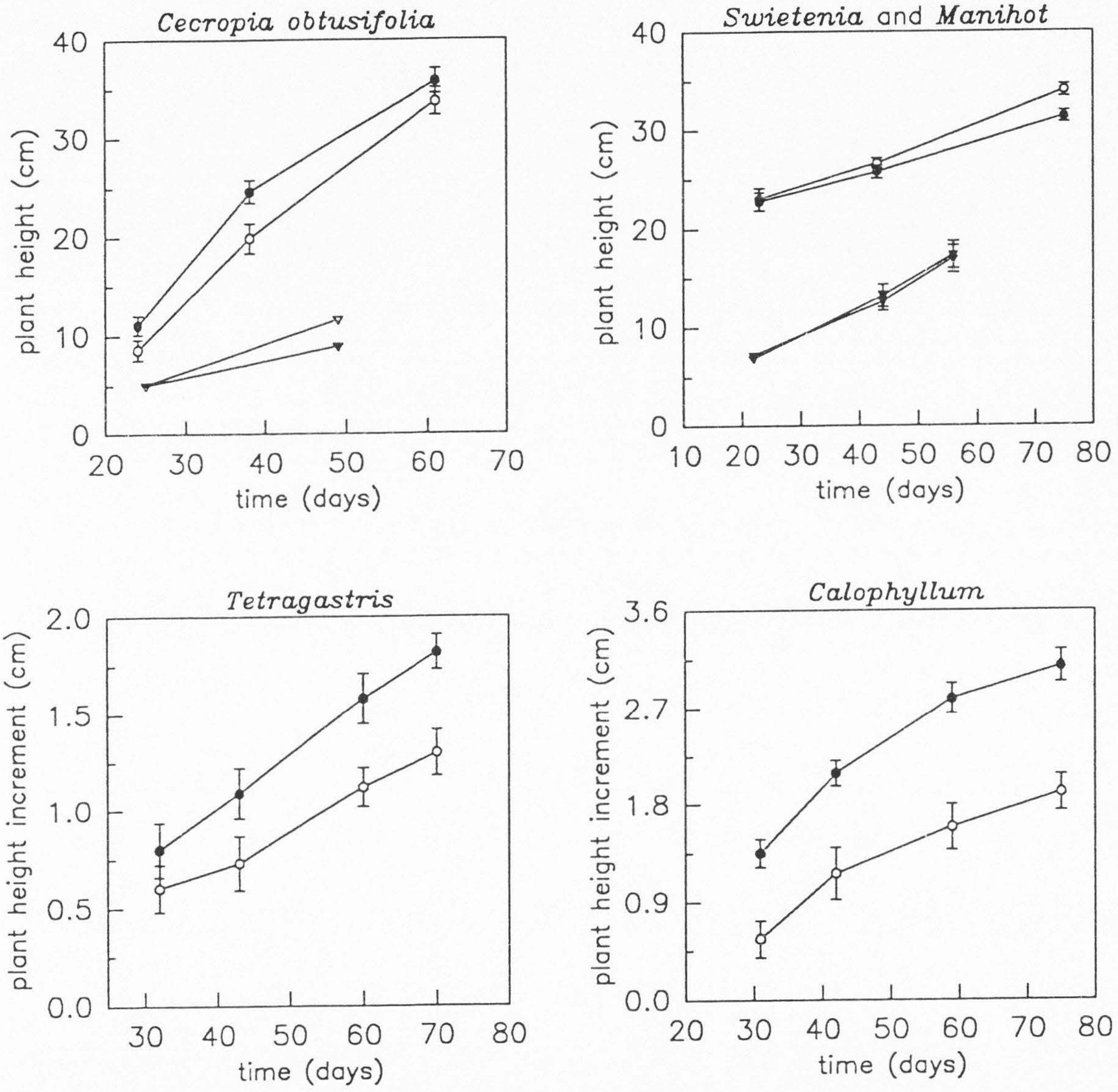

Fig. 1 - Plant height under natural solar UV-B (open symbols) or no UV-B (closed symbols) radiation. Height is shown as either absolute plant height (<u>Swietenia</u>, <u>Manihot</u>, two trials of <u>Cecropia</u> <u>obtusifolia</u>) or plant height increment (<u>Tetragastris</u>, <u>Calophyllum</u>). <u>Cecropia</u> trial 1 and trial 2 are represented as circles and triangles, respectively, as are <u>Swietenia</u> and <u>Manihot</u>. N=5 pairs of plants per treatment per species with only nine plants represented among the five pairs for <u>Swietenia</u> and <u>Manihot</u> in the no UV-B groups. Vertical bars denote ± SE. P values for significant differences are noted in the text using repeated measures analysis of variance (ANOVAR) over time.

Table 1. Internode distance, leaf number, and leaf blade length for *Cecropia* (trial 1 and 2), *Swietenia*, and *Manihot* grown under natural solar UV-B (+UV-B) or no UV-B (-UV-B) radiation with the last day shown coinciding with final plant harvest[a]

Species	Day	UV-B level	Internode distance	# of leaves	Blade length
Cecropia obtusifolia (trial 1)	24	+UV-B	2.3	8.2	12.4
		-UV-B	3.5	8.6	14.4
	38	+UV-B	5.1 (+)	11.3	18.5 (+)
		-UV-B	6.4	11.3	22.4
	61	+UV-B	6.4	11.9	17.3
		-UV-B	6.0	12.3	17.8
Cecropia obtusifolia (trial 2)	25	+UV-B	-	6.8	-
		-UV-B	-	6.8	-
	49	+UV-B	-	9.7	4.4
		-UV-B	-	9.4	3.0
Swietenia macrophylla	23	+UV-B	0.6	3.6	9.4
		-UV-B	0.5	3.1	8.9
	43	+UV-B	1.0	6.4	14.8
		-UV-B	0.8	5.9	15.3
	75	+UV-B	2.9	11.1	43.8
		-UV-B	2.4	10.3	37.7
Manihot esculenta	22	+UV-B	-	8.1	6.6
		-UV-B	-	8.1	6.9
	43	+UV-B	2.4	11.8	7.5
		-UV-B	2.5	12.0	7.3
	56	+UV-B	2.0	15.9	8.9
		-UV-B	2.2	15.9	9.5

[a] The two-node internode distances and the leaf blade lengths are measurements of new internodes and leaves that developed since the last measurement date and not measurements on the same internode distance or leaf over time. Values are the means of five plants per treatment per species with only nine plants represented among the five pairs for the no UV-B groups of *Swietenia* and *Manihot*. + = significant difference of $P<0.10$ over time for a particular species growth variable using repeated measures ANOVA. Dashes (-) indicate no data recorded on a certain day.

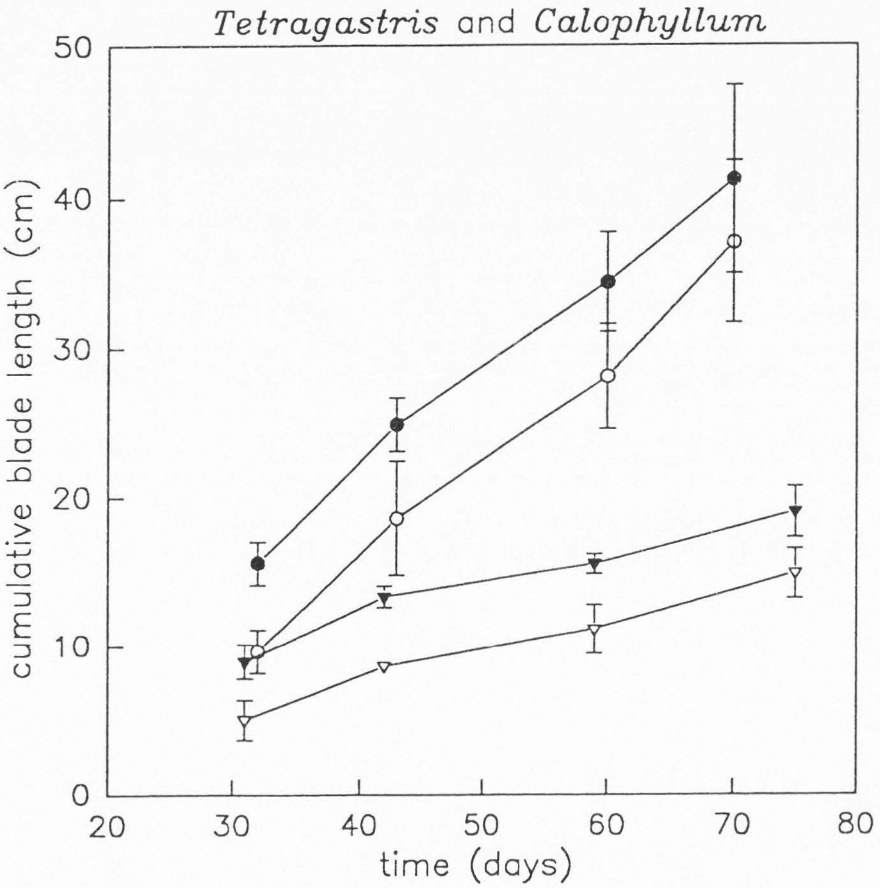

Fig. 2 - Cumulative blade length for Tetragastris (circles) and Calophyllum (triangles) under natural solar UV-B (open symbols) or no UV-B (closed symbols) radiation. ANOVAR over time was used with five pairs of plants per treatment per species; vertical bars denote ± SE. P values for significant differences are noted in the text.

area ($P<0.05$) measured only at day 38 and blade width over the entire experiment ($P<0.05$) concurred with blade length for the two species, respectively (data not shown). No differences in leaf number were seen for any of the species.

In contrast to the morphological measurements, total plant dry mass including belowground dry matter was generally unaffected by UV-B (Fig. 3). Only the first Cecropia trial showed a significant change with total plant dry mass being reduced under ambient UV-B ($P<0.10$) although Tetragastris, Calophyllum, and Manihot showed nonsignificant +UV-B reductions of 17%, 20%, and 14%, respectively. With respect to individual plant components, Cecropia trial 1 ($P<0.10$) and Calophyllum ($P<0.10$) both showed significant reductions in root mass under ambient UV-B (Table 2). Calophyllum also showed a +UV-B reduction ($P<0.10$) in leaf dry mass for leaves produced during the experiment. This follows from the reduction of blade length and no change in the leaf mass per area ratio (LMA). Significant increases in LMA were evident in +UV-B plants for Tetragastris and the first trial of Cecropia (Fig. 4). No differences in root/shoot were noted, indicating a lack of UV-B effect on dry matter partitioning.

UV-B Absorbing Compounds

The UV-B absorbing compounds significantly increased in 4 of 5 species, including both trials of Cecropia when expressed on a leaf area and dry mass basis in plants

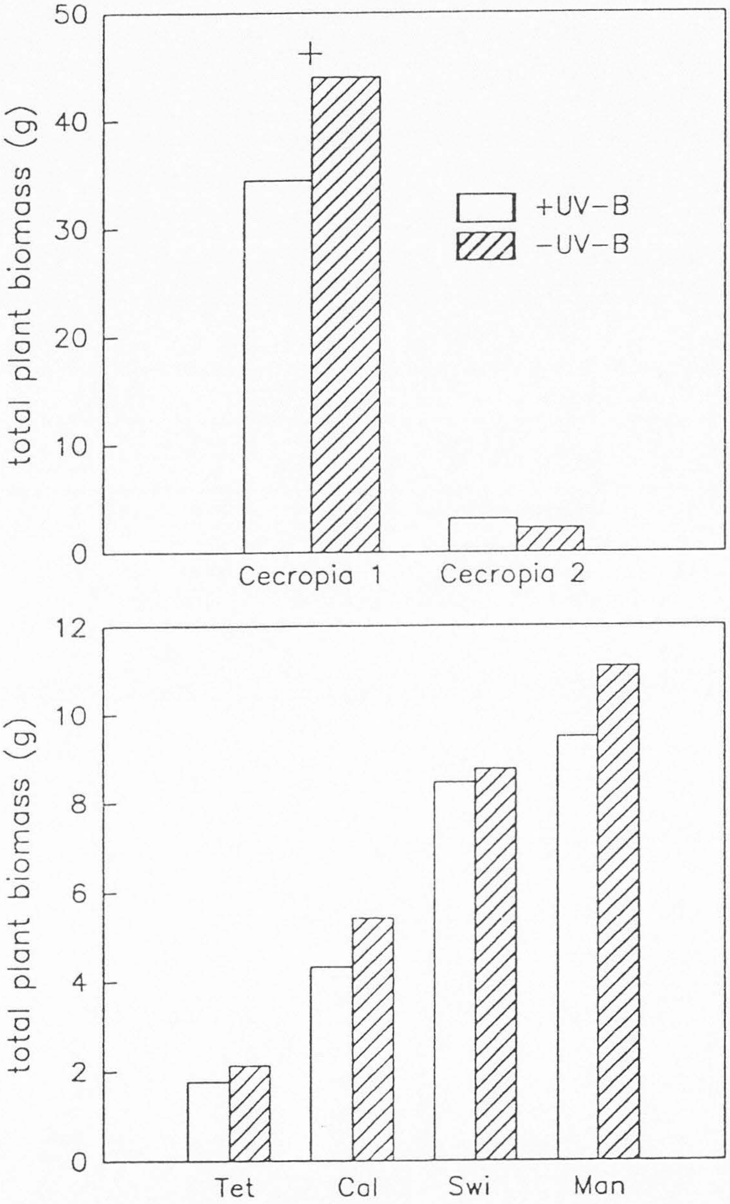

Fig 3. - Total plant dry biomass including roots under natural solar UV-B (open bars) and no UV-B (closed bars) radiation. The first three letters of the genus name are used as abbreviations. N=5 pairs of plants per treatment per species with only nine plants represented among the five pairs for _Swietenia_ and _Manihot_ in the no UV-B groups. + = significant at $P<0.10$ as determined by one-way ANOVA for comparisons between natural solar UV-B and no UV-B pairs of plants.

Table 2. Dry biomass (g) partitioning under natural solar UV-B (+UV-B) or no UV-B (-UV-B) radiation [a]

Species	UV-B level	Leaves[b]	Petiole	Stem	Shoot	Root	R/S
Cecropia (trial 1)	+UV-B	11.69	1.69	5.20	18.58	15.87b	0.86
obtusifolia	-UV-B	13.25	1.83	6.68	21.76	22.22b	1.00
Cecropia (trial 2)	+UV-B	1.77	-	0.39	2.30	0.78	0.35
obtusifolia	-UV-B	1.27	-	0.25	1.59	0.57	0.36
Tetragastris	+UV-B	0.31	-	0.57	1.33	0.43	0.32
panamensis	-UV-B	0.40	-	0.65	1.54	0.58	0.38
Calophyllum	+UV-B	0.59a	-	1.06	2.69	1.64a	0.63
longifolium	-UV-B	0.98b	-	1.21	3.33	2.08b	0.64
Swietenia	+UV-B	3.17	0.53	2.40	6.74	1.73	0.26
macrophylla	-UV-B	2.86	0.48	2.49	6.50	1.79	0.28
Manihot	+UV-B	2.89	0.69	1.27	4.85	4.63	0.90
esculenta	-UV-B	3.18	0.82	1.28	5.28	5.81	1.03

[a] R/S = root to shoot ratio. Values are means of five pairs of plants per treatment per species with only nine plants represented among the five pairs for the no UV-B groups of *Swietenia* and *Manihot*. Different letters indicate a significant difference at $P<0.10$ according to one-way ANOVA. Letters are only shown if significant differences were observed.

[b] Only dry leaf biomass from leaves developed during the experimental period is reported for *Tetragastris* and *Calophyllum*.

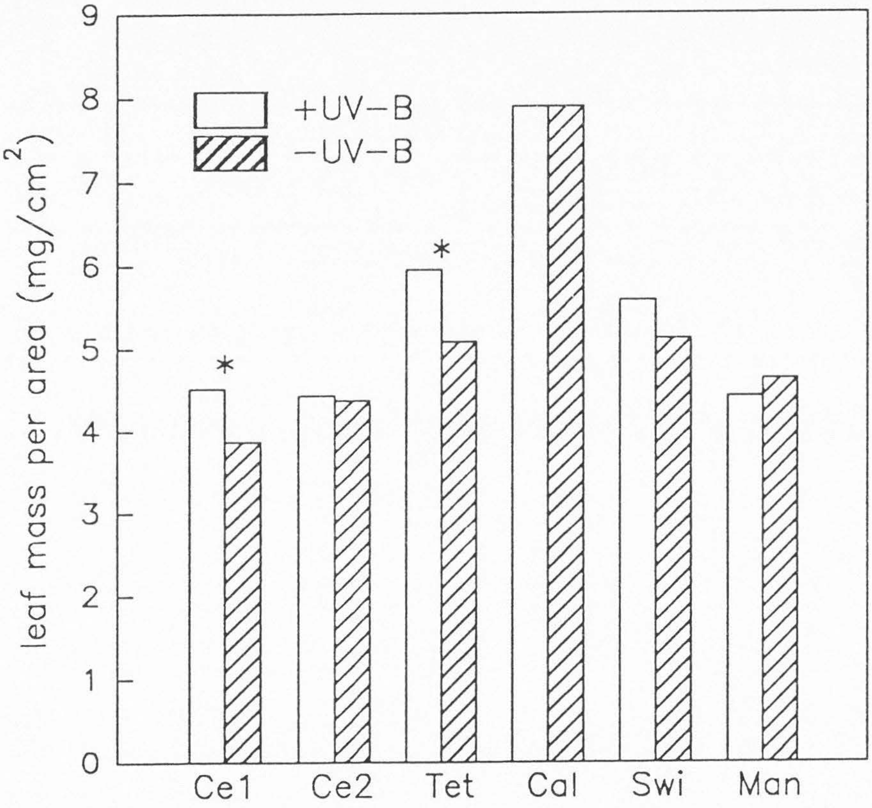

Fig. 4 - Leaf mass per area for plants receiving natural solar UV-B (open bars) or no UV-B (hatched bars) radiation. Leaf discs were collected at the final plant harvest except for Cecropia trial 1 at mid-experiment. Measurements at final harvest showed a similar trend, but are not presented due to sampling error. Genus abbreviations are similar to fig. 3. N=5 pairs of plants per treatment per species. * = significant at $P<0.05$ as determined by one-way ANOVA between natural solar UV-B and no UV-B pairs of leaf discs.

exposed to natural, near-ambient UV-B (Fig. 5). The shade-tolerant Calophyllum showed a nonsignificant increase of 11% by area and 13% by mass. The increase in absorbance on a dry weight basis shows a direct increase per milligram of leaf material of UV-B absorbing compounds. On a leaf area basis, indirect flavonoid increases often occur based on an increase in LMA.

Chlorophyll Fluorescence

No significant +UV-B reductions occurred in predawn, dark-adapted Fv/Fm or light-adapted fluorescence yield (Φ) at solar noon and late afternoon (Table 3). However, a slight increase in Fv/Fm was seen for cassava ($P<0.10$) and the first trial of Cecropia ($P<0.10$) on 25-day-old leaves on day 39 with UV-B exposure. Minimal (Fo or Ft) and maximal (Fm or Fm′) fluorescence parameters did not differ except for increases in Fo ($P<0.10$) and Fm ($P<0.05$) in the +UV-B treatment for the second Cecropia trial (data not shown). The difference in Fo and Fm for this trial without a change in Fv/Fm could reflect a slightly higher chlorophyll content in the +UV-B leaves. A minor UV-B*leaf interaction occurred for the predawn, Cecropia trial 1 measurements with a 20-day-old leaf showing a higher Fm in the no UV-B group (1.742 for -UV-B vs. 1.639 for +UV-B; relative units), while the next youngest leaf had a somewhat lower Fm in the -UV-B plants (1.630 for -UV-B vs. 1.68 for +UV-B) at day 23.

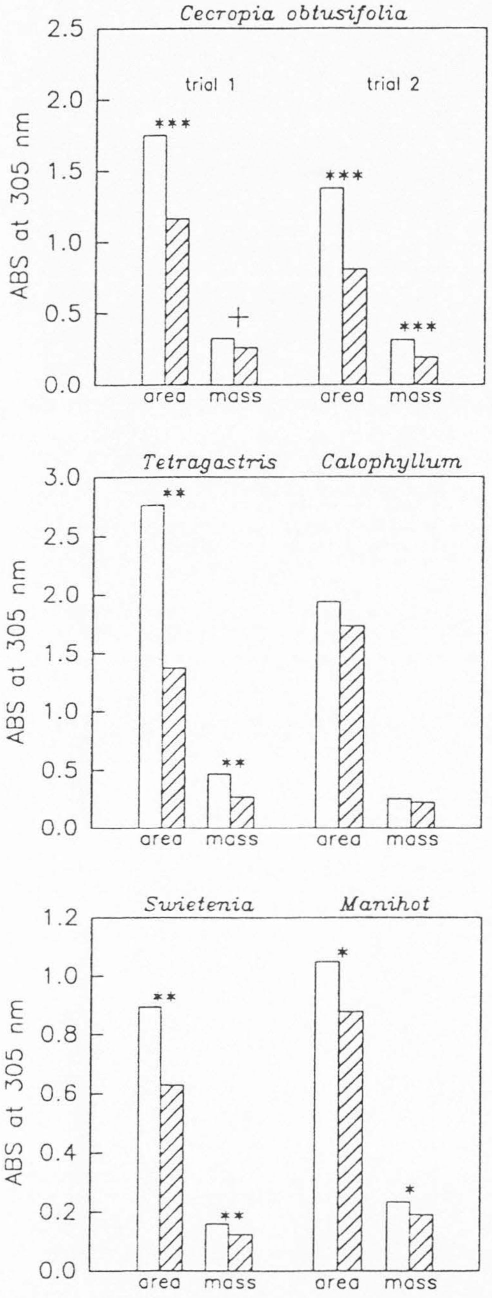

Fig. 5 - UV-B absorbing compounds (absorbance at 305 nm) for leaf disc pigment extracts under natural solar UV-B (open bars) or no UV-B (hatched bars) radiation on a leaf area and leaf mass basis. N=5 pairs of leaf discs per treatment per species except for Swietenia and Manihot where n=9 leaf discs per treatment. Pair information was improperly recorded for these samples. *** = significant at P<0.001, ** = significant at P<0.01, * = significant at P<0.05, + = significant at P<0.10.

Table 3. Chlorophyll fluorescence results for variable-to-maximal fluorescence (Fv/Fm) at predawn and for quantum yield (Φ) at solar noon and during the late afternoon (17:00 hr, solar) under natural solar (+UV-B) or no UV-B (-UV-B) radiation[a]

		Predawn Fv/Fm		Noon Φ		Late afternoon Φ	
Species	Day	+UV-B	-UV-B	+UV-B	-UV-B	+UV-B	-UV-B
Cecropia (trial 1)	23	0.796	0.788	0.239	0.256	0.715	0.705
obtusifolia	39	0.820a	0.810b	-	-	-	-
	54	0.805	0.791	-	-	-	-
Cecropia (trial 2) *obtusifolia*	49	0.801	0.794	-	-	-	-
Tetragastris	24	0.662	0.677	-	-	0.503	0.487
panamensis	71	0.631	0.618	-	-	-	-
Calophyllum	23	0.650	0.690	-	-	0.482	0.530
longifolium	76	0.722	0.697	-	-	-	-
Swietenia	64	0.782	0.777	0.233	0.217	0.672	0.662
macrophylla	67	-	-	0.224	0.173	-	-
Manihot	52	0.829a	0.820b	0.327	0.277	0.740	0.731
esculenta	55	-	-	0.311	0.307	-	-

[a] The dashes indicate no measurement taken for a species at a certain time. Values are means of five pairs of leaves per treatment per species with only nine plants represented among the five pairs for *Swietenia* and *Manihot* in the no UV-B group. For the last two measurement days of *Cecropia* trial 1, N=8 plants per treatment as pair information was not recorded. See table 2 for different letter significance.

The midday leaf temperature and PFD were typically high for the chlorophyll fluorescence measurements. Average leaf temperature was 34-35°C for Cecropia obtusifolia. Cassava and mahogany had leaf temperatures of 37-39°C on April 4 and 35-37°C on April 7 with comparisons between +UV-B and -UV-B plants always being within one degree centigrade. The PFD averaged 1200-1300 μmol m^{-2} s^{-1} for cassava and Cecropia at the site of fluorescence measurement with differences in PFD for ± UV-B comparisons not being greater than 40 μmol m^{-2} s^{-1}. Mahogany midday measurements showed a 100 μmol m^{-2} s^{-1} difference between treatments, but leaf temperature was very similar and no relationship between PFD and yield occurred over the measured range of PFD.

Carotenoid and Chlorophyll Measurements

Xanthophylls are a category of carotenoids consisting of violaxanthin (V), antheraxanthin (A), and zeaxanthin (Z) that interconvert in a light-dependent cycle. Increasing visible light results in the de-epoxidation of violaxanthin to zeaxanthin via the intermediate antheraxanthin with zeaxanthin being important in dissipating heat energy resulting from excess PFD (Demmig-Adams, 1990). No differences in total chlorophyll content, chl a/b ratio, or carotenoids including xanthopylls were seen between +UV-B plants and -UV-B treatments with only the first Cecropia trial measured. Additionally, no UV-B*time interaction between noon and predawn occurred for the xanthophyll pigments. The epoxidation state ((0.5A + V)/(V + A + Z)) of the xanthophyll cycle, a measure of the relative amount of zeaxanthin, was measured to be 0.21 (+UV-B) and 0.18 (-UV-B) at noon on March 6 under clear skies and 0.86 (+UV-B and UV-B) at predawn on March 7.

DISCUSSION

This study provides the first evidence that tropical plants respond to natural solar UV-B radiation. Additionally, little previous work has focused on woody species in natural communities, with only the Pinaceae being intensively studied (Kossuth and Biggs, 1981; Sullivan and Teramura, 1988, 1989, 1992; Naidu et al., 1993). Significant effects were typically seen for increased UV-B absorbing compounds and altered plant morphology and not for plant biomass or photosynthetic reductions.

Ultraviolet-B radiation has been suggested to cause subtle changes in plant morphology, such as reduced blade length, internode length, and plant height, without significant reductions in plant biomass necessarily occurring (Barnes, Flint, and Caldwell, 1990). Sullivan, Teramura, and Ziska (1992) have recently found one-half of endemic, subtropical Hawaiian species to show reduced plant height under high UV-B radiation in a greenhouse. In the same study, total plant biomass was rarely significantly affected although the large variation in these wild plant populations made statistical differences difficult to detect. The lowland Panamanian tropical rainforest species in this study showed remarkably similar results. However, if a nonsignificant UV-B-induced reduction in biomass does exist as indicated by the three native, Panamanian natural community species, reductions in primary productivity may still be of importance. As in the present study, the plant height of Manihot esculenta has previously been shown not to be affected by UV-B radiation under field conditions. In contrast, a strong shift in carbon allocation from roots to shoots was seen in the field under artificial, supplemental UV-B radiation in Maryland (USA) (Ziska et al., 1993), but not under natural UV-B in Panama. Direct comparisons between studies are difficult, however, because of the differences in the UV-B levels employed.

Studies with ultraviolet-B radiation in natural sunlight have previously shown an amelioration of UV-B effects over time. Becwar, Moore, and Burke (1982) saw a retarding of plant height in wheat at days 14 and 31 under ambient solar UV-B, but not at day 50. A similar reduction of UV-B effect has been observed for dry weight in rye and corn over a 5-d period in small seedlings grown outdoors in sun-lit growth chambers receiving either near-ambient or partially reduced UV-B radiation (Tevini, Mark, and Saile, 1989). Plant height for the first trial of Cecropia showed a similar reduction of any UV-B-induced effect over time. The mechanism of this amelioration of UV-B effects is not clear but may involve the action of a UV-B photoreceptor or interaction with indoleacetic acid (IAA) (Curry, Thimann, and Ray, 1956). However, the UV-B radiation requirement for IAA photooxidation has recently been estimated as 1000-fold greater than for the UV-B-induced phototropic base curvature response of the oat seedlings used in that study (Ensminger, 1993). UV-B photoreceptors have been shown to influence a number of plant processes, including shoot elongation (Ballaré, Barnes, and Kendrick, 1991), anthocyanin synthesis (Wellman, 1983) and cotelydon curling (Wilson and Greenberg, 1993).

No significant reductions in chlorophyll fluorescence occurred under solar UV-B reduction. The insensitivity of photosystem II under field conditions was not unexpected. In the field, chlorophyll fluorescence has shown only minor temporal effects or no effect at all under supplemental UV-B (Naidu et al., 1993; Ziska et al., 1993; Caldwell, Flint, and Searles, in press) or under filtered natural sunlight (Tevini, Grusemann, and Fieser, 1988). Additionally, no significant difference was seen in tropical phytoplankton photosynthesis using 14-C labelling between surface water samples with or without natural solar UV-B along a transect from Valparaiso, Chile to San Diego, California (USA) (Helbling et al., 1992). Growth chamber experiments typically result in large reductions in photosynthesis probably because of low PFD and often unrealistic UV-B levels (Teramura, 1986). Again, photomorphological differences in plant height without photosynthetic inhibition may be mediated by a specific UV-B photoreceptor (Ballaré, Barnes, and Kendrick, 1991).

Even the shade-tolerant tree species (Tetragastris and Calophyllum) did not show photosystem II damage despite being exposed to a sudden increase in UV-B to simulate gap opening. The concomitant, photoinhibitory increase in visible light did not lead to a positive synergistic effect with UV-B. This lack of synergistic effect has been demonstrated between UV-B and other environmental stress factors as well, including water stress (Teramura, Sullivan, and Lydon, 1990) and nutrient deficiency (Murali and Teramura, 1987). With further relevance to interactive stresses, concomitant high PFD and high temperature have been suggested to result in greater UV-B reduction of Fv/Fm at midday for cassava (Ziska et al., 1993). No reductions in chlorophyll fluorescence were seen in this study for any species despite leaf temperatures in excess of 35°C at midday.

An increase in UV-B absorbing compounds such as flavonoids and in leaf mass per area has been shown to be an important effect of UV-B radiation (Caldwell, Robberecht, and Flint, 1983; Flint, Jordan, and Caldwell, 1985; Barnes, Flint, and Caldwell, 1987; Warner and Caldwell, 1983). However, tropical species primarily from high elevation and alpine areas have shown little or no ability to increase their flavonoid level as mentioned earlier. The UV-B stimulation of epidermal compounds can provide an attenuation of UV-B reaching chloroplasts in the mesophyll layer and reduced photosystem II damage (Tevini, Braun, and Fieser, 1991). The increased level of UV-B absorbing compounds in all of the tropical species studied except for Calophyllum may reflect the importance of these compounds in preventing photosynthetic damage. Leaf mass per area also may have influenced possible UV-B-induced damage to the photosynthetic apparatus as Tetragastris and the first trial of Cecropia showed an increase in both leaf mass per area and UV-B absorbing compounds. However, it is not known whether the lower level of UV-B absorbing pigments and LMA in leaves receiving no UV-B would have been adequate to protect against photosynthetic damage if the no UV-B leaves had been exposed to ambient UV-B radiation.

Besides attenuation of UV-B by flavonoids (i.e., UV-B absorbing compounds), carotenoid pigments have been hypothesized to be important in ameliorating UV-induced damage to photosynthesis possibly by the scavenging of chlorophyll-destroying oxygen radicals (Larson, Garrison, and Carlson, 1990; Strid, Chow, and Anderson, 1990; Lovelock, Clough, and Woodrow, 1992). However, this hypothesis has not been supported, as a direct UV-induced increase in carotenoids was not detected in any of these studies. Although no change in carotenoids per unit of chlorophyll was reported, Middleton and Teramura (1993) have suggested that carotenoids are directly linked to UV-B photoprotection based on a positive correlation between leaf carotenoid level and chlorophyll content with increasing UV-B radiation. In terms of the xanthophyll cycle, Pfündel, Pan, and Dilley (1992) demonstrated that extreme UV-B radiation doses can cause an inhibition of the violaxanthin de-epoxidation reaction (i.e., conversion of violaxanthin to antheraxanthin and zeaxanthin) and thus possibly an increased susceptibility to excess visible light. Under natural solar UV-B radiation, no indication of an increase in total carotenoids or inhibition of zeaxanthin formation occurred for Cecropia obtusifolia.

Because of the difficulty in extrapolating from isolated plant studies to the ecosystem level, speculation on the effects of UV-B radiation in tropical rainforest processes such as forest treefall gap dynamics would be tenuous at best. Barnes et al. (1988) and Ryel et al. (1990) have shown a change in competitive balance between wheat (Triticum aestivum) and wild oat (Avena fatua) under enhanced UV-B radiation. This difference was based primarily on altered plant morphology and not on direct induced effects on leaf photosynthesis (Beyschlag et al., 1988) or reduced biomass. Such effects on interspecific competition may be important in natural communities as well (Gold and Caldwell, 1983). Further work is needed to determine how UV-B radiation affects interspecific competition and secondary succession in tropical forests. Potential UV-B interactions with the expected global elevation of CO_2 and temperature should also be pursued in natural communities.

In conclusion, tropical plants including native, lowland rainforest species do respond to natural solar UV-B radiation. Increases in leaf UV-B absorbing compounds and alterations in morphological parameters such as plant height were especially evident in comparison to the largely nonsignificant effects on biomass and Photosystem II function. Previously, numerous studies have suggested that terrestrial species at low latitudes are very capable of withstanding high solar UV-B flux (Robberecht, Caldwell, and Billings, 1980; Caldwell et al., 1982; Barnes, Flint, and Caldwell, 1987; Teramura, Ziska, and Sztein, 1991; Sullivan, Teramura, and Ziska, 1992). Based on the results from the native, lowland Panamanian rainforest species presented here, tropical forest species are not as tolerant of UV-B radiation as might have been suspected. It is proposed that increased UV-B radiation with ozone depletion will be influential for tropical forest species, assuming a larger UV-B dose is more biologically effective. The discovery of air rich in thin sheets of ozone-destroying chlorine monoxide in 1992 over Cuba (Kerr, 1992) and the recent startling indication of permanent ozone depletion near the equator from the total ozone mapping system (TOMS) (Madronich and de Gruijl, 1993) suggest the very real possibility of increased UV-B at the lower latitudes as part of the future global environment.

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APPENDIX

Table 4. Analysis of Variance of UV-B effect on repeated plant morphological measurements over time. The between subject effects are shown below. ANOVAR was used for all of the species except Cecropia obtusifolia trial 2 where data was collected on two dates. Prior to examining the ANOVAR results, the hypothesis of compound symmetry of the covariance matrix was tested using Mauchly's criterion. Contrasts between individul means were made a posteriori to allow for further analysis of the effect of time on UV-B effect (results not shown). The following symbols represent significance levels: +=0.10, *=0.05, **=0.01, ***=0.001.

Cecropia obtusifolia trial 1

source of variation	df	MS	F	source of variation	df	MS	F
plant height				internode distance			
UV	1	143.53	3.42+	UV	1	8.3626	3.42+
pair(UV)	8	41.94	2.18	pair(UV)	8	2.4343	2.01
error	10	19.24		error	10	1.2127	
# of leaves				blade length			
UV	1	1.0667	0.51	UV	1	67.628	4.65+
pair(UV)	8	2.0833	1.20	pair(UV)	8	14.536	1.97
error	10	1.7333		error	10	7.385	

Cecropia obtusifolia trial 2

source of variation	df	MS	F	source of variation	df	MS	F
plant height				# of leaves			
UV	1	19.460	3.86	UV	1	0.225	0.175
pair(UV)	8	5.039	1.79	pair(UV)	8	1.2875	0.873
pot(UV*pair)	10	2.820	1.07	pot(UV*pair)	10	1.475	2.493*
time	1	271.96	102.9***	time	1	75.625	127.8***
UV*time	1	18.360	6.94**	UV*time	1	0.225	0.380
error	18	2.643		error	18	0.5916	

(Table continued)

Cecropia obtusifolia trial 2 (cont.)

source of variation	df	MS	F
blade length			
UV	1	3.6000	0.77
pair(UV)	8	4.9895	1.17
pot(UV*pair)	10	4.2755	2.78*
time	1	191.84	124.78***
UV*time	1	7.921	5.15*
error	18	1.5375	

Tetragastris panamensis

source of variation	df	MS	F
plant height			
UV	1	2.9645	6.73*
pair(UV)	8	0.4401	0.71
error	10	0.6157	

source of variation	df	MS	F
blade length			
UV	1	642.97	2.48
pair(UV)	8	258.79	0.92
error	10	279.99	

Calophyllum longifolium (pair was dropped as an error term because it was much smaller than the residual error. Pair df was still used for determining the significance level.)

source of variation	df	MS	F
plant height			
UV	1	21.012	11.17**
error	19	1.880	

source of variation	df	MS	F
blade length			
UV	1	369.8	5.63*
error	19	65.68	

(Table continued)

Swietenia _macrophylla_

source of variation	df	MS	F	source of variation	df	MS	F
plant height				internode			
UV	1	27.885	2.21	UV	1	0.1875	3.64*
pair(UV)	8	12.639	1.04	pair(UV)	8	0.2399	1.05
error	9	12.182		error	9	0.2281	
# of leaves				blade length			
UV	1	3.4090	1.03	UV	1	157.16	1.04
pair(UV)	8	2.1417	1.14	pair(UV)	8	151.80	1.14
error	9	2.7222		error	9	133.44	

Manihot _esculenta_

source of variation	df	MS	F	source of variation	df	MS	F
plant height				internode			
UV	1	1.6378	0.07	UV	1	0.1656	0.53
pair(UV)	8	23.546	1.00	pair(UV)	8	0.9970	3.20*
error	9	23.430		error	9	0.3119	
# of leaves				blade length			
UV	1	0.1363	0.01	UV	1	0.1856	0.03
pair(UV)	8	14.3375	1.50	pair(UV)	8	5.5768	1.68
error	9	9.5370		error	9	3.3190	

Table 5. Analysis of variance of the UV-B effect on plant biomass. Each plant was separated into leaves, petioles, stem, and shoot for analysis.

<u>Cecropia</u> <u>obtusifolia</u> trial 1

source of variation	df	MS	F	source of variation	df	MS	F
leaves				petioles			
UV	1	12.277	1.45	UV	1	0.0966	0.31
pair(UV)	8	8.4836	2.17	pair(UV)	8	0.3128	2.19
error	10	3.9189		error	10	0.1426	
stem				shoot			
UV	1	10.833	3.05	UV	1	50.498	1.82
pair(UV)	8	3.5469	3.11*	pair(UV)	8	27.715	2.51+
error	10	1.1383		error	10	11.059	
root				root/shoot			
UV	1	202.12	4.58+	UV	1	0.1328	3.19
pair(UV)	8	44.158	1.58	pair(UV)	8	0.0417	1.14
error	10	28.172		error	10	0.0366	
total plant							
UV	1	454.67	3.54+				
pair(UV)	8	128.47	1.97				
error	10	65.376					

(Table continued)

Cecropia obtusifolia trial 2

source of variation	df	MS	F	source of variation	df	MS	F
leaves				stem			
UV	1	1.2450	1.50	UV	1	0.1051	2.02
pair(UV)	8	0.8297	1.70	pair(UV)	8	0.0503	2.18
error	10	0.4876		error	10	0.0238	
shoot				root			
UV	1	2.4492	1.65	UV	1	0.2020	1.32
pair(UV)	8	1.5128	1.81	pair(UV)	8	0.1532	1.15
error	10	0.8333		error	10	0.1332	
root/shoot				total plant			
UV	1	0.0009	0.14	UV	1	4.1233	1.62
pair(UV)	8	0.0067	0.79	pair(UV)	8	2.5424	1.65
error	10	0.0086		error	10	1.5392	

Tetragastris panamensis

source of variation	df	MS	F	source of variation	df	MS	F
leaves				stem			
UV	1	0.04232	1.37	UV	1	0.03916	0.86
pair(UV)	8	0.03085	0.96	pair(UV)	8	0.04580	0.49
error	10	0.03218		error	10	0.09336	
shoot				root			
UV	1	0.22323	0.95	UV	1	0.11295	1.38
pair(UV)	8	0.23601	0.50	pair(UV)	8	0.08212	1.32
error	10	0.46852		error	10	0.06242	

(table continued)

Tetragastris panamensis (cont.)

source of variation	df	MS	F
root/shoot			
UV	1	0.01562	1.47
pair(UV)	8	0.01064	1.14
error	10	0.00935	

source of variation	df	MS	F
total plant			
UV	1	0.65377	1.13
pair(UV)	8	0.57662	0.70
error	10	0.82239	

Swietenia macrophylla

source of variation	df	MS	F
leaves			
UV	1	0.65291	1.16
pair(UV)	8	0.56069	0.66
error	9	0.83037	
stem			
UV	1	0.00077	0.001
pair(UV)	8	1.00561	1.77
error	9	0.57759	
root			
UV	1	0.00181	0.007
pair(UV)	8	0.27640	0.83
error	9	0.33049	
total plant			
UV	1	0.1920	0.004
pair(UV)	8	4.4772	0.672
error	9	6.5444	

source of variation	df	MS	F
petiole			
UV	1	0.01582	0.39
pair(UV)	8	0.03994	0.92
error	9	0.04352	
shoot			
UV	1	0.94102	0.22
pair(UV)	8	0.42834	1.42
error	9	3.04931	
root\shoot			
UV	1	0.00147	0.23
pair(UV)	8	0.00641	2.53
error	9	0.00258	

(Table continued)

__Manihot Esculenta__

source of variation	df	MS	F
leaves			
UV	1	0.0995	0.05
pair(UV)	8	1.9795	1.19
error	9	1.6774	
stem			
UV	1	0.0109	0.04
pair(UV)	8	0.2756	0.88
error	9	0.3103	
root			
UV	1	5.4401	0.54
pair(UV)	8	10.133	0.95
error	9	10.681	
total plant			
UV	1	7.4589	0.26
pair(UV)	8	28.520	1.04
error	9	27.452	

source of variation	df	MS	F
petiole			
UV	1	0.0352	0.24
pair(UV)	8	0.1497	1.09
error	9	0.1381	
shoot			
UV	1	0.1589	0.03
pair(UV)	8	5.1683	1.07
error	9	4.8244	
root/shoot			
UV	1	0.1966	1.22
pair(UV)	8	0.1615	1.55
error	9	0.1055	

__Calophyllum longifolium__ (pair was dropped as an error term because it was much smaller than the residual error. pair df is still used for determining the significance level.)

source of variation	df	MS	F
ln(new leaves + 1)			
uv	1	0.21200	4.41+
error	18	0.04804	(pair=8)

source of variation	df	MS	F
ln(stem + 1)			
uv	1	0.02199	1.31
error	18	0.01676	(pair=8)

(Table continued)

Calophyllum longifolium (cont.)

source of variation	df	MS	F
ln(shoot)			
uv	1	0.19052	2.06
error	18	0.09247	(pair=8)
root			
uv	1	0.99012	4.57+
error	18	0.21688	(pair=8)

source of variation	df	MS	F
ln(total plant)			
uv	1	0.203971	2.67
error	18	0.076410	(pair=8)
root/shoot			
uv	1	0.00045	0.063
pair(uv)	8	0.00711	0.996
error	10	0.00711	

Table 6. Analysis of Variance of the UV-B effect on the chlorophyll fluorescence of five tropical plant species. Either dark-adapted Fv/Fm or light-adapted quantum yield was measured.

<u>Cecropia</u> <u>obtusifolia</u> trial 1

predawn Fv/Fm-day 23

source of variation	df	MS	F
uv	1	7.2E-6	0.024
pair(uv)	8	0.0003	0.761
pot(uv*pair)	10	0.00039	2.141
leaf	1	0.00197	10.653**
uv*leaf	1	0.00043	2.315
error	18	0.00018	

predawn Fv/Fm-day 39

source of variation	df	MS	F
uv	1	0.00038	3.70+
error	14	0.00010	

predawn Fv/Fm-day 54

source of variation	df	MS	F
uv	1	0.00067	2.90
error	14	0.00023	

Noon quantum yield-day 23

source of variation	df	MS	F
uv	1	0.00151	0.515
pair(uv)	8	0.00293	0.439
error	10	0.00669	

late afternoon quantum yield-day 23

uv	1	0.00053	0.739
pair(uv)	8	0.00072	2.149
error	10	0.00033	

<u>Cecropia</u> <u>obtusifolia</u> trial 2

predawn Fv/Fm-day 49

source of variation	df	MS	F
uv	1	0.00021	0.837
pair(uv)	8	0.00025	3.124*
error	10	0.00008	

(Table continued)

Tetragastris panamensis

predawn Fv/Fm-day 24

source of variation	df	MS	F
uv	1	0.00022	0.215
pair(uv)	8	0.00105	0.497
pot(uv*pair)	10	0.00212	3.857**
leaf	1	0.00507	9.220**
uv*leaf	1	0.00081	1.582
error	16	0.00055	

late afternoon quantum yield-day 24

source of variation	df	MS	F
uv	1	0.00063	0.089
pair(uv)	8	0.00703	1.813
pot(uv*pair)	10	0.00389	0.902
leaf	1	0.02169	5.015*
uv*leaf	1	0.00062	0.145
error	17	0.00432	

predawn Fv/FM-day 71

source of variation	df	MS	F
uv	1	0.00029	0.122
pair(uv)	7	0.00241	2.52
error	4	0.00100	

Calophyllum longifolium

Predawn Fv/Fm-day 23

source of variation	df	MS	F
uv	1	0.00537	1.90
pair(uv)	8	0.00283	1.52
error	9	0.00189	

Predawn Fv/Fm-day 76

source of variation	df	MS	F
uv	1	0.0016	2.55
pair(uv)	5	0.0006	1.45
error	5	0.0004	

(Table continued)

Calophyllum longifolium (cont.)

late afternoon quantum yield-day 76

source of variation	df	MS	F
uv	1	0.01747	1.463
pair(uv)	8	0.00774	2.051
error	9	0.00708	

Swietenia macrophylla

predawn Fv/Fm-day 64

source of variation	df	MS	F
uv	1	0.00537	1.895
pair(uv)	8	0.00283	1.517
error	9	0.00189	

Midday quantum yield-day 64 and day 67

source of variation	df	MS	F
uv	1	0.00118	2.337
pair(uv)	8	0.00505	0.978
pot(uv*pair)	9	0.00516	1.806
day	1	0.00662	2.315
uv*day	1	0.00288	1.007
error	17	0.00286	

late afternoon quantum yield-day 64

source of variation	df	MS	F
uv	1	0.00049	0.285
pair(uv)	8	0.00164	0.617
error	8	0.00266	

(Table continued)

Manihot esculenta

Predawn-day 52

source of variation	df	MS	F
uv	1	0.00038	4.503
pair(uv)	8	0.00008	1.183
error	9	0.00007	

Midday quantum yield-day 52 and day 55

source of variation	df	MS	F
uv	1	0.00836	1.721
pair(uv)	8	0.00486	0.586
pot(uv*pair)	9	0.00828	1.420
day	1	0.00049	0.084
uv*day	1	0.00487	0.837
error	17	0.00583	

Late afternoon-day 52

source of variation	df	MS	F
uv	1	0.00016	1.463
pair(uv)	8	0.00011	2.051
error	7	0.00005	

Table 7. Analysis of variance of the UV-B effect on leaf mass per area (LMA).

Cecropia obtusifolia trial 1

source of variation	df	MS	F
uv	1	2.1846	10.534*
pair(uv)	8	0.2073	1.48
error	10	0.1401	

Cecropia obtusifolia trial 2

source of variation	df	MS	F
uv	1	0.01568	0.81
pair(uv)	8	0.26927	0.41
error	10	0.23292	

Tetragastris panamensis

source of variation	df	MS	F
uv	1	2.9415	5.521*
pair(uv)	7	0.5328	1.483
error	4	0.3678	

Calophyllum longifolium

source of variation	df	MS	F
uv	1	0.0398	0.023
pair(uv)	5	0.7131	4.987*
error	5	0.3468	

Swietenia macrophylla

source of variation	df	MS	F
uv	1	0.10745	0.557
pair(uv)	7	0.19247	0.872
error	8	0.22073	

Manihot esculenta

source of variation	df	MS	F
uv	1	1.7764	3.214
pair(uv)	8	0.3664	3.340*
error	9	0.1126	

Table 8. Analysis of variance of leaf phenolics by absorbance per square centimeter (ABS/cm^2) and by absorbance per milligram (ABS/mg). If the pair(uv) error term was drastically smaller than the residual error, the model was run without the pair(uv) term. The degrees of freedom associated with the pair term (indicated in parentheses) was still used in determining the P value.

Cecropia obtusifolia trial 1

ABS/cm^2 source of variation	df	MS	F	ABS/mg source of variation	df	MS	F
uv	1	1.74109	31.23***	uv	1	0.01030	4.32+
error	18	0.05574	(pair=8)	error	8	0.00238	(pair=7)

Cecropia obtusifolia trial 2

ABS/cm^2 source of variation	df	MS	F	ABS/mg source of variation	df	MS	F
uv	1	1.62906	100.48***	uv	1	0.07494	47.39***
error	18	0.01622	(pair=8)	error	18	0.00158	(pair=8)

Tetragastris panamensis

ABS/cm^2 source of variation	df	MS	F	ABS/mg source of variation	df	MS	F
uv	1	6.94771	14.91**	uv	1	0.1557	12.41**
error	11	0.48967	(pair=7)	error	11	0.0125	(pair=7)

(Table continued)

Calophyllum longifolium

ABS/cm^2

source of variation	df	MS	F
uv	1	0.11179	2.322
pair(uv)	6	0.04788	0.989
error	4	0.04843	

ABS/mg

source of variation	df	MS	F
uv	1	0.00222	1.524
pair(uv)	6	0.00146	1.761
error	4	0.00085	

Swietenia macrophylla

ABS/cm^2

source of variation	df	MS	F
uv	1	0.31627	19.88***
error	16	0.01591	

ABS/mg

source of variation	df	MS	F
uv	1	0.00638	19.38***
error	16	0.00032	

Manihot esculenta

ABS/cm^2

source of variation	df	MS	F
uv	1	0.13594	4.38*
error	17	0.03100	

ABS/mg

source of variation	df	MS	F
uv	1	0.00870	6.24*
error	17	0.00139	