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Interactive Effects of Ultraviolet-B Radiation and Temperature on Cotton Physiology, Growth, Development and Hyperspectral Reflectance¶

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ABSTRACT

Current conditions of 2–11 kJ m^{-2} day^{-1} of UV-B radiation and temperatures of >30°C during flowering in cotton cultivated regions are projected to increase in the future. A controlled environment study was conducted in sunlit growth chambers to determine the effects of UV-B radiation and temperature on physiology, growth, development and leaf hyperspectral reflectance of cotton. Plants were grown in the growth chambers at three day/night temperatures (24/16°C, 30/22°C and 36/28°C) and three levels of UV-B radiation (0, 7 and 14 kJ m^{-2} day^{-1}) at each temperature from emergence to 79 days under optimum nutrient and water conditions. Increases in main stem node number and the node of first fruiting branch and decrease in duration to first flower bud (square) and flower were recorded with increase in temperature. Main effects of temperature and UV-B radiation were significant for net photosynthetic rates, stomatal conductance, total chlorophyll and carotenoid concentrations of uppermost, fully expanded leaves during squaring and flowering. A significant interaction between temperature and UV-B radiation was detected for total biomass and its components. The UV-B radiation of 7 kJ m^{-2} day^{-1} reduced boll yield by 68% and 97% at 30/22°C and 36/28°C, respectively, compared with yield at 0 kJ m^{-2} day^{-1} and 30/22°C. No bolls were produced in the three temperature treatments under 14 kJ m^{-2} day^{-1} UV-B radiation. The first-order interactions between temperature, UV-B radiation and leaf age were significant for leaf reflectance. This study suggests a growth- and process-related temperature dependence of sensitivity to UV-B radiation.

INTRODUCTION

Crop growth, development and, finally, yield are intricately connected to weather. Past and present-day anthropogenic activities are clearly causing major changes in the atmospheric chemistry and climate. The atmospheric concentration of carbon dioxide ($[CO_2]$) has increased approximately by 30% since the mid-18th century, and projections indicate that $[CO_2]$ could increase from current levels of approximately 360 μL L^{-1} to between 540 and 970 μL L^{-1} by the end of the 21st century (1). Atmospheric concentrations of other greenhouse gases (methane, tropospheric ozone, nitrous oxide, chlorofluorocarbons [CFC] etc.) have also increased as a result of anthropogenic activities (1). Furthermore, global circulation models project that the increase in global surface air temperature could range from 1.4°C to 5.8°C because of a projected increase in the concentrations of greenhouse gases (1).

The CFC, in addition to their contribution to global warming, also deplete the earth's protective stratospheric ozone layer (2,3). Continued depletion of the earth's stratospheric ozone layer is of concern because the ozone column is the primary attenuator of solar UV-B radiation (280–320 nm). Reductions in the ozone column have led to substantial increases in UV-B radiation at the earth's surface, with the amount and intensity dependent on atmospheric and geographic factors (4,5). Because CFC can remain in the upper atmosphere for 40–150 years (2), it is probable that terrestrial plants will experience increased levels of UV-B radiation for many more years even with full compliance to the Montreal Protocol and its amendments and adjustments (6,7). Failure by member countries to comply would delay or could prevent the recovery process. Because crop ecosystems are very responsive to these two important global climate change factors, temperature and UV-B radiation, understanding the interactive effects of these factors on crops is important for developing suitable management practices for future climate conditions.

Temperature effects have been studied extensively in many plants, including cotton, but relatively few experiments have addressed the influence of UV-B on cotton. Studies on the interactive effects of UV-B radiation and temperature on crop growth and development are few (8,9). The UV-B–induced reduction in seedling growth of maize and sunflower was alleviated by 4°C increase in temperature from 28°C (10). In contrast, in cowpea plants, UV-B damage was greater for plants grown at 30°C than for plants grown at 20°C (11). Plant reproductive processes were more sensitive than vegetative growth processes to both high temperature (12) and UV-B radiation (13). Therefore, studies are needed to understand the interactive effects of these environmental factors on crop reproductive processes and yield.

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Abbreviations: ANOVA, analysis of variance; CA, cellulose diacetate; CFC, chlorofluorocarbons; C_i, intercellular $[CO_2]$; $[CO_2]$, carbon dioxide concentration; DAE, days after emergence; g_s, stomatal conductance; Pn, net photosynthetic rate; SPAR, Soil–Plant–Atmosphere–Research.

Table 1. Temperature and UV-B radiation effects on Pn, g_s, C_i, and fluorescence (Fv′/Fm′) of cotton uppermost, fully extended, main stem leaves. Data are means of measurements at 24 and 52 DAE*

Temperature, day/night (°C)	UV-B (kJ m^{-2} day^{-1})	Pn (μmol m^{-2} s^{-1})	g_s (mol m^{-2} s^{-1})	C_i (μmol mol^{-1})	Fv′/Fm′
24/16	0	29.5	0.237	144.7	0.314
	7	29.8	0.335	191.9	0.316
	14	29.9	0.245	117.5	0.290
30/22	0	40.9	0.594	229.3	0.332
	7	41.5	0.560	219.0	0.347
	14	36.8	0.578	236.8	0.322
36/28	0	43.1	0.698	240.2	0.336
	7	43.1	0.657	231.5	0.340
	14	34.1	0.570	241.3	0.256
SED					
T		1.48***	0.045***	16.5***	0.0069*
UV-B		1.48**	NS	NS	0.0069***
T × UV-B		NS	NS	NS	0.0120*

*Significance levels are indicated by ***, **, * and NS, representing $P < 0.001$, $P < 0.01$, $P < 0.05$ and $P > 0.05$, respectively.
SED is standard error of difference of means.

In cotton, crop developmental events defined as sequence of discrete phenological events, such as emergence to square, square to flower and flower to open boll, leaf addition on the main stem and branches and duration of expansion of internodes and leaves, were affected by temperature (12,14,15). These developmental events increased either curvilinearly or linearly as temperature increased from a minimum of about 15°C, depending on the phenological event (12,14–16). Growth rates of cotton organs or processes such as leaf area expansion, stem elongation, boll retention and biomass accumulation were also increased with the increase in temperature, having a base temperature of 15°C and optimum temperature of 27–29°C. High temperatures, on the other hand, affected the boll retention processes more severely than the vegetative growth (12). Temperatures higher than optimum are frequently observed during flowering in many regions of the U.S. Cottonbelt, and projected high temperatures will have a profound influence on cotton reproductive efficiency (17,18).

The influence of UV-B radiation on cotton growth and development has been recently studied. Exposure of cotton plants to UV-B radiation reduced the canopy size by decreasing stem extension and leaf area expansion rates and branch lengths (19). However, major phenological events, such as emergence to first square or flower and adding leaves to the main stem, were not affected (13,19,20). Biomass production and leaf and canopy photosynthesis remained insensitive to elevated UV-B treatments (13). Also, enhanced UV-B radiation accelerated many of the parameters associated with the leaf ageing process (20). Similar to high temperature, UV-B has also been reported to damage reproductive processes and capacity of cotton to set fruit, resulting in lower yield (13,20,21). Cotton and other crops cultivated between 40°N and 40°S are already experiencing UV-B radiation of 2–11 kJ m^{-2} day^{-1} and often higher than optimum temperatures for boll or fruit retention, depending on the location (18,22). Earlier studies have shown that elevated CO_2 levels (>700 μL L^{-1}) do not ameliorate the harmful effects of either high temperature (23) or elevated UV-B radiation (13).

Environmental factors have also been shown to change leaf internal structure (24,25). Leaf anatomy, thickness and cell wall–air interfaces were modified on exposure to water deficit (25) and UV-B radiation (26) in cotton. High temperature was known to cause disorganization of leaf internal structure (27). The partitioning of incoming radiation into reflectance, absorption and transmittance would be modified by changes in leaf structure and biochemistry (28). Elevated UV-B increased leaf reflectance in the regions of hyperspectral curve associated with chlorophyll and tissue reflectance (20). Thus, detecting changes in leaf reflectance due to environmental stress would enhance our ability to detect climate change impacts.

Cotton is a major economic crop grown on over 32 Mha worldwide and over 5 Mha in the United States (29), therefore, it is important to understand the combined effects of elevated temperature and UV-B radiation on cotton. In this study, we examined the interactive effects of temperature and UV-B radiation on cotton physiology, growth and development, including the reproductive processes. The modifying effect of temperature and UV-B radiation on leaf hyperspectral reflectance was also studied.

MATERIALS AND METHODS

Soil–Plant–Atmosphere–Research chamber facility. An experiment was conducted at the Mississippi Agriculture and Forestry Experiment Station, Mississippi State (88.8° W longitude, 33.5° N latitude and 85 m amsl), MS, in 2002 using controlled environment chambers known as Soil–Plant–Atmosphere–Research (SPAR) chambers. Details of operation and control of SPAR chambers have been described by Reddy *et al*. (30). The SPAR chambers are located outdoors and use solar radiation as the light source; temperature and [CO_2] can be controlled. Each SPAR chamber consists of a steel soil bin (1 m deep by 2 m long by 0.5 m wide) to accommodate the root system, a Plexiglas chamber (2.5 m tall by 2.0 m long by 1.5 m wide) to accommodate aerial plant parts, a heating and cooling system and an environmental monitoring and control system. The Plexiglas allows 97% of the visible solar radiation to pass without spectral variability in absorption and blocks solar UV radiation (100% of UV-B and 88% of UV-A).

Air ducts located on the northern side of each SPAR chamber connect the heating and cooling devices to each unit. Conditioned air is passed through the plant canopy with sufficient velocity to cause leaf flutter (4.7 km h^{-1}) and is returned to the air-handling unit just above the soil level. Chilled ethylene glycol is supplied to the cooling system via several parallel solenoid valves that open or close depending on the cooling requirement. Two electrical-resistance heaters provide short pulses of heat, as needed, to fine-tune the air temperature. Air temperature, [CO_2] and soil watering in each SPAR chamber, as well as continuous monitoring of all-important

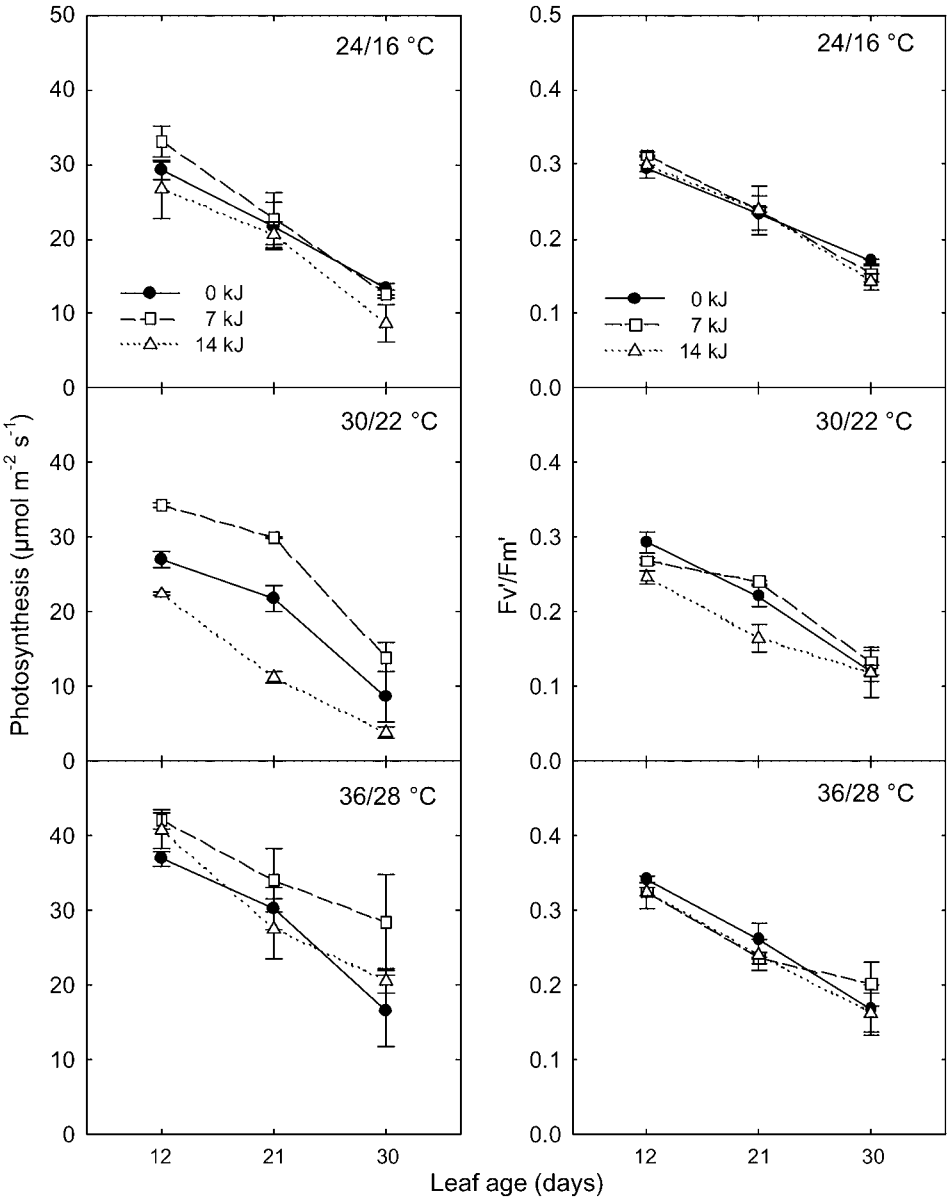

Figure 1. Photosynthesis and fluorescence (Fv′/Fm′) of cotton main stem leaves aged 12, 21 and 30 days as affected by temperature and UV-B radiation. Data are means ± SE (n = 3).

environmental and plant gas exchange variables, were controlled by a dedicated computer system. The data acquisition and control system consisted of three HP3497A data acquisition or control units, each with 100-channel measuring capacity, a Racal-Dana Model 1200 digital output system with 130 individually addressed output channels and a John Fluke Model 1120A digital input system. These components are connected via an IEEE-488 interface to a computer that is used as the system controller. These components are networked to provide automatic acquisition of the data from the SPAR units, control of the SPAR environments and storage of collected data in an online data warehouse.

Treatments. Cotton (*Gossypium hirsutum* L.) cv. NuCOTN 33 B, a midseason Upland Bt. variety, was sown on 24 July 2002 in the SPAR soil bins filled with fine sand. Each SPAR chamber had 11 rows of five plants per row, with each row 182 mm apart. Emergence was observed 5 days after sowing. Six rows of plants were harvested 14 days after emergence (DAE), and two rows were harvested 22 DAE to avoid competition and to determine aboveground biomass and total leaf area during the early growth period, and thus three rows of 15 plants m^{-2} were retained till 79 DAE. These rows were 667 mm apart with 100 mm between plants. Plants were irrigated three times a day with half-strength Hoagland's nutrient solution delivered at 0800, 1200 and 1700 h with an automated, computer-controlled drip system to provide favorable nutrient and water conditions for plant growth (31). Variable-density shade cloths placed around the edges of plants at emergence were adjusted regularly to match plant heights, simulating the presence of other plants and eliminating the need for border plants.

The chambers were maintained at 30/22°C (day/night) until seedling emergence (5 days). Thereafter, day/night air temperatures in the chambers were maintained at 24/16°C (low), 30/22°C (optimum) or 36/28°C (high), until the plants were harvested 79 DAE, at each UV-B treatment as described later. Air temperature in each SPAR unit was monitored and adjusted every 10 s throughout the day and night and maintained within ±0.5°C of the set points. The daytime temperature was initiated at sunrise and returned to the nighttime temperature 1 h after sunset. The average temperatures during the experiment were 20.3 ± 0.75 for 24/16°C, 25.6 ± 0.65 for 30/22°C and 31.2 ± 0.68 for 36/28°C. The [CO_2] in each SPAR chamber was monitored and adjusted every 10 s throughout the day and maintained at 360 ± 10 μL L^{-1} during the daylight hours using a dedicated LI-6250 CO_2 analyzer (LI-COR, Inc., Lincoln, NE).

The UV-B radiation treatments of zero (control, no UV-B) and a total daily dose of biologically effective UV-B radiation of 7 kJ m^{-2} day^{-1} (ambient) and 14 kJ m^{-2} day^{-1} (high) were imposed from emergence at

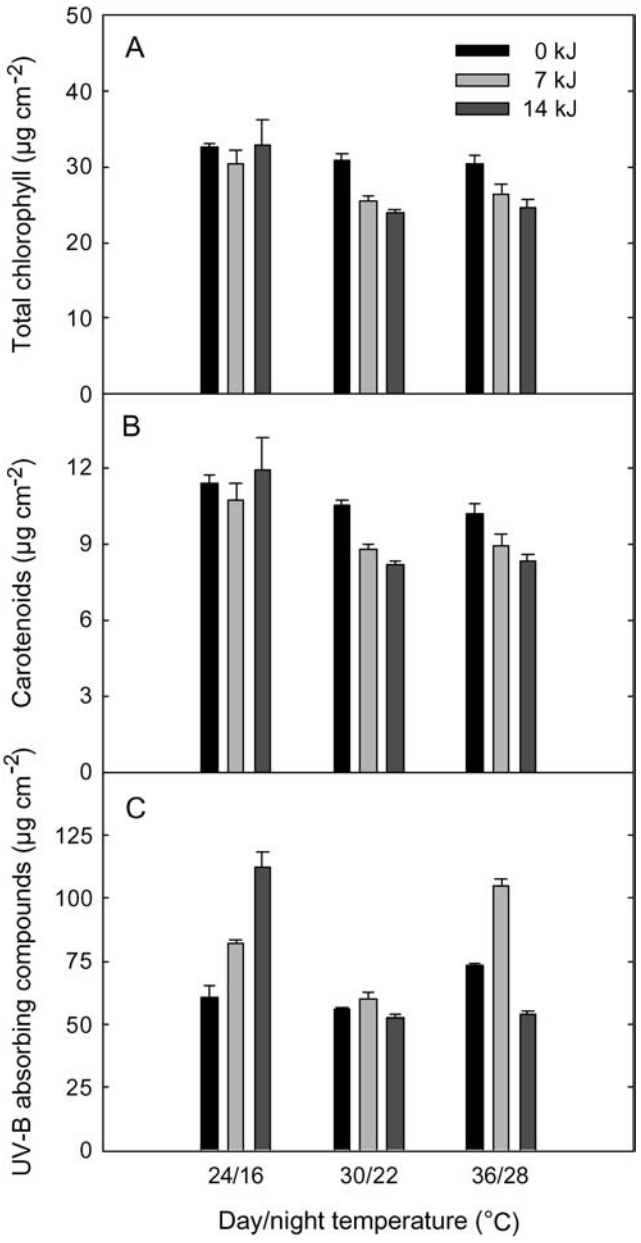

Figure 2. Chlorophyll, carotenoid and UV-B–absorbing compounds of cotton leaves measured at 66 DAE as affected by temperature and UV-B radiation. Data are means ± SE (n = 3).

each temperature level. The UV-B doses imposed in the experiment simulated ambient and 30% depletion of stratospheric ozone based on the empirical model of Green *et al.* (32). The 7 kJ m^{-2} day^{-1} UV-B radiation treatment is near the natural solar UV-B levels during June–July in Mississippi (http://toms.gsfc.nasa.gov/ery_uv/ery_uv1.html; http://uvb.nrel.colostate.edu/UVB/). Although the square-wave UV-B supplementation systems in controlled environments provide disproportionate spectral conditions on cloudy days than those that occur in field conditions, they are particularly useful for quantifying the growth and developmental responses of plants to UV-B and allow modeling its impact without the interacting effects of other variables.

The UV-B radiation was delivered to plants for 8 h from 0800 to 1600 h by UV-313 lamps (Q-Panel Company, Cleveland, OH) driven by 40 W dimming ballasts. To filter UV-C radiation (<280 nm), the lamps were wrapped with solarized 0.07 mm cellulose diacetate (CA) film (JCS Industries Inc., La Mirada, CA). The CA on the lamps was changed at 3–4 day interval to account for the degradation of the CA properties. The UV-B energy delivered at the top of the plant canopy was checked daily with a UVX digital radiometer (UVP Inc., San Gabriel, CA) and calibrated against an Optronic Laboratory (Orlando, FL) Model 754 Spectroradiometer, which was used to initially quantify lamp output. The lamp power was adjusted, as needed, at 1000 h each day to maintain the respective UV-B radiation levels. The distance from lamps to the plant tops was maintained at 0.5 m throughout the experiment. Unilluminated lamps with frame were placed in the control units to simulate equivalent shading. The average daily biologically effective UV-B radiation during the experiment was 6.85 ± 0.03 for 7 kJ m^{-2} day^{-1} and 13.56 ± 0.05 for 14 kJ m^{-2} day^{-1}. The average total daily incoming solar radiation during the experimental period was 17.6 ± 0.67 MJ m^{-2} day^{-1}.

Measurements. Nodes were counted, and plant heights were measured on all plants at the final harvest. Leaf area was measured using the LI-3100 leaf area meter (LI-COR). Dates of appearance of squares (flower buds 3 mm in size) and flowers were recorded on all plants when >50% of the plants had their first square or flower. Number of branches and branch lengths were also recorded on all plants along with number of fruiting sites (where a square was produced), squares and bolls retained at the end of the experiment. Plant component dry weight was measured after oven drying at 75°C until it weighed constant during a period of 48 h.

Leaf net photosynthesis (Pn), stomatal conductance (g_s), intercellular [CO_2] (C_i) and chlorophyll fluorescence (Fv′/Fm′) were measured using LICOR-6400 (LI-COR) with an integrated fluorescence chamber head (LI-6400-40 leaf chamber flurometer) on each of the selected leaves, fourth or fifth leaf from the terminal. To study the interactive effects of UV-B and temperature on leaf ageing, Pn and chlorophyll fluorescence of leaves positioned fourth, seventh and 10th nodes from the top on the main stem that aged 12, 21 and 30 days, respectively, from leaf unfolding, were measured. The leaf Pn measurements were made using a red–blue light source (LI-6400-02B) and adjusted to provide a fixed photosynthetic photon flux density of 1500 µmol photons m^{-2} s^{-1}. Cuvette block temperature was maintained to match the treatment daytime temperature using a computer-controlled Peltier module mounted on the cuvette. Relative humidity inside the cuvette was maintained at approximately 40%. The airflow entering the cuvette was maintained at [CO_2] of 360 µL L^{-1}.

After measuring Pn, the Fv′/Fm′, which is the efficiency of energy harvesting by oxidized (open) Photosystem II reaction centers in the light, was calculated using the following equation:

$$Fv'/Fm' = (Fm' - Fo')/Fm'$$

The Fo′ (minimum fluorescence of a light-adapted leaf that has momentarily been darkened) and Fm′ (maximum fluorescence during a saturating light flash) were measured using a saturating flash intensity of >6000 µmol m^{-2} s^{-1} and flash duration of 0.8 s.

Leaf hyperspectral reflectance was measured on the leaves used for Pn measurement by ASD FieldSpec FR spectroradiometer (Analytical Spectral Devices Inc., Boulder, CO) with a spectral range of 350–2500 nm. The sensors include one 512-element photodiode array and two thermoelectrically cooled, ''graded index,'' extended range InGaAs photodiodes. The sampling interval is 1.4 nm for 350–1000 nm and 2 nm for 1000–2500 nm and has a spectral resolution of 3 nm from 350 nm, 10 nm from 700 nm and 10 nm from 1500 nm, and data were stored on a laptop computer connected to the instrument. The contact reflectance probe with an internal light source and a fiber-optic input socket was used to measure leaf reflectance spectra. The instrument was optimized by placing the light probe against the Spectralon panel for the specific measuring conditions and was also corrected for the electric current generated by thermal electrons within the ASD. The reflectance of the ASD instrument was set to 100% by measuring the reflectance Spectralon reference panel (white reference). The white reference was measured at 5 min interval to check the instrument stability for 100% reflectance. A single hyperspectral reflectance curve was obtained from each selected leaf when attached to the plant. To measure leaf reflectance, the leaf was sandwiched between the nonreflecting, polyurethane black body and the light probe. This ensured that no extraneous light entered the sensor during these measurements. The reflectance values were averaged in the spectral regions 400–700 (photosynthetically active radiation), 400–520 (blue), 520–600 (green), 630–690 (red), 760–900 (near infrared), 900–1350 (tissue reflectance) and 1600–1850 nm (water band).

Pigment extraction was made from the leaves used for photosynthetic and hyperspectral measurements. The photosynthetic pigments (chlorophyll

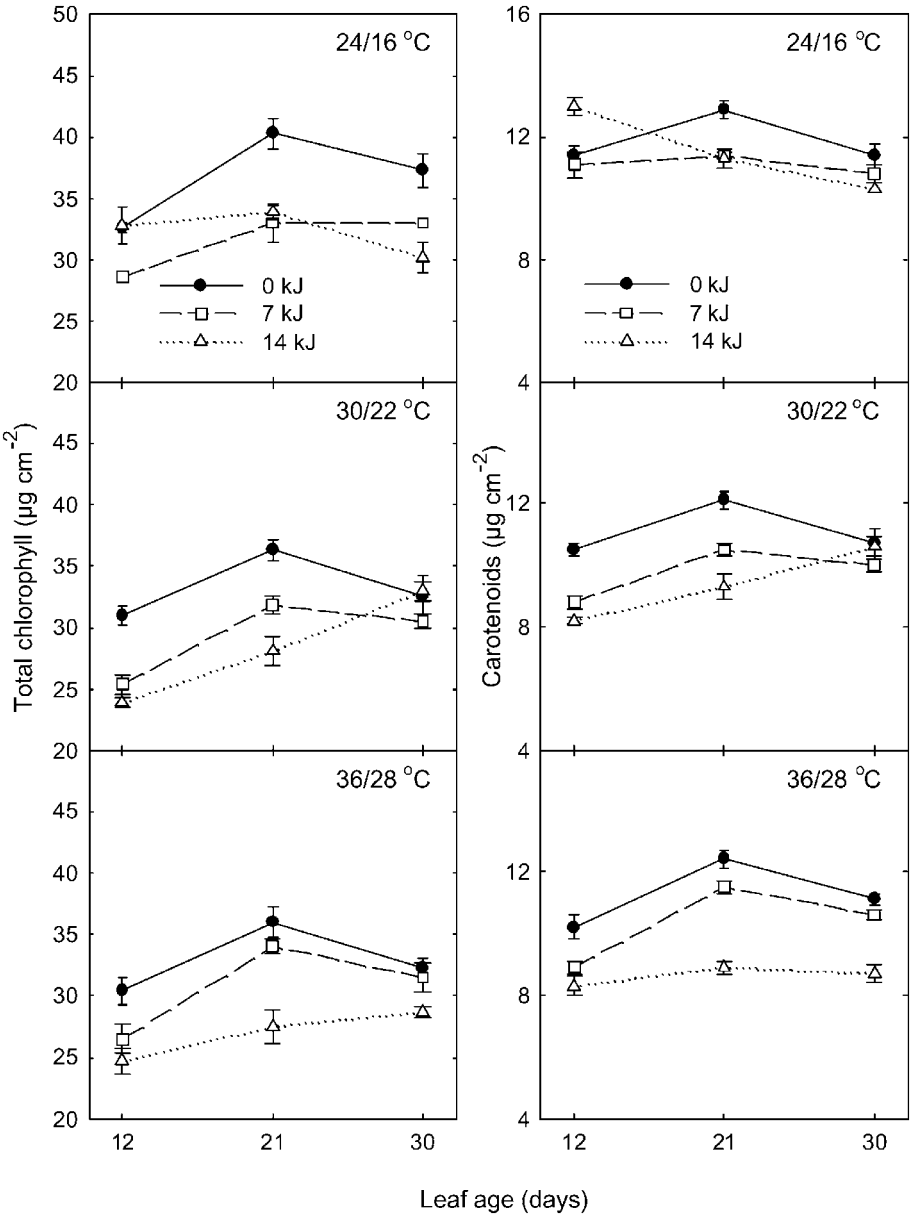

Figure 3. Chlorophyll and carotenoid concentrations of cotton leaves aged 12, 21 and 30 days as affected by temperature, UV-B radiation and leaf age. Data are means ± SE (n = 3).

a, chlorophyll *b* and carotenoids) were extracted by placing five 38.5 mm^2 leaf discs in a vial with 5 mL of dimethyl sulfoxide and extracting for 24 h. The absorption of the extracts was determined, at 664, 648 and 470 nm, using the Bio-Rad UV/VIS spectrophotometer with a resolution of 1 nm by scanning from 200 to 900 nm. The equations by Lichtenthaler (33) were used to obtain the pigment concentrations. The pigment concentrations were expressed on a leaf area basis (µg cm^{-2}).

The UV-B–absorbing compounds were extracted from five 38.5 mm^2 leaf discs placed in a vial with 10 mL of extractant, a mixture of methanol, water and hydrochloric acid in 79:20:1 ratio (34). The vials were incubated at room temperature for 24 h in dark to allow for complete extraction of UV-B–absorbing compounds. The absorbance of the extracts from different treatments was measured at 300 nm (20). The content of UV-B–absorbing compounds was calculated using the equation, $C = 16.05 \times A$, where A is absorbance at 300 nm and C is concentration of UV-B–absorbing compound (µg mL^{-1} of extract) and expressed as equivalents of *p*-coumaric acid.

Analysis of data. Statistical analysis was conducted by using two-way analysis of variance (ANOVA) (35). The least significant difference tests at $P = 0.05$ were used to distinguish treatment differences for the growth and physiological parameters measured in the study. The standard errors of each mean were also calculated and presented in the graphs as error bars. A factorial analysis was carried out on the averaged reflectance values to study the effect of temperature, UV-B radiation and leaf age. The values from three leaves in each treatment were used as replications.

RESULTS AND DISCUSSION

Leaf photosynthesis

The leaf temperatures were not affected by the UV-B radiation treatment but differed with temperature treatment. The average leaf temperatures recorded were 27.1°C, 29.7°C and 32.9°C when the air temperatures in the leaf chamber were 25.5°C, 30.5°C and 35.9°C, respectively. The vapor pressure deficit in the leaf chamber ranged between 1.5 and 2.2 kPa and did not differ ($P > 0.05$) between the treatments.

Table 2. Effects of temperature and UV-B radiation on cotton plant height, main stem node number, leaf area, vegetative, fruiting and total branch number and total branch length. Data are means of 15 plants per treatment measured at 79 DAE*

Temperature day/night (°C)	UV-B (kJ m^{-2} day^{-1})	Plant height (cm $plant^{-1}$)	Main stem nodes (no. $plant^{-1}$)	Total leaf area (cm^2 $plant^{-1}$)	Vegetative branches (no. $plant^{-1}$)	Fruiting branches (no. $plant^{-1}$)	Total branches (no. $plant^{-1}$)	Total branch length (cm $plant^{-1}$)
24/16	0	58.1	12.2	2337	6.3	6.1	12.8	199
	7	70.1	14.9	2977	8.1	8.6	16.7	264
	14	49.2	14.4	1947	6.2	8.9	15.1	158
30/22	0	160.5	20.5	7752	4.5	14.9	19.3	548
	7	148.5	21.0	6759	4.2	16.0	20.3	583
	14	125.0	22.9	4508	0.1	18.9	19.0	375
36/28	0	149.5	24.6	7179	1.3	18.4	19.7	579
	7	154.3	25.7	6945	1.0	18.7	19.7	577
	14	122.8	29.1	4961	0.0	20.3	20.3	367
SED								
T		2.53***	0.35***	357.6***	0.43***	0.46***	NS	29.8***
UV-B		2.53***	0.35***	357.6***	0.43***	0.46***	0.69***	29.8***
T × UV-B		4.38***	0.61***	619.4***	0.74***	NS	NS	NS

*Significance levels are indicated by ***, **, * and NS, representing $P < 0.001$, $P < 0.01$, $P < 0.05$ and $P > 0.05$, respectively.
SED is standard error of difference of means.

Both temperature and UV-B radiation significantly affected Pn of uppermost, fully expanded leaves during squaring and flowering, but there was no temperature × UV-B interactive effect (Table 1). Averaged across the three temperature treatments and compared with the control plants of no UV-B, the 7 kJ m^{-2} day^{-1} UV-B–treated plants had photosynthesis similar to that of the control, but plants exposed to 14 kJ m^{-2} day^{-1} UV-B had 11% ($P < 0.05$) decrease in leaf photosynthesis. The leaf Pn did not differ between the 30/22°C and 36/28°C treatments. Low temperature, however, significantly decreased cotton leaf photosynthesis. Averaged across the UV-B treatments, the Pn of the three temperature treatments of 24/16°C, 30/22°C and 36/28°C were 30, 40 and 40 μmol m^{-2} s^{-1}, respectively.

Although leaf Pn of 14 kJ m^{-2} day^{-1} UV-B–treated plants under optimum and high temperature was significantly lower than the Pn of 0 and 7 kJ m^{-2} day^{-1} UV-B–treated plants, neither g_s nor C_i was affected by UV-B radiation. Therefore, decreased leaf photosynthesis due to high UV-B radiation was not associated with either g_s or C_i but with the chlorophyll *a* fluorescence (Fv′/Fm′) as observed in other crops on exposure to UV-B radiation (36–38). In contrast, under the low temperature, lower leaf photosynthesis was mainly related to decreased g_s and Ci, rather than the Fv′/Fm′ (Table 1). Similar observations were made in cotton (39) and corn (40).

To determine leaf age and UV-B radiation effects on photosynthesis at each temperature level, main stem leaves 4, 7 and 10 from plant terminal aged 12, 21 and 30 days from leaf unfolding, respectively, were selected in all treatments at 65 DAE (Fig. 1). These leaves were located in the upper, middle and lower canopies of plants, respectively. Both leaf photosynthesis and Fv′/Fm′ rapidly declined with changes in leaf positions from the upper to lower canopy, a typical phenomenon that indicates leaf senescence (41,42). UV-B radiation did not affect Pn of 12, 21 and 30 day old leaves on plants grown in either the low (24/16°C) or high (36/28°C) temperature, except for lower-canopy leaves in

Table 3. Effects of temperature and UV-B radiation on cotton total fruiting sites and retained bolls per plant. Data are means of 15 plants per treatment*

Temperature day/night (°C)	UV-B (kJ m^{-2} day^{-1})	First fruiting node (no.)	Days to square	Days to flower	Total fruiting sites (no. $plant^{-1}$)	Retained bolls (no. $plant^{-1}$)
24/16	0	5.8	38.0	—	24.1	—
	7	6.3	37.0	—	40.1	—
	14	5.9	36.5	—	25.9	—
30/22	0	5.1	20.6	43.0	58.4	16.8
	7	5.3	21.9	44.3	63.1	10.5
	14	5.9	23.1	—	71.1	—
36/28	0	5.8	20.3	40.1	70.1	5.9
	7	6.1	21.1	40.0	67.3	0.5
	14	7.1	21.3	—	66.0	—
SED						
T		0.15***	0.48***	0.27***	NS	0.58***
UV-B		0.15***	0.48 (NS)	0.27***	3.79***	0.58***
T × UV-B		0.27***	0.84*	0.47***	NS	1.02***

*Significance levels are indicated by ***, **, * and NS, representing $P < 0.001$, $P < 0.01$, $P < 0.05$ and $P > 0.05$, respectively. ''—'' indicates treatments that did not reach flowering.
SED is standard error of difference of means.

Table 4. Effects of temperature and UV-B radiation on cotton dry weight of leaf, stem, root, boll and total biomass at final harvest. Data are means of 15 plants per treatment measured at 79 DAE*

Temperature day/night (°C)	UV–B (kJ m^{-2} day^{-1})	Dry weight (g $plant^{-1}$)				
		Leaf	Stem	Root	Boll	Total biomass
24/16	0	13.6	13.1	2.9	—	29.6
	7	15.0	16.7	3.3	—	35.4
	14	9.6	8.9	1.8	—	19.1
30/22	0	34.9	52.5	7.7	22.5	117.7
	7	28.6	46.2	6.9	7.2	87.7
	14	17.9	24.6	4.1	—	47.7
36/28	0	33.8	53.8	7.6	5.3	101.9
	7	29.4	46.8	4.1	0.6	85.5
	14	20.5	26.2	4.5	—	51.6
SED						
T		1.61***	2.58***	0.35***	0.72***	4.57***
UV-B		1.61***	2.58***	0.35***	0.72***	4.57***
T × UV-B		2.79*	4.47***	0.61*	1.24***	7.91***

*Significance levels are indicated by ***, **, * and NS, representing $P < 0.001$, $P < 0.01$, $P < 0.05$ and $P > 0.05$, respectively. — indicates treatments that did not reach flowering.
SED is standard error of difference of means.

the high temperature in which the 7 kJ m^{-2} day^{-1} UV-B–treated plants had significantly higher photosynthesis than the control. Under the optimum growth temperature (30/22°C), however, UV-B radiation of 7 kJ m^{-2} day^{-1} stimulated, whereas UV-B radiation of 14 kJ m^{-2} day^{-1} inhibited, photosynthetic rates of leaves at all the three positions at the measuring time. These results are in agreement with the earlier observations (20). The leaves only at the midcanopy of the 14 kJ m^{-2} day^{-1} UV-B–treated plants, grown in the optimum temperature, had lower Fv′/Fm′ than the control leaves. The Fv′/Fm′ of the other leaves was not affected by UV-B radiation within any temperature (Fig. 1).

Pigment and UV-B–absorbing compounds

No significant interaction of UV-B and temperature was observed for the photosynthetic pigment concentrations at 66 DAE (Fig. 2A,B). Significant ($P < 0.05$) main effects of temperature and UV-B were observed for both total chlorophyll and carotenoid concentrations. Compared with leaves under 24/16°C, leaves exposed to 36/28°C had 19% less carotenoid concentrations and 15% less total chlorophyll concentrations. Similar observations were made by Jenkins *et al.* (43) in *Arabidopsis*, where both high temperature and UV-B radiation reduced chlorophyll content. In contrast, Mark and Tevini (44) did not find any change in chlorophyll concentration due to leaf exposure to a 4°C higher temperature in maize and sunflower seedlings. High levels of UV-B reduced the carotenoid and total chlorophyll concentrations by about 11 and 13%, respectively (Fig. 2A,B), compared with the control, and it is widely established that exposure to high UV-B reduces photosynthetic pigment content (16). The reduction in chlorophyll can be attributed to breakdown of structural integrity of chloroplasts (45–47).

A significant interaction between temperature and UV-B radiation was observed for UV-B–absorbing compounds (Fig. 2C). The increase in UV-B–absorbing compounds with increased UV-B levels at low temperature was negated by increased temperatures. However, at 24/16°C, UV-B–absorbing compounds increased by 36% when UV-B levels increased from 0 to 7 kJ m^{-2} day^{-1}, and a further increase of UV-B levels to 14 kJ m^{-2} day^{-1} increased UV-B–absorbing compounds by another 36%. At 30/22°C, there were no significant differences between UV-B levels for UV-B–absorbing compounds. But at temperatures of 36/28°C, UV-B–absorbing compounds increased by 42% when UV-B levels increased from 0 to 7 kJ m^{-2} day^{-1}, and a further increase in UV-B to about 14 kJ m^{-2} day^{-1} decreased UV-B–absorbing compounds by 48%. The results suggest that cotton plants were not sensitive to UV-B levels at optimum temperatures but were more sensitive to UV-B radiation at both low and high temperatures as indicated by the increase in UV-B–absorbing compounds (Fig. 2C).

Total chlorophyll and carotenoids measured on 12, 21 and 30–day old leaves to determine UV-B and temperature effects on leaf age are presented in Fig. 3. Effects of UV-B and temperature on pigment concentrations varied significantly with leaf age. Significant interactions were observed for all the three factors. In most cases, higher total chlorophyll and carotenoid concentrations were observed on 21 day old leaves and the highest being at 0 kJ m^{-2} day^{-1} UV-B at 24/16°C. The low pigment concentrations observed in 12 day old leaves at 7 and 14 kJ m^{-2} day^{-1} UV-B levels in the two high-temperature treatments were not observed in older leaves of the canopy. The 12 day old leaves that received 0 kJ m^{-2} day^{-1} had pigment concentrations similar to those of 21- and 30 day old leaves that received 7 and 14 kJ m^{-2} day^{-1} UV-B treatments.

Plant growth and development

A significant interaction ($P < 0.001$) was recorded between temperature and UV-B radiation for plant height, main stem node number and leaf area (Table 2). Increase in UV-B radiation at a given growth temperature caused a reduction in plant height. Irrespective of UV-B radiation, plants grown at 24/16°C were shortest with smallest leaf area and fewer nodes. At a given temperature, plants grown at 0 and 7 kJ m^{-2} day^{-1} were similar for plant height and leaf area, but at 14 kJ m^{-2} day^{-1} UV-B, a 15–20% decrease in plant height and a 30–42% of lower leaf area was recorded. Inhibition of growth and shoot length can be

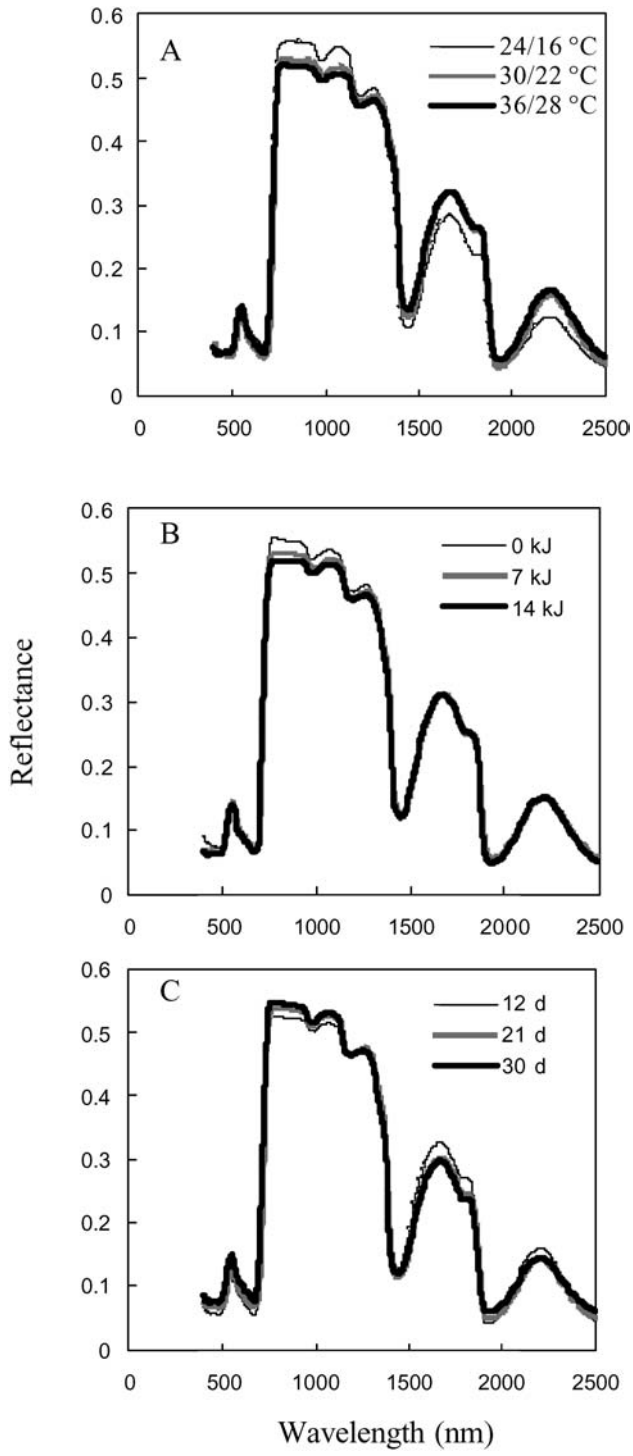

Figure 4. Changes in hyperspectral reflectance of the fourth leaf (top most, fully expanded) of cotton as affected by (A) temperature, (B) UV-B and (C) leaf age. Each spectral curve is an average of three leaves collected from separate plants grown under similar conditions.

attributed to the destruction of endogenous auxin levels by UV-B (48). For main stem node number, the interaction was additive because main stem node number increased with increase in both temperature and UV-B radiation (Table 2). The minimum node number recorded was 12 per plant at 24/16°C and 0 kJ m^{-2} day^{-1} UV-B, and the maximum node number recorded was 29 per plant at 36/28°C and 14 kJ m^{-2} day^{-1} UV-B. Our results are in agreement with those by Reddy *et al*. (49,50) with respect to temperature, but the role of UV-B in increasing node number needs further investigations.

Regarding branching, interaction between temperature and UV-B was significant only for vegetative branches (Table 2). Vegetative branches decreased while fruiting branches increased as temperature and UV-B increased. On an average, vegetative branches decreased by 50% with increase in UV-B from 0 to 14 kJ m^{-2} day^{-1}, but a 90% decrease was recorded with increased temperature from 24/16°C to 36/28°C. In contrast, average reproductive branches increased 18% with increase in UV-B from 0 to 14 kJ m^{-2} day^{-1}, whereas the increase was 142% with the increase in temperature from 24/16° to 36/28°C. The higher vegetative branch number can be attributed to greater accumulation and availability of resources at low temperature (49,50). The main effects of temperature and UV-B were significant ($P < 0.001$) for total plant branch length (Table 2). The branch lengths at 0 and 7 kJ m^{-2} day^{-1} were on par with each other, but the branch lengths at 14 kJ were 37% shorter than others. A similar decrease in branch length was observed in our previous studies (16). Among the temperature treatments, branch lengths on plants grown at 30/22°C and 36/28°C were not different; however, plants grown at 24/16°C had 60% shorter branches. The short branches at low temperatures can be attributed to slower growth rates and shorter internodal lengths (50).

First fruiting branch was on the fifth main stem node in the 0 kJ m^{-2} day^{-1} UV-B and 30/22°C treatment, whereas it was on the seventh node in the 14 kJ m^{-2} day^{-1} UV-B and 36/28°C treatment (Table 3). This can be attributed to faster node addition rate in high-temperature treatments. Days to square were affected by both temperature and UV-B radiation (Table 3). The first square emerged by 37–38 DAE at 24/16°C, 21–23 days at 30/22°C and 21 days at 36/28°C. Plants grown under 14 kJ m^{-2} day^{-1} did not reach flowering. Plants exposed to 0 and 7 kJ m^{-2} day^{-1} flowered at 40 DAE under 36/28°C and 44 DAE under 30/22°C. The number of branches (vegetative and fruiting) affected the number of potential fruiting sites because only the main effects of temperature were significant (Table 3). Increase in temperature resulted in more fruiting sites (50), with no differences between 30/22°C and 36/28°C treatments. The highest boll number was seen on plants grown under 30/22°C, with 17 per plant at 0 kJ m^{-2} day^{-1} of UV-B and 11 per plant at 7 kJ m^{-2} day^{-1} of UV-B (Table 3). High temperatures (36/28°C and 0 kJ m^{-2} day^{-1} of UV-B) caused fewer bolls (six per plant). However, increase in UV-B to 7 kJ m^{-2} day^{-1} at 36/28°C severely reduced bolls to 0.5 per plant. The decrease in boll number per plant at high temperature and UV-B was due to both square and boll drop observed in these treatments.

Total biomass production and partitioning

A significant interaction between temperature and UV-B radiation was detected for total biomass and its components (Table 4). The increase in biomass due to increased temperature was negated by increased UV-B radiation at a given temperature. In plants grown at 24/16°C, about 90% of total biomass was allocated 50% each to leaves and stems. In plants grown at 30/22°C, 74%, 85% and 89% of total biomass was apportioned to leaves and stems at 0, 7, and 14 kJ m^{-2} day^{-1} of UV-B, respectively. Similarly, at 36/28°C, 86%,

Table 5. ANOVA showing standard errors of difference of means and significance levels for the effect of temperature (T), UV-B and leaf age and their interactions on average reflectance of in different regions of cotton leaf hyperspectral reflectance associated with leaf anatomical, pigment and physiological properties*

		Hyperspectral reflectance regions						
Source of variation	df	PAR (400–700 nm)	Blue (400–520 nm)	Green (520–600 nm)	Red (630–690 nm)	Near infrared (760–900 nm)	Internal tissue (900–1300 nm)	Tissue water (1600–1850 nm)
T	2	0.002*	0.003 (NS)	0.004 (NS)	0.003***	0.008***	0.006***	0.005***
UV-B	2	0.002 (NS)	0.003*	0.004 (NS)	0.003 (NS)	0.008***	0.006**	0.005 (NS)
Leaf age	2	0.002***	0.003***	0.004***	0.003***	0.008*	0.006 (NS)	0.005***
T × UV-B	4	0.004***	0.005***	0.007***	0.006**	0.013***	0.011***	0.009***
T × Leaf age	4	0.004*	0.005*	0.007**	0.006*	0.013 (NS)	0.011 (NS)	0.009***
UV-B × Leaf age	4	0.004*	0.005*	0.007*	0.006 (NS)	0.013 (NS)	0.011 (NS)	0.009 (NS)
T × UV-B × Leaf age	8	0.007 (NS)	0.009 (NS)	0.013*	0.011 (NS)	0.023 (NS)	0.018 (NS)	0.015 (NS)

*Significance levels are indicated by ***, **, * and NS, representing $P < 0.001$, $P < 0.01$, $P < 0.05$ and $P > 0.05$, respectively.

90% and 91% of total biomass was apportioned to leaves and stems exposed to 0, 7 and 14 kJ m^{-2} day^{-1} UV-B, respectively. Of the total aboveground biomass, 50% was allocated to each of leaves and stems under 24/16°C, irrespective of UV-B radiation. However, under 30/22°C and 36/28°C, 40% of the aboveground biomass was apportioned to leaves and 60% to stems, irrespective of the UV-B radiation. Both temperature and UV-B also affected the root biomass (Table 4). The root weights of the three UV-B treatments at 24/16°C were not significantly different, but at 30/22°C and 36/28°C, a 45% reduction in root biomass resulted on exposure to 14 kJ m^{-2} day^{-1} UV-B compared with plants grown at 0 kJ m^{-2} day^{-1}. At a given temperature, the decrease in leaf area and leaf Pn with increased UV-B would have resulted in lower canopy Pn resulting in lower biomass. A decrease in canopy Pn and biomass with a significant positive relation was reported by Reddy *et al*. (19).

Bolls were not observed on plants grown at all UV-B doses under 24/16°C and at 14 kJ m^{-2} day^{-1} of 30/22°C and 36/28°C because the plants failed to produce or retain flowers (Table 3). At 24/16°C, the squares produced but did not form flowers because of slower development rate. Squares were dropped 2–3 days after they were formed in the 14 kJ m^{-2} day^{-1} treatment. We hypothesize that decrease in availability of assimilates, due to reduced leaf area and lower Pn, caused square loss in cotton. Maximum boll biomass was recorded in the treatment with 30/22°C and no UV-B radiation, which accounted for 20% of the total plant biomass. Only 8%, 5% and 1% of the total biomass was partitioned to bolls in 30/22°C and 7 kJ m^{-2} day^{-1}, 36/28°C and 0 kJ m^{-2} day^{-1} and 36/28°C and 7 kJ m^{-2} day^{-1} treatments, respectively. The small values partitioned to bolls in these plant treatments can be attributed to the limited time allocated to this experiment.

Leaf hyperspectral reflectance

The leaf hyperspectral reflectance curves from 400 to 2500 nm, as affected by temperature; UV-B radiation and leaf age are shown in Fig. 4A,B,C, respectively. The ANOVA of leaf reflectance showed the treatment-specific differences in identified regions of the hyperspectral curve (Table 5). The first-order interactions were significant among temperature, UV-B and leaf age (Table 5). Only the main effects of the treatments on leaf reflectance are presented in Fig. 5. Plants grown in different temperature treatments were not significantly different for reflectance in the blue and green regions of the spectrum (Fig. 5A,D). Significantly higher leaf reflectance in the red, infrared and tissue regions and lower reflectance in the tissue water region were recorded at low temperature (24/16°C). The increase in UV-B treatments significantly decreased reflectance in the blue, near infrared and tissue regions of the spectral curve (Fig. 5B,E), indicating the role of UV-B in modifying the tissue arrangement of cotton leaves (20,26). The increase in leaf age increased reflectance in all the regions of hyperspectral curve except in the tissue-specific region (Fig. 5C,F). Internal leaf anatomy has been reported to change in senescing leaves (51,52), and senescence is known to produce significant spectral fluctuations in the chlorophyll (450–750 nm), internal cellular geometry (750–1400 nm) and water content (1400–2500 nm) regions (51).

CONCLUSIONS

Of the various growth and development processes, square and boll retention were most sensitive to temperature and UV-B radiation. Positive interactions were found on main stem nodes and total leaf area, and total fruiting sites. Leaf photosynthesis increased with increase in temperature, but was reduced only by extreme UV-B radiation at high temperature. The UV-B radiation reduced reflectance in the blue region of the spectrum due to presence of UV-B–absorbing compounds. The interaction between temperature and UV-B radiation was additive on boll retention, causing severe boll loss. Therefore, in current and future environments, severe yield losses would occur in the presence of high temperature and UV-B radiation. Future studies are needed to understand the underlying mechanisms for square and boll drop under these extreme environmental conditions. We conclude that future research should be focused on identifying and using high-temperature– and UV-B–tolerant cultivars, which would be beneficial under both present and future climates.

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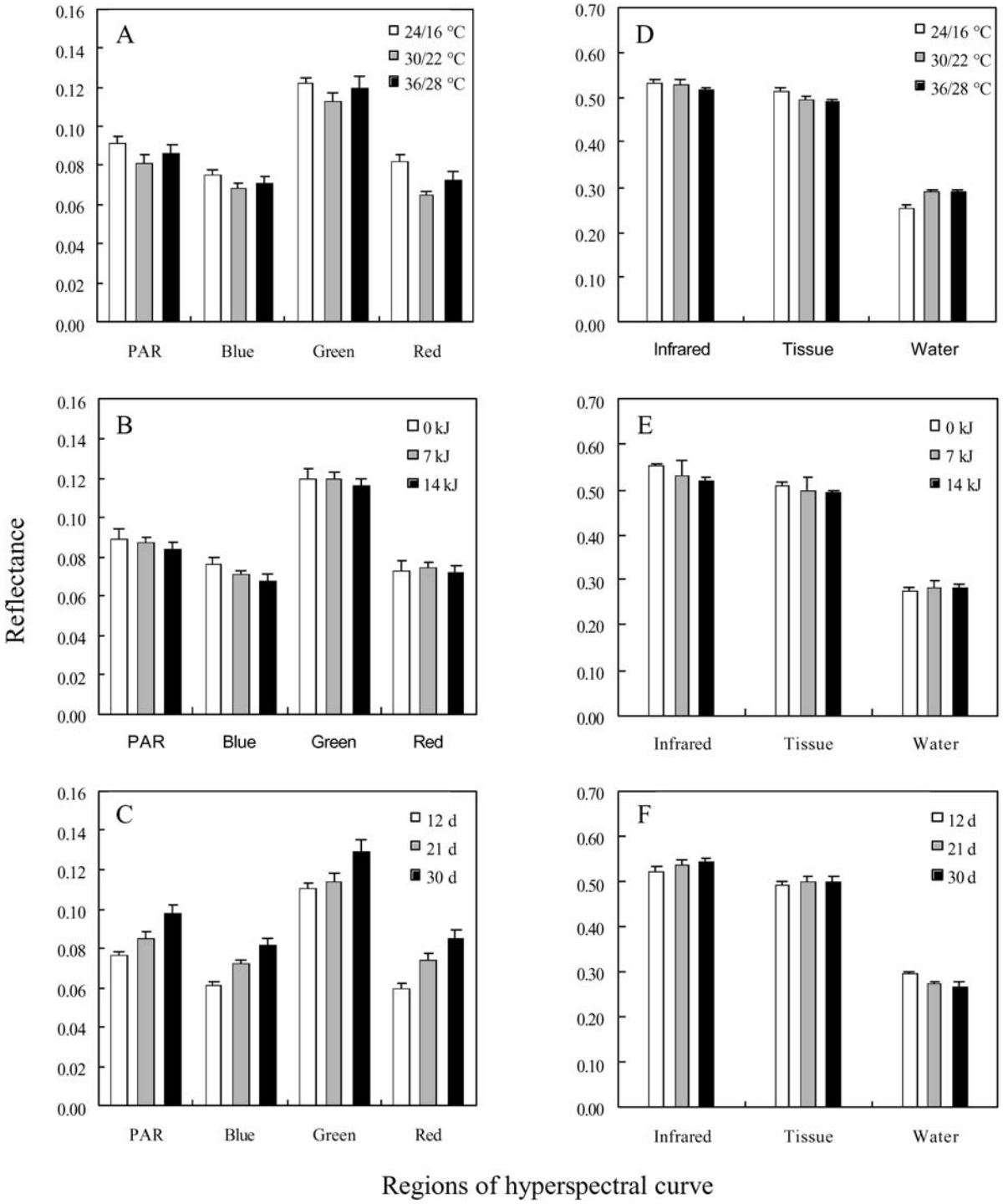

Figure 5. Effect of temperature (A, D), ultraviolet-B radiation (B, E) and leaf age (C, F) on regions of hyperspectral curve of cotton leaves; PAR = 400–700 nm; blue = 400–520 nm; green = 520–600 nm; red = 630–690 nm; near infrared = 760–900 nm; tissue = 900–1300 nm; water = 1600–1850 nm associated with leaf physiological characters. Data are means ± SE (n = 3).

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