

Cotton Fiber Length Is Affected by Far-Red Light Impinging on Developing Bolls

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ABSTRACT

Cotton (*Gossypium hirsutum* L.) fiber length is an important component of quality as defined by the textile industry. The fibers are single elongated cells that extend from the seed coat during development within bolls. It was hypothesized that elongating cotton fibers would be responsive to the far-red (FR) to red light (R) photon ratio (FR/R) reflected to developing bolls. To test the hypothesis, plants were grown in trickle irrigated field plots over different colored soil covers that reflected high FR/R and low photosynthetic photon flux (PPF), or low FR/R and high PPF to developing bolls. Newly opened flowers were tagged over three replicate plots of each of four colors. After the flowers were fertilized, randomly selected young bolls were covered with aluminum foil to shield them from light during development. Others served as unshielded controls. The FR/R and PPF impinging on developing bolls affected biomass per area of carpel walls and the amount of FR transmitted into the bolls to the developing fibers. Fiber lengths were determined after the bolls matured. Fibers in unshielded bolls that developed over green and red (higher reflected FR/R) soil covers were significantly longer than those that developed over aluminum and white (higher reflected PPF). The difference in length of fiber developed in unshielded versus shielded bolls was greater over green and red than over aluminum and white indicating a greater response to increased FR/R than to increased PPF impinging on the developing bolls. These results suggest that reflected FR should be considered along with other environmental factors when developing innovative production systems that involve nontraditional row spacing, plant population densities, or soil surface conditions that can affect the photon ratios to which the developing plants are exposed.

FIBER LENGTH is an important component of cotton quality because it affects use by the textile industry. Length and perimeter of fiber from the same cotton cultivar may vary from year to year at a given location (22, 29). Environmental factors known to influence fiber quality include water stress (shortage or excess), temperature, soil nutrient availability, insects, diseases, and plant population density (8, 10, 21, 22, 29). Canopy architecture and the interception of photosynthetically active light have also received considerable attention (1, 7, 9, 11, 23). However, experiments with other crop and test plants have shown that the spectral distribution of light acts through natural photomorphogenic pigments, such as phytochrome, within growing plants to regulate where the photosynthate is allocated and how it is used under field conditions (2, 3, 13, 19, 20). The relative amounts of far-red (FR) and red light (R) received by the phytochrome system in growing plants affects the photoequilibrium between the R-absorbing form (Pr) and the FR-absorbing form (Pfr). Thus, the

phytochrome system functions as a sensor of the light environment and a regulator of morphological development.

Under field conditions, the FR/R photon ratio can be changed by the number and nearness of other plants (green leaves reflect much FR but little R) or by the FR/R ratio reflected from dead plant residue or different colored soil surface covers (14). Thus, the FR/R ratio can be influenced by crop management systems, and it affects morphological characteristics of the plant parts that are developing when the light ratio is received. For example, the size and number of early crop tomatoes (*Lycopersicon esculentum* Mill.) were greater when grown over a specially formulated FR-reflecting, plastic mulch (18). Similarly, the FR/R ratio in upwardly reflected light received by field-grown cotton plants was also shown to influence the number of bolls and yield per plant (15). The latter study suggested a possible effect on fiber length, but there was no attempt to determine which part or parts of the plant were most responsive to the reflected FR.

Some reports in the older botanical literature are highly relevant to the present study of cotton fiber elongation. For example, seedlings that received FR (a high FR/R ratio) developed longer and slimmer hypocotyls than plants that received R (a low FR/R ratio) in controlled environments (6). Cells within FR-treated hypocotyls were also longer and slimmer than those in R-treated hypocotyls (24). These observations from the 1950s and 1960s are important in the present study because each cotton fiber is a single elongated cell.

In the present study, it was hypothesized that cotton fiber length would also be responsive to FR received during its elongation stage. Knowledge of potential effects of FR on developing cotton fiber and on final fiber length and quality is important as we consider non-traditional production systems that affect the amount and color of reflected light.

The objectives of this study were to determine (i) whether length of developing cotton fiber can be affected by FR impinging on the developing bolls and, if so, (ii) whether any part of the FR spectrum can penetrate through the carpel walls and reach the fibers during their development inside the bolls.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Cotton (cv. PD-1) plants were grown in irrigated field plots of Norfolk loamy sand (Typic Kandidults) at the Coastal Plains Soil, Water, and Plant Research Center near Florence, SC, in 1992 and 1993. Different colored panels on the soil surface were used to determine whether reflected FR and visible light

Abbreviations: FR, far-red light; FR/R, far-red to red photon ratio; Pfr, the far-red-absorbing form of phytochrome; Pr, the red-absorbing form of phytochrome; PPF, photosynthetic photon flux; R, red light.

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could affect fiber quality. The experimental design was a randomized complete block with three replications of four different colored soil surface covers.

Each year, plots were fertilized according to recommendations of the Clemson University Cooperative Extension Service and ridge rows were prepared at 1-m intervals. Trickle irrigation tubes were placed on top of the ridges and the plots were covered with 6- by 30-m sheets of black plastic. The 6-m width covered four of the ridged rows, and there was an uncovered row between adjacent plastic-covered plots. There were three such plots each year. Each plot contained subplots that were painted with exterior enamel to provide the desired reflection spectra. The sequence of colors was randomized within each plot. Green was included because its reflection spectrum was similar to reflection from green leaves. Exterior enamels were used because they provided an economical and repeatable method to obtain the desired reflection spectra for small plot studies. However, reflection spectra had to be measured from each batch of paint because two batches of paint of a given color may appear identical to human eyes but quite different to plants if they reflect different amounts of FR. The same batches of green, aluminum, and white paint were used each year. Reflection from red was nearly identical in the visible spectrum (400–700 nm) both years. However, the batch used in 1993 reflected more FR and a higher FR/R photon ratio.

Each subplot (color) consisted of four parallel rows (1 m apart). A series of 5-cm diameter holes were cut in the plastic at the top of each ridge to provide within-row plant spacing of 60 cm. These spatial arrangements assured that plants would be exposed to light reflected from the different colored soil covers during plant growth and fiber development. Four seeds were sown in each hole on 19 May 1992 and on 18 May 1993. When seedlings were in the cotyledon stage, all but one per hole were removed by cutting below the cotyledons. The paints were then applied to the plastic covers with rollers. In this manner, roots of the remaining plants were not damaged, and the seedlings were exposed to the various patterns of reflected light from the cotyledon stage to maturity.

Upwardly Reflected Light

The quantity and spectral distribution of upwardly reflected light were measured 30 cm above each colored surface with a LI-COR LI-1800 portable spectroradiometer (LI-COR Inc., Lincoln, NE) equipped with a remote hemispherical light collector on a 1.5-m fiber optic probe. Measurements were made at 5-nm intervals between 400 and 800 nm. A reference spectrum was obtained by measuring incoming sunlight at the same wavelengths. Incoming and reflected light measurements were taken on cloudless days at solar noon \pm 30 min each year. The reflected light values were calculated as percentages of incoming sunlight at each measured wavelength.

Values for R were measured at 645 ± 5 nm. That is the approximate action peak in green plants because of competitive absorption by chlorophyll at 660 nm, which is also the *in vitro* absorption peak for Pr (17). Values for FR were measured at 755 ± 5 nm which is the beginning of the reflection plateau (percentage-wise) from green leaves (13), instead of 735 nm which is the *in vitro* absorption peak for Pfr (5). Furthermore, prolonged exposure to FR at 735 nm results in a R response because of the overlapping of Pr absorption into that waveband (16). At 755 nm, the Pr absorption is extremely low, resulting in the FR response to prolonged reflection from nearby plants, as observed in nature (13).

The FR/R ratios in upwardly reflected light were expressed relative to the FR/R ratio in incoming sunlight measured at the same time and place. The rationale for this approach was

that field plants normally grow in sunlight with constantly changing characteristics, and plants are able to sense and respond morphologically to reflected light that differs in spectral distribution from incoming sunlight (19, 20). Reflection from the green, aluminum, and white paints were consistent between years. The red paint used in 1993 reflected more FR than that used in 1992. It reflected a spectrum similar to that reflected by the SRM-Red-1995 formulation used in experiments with tomato (18).

Sampling and Measurements

In 1992, cotton was harvested by hand on 3 October from newly opened bolls that were 25 to 35 cm above the colored soil covers. The samples were taken from the middle two rows in each subplot, and stored in darkness at room temperature until June of 1993 when 100 seeds (with fiber attached) were randomly selected from each of the samples. The seeds were separated from others within a locule using a gentle stream of air. Fibers were extended by brushing with a soft-bristle tooth brush. Each seed with its fibers extended was stored in a piece of folded paper until all were ready to be measured. Measurements from seed surface to tip of longest fibers were taken with a standard laboratory mm scale. Three measurements were taken per seed. All processing and measurements were made by the same person. After the measurements, seeds (with fibers still attached) were returned to darkness at room temperature for possible microchemical or ultrastructural studies of the secondary walls.

In addition to the samples prepared for hand measurements, other cotton harvested in 1992 from the same plots was bulked, ginned, and measured for fiber length and perimeter by an automated procedure (Advanced Fiber Information System, AFIS). This was done to determine whether the automated measurements of fiber length from bulked and ginned cotton grown over the various reflectors would be consistent with the hand measurements. It also provided measurements of fiber perimeter.

Unshielded versus Shielded Bolls

The 1993 experiment compared cotton fibers that developed in unshielded (control) bolls with fibers that developed in bolls that were shielded from the light reflected from four colors of soil covers (the same green, aluminum and white used in 1992 plus a red that reflected a FR/R ratio similar to that reflected by the green). Twenty newly opened flowers located about 30 cm above each of the four colors were tagged in each of the three replicate plots on 22 July. Ten fertilized flowers were randomly selected from each set and remained tagged as the controls (unshielded). The other 10 from each group of 20 were covered with aluminum foil on 26 July to shield them from light during their development. The 15- by 15-cm aluminum foil envelopes were folded from 15- by 30-cm pieces of household aluminum foil. The two sides were folded three times to block entry of light, but loose enough to allow gaseous exchange. The entry end of each envelop was folded around the branch on which the boll was developing. The shields remained in place until 3 September when they were removed and bolls, including the unshielded controls, were harvested. Seed cotton from control and shielded bolls was stored in darkness at room temperature until the fibers were measured. All seed separation, fiber brushing, and hand measurements were done by the same person who had processed and measured the 1992 samples.

Transmission of FR into Developing Bolls

Preliminary measurements of light transmission through developing carpel walls were done in 1993 with the spectroradi-

diometer described earlier in this section. The measurements were made near solar noon on a cloudless day. Other more extensive measurements were made in 1996 to determine whether growth over the green versus white reflectors could affect thickness of carpel walls and transmission of visible and/or FR into the bolls to the developing fibers. For these measurements of transmission through carpel walls, a layer of clear plastic (Saran Wrap, Dow Chemical Co., Indianapolis, IN) was wrapped around the light collector (at the end of the 1.5-m fiber optic probe) which was clamped to a ring stand and aimed at the sun to obtain the spectrum of incoming light. The clear plastic allowed transmission of light and kept all measured carpel walls in the same position relative to the window of the light collector. Similar ages of developing bolls were selected from those over green versus white surfaces. Carpel walls were removed from developing bolls and pressed over the light collector window (less than 1 cm in diameter) to measure light transmission at 5-nm intervals from 400 to 800 nm. The transmitted light at each measured wavelength was expressed as percentage of light at the same wavelength in the incoming sunlight.

After it was evident that carpel walls from similar size bolls developing over green versus white reflectors differed in the amount of FR transmitted to the developing fibers, dry weights per area of carpel wall were determined from six replicate bolls per color. Of the four or five carpels per boll, one carpel wall was used to obtain the light transmission spectra described above. The other carpel walls were measured for area by outlining their perimeters on paper, cutting these, and measuring areas of the paper cut-outs on a LI-COR Model 3100 Area Meter (LI-COR, Inc.). After the carpel walls were outlined, they were freeze-dried and weighed. The dry weights per square centimeter were calculated to determine whether reflected light (over the green versus white surfaces) received by the developing bolls influenced the biomass per area in a way that might affect the amount of FR penetration into bolls to the developing fibers.

Data Analyses

Data on fiber length were analyzed by analyses of variance (ANOVA) as outlined by SAS Institute (26).

RESULTS AND DISCUSSION

Reflected Light

The PPF values and the FR/R photon ratios in upwardly reflected light (relative to those in incoming sunlight) measured about 30 cm above the different colors of paints in 1992 are summarized in Table 1. The PPF values indicate that bolls developing over aluminum and white surfaces received substantially more photosynthetic light. They also received reflected FR/R ratios that were very similar to the FR/R ratio in incoming sunlight during fiber development. On the other hand, bolls developing over the green and red surfaces received less reflected photosynthetic light but higher FR/R ratios. The batch of red paint used in 1993 reflected the same visible spectrum but more FR (than the batch of red paint used in 1992) and a FR/R ratio similar to that reflected by the green. This experimental system allowed comparison of fiber responses to increased photosynthetic light (over aluminum and white) and to increased FR/R photon ratios (over green and red). Additionally, the green surface (which reflected a spectrum quite similar to that reflected from leaves of growing plants) was analogous to studying

Table 1. Approximate upwardly reflected photosynthetic photon flux (PPF) and FR/R ratios, and mean lengths of cotton fiber that developed in unshielded bolls on plants grown in full summer sunlight over different colored surfaces in 1992.

Characteristic	Color of reflector on soil surface			
	Green	Red	Aluminum	White
Reflected PPF (%)†	11	15	38	39
Reflected FR/R (ratio)†	1.28	1.09§	1.00	0.98
Fiber length (mm)‡	35.9a	34.7a	31.8b	31.4b

† In upwardly reflected light relative to incoming sunlight at the same time and place. Values are means for at least five measurements.

‡ Measurements were from seed to tip of longest fibers. There were three such measurements on each of 100 seeds per each of three replications per color. Fiber length data are means for the three replications per color. Values followed by the same letter do not differ significantly at $P = 0.05$.

§ The red used in 1993 reflected more FR, and a FR/R ratio similar to that reflected from green.

response to reflection from nearby plants, which can be varied by plant population densities (13). Plant responses to reflected FR are expected to become increasingly important with development of new crop production systems that utilize closer rows, higher population densities, and plant residues on the soil surface.

Fiber Characteristics

1992

Fibers that developed in bolls over the green and red soil covers were significantly longer than those that developed over aluminum and white (Table 1). These hand measurements were from the seed surface to the tips of the longest fibers per seed. Thus, the values are higher than those normally obtained for ginned cotton using automated procedures. In a companion study with ginned cotton, an automated procedure (AFIS) was used to determine fiber characteristics of bulked samples grown over the same four colors. Data from fibers grown over green versus red did not differ significantly at $P = 0.05$. Similarly, data from fibers grown over aluminum versus white did not differ significantly. Therefore, data from green and red were pooled, as were data from aluminum and white. As shown in Table 2, fibers that developed over the high FR/R reflectors (green and red) were longer than those that developed over the high PPF reflectors (aluminum and white). As expected, the fiber length values with ginned samples were lower than those obtained by hand measurement of the longest fibers attached to seed. However, the important point is that both the hand and the automated

Table 2. Length and perimeter of ginned cotton from bulked samples grown over the green and red versus aluminum and white reflectors in 1992, using AFIS procedures.

Fiber character	Color of reflector on soil surface	
	G+R†	A+W
Length (mm)‡	33a§	31b
Perimeter (AFIS units)	51.0b	52.3a

† G+R, pooled samples grown over green and red; A+W, pooled samples grown over aluminum and white reflectors.

‡ Upper quartile length by AFIS procedures.

§ Values are means for 10 measurements, converted to nearest mm after statistical analysis. Those in the same row followed by different letters differ significantly at $P = 0.05$.

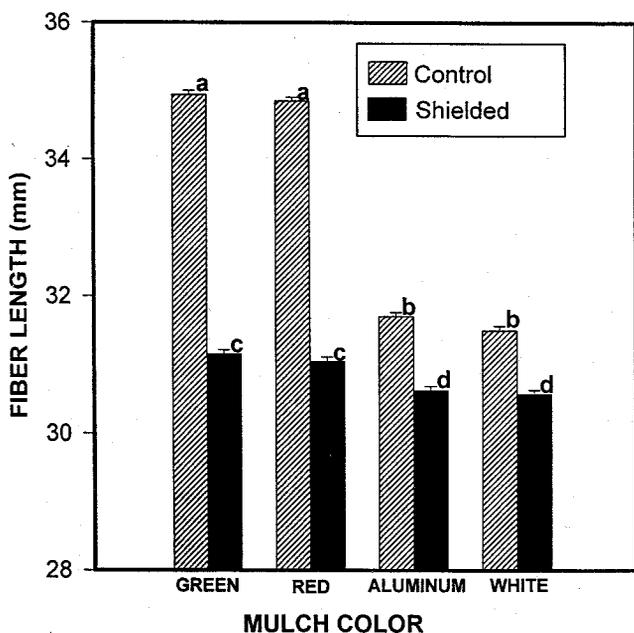


Fig. 1. Length of fibers developed in unshielded (control) versus shielded bolls on cotton plants in trickle irrigated field plots over green, red, aluminum, and white surfaces in 1993. Values are means for the hand measurements \pm standard errors. Those with the same letter do not differ significantly at $P = 0.05$ by ANOVA procedures (26).

measurements of fiber length showed that fibers developed over the higher FR/R reflectors were significantly longer. Values obtained for fiber perimeter by AFIS procedures indicated that those grown over the high FR/R reflectors (green and red) were also slimmer.

In this experiment, fibers were longer (and slimmer) when bolls developed over the colors that reflected the higher FR/R photon ratios (Tables 1 and 2). This response was consistent with my hypothesis based on the longer and slimmer cells developed in seedling hypocotyls that received extra FR during development. However, the experiment did not indicate whether the FR/R action was due to its impinging on the developing bolls, on nearby leaves as was the case in floral induction observed by Parker et al. (25), or on both the developing bolls and the nearby leaf and stem tissue. Thus, it was necessary to include measurement of fiber lengths from

shielded versus unshielded (control) bolls grown over the same colors in the second year of the investigation.

1993

Length of fiber developed in unshielded (control) versus shielded bolls over green, red, aluminum, and white are shown in Fig. 1. The treatment combination allowed comparison of both additional photosynthetic light and increased FR/R ratio on elongation of developing fibers. As in 1992, length of fibers that developed in unshielded bolls over green and red were longer than those that developed over aluminum and white. Fiber length differed more between control and shielded bolls when plants were grown over the high FR/R reflectors (3.79 and 3.78 mm for green and red, respectively) than when grown over surfaces that reflected more photosynthetic light (1.07 and 0.94 mm for aluminum and white, respectively). Thus, a higher FR/R ratio impinging on developing bolls was more effective than increased photosynthetic light impinging on the developing bolls. However, when considering only the shielded bolls (see Fig. 1), longer (at $P = 0.05$) fibers over green and red (higher FR/R ratio) than over aluminum and white suggests that the higher FR/R received by nearby unshielded parts of the same plants contributed a small additional amount to fiber elongation.

Spectral Transmission through Carpel Walls to Developing Fibers

Because fiber length responded to FR impinging on the developing bolls, light transmission through carpel walls of developing bolls was measured to test the possibility that FR might penetrate into the bolls and impinge directly on the developing fibers. Transmission of FR and visible light through the carpel walls of immature bolls from plants growing over green versus white are shown in Fig. 2. Although FR beyond 750 nm was transmitted through carpel walls that were developing over both the green and the white reflectors, more transmitted through carpel walls that were developing over the green. It should be noted that the waveband transmitted through the carpel walls of developing cotton bolls was similar to the FR waveband reflected by green leaves (Fig. 3). They both plateau (percentagewise) at about 750 nm, which is well beyond the in vitro absorption

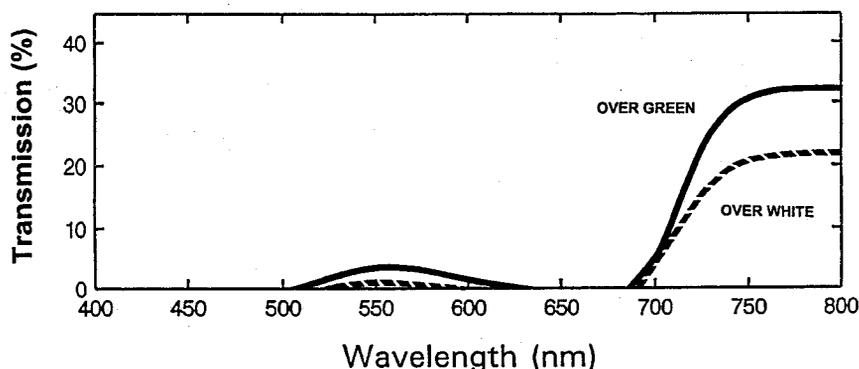


Fig. 2. Percentage of incident visible and FR light transmitted through carpel walls of immature cotton bolls that were developing over green versus white reflectors.

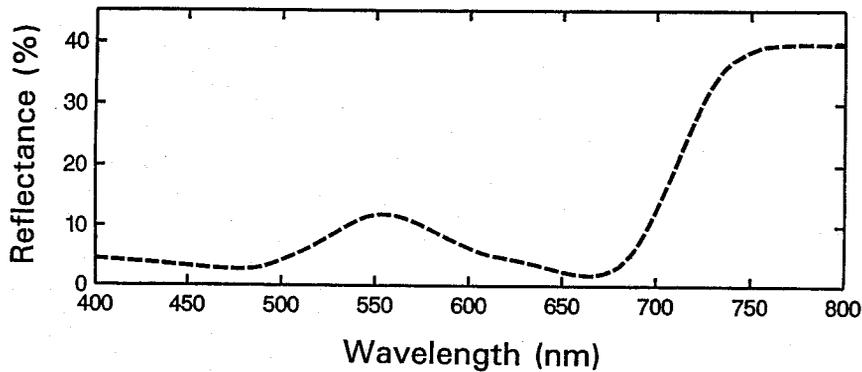


Fig. 3. Percentage of incident visible and FR light reflected from the surface of a recently expanded green leaf from a cotton plant growing over soil. Reflection from the leaf was similar to that from the green reflectors used in these experiments.

peak for Pfr and where there is little competitive absorption by Pr (14, 17). Thus, the number and nearness of leaves on competing plants affects the quantity of FR reflected to the developing parts including bolls, whose carpel walls allow transmission of FR. Clearly, FR can enter an immature boll and reach the elongating fibers. This is highly relevant because the effective amount of FR within the plant tissues (developing fiber and seed in this case) may be even greater than the amount impinging on the surface of those tissues because of photon scattering and reflection, as was measured in fleshy leaves by Vogelmann and Björn (28). The biophysics of FR photon movement and absorption within plant tissue is described in a review article by Vogelmann (27). Nevertheless, the present indication that FR can penetrate into developing cotton bolls to affect dimensions of developing fibers appears to be a new discovery that may also affect fiber and seed chemistry.

A possible explanation for the greater penetration of FR into cotton bolls developing over green is that thickness of the carpel walls on the developing bolls might be influenced by both the quantity of light (including blue light) and the FR/R ratio. In the present study, carpel walls developing over green versus white soil covers averaged 26.8 and 32.3 mg dry weight per square centimeter, respectively. The difference in weight per area suggests that carpel walls were thinner when they received very little reflected blue light and the higher FR/R ratio during their development. This concept is supported by a study of the biomass per area of cotton leaves that developed in a field over green versus white soil covers (4). In that study of affects of upwardly reflected color on the developing photosynthetic system, cotton leaves developing over green reflectors were thinner than those developing over white; they also transmitted more light.

Now that the elongation responsiveness of developing cotton fiber to FR has been established, the next step will be to determine effects on physical and chemical characteristics of the secondary walls, and effects on fiber strength and elasticity. This is beyond the scope of the present experiment, but the analyses have begun.

In summary, the same elevated FR/R ratio that acts through the natural phytochrome system within a seedling and influences cell length and slimness in developing hypocotyls can also influence length of a developing

cotton fiber, which is a single elongating cell. The information on cotton fiber elongation responses to FR reflected to and penetrating into the developing bolls is highly relevant because nearby green leaves reflect FR. Therefore, the amount of FR received by a cotton plant (and a developing boll on that plant) can be affected by the number, size, and nearness of other plants, including those on the soil surface. This should be considered along with other environmental factors as we develop new field production procedures that result in nontraditional plant spacing and population densities.

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