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# Influence of Light and Temperature Environments Preceding and During the Dark Period on Floral Initiation in Pima Cotton (*Gossypium barbadense* L.)[1]

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## ABSTRACT

**Floral induction in American Pima cotton, (*Gossypium barbadense* L.), cultivars 'Pima S-3' and 'Pima S-4', occurred earlier (at lower node levels on the main stem) under short winter days than under long summer days when plants were grown under natural light and a controlled temperature program.**

**The intensities of red and far red light in natural sunlight were measured to determine if there were daily or seasonal changes in spectral quality that might be related to variations in floral initiation. Red light predominated during the day, but the balance shifted to a predominance of far red light between sunset and darkness. This relationship was not seasonal.**

**Total exclusion of all light from plants during the 1.5-hour period before sunset accelerated floral induction if the temperature during this period was above 30 C, but either had no effect or retarded induction if the temperature was below 30 C. Shortening or interrupting the natural dark period with low intensity lights of different spectral composition retarded induction. The intensity of blue light, but not red or far red light, appeared to be related to induction. The increase in the node number of the first fruiting branch was almost directly proportional to the time interval between sunset and the interruption of the dark period.**

**Floral induction was most sensitive to photoperiod and to high temperatures during sunset during the 2nd week of development after germination.**


*Additional index words:* **Photoperiod, Flowering, Spectral quality, Red light, Far red light, Blue light.**

MOST cultivars of cotton grown in the U. S. are commonly believed to be day-neutral in response to photoperiod. Recent observations of American Pima cotton (*Gossypium barbadense* L.), cultivars 'Pima S-3' and 'Pima S-4', indicate that these cultivars do respond to photoperiod with some of the characteristics of short-day plants. These studies were designed to determine the effects of photoperiod and of light and temperature preceding and during the dark period on floral initiation in Pima cotton.

A range of photoperiodic responses have been reported among the *Gossypium* species (5, 6, 7, 8, 13). Konstantinov (6) classified the Egyptian cottons, which include the ancestors of cultivars Pima S-3 and Pima S-4, among the forms that show a slight photoperiodic reaction. Lewis and Richmond (8) studied Pima cotton, cultivar S-1, and Lengupa, day-neutral and short-day stocks of *G. barbadense,* respectively, and determined that a single gene pair controlled flowering under long-day conditions. The short-day nonflowering reaction was dominant.

Moraghan, Hesketh, and Low (12) and Low, Hesketh, and Muramoto (9) have recently shown that short days induce flowering of several *G. barbadense* cultivars earlier (at a lower node on the main stem) than do long days. Berkley (1) observed that photoperiod affected floral initiation of *G. hirsutum* L. at high temperatures but not at low temperatures. Several workers have shown that high night temperatures delay floral initiation in both *G. barbadense* and *G. hirsutum* (10, 11, 12).

Photoperiodic control of floral induction, as well as many other aspects of morphological development, has been associated with the effects of red and far red light acting on the phytochrome system (3). The relative intensities of these two wavelengths of light are important factors in establishing the equilibrium levels of the red- and far red-absorbing forms of phytochrome. The equilibrium between the two forms of phytochrome at the end of the photoperiod affects flowering in some plants. Small changes in the ratio of red to far red light, at low intensities, have been shown to alter the equilibrium between the two forms of phytochrome sufficiently to cause measurable morphological changes (4).

## MATERIALS AND METHODS

### Growth Chamber and Greenhouse Studies

Seeds were germinated for 2 days in darkness over aerated nutrient solution at 32 C. On the 3rd day the seed coats were removed and the seedlings were illuminated for 24 hours with a 300-watt incandescent lamp (2,150-3,230 lux). The seedlings were transplanted on the 4th day to frames and placed in aerated nutrient solutions in the greenhouse or in Plexiglass[3] growth chambers illuminated by sunlight. Unless otherwise indicated, temperatures in the growth chambers were set at 35 and 25 C during the day and night, respectively. Greenhouse heat and evaporative cooling controls were set at approximately 26 and 39 C, respectively.

### Outdoor Daylength Studies

Plants were grown in sand in benches outdoors. Nutrient solution was added twice weekly. Daylength was extended by illuminating plants during the night with lights mounted on one side of the bench and shielded from neighboring benches. Outdoor temperatures prevailed.

Daylength was shortened by covering plants with aluminum covers that totally excluded light. The covers were motor operated and could be programmed to completely cover the plants for any desired period. Plexiglass[3] cabinets slightly smaller than the covers were placed over the benches during winter. Within these cabinets, the temperature was controlled at 35 and 25 C during the day and night, respectively. The cabinets were removed during the summer, allowing outdoor temperatures to prevail. During the summer, ambient temperatures were maintained around the plants by mechanical ventilation whenever the covers were over the plants.

Far red light was obtained from eight 150-watt floodlamps filtered through 9 cm water, 0.6 cm clear Plexiglass[3] and a 0.6 cm-FRF-700 filter (Westlakes Plastics Co.[3]).

An IL 150 Plant Growth Photometer (International Light, Inc.[3]) was used to measure irradiance from artificial lights. This

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[3] Mention of a proprietary product does not constitute a guarantee or warranty of the product by the U. S. Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.

instrument has separate sensors for the blue (350 to 575 nm), red (600 to 850 nm), and far red (700 to 950 nm) regions of the spectrum. Maximum sensitivities for these sensors are at 460, 640, and 730 nm, respectively. A modified Model SR Spectroradiometer (Instrumentation Specialties Co.[3]) with a slit bandwidth of 30 nm was used to measure the spectral distribution of sunlight.

## RESULTS AND DISCUSSION

Pima S-3 and Pima S-4 plants were grown in all tests. No consistent differences were found between the two cultivars. All results, therefore, are presented as averages of the two cultivars.

Floral induction occurred earlier (at a lower main stem node) in February than in May or summer when plants were grown under a controlled temperature program in growth chambers illuminated by sunlight (Table 1). This temperature program, consisting of a minimum temperature of 26 C between 1800 and 0800 hours and a peak maximum temperature of 36 C between 1300 and 1400 hours, duplicated temperatures in the greenhouse on a clear day in February.

Since the same temperature program was used in February as in spring and summer this elevation in node level of induction was probably due to changes in light intensity, light quality, photoperiod, or some combination of these factors. *A priori,* it can easily be determined that between February 1 and June 20, daylength increased from about 10.5 to 14.3 hours and noon solar altitude, which is directly related to light intensity, from 45 to 80 degrees. While the time-light relationship was changing, however, the time-temperature relationship remained constant, and thus, as daylength increased, the minimum temperature of 26 C (1800 to 0800 hours) prevailed during the beginning and the end of the photoperiod.

The intensities of red and far red light in sunlight were measured to determine if there were any daily or seasonal changes in spectral quality that might be related to variations in floral initiation. Natural radiation at 660 and 730 nm was measured several times a day on 2 days per week over a 6-month period. Measurements for a typical day are shown in Table 2. Generally, the ratio of red to far red light was 1.0 to 1.25 during the day. This ratio declined to about 0.75 to 0.85 during the 20- to 30-min period after sunset. The reverse change occurred during sunrise. Light intensity at these wavelengths was approximately 1% of full sunlight at sunset and about 0.25 to 0.50% of full sunlight 20 min after sunset. These relationships were not seasonal.

To determine if the daily change in the balance of red and far red light is related to floral initiation, I altered the photoperiod and the balance of red and far red light at the end of the photoperiod.

The photoperiod was shortened by covering the plants to exclude all light for 1.5 hours prior to natural darkness. The results (Table 3) indicate a relationship between the light environment and temperature. When the temperature was below 30 C, shortening the photoperiod either had no effect or raised the node number of the first floral branch. Shortening the photoperiod for an equivalent period of time during the sunrise period generally had no effect.

The general change in node level of the first floral branch between winter and summer noted in Table 1 also occurred here (Table 3). The first floral branch of untreated plants occurred at the fourth to fifth node in the short days of winter, but at the seventh to eighth node in summer.

Floral initiation was unaffected when a natural photoperiod of about 11.5 hours or more (sunset temperatures greater than 30 C) was reduced to 10 hours, if the added period of darkness commenced at sunrise. The first fruiting branch, however, was initiated several nodes lower on the main stem (Table 4) if the dark period extension immediately preceded sunset, or was divided between sunrise and sunset. Low-intensity artificial illumination reversed the effect of darkness imposed during sunset (Table 4).

Total light energy was measured during the August 1969 experiment. In a typical week the 10-hour photoperiod that ended at sunset provided only 1% more

**Table 1. Floral induction in Pima cotton plants grown under the same temperature program in different months of the year. Tests conducted in growth chambers illuminated by sunlight. Data are averages of at least 31 plants per treatment.**

| Date of test | Node number of first fruiting branch |
|---|---|
| February | 5.6 a† |
| April | 6.1 ab |
| May | 6.7 b |
| June-July | 7.0 b |
| August-September | 6.9 b |

† Means followed by a letter in common are not statistically different at the .05 level of probability (Duncan's new multiple range test).

**Table 2. Ratios of intensities of red and far red light in natural sunlight at different times of the day on 11 February 1969. Sunrise, 0716 hours; sunset, 1809 hours.**

| Time of day | Intensity of red radiation / Intensity of far red radiation | Time of day | Intensity of red radiation / Intensity of far red radiation |
|---|---|---|---|
| 0704 | 0.71 | 1200 | 1.01 |
| 0710 | 0.93 | 1500 | 1.05 |
| 0720 | 0.92 | 1700 | 0.99 |
| 0730 | 1.03 | 1730 | 0.96 |
| 1000 | 1.14 | 1800 | 0.89 |
| | | 1830 | 0.80 |

**Table 3. Floral induction in Pima cotton plants under shortened photoperiod. Data are averages of at least 27 plants per treatment.**

| Temperature during period of shade | Environment† | Month of test | Time of dark period extension (S=sunset) | Node no. of first fruiting branch |
|---|---|---|---|---|
| below 30 C | G | Nov-Dec | (S-1 hr) to (S+1 hr) | 5.1**‡ |
| | | | Natural photoperiod | 4.3 |
| | O | May | (S-1.5) to (S+0.25) | 7.5 |
| | | | Natural photoperiod | 6.8 |
| | C | Dec-Jan | (S-1.5) to (S+0.25) | 4.4 |
| | | | Natural photoperiod | 4.1 |
| 30 C or above | O | June-July | (S-1.5) to (S+0.25) | 6.9** |
| | | | Natural photoperiod | 7.7 |
| | C | Dec-Jan | (S-1.5) to (S+0.25) | 4.1* |
| | | | Natural photoperiod | 4.8 |

† C = controlled, 35 C day, 25 C night; G = greenhouse; O = outdoor ambient.
‡ Means followed by * and ** differ from "natural photoperiod" treatments at .05 and .01 levels of probability, respectively.

**Table 4. Floral induction in Pima cotton plants given a 10-hour natural photoperiod at different times during a longer natural day. Photoperiod extended in one treatment with 75-watt incandescent bulb (15 to 25 ft-c). Temperature at sunset exceeded 30 C in May 1970, and 35 C in August 1969, July 1970, and August 1970. Data are averages of at least 19 plants per treatment.**

| Light treatment† (Sunrise / Sunset) | Node number of first fruiting branch 8/69 | 5/70 | 7/70 | 8/70 | Mean difference, treated - control |
|---|---|---|---|---|---|
|  | 11.7 a‡ | 4.7 b | 12.3 b | 6.4 a | -4.3 |
|  | -- | 7.4 a | 17.4 a | 9.6 ab | -0.7 |
|  | 16.5 b | 7.6 a | -- | -- | +0.7 |
|  | 11.5 a | -- | -- | -- | -4.4 |
| | 15.9 b | 6.9 a | 17.7 a | 12.1 b | |

† ▭ = Sunlight ▦ = Artificial illumination ■ = Dark
‡ Within each column, means followed by a letter in common are not statistically different at the .05 level of probability (Duncan's new multiple range test).

light energy than the 10-hour photoperiod that began at sunrise. On the other hand, the photoperiod that was approximately centered in the day yielded about 9% more energy than the photoperiod that ended at sunset.

It appears, therefore, that during periods of high temperatures at sunset, Pima S-3 and Pima S-4 plants are sensitive to photoperiod. Reversal of the effect of darkness imposed during sunset by illumination with low-intensity light and the light energy relationships during the 1969 test both indicate that the observed phenomena are related to the length of the illumination period and not to the total energy received.

The marked effect of darkness imposed during sunset suggests that light quality, either directly or in conjunction with the temperature environment, controls floral initiation. Placing plants in darkness during this period alters the balance of red and far red radiations that the plants receive. This balance at the end of the photoperiod is believed to be a factor affecting floral initiation in some truly photoperiodic plants.

The role of spectral distribution at the end of the photoperiod was investigated by extending the light period with artificial lights of different spectral composition (Table 5). All of the tested sources delayed floral initiation. The amount of delay does not appear to be related to the intensity of either the red or the far red radiation. Node numbers of the first fruiting branches observed in the February test, however, agree in rank order with the intensities of blue radiation in the light sources. Similar relationships between color quality of light and effect on floral initiation were observed when these lights were used to illuminate the plants in the middle of the natural dark period (Table 5).

Recently, Brown and Klein (2) showed that both blue and far red wavelengths induced flowering in the long day plant *Arabidopsis thaliana* (L.) Heynh. The longer delays in initiation in Pima S-3 and Pima S-4 observed with greater intensities of blue light suggest that blue light may be involved in the regulation of flowering in cotton.

Ilumination in the middle of the natural dark period delayed floral initiation more than did illumination at the beginning of the dark period (Table 5). When separate groups of plants were illuminated during each quarter of the night, the increase in the node number of the first fruiting branch appeared to be directly related to the time interval between sunset and the period of illumination (Table 6).

The time course of development of floral induction was investigated by exposing plants at different stages of development to a high temperature during the sunset period or to an extended photoperiod. The temperature treatment was applied to cultivar S-4 only. High temperatures during the sunset period caused plants to flower almost five nodes higher on the main stem. When this treatment was applied for only 1 week, floral initiation was most sensitive to it during the 2nd week after germination (Table 7).

The number of nodes initiated was determined by dissection following each week of treatment. Without exception, floral initiation occurred at nodes that developed during the 2nd week if the temperature at sunset was low during that week. In almost every case, if the temperature was high at sunset during the 2nd week, induction was delayed until the 3rd week (Table 7).

Extension of the photoperiod for 2 hours during successive 3-day periods produced the greatest delay in induction when this treatment was applied on the 8th through the 10th days following germination (Table 8).

The evidence acquired thus far indicates that floral initiation in Pima cotton is influenced by the light and temperature environments, specifically, by the length of the photoperiod and by the temperature at the end of the photoperiod. These factors, however, do not exert complete control over floral initiation.

**Table 6. Floral initiation in Pima cotton plants illuminated with 75-watt incandescent lamp (20 to 30 ft-c) for 1.5 hours at different times during a 12-hour natural dark period. Test conducted in greenhouse. Data are averages of 16 plants per treatment.**

| Part of dark period during which plants were illuminated | Node number of first fruiting branch |
|---|---|
| First quarter | 4.9 c† |
| Second quarter | 5.3 bc |
| Third quarter | 5.9 ab |
| Fourth quarter | 6.4 a |
| Check - no illumination | 4.2 d |

† Means followed by a letter in common are not statistically different at the .05 level of probability (Duncan's new multiple range test).

**Table 7. Floral initiation in Pima cotton plants, cultivar S-4, exposed to a short period of high temperature at sunset during different weeks of growth. Data are averages of at least 14 plants per treatment.**

| Temperature treatments week† | | | Node of first fruiting branch | No. of nodes developed at end of week | | |
|---|---|---|---|---|---|---|
| 1 | 2 | 3 | | 1 | 2 | 3 |
| C | C | C | 10.6 d‡ | 8.0 | 12.5 | 16.5 |
| W | W | W | 14.6 a | 8.5 | 13.5 | 16.0 |
| C | W | W | 14.3 a | 8.0 | 11.8 | |
| W | C | W | 11.3 c | 8.5 | 12.8 | |
| W | W | C | 12.6 b | 8.5 | 13.5 | 16.3 |
| W | C | C | 11.6 c | 8.5 | 12.8 | |
| C | W | C | 12.5 b | 8.0 | 11.8 | |
| C | C | W | 10.4 b | 8.0 | 12.5 | 15.5 |

† C = 35 C sunrise (R) to sunset (S), 25 C S to R; W = 35 C R to (S - 0.5), 45 C at (S + 0.5), 25 C (S + 2.5) to R; all temperature changes at rate of 10 C per hour. ‡ Means followed by a letter in common are not statistically different at the .05 level of probability (Duncan's new multiple range test).

**Table 5. Floral induction in Pima cotton plants illuminated by lights of different spectral characteristics. Illumination period was 1.75 hours except in March (1.5 hours) and October (2.0 hours) tests. Treatments were applied in greenhouse during entire growth period after 3-day germination period. Data are averages of at least 14 plants per treatment.**

| | Intensity, $\mu w\ cm^{-2}\ nm^{-1}$ | | | Node number, 1st floral branch | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Illuminated following sunset | | | | Illuminated in middle of night | | | | | |
| Light source | Blue | Red | Far red | Feb | Mar | Jun-Jul | Oct | Jan-Feb | Apr | Jun-Jul | Aug-Oct | Oct | Dec |
| Incandescent 1 | 3.1 | 20.0 | 22.0 | 6.6 d† | | | | | | | | | |
| Black light 1 | 2.6 | 0.5 | 0.5 | | | | | | | | | | 10.7 b |
| Incandescent 2 | 2.4 | 14.0 | 17.0 | | 4.9 b | | 6.2 b | | 8.6 c | | | | |
| Grolux | 2.1 | 3.1 | 0.5 | 6.1 c | | 9.6 a | | 7.6 b | 9.0 d | 15.4 b | 14.7 a | | |
| Incandescent 3 | 1.6 | 10.0 | 12.0 | | | | | 7.6 b | 8.0 c | | | | |
| Black light 2 | 0.7 | 0.5 | 0.5 | 6.2 c | | | | | | | | 9.3 c | 10.8 b |
| Black light 3 | <0.5 | 0.5 | 0.5 | | | | | | | | | 8.3 b | |
| Far red | <0.5 | 6.0 | 22.0 | 5.7 b | | | | | 6.7 b | | | | |
| Check | | | | 5.3 a | 4.2 a | 9.1 a | 5.2 a | 5.3 a | 5.8 a | 9.1 a | 12.4 a | 7.7 a | 5.3 a |

† Within each column, means followed by a letter in common are not statistically different at the 0.05 level of probability (Duncan's new multiple range test).

**Table 8. Floral initiation in Pima cotton plants illuminated with two 150-watt floodlamps (500 to 1000 ft-c) for 2 hours following sunset on three successive days. Test conducted in greenhouse. Data are averages of at least 30 plants per treatment.**

| Days of treatment† | Node no. of first fruiting branch |
|---|---|
| 2-4 | 4.7 bcd‡ |
| 5-7 | 4.9 bc |
| 8-10 | 5.5 a |
| 11-13 | 5.1 ab |
| 14-16 | 4.3 d |
| Check - no illumination | 4.5 cd |

‡ Means followed by a letter in common are not statistically different at the .05 level of probability (Duncan's new multiple range test). † Day 1 = first day after 3-day germination period.

Thus, the photoperiod response can be classified as quantitative rather than qualitative.

The effects of photoperiod and temperature on floral initiation are greatest during the 1st and the 2nd weeks of plant development. During this period of sensitivity floral initiation is retarded by short dark periods or by high temperatures preceding or during the dark period. Conversely, long dark periods and low temperatures preceding and during the dark period promote floral initiation. The retarding effect on floral initiation of high temperatures during the sunset period can be reversed by placing the plants in darkness during this period. Additional evidence will be needed to determine if the phytochrome system is involved in the observed sensitivity to photoperiod and temperature.

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