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Twilight Effect: Initiating Dark Measurement in Photoperiodism of *Xanthium*[1]

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ABSTRACT

Six experiments studied the effects of low levels of red and far-red light upon the initiation of measurement of the dark period in the photoperiodic induction of flowering in *Xanthium strumarium* L. (cocklebur), a short-day plant, and compared effects with those of comparable light treatments applied for 2 hours during the middle of a 16-hour inductive dark period. Red light, or red plus far-red, at levels that inhibit flowering when applied during the middle of the inductive dark period, either had no effect on the initiation of dark measurement (*i.e.*, were perceived as darkness), or they delayed the initiation of dark measurement by various times up to the full interval of exposure (2 hours). Far-red light alone had virtually no effect either at the beginning or in the middle of the dark period. These results confirm that time measurement in the photoperiodic response of short-day *Xanthium* plants is not simply the time required for metabolic dark conversion of phytochrome. Results also suggest that the pigment system (phytochrome?) and/or responses to it may be significantly different as they function during twilight (initiation of dark measurement), and as they function during a light break several hours later. Possible mechanisms by which cocklebur plants detect the change from light to darkness are discussed.

Comparing experimental results with spectral light measurements during twilight and with measurements of light from the full moon led to two conclusions: First, light levels pass from values perceived by the plant as full light to values perceived as complete darkness in only about 5.5 to 11.5 minutes, although twilight as perceived by the human eye lasts well over 30 minutes. Second, cocklebur plants probably do not respond to light from the full moon, even when most sensitive, 7 to 9 hours after the beginning of darkness.

Time measurement in photoperiodism continues to be a mystery. Several important questions remain unanswered: How does the plant detect light and darkness in photoperiodism? What is the nature of the clock that measures the lengths of the light and/or the dark periods? How is the clock coupled to the system that detects light and darkness? How do these mechanisms function under natural conditions?

Since 1952 when Borthwick *et al.* (2) discovered that the inhibitory effects of a R[2] interruption of an inductive dark period for *Xanthium* could be reversed by subsequent exposure to FR, we have known that the phytochrome system was involved and have assumed that this system detected light and darkness in photoperiodism. Borthwick *et al.* (2) suggested that the phytochrome system was primarily in Pfr at the end of the light period and that it was metabolically converted to Pr during the dark period. They further suggested that the time required for this metabolic conversion constituted measurement of the critical dark period for photoperiodic induction of flowering in *Xanthium* (a short-day plant) or inhibition of flowering in long-day plants (*i.e.*, they assumed that pigment conversion was equivalent to time measurement).

This assumption was largely abandoned during the late 1950s and 1960s (reviews in 2, 10, 20, and 30). Some important reasons relating to the present topic are as follows:

1. The quantity of light required to saturate the inhibitory processes during an inductive dark period is essentially constant after the first 2 h of darkness (23). This suggests that metabolic dark conversion is complete in less than 2 h, a time much shorter than the critical night.

2. Low levels of light applied during an entire inductive "dark" period, which are capable of inhibiting subsequent flowering by about half, did not change the critical night (21). If inhibition of flowering is caused by Pfr produced by the low light levels, metabolic conversion of Pfr to Pr cannot go to completion, and thus cannot be equivalent to time measurement.

3. There is much evidence that time measurement in photoperiodism is not equivalent to a simple completion of a metabolic reaction such as pigment conversion (*i.e.*, that time measurement is not analogous to an hourglass), but that time measurement is dependent upon an oscillating timer mechanism (analogous to a pendulum). An oscillating-type clock controls leaf sleep movements and other circadian rhythms in plants (reviewed in 10 and 30). One basic observation is that sensitivity to light (*i.e.*, inhibition of induction) oscillates with about a 24-h periodicity during extended dark periods given to short-day plants (*e.g.*, 5, 9). Also, maximum flowering in soybeans occurs when day and night total about 24 h of multiples thereof (9). One assumption (based mostly on work with short-day plants) is that a circadian rhythm controls the plant's sensitivity to light (to Pfr), oscillating between a state during which light (Pfr) inhibits induction of flowering and a state when light (Pfr) promotes induction. In *Xanthium*, the rhythm appears to oscillate with (to "track") the normal conditions of oscillating light and darkness, but if plants are exposed to continuous light, the rhythm, after an initial minor oscillation (maximum about 12 h after the onset of light), goes into a "suspended" condition, in which it remains until the onset of a dark period (18). Thus, dusk (change from light to darkness) seems to initiate the measurement of the light inhibitory period in the floral induction of the short-day plant, *Xanthium*. It is not clear whether the photoperiodism clock and the clock controlling circadian leaf movements are identical (they certainly have similar characteristics), but manifestations of the two clocks are experimentally separable (1, 10, 13, 22, 24, 25).

This paper reports the results of six experiments designed to

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[2] Abbreviations: R, red light; FR, far-red light; HIR, high irradiance response.

study the light levels required to inhibit the initiation of dark measurement (the dusk or twilight effect) in photoperiodic induction of flowering in *Xanthium,* compared with the light levels required to inhibit photoperiodic induction when the light is applied during the most sensitive time (around the time of the critical night). The experiments also bring up two ecological questions. First, when during natural twilight does the plant stop reacting as if it were in the light and begin to react in its "dark mode?" As a variant of this question we might ask if the transition is as gradual as twilight itself or more sudden? Second, can a plant respond photoperiodically to moonlight? To provide tentative answers, light levels and spectra during twilight and from a full moon were measured and compared with levels and spectra from the light sources used in the experiments.

MATERIALS AND METHODS

Burs of *Xanthium strumarium* L. (Chicago strain) were germinated for over a week in moist sand, and then seedlings were transplanted to 10-cm, square, plastic pots containing a greenhouse soil, which consisted of loam, sand, and peat moss (3:1:1 by volume). Culture and experimentation were much as previously described (20). Plants were fertilized with a few pellets of ammonium nitrate once a week and maintained in the vegetative condition by long days: cool-white fluorescent lamps, producing about 6 to 16 μE m^{-2} s^{-1} from 400 to 700 nm (500–1200 lux) at the leaf level, from 4:00 MST to 8:00 MST and then from 16:00 MST to 24:00 MST plus natural daylight in a corrugated fiberglass greenhouse. Thermostats were set at 26 C, but temperatures dropped below at night in early spring and were above during daytime in early summer.

To obtain total darkness, plants were moved into a booth made of black plastic film and in the room with the illuminating box described below. When it was necessary to move plants in the dark, a flashlight with the lens replaced by a layer of green and a layer of blue Plexiglas (3.175 mm) was used (spectral distribution in Fig. 1). Plants were placed in the dark or under the lights at various times during the late afternoon and evening, and then all were returned to the greenhouse at 8:00 a.m. the next morning.

Nine days after exposure to the single inductive dark period, plants were examined with a dissecting microscope to classify their apical meristems according to a series of floral stages previously described (19, 20). Each point in the figures represents the average floral state of 10 plants that were given the same treatment.

Low intensity light for the experimental treatments was provided by a specially constructed metal box that included a number of incandescent (25 and 100 w) and small fluorescent (8 w) lamps, each controlled by a separate switch. Six layers of white translucent Plexiglas (3.175 mm thick, 4.5 cm apart) were inserted in a rack below the lights to diffuse the light so that levels were highly uniform over an area of about 1.5 m^2. (Plexiglas was purchased many years ago and catalog numbers have been lost, but comparisons were made with recently obtained samples. Translucent white was probably "White W-2447," manufactured by Rohm and Haas.) Red light was obtained by adding two 3.175-mm, translucent, red, Plexiglas filters (probably a translucent version of Red 2423) and using only the fluorescent lamps. A mixture of R and FR was obtained by using the same filters but incandescent instead of fluorescent lamps. FR was obtained by filtering incandescent light through the six white filters and then through 3.175-mm, clear, FR transmitting Plexiglas (FRF-700, Westlake Plastics Co., Lenni Mills, PA). In five of the six basic experiments, two light levels were used, and these were obtained by putting plants on a shelf (leaves close to filters) or on the floor a greater distance away. Light levels for the six experiments are listed in Table I, and Figure 1 shows relative spectral energy distributions for the three sources plus the safelight.

Light levels and spectral distributions were measured with equipment from the laboratory of Martyn Caldwell at Utah State University, calibrated by Stephan D. Flint in that laboratory. Equipment was a model 2900 Auto-Photometer, manufactured by Gamma Scientific, Inc., San Diego, CA. It uses a photomultiplier tube (Hamamatsu R 928) in a high-resolution diffraction-grating monochromator (modified model 700-31), and a microprocessor to control the spectral scans. Spectral levels of light sources, of full sunlight, and during twilight were measured with a cosine receptor attached to the instrument. Levels of moonlight used an unshielded fiber-optics probe aimed directly at the moon (the cylindrical probe forming a circular shadow). Sensitivities of the instrument had to be decreased for full sunlight by reducing voltage across the photomultiplier tube; for the light sources, twilight, and moonlight, voltages were increased above calibration voltage to increase sensitivity. Changes in sensitivity were empirically determined by making several scans of a constant light source (the box described above without the colored filters).

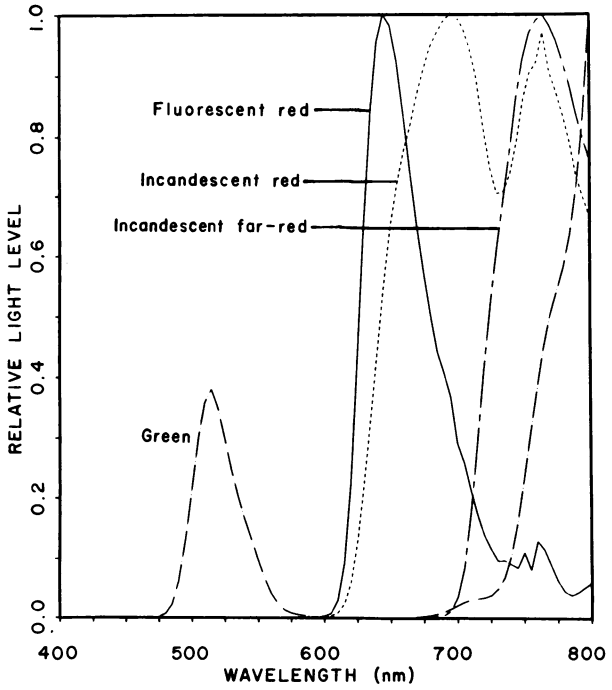

FIG. 1. Spectra from 400 to 800 nm for the three light sources used in these experiments plus the safelight. The four curves were normalized so their high points are all at the same level. Although the safelight transmits considerable near IR when incandescent lamps are used (fluorescent lamps would have been better), those wavelengths were without effect in these experiments, and the flashlight was aimed mostly at tags in the pots and not at the leaves. (Figure computer drawn.)

Each spectral scan begins at 800 nm and progresses at 5 nm intervals to 400 nm, and an entire scan requires 10 min with 2 min more for the instrument to reset to 800 nm. Thus, for twilight measurements, light levels were falling during the time of the scans. To correct for this, curves were "tilted" mathematically so they were proportionately lower in the red and higher in the blue ends of the spectrum. To achieve this, each point was adjusted by the relationship:

$$D = D_1 - \frac{10}{12}(D_1 - D_2)\left(\frac{N - 40.5}{81}\right)$$

Where: D = corrected light level for a given wavelength and time; D_1 = the uncorrected light level for the same wavelength and time; D_2 = the uncorrected light level measured at the same wavelength 12 min after measurement of D_1; N = number from 1 to 81, corresponding to wavelengths as follows: 1 = 400 nm, 2 = 405

Table I. *Light Levels and Summarized Flowering Responses for the Experiments Shown in Figures 1, and 3 to 8*

Light Source	Approximate Filter to Leaves Distance	Light Levels[a]					Delay of Dark Time Initiation[b]	Inhibition of Flowering[b]
		660 nm	730 nm	$\frac{660}{730}$	600–697 nm	700–800 nm		
	cm	*mw m^{-2} nm^{-1}*			*mw m^{-2}*		%	%
Fig. 2: red filters 6 fluorescents	50	0.090	0.011	8.34	5.73	1.402		50
Fig. 3: red filters 12 fluorescents	6	0.293	0.033	8.80	18.65	4.15	60	100
	100	0.076	0.009	8.26	5.01	1.21	0	75
Fig. 4: red filters 8 fluorescents	6	0.194	0.026	7.34	12.46	2.84	100	100
	100	0.050	0.006	8.96	3.34	0.79	40	100
Fig. 5: red filters 4 fluorescents	6	0.100	0.011	9.15	6.11	1.43	0	62
Fig. 6: red filters 10 incandescents	6	1.358	1.233	1.10	91.2	153.0	100	100
	100	0.368	0.357	1.03	24.8	44.2	37	100
Fig. 7: red filters 4 incandescents	6	0.562	0.516	1.09	37.6	62.6	75	100
	100	0.148	0.142	1.05	10.01	17.63	0	81
Fig. 8: far-red filter All incandescents (10 (25 w), 4 (100 w))	6	0.013	6.089	0.0021	1.91	719.0	0	0
	100	0.004	1.733	0.0022	0.675	200.0	0	0

[a] Significant figures were provided by the instrument, but actual values would vary considerably (note variability in ratios of 660/730), depending on exact distances of leaves from the filter, leaf angles, plant position, variations in line voltages, etc.

[b] Flowering responses are rough estimates of the delay in the initiation of dark measurement (light during first 2 h) and inhibition of flowering by light during the middle 2 h of a 16-h dark period.

nm, 3 = 410 nm, 81 = 800 nm. Other details of light measurements are given in the captions of Figures 9 to 11 and in Table II.

RESULTS

Red Light in Middle of the Dark Period and Critical Night. Light, with an irradiance sufficient to partially inhibit flowering when applied from the 7th to the 9th h after the beginning of darkness, was given to plants with dark periods of various lengths. Results of one experiment (of two performed) are shown in Figure 2, which also serves as an introduction to the following six figures. The control curve is drawn to fit the data (○) obtained by exposing plants to uninterrupted dark periods of various lengths. Extrapolation of this curve to zero (vegetative plants) yields the critical dark period. Curves labeled A, B, and "theoretical" are derived from the control curve based upon various models of how plants might respond to 2 h of light given either from the 7th to the 9th h as in this experiment or during the first 2 h of the dark period as in the next six experiments. The "theoretical" curve would result if 2 h of light at the beginning were perceived as full daylight, simply delaying the onset of dark measurement by 2 h. Curves A and B represent two possible responses if the light level during the first 2 h is too high to be perceived as darkness but too low to be perceived as full daylight. Curve A would result if the light reduced the flowering response uniformly by 50%. Curve B would result if the flowering response remains constant but critical night is shifted by 50% of the 2 h of light exposure at the beginning of the dark period (*i.e.*, half way to the "theoretical" curve). Data points (●) were obtained by exposing plants to dim R for 2 h from the 7th to 9th h after beginning of the dark period. These points closely approximate curve A. Results are virtually identical to these obtained when threshold light is given during the entire dark period rather than only from the 7th to 9th h (21).

Red Light. Figures 3 to 5 and Table I show the results of experiments with the 8-w fluorescent lamps, which produced nearly pure filtered R at five levels. Figure 3 shows results with the highest light levels. Plants on the shelf (about 6 cm from filter) were completely inhibited (100% were vegetative) by the light level applied during the middle of the 16-h dark period, but that same level during the first 2 h was not bright enough to be perceived as full daylight (initiation of dark measurement—the critical night—shifted about 1.2 h of the theoretical 2 h, or about 60%). Plants on the floor (about 100 cm from filter) were inhibited about 75% by light in the middle of a 16-h dark period, but they perceived that light as darkness (were not affected by it) during the first 2 h of the inductive dark period. Figure 4 shows results with intermediate light levels. Plants both on the floor and on the shelf were completely inhibited by light during the middle of the dark period, whereas those on the shelf perceived the light as full daylight at the beginning of the dark period and those on the floor were delayed about 40% in initiation of dark measurement. Inhibitions were higher than those of Figure 3, although light levels were lower. This unexpected sensitivity of the plants used for the experiment of Figure 4 suggests that plants change in sensitivity, perhaps in response to unknown environmental factors (temperatures, greenhouse light levels, etc.?). In the experiment of Figure 5 (lowest light levels), plants were placed on the shelf but not on the floor. Flowering of plants exposed during the middle of the dark period was reduced by about 62%, but plants exposed at the beginning of the dark period were not influenced at all by that light level.

R Plus FR. Figures 6 and 7 and Table I show results with light produced by 10 and by 4, 25-w, incandescent bulbs and filtered through the two red filters; that is, a mixture of R and FR. At the highest levels (Fig. 6), plants both on the shelf and on the floor were completely inhibited by light given during the middle 2 h of the 16-h dark period. Plants on the shelf were also completely delayed in their initiation of dark measurement, but plants on the floor were delayed only about 37%. Only four incandescent bulbs were used in the experiment of Figure 7. Plants on the shelf were again inhibited 100% by light in the middle of the dark period but delayed only about 75% by light at the beginning of induction. Plants on the floor were inhibited a full 81% in the middle of the dark period but not at all at the beginning of the dark period. Note that the addition of FR requires R (660-nm) levels 6 or 7 times higher, for approximately equivalent inhibitory effects at either time.

FR Alone. Figure 8 and Table I show results with relatively

Table II. *Conditions During Readings of Figures 9 to 11*

Tape No.	Time[a] and Date	Notes on Light Conditions	Phototube Voltage	Light Levels 400 to 800 nm
				$mw\ m^{-2}$
	July 28, 1980			
19	12:40	Full sunlight, sun close to zenith	235	507,000
20	12:55	Full sunlight	235	477,000
	July 26, 1980			
17	14:56	Full sunlight	235	308,000
18	15:09	Full sunlight	235	286,000
	July 26, 1980			
16	14:34	Skylight	279	36,200
21	19:15	Open sky (shade), sun on ground 50 m away	279	10,400
22	19:27	open sky	279	6,640
23	19:39	Sun on top of nearby building, hazy on mountains	279	3,280
24	19:51	Sun nowhere visible, not even on mountains	279	1,080
25	20:03	Added shade around receptor (all but about 90° of sky); twilight	400	121
26	20:15	Can still read, but it's now real twilight	400	11.78
27	20:27	Difficult to read; moon just came over Mt. Logan	400	1.229
28	20:39	Can barely read, Vega and few other stars visible, reading is now close to noise level; barely see cosine receptor	400	0.203
	July 28, 1980			
29	19:31:20	Sun still on high clouds (on Hyrum mountains at beginning), using 90° solid angle shade around cosine receptor	400	4,550
	July 28, 1980			
30	19:43:20	Still light on clouds, but mountain darkening, clouds clearing	400	1,008
31	19:55:20	Getting dark (still quite light), clouds in west fairly dark but clearing a bit	400	180
32	20:07:20	Still easy to read, clouds about gone, some in west, getting dark now	400	20.2
33	20:19:20	Difficult to read, clouds on horizon, clouds gone overhead, bright stars visible	400	1.738
34	20:31:20	Can't read now; really dark	400	0.1092
	July 26, 1980			
35	21:51	Light of the full moon	600 fiber optic probe	0.780
37	22:17	Light of the full moon	400	0.829
38	22:31	Light of the full moon	600	0.879
	July 27, 1980			
39	24:21	Moon close to due south during first scan, elevation 30° above horizon	600 fiber optic probe	0.885
40	24:34	Light of the full moon	600	0.870
41	24:48	Light of the full moon	600	0.890
42	1:00	Light of the full moon	600	0.919
43	1:16	Light of the full moon	600	0.897

[a] Mountain Standard Time at beginning of scan.

high levels of FR. There is no significant effect at any time, neither at the beginning nor in the middle of the inductive dark period, although there is a slight tendency for the plants irradiated at the beginning of the dark period to flower more than controls.

It is clear from Figures 3 to 8 and Table I that plants are always more sensitive during the middle of the dark period than at the beginning, although the unexpected reversal in sensitivities between Figures 3 and 4 make it impossible to state an exact range of R levels that are effective at either time; such a range apparently depends upon conditions of the plant.

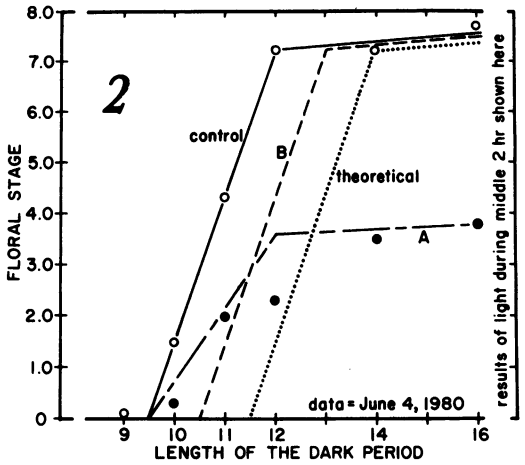

FIG. 2. Generalized form for presentation of the experimental results discussed in this paper. The curve labeled "control" is typical of results obtained by subjecting *Xanthium* plants to a single dark period of various lengths; it is based on an actual experiment performed on June 4, 1980, and its intersection with the abscissa is the critical night (about 9.5 h in this case). The curve labeled "theoretical" is the control curve shifted two hours to the right. Curves A and B represent possible results with light levels during the first 2 h of the inductive dark period that are too bright to be perceived as darkness and too dim to be perceived as full daylight. Curve A is half of the control curve, and curve B is the control curve shifted 1 h to the right. Data points close to curve A represent plants that received dark periods as shown but 2 h of dim R from the 7th to the 9th h after beginning of dark period. Light levels are given in Table I.

Light Levels and Spectra During Twilight and of Moonlight. Beginning with the full moon of February 29, 1980, several attempts were made to measure spectra and light levels during twilight and of the full moon. Figure 9 shows results of one twilight study on July 26, 1980; Figure 10 shows light levels at 660 nm as a function of time for scans on July 26 and 28, 1980; and Figure 11 shows spectra of moonlight during the two nights of July 26, and 27 to 28, 1980. (This is close to the time of year when cocklebures are naturally induced at 40° latitude.) Table II lists conditions during these light measurements, photomultiplier-tube voltages for each spectral scan, and integrated light energies. Times shown in Table II are for beginning of the scans, but integrated light energies during twilight would best express total energy at the middle of the scan (*i.e.*, 5 min after beginning). Note also values for full sunlight falling on the horizontal cosine receptor (Figs. 9 and 10). Readings during twilight were taken in the shade of the Agricultural Science Building (east side) on the Utah State University campus, and many of them (Table II) utilized a shade that allowed only light from a 90° solid angle of sky (inverted cone with zenith at center) to fall on the cosine receptor, shading out light from nearby windows. Readings of moonlight from the fiber-optics probe (shaded to 16° solid angle) varied within about 10%, depending upon whether the probe was aimed exactly at the moon. Inasmuch as curves in Figure 11 represent average values for a given night, they must be slightly low; errors in aiming the instrument could only result in lowered readings.

DISCUSSION AND CONCLUSIONS

The main point of the experiments was to see if light levels effective in inhibiting flowering during the middle of an inductive dark period were lower than the lowest levels effective in delaying the initiation of dark measurement at the beginning of the dark period. In several cases (Figs. 3, 5, and 7), inhibition during the middle of a 16-h dark period was easily observed when the same light levels had little or no effect at the beginning of darkness, and when light caused some delay at the beginning, inhibition in the middle was complete or nearly so (Figs. 3, 4, 6, and 7). This difference in sensitivity is shown in Figure 10, based only on experiments with a mixture of R and FR (R:FR ratios similar to natural twilight and moonlight; experiments with white light approximating skylight would have been appropriate). Preliminary results (22) also suggest that still higher levels are required to influence the patterns of leaf sleep movements (time measurement in circadian rhythms).

Over an appropriate range, the delay in initiation of dark measurement is roughly proportional to light levels and matches the model of curve B in Figure 2. When low levels of light are given during an entire dark period, curves such as curve A in Figure 2 are obtained (21). This is also true when low light levels are given between the 7th and 9th h of darkness (Fig. 2). It is clear that the lowest light levels capable of inhibiting during the most sensitive time do not influence the initiation of dark measurement.

The relationship between initiation of dark timing and subsequent light inhibition remains unclear. Papenfuss and Salisbury (18) showed that light inhibition is not always a matter of stopping and restarting timing of the dark period. This happens only after plants have been in the dark for about 6 h when light does reset the clock. Before then, light seems to inhibit flowering without affecting timing; after, light can apparently both reset timing and otherwise inhibit flowering. At the beginning of darkness, light can obviously delay the initiation of dark measurement. In general, it appears that the clock in some way controls the sensitivity to light, rather than the amount of pigment in any particular form at any given time controlling sensitivity. Perhaps the clock controls the sensitivity of some target system that responds to the pigment. Consider some possible mechanisms of dark detection, along with the other two questions stated in the introduction:

How Does the Plant Detect the Beginning of Darkness? Light and darkness must be detected *via* some pigment system, and it is reasonable to suspect that the system might be phytochrome, although R-FR reversibility at the beginning of darkness has probably not been clearly demonstrated and might be difficult or impossible to show. Based on the well known summary equation for phytochrome functioning, at least four ways can be imagined that phytochrome could control the initiation of dark measurement:

$$\text{Synthesis} \rightarrow \text{Pr} \underset{\text{FR}}{\overset{\text{R}}{\rightleftharpoons}} \text{Pfr} \rightarrow \text{Action}$$

$$\text{Pfr} \rightarrow \text{Destruction}$$

metabolic dark conversion

First. When Pfr falls to some critical level, dark timing might be initiated. As long as the plant is in the light, Pfr is being generated, but when light levels are low enough, Pfr might begin to drop to the critical level, thanks to metabolic dark conversion and/or destruction. This is the traditional view and has been implicit in our thinking since Borthwick *et al.* (2) suggested that dark conversion might provide the timing mechanism in photoperiodism. Indeed, Borthwick *et al.* (2) reported an initial test of the idea: High levels of FR (sunlight through a Corning red purple ultra filter for the last 30 min of the light period) were reported to shorten the critical night by about 2 h (R lengthened it about 30 min). The immediate conversion of Pfr to Pr by FR should remove the necessity of metabolic dark conversion—a conclusion that would apply even if lowering the Pfr level were not the timing mechanism itself but only initiated timing. But Borthwick and coworkers were never able to repeat these results (personal communication), and they are not repeated by the data of Figure 8 in this paper (or by other unpublished experiments of mine and of others). Is it possible that metabolic dark conversion and/or destruction is so rapid (*e.g.*, only 5 to 10 min) that it cannot be

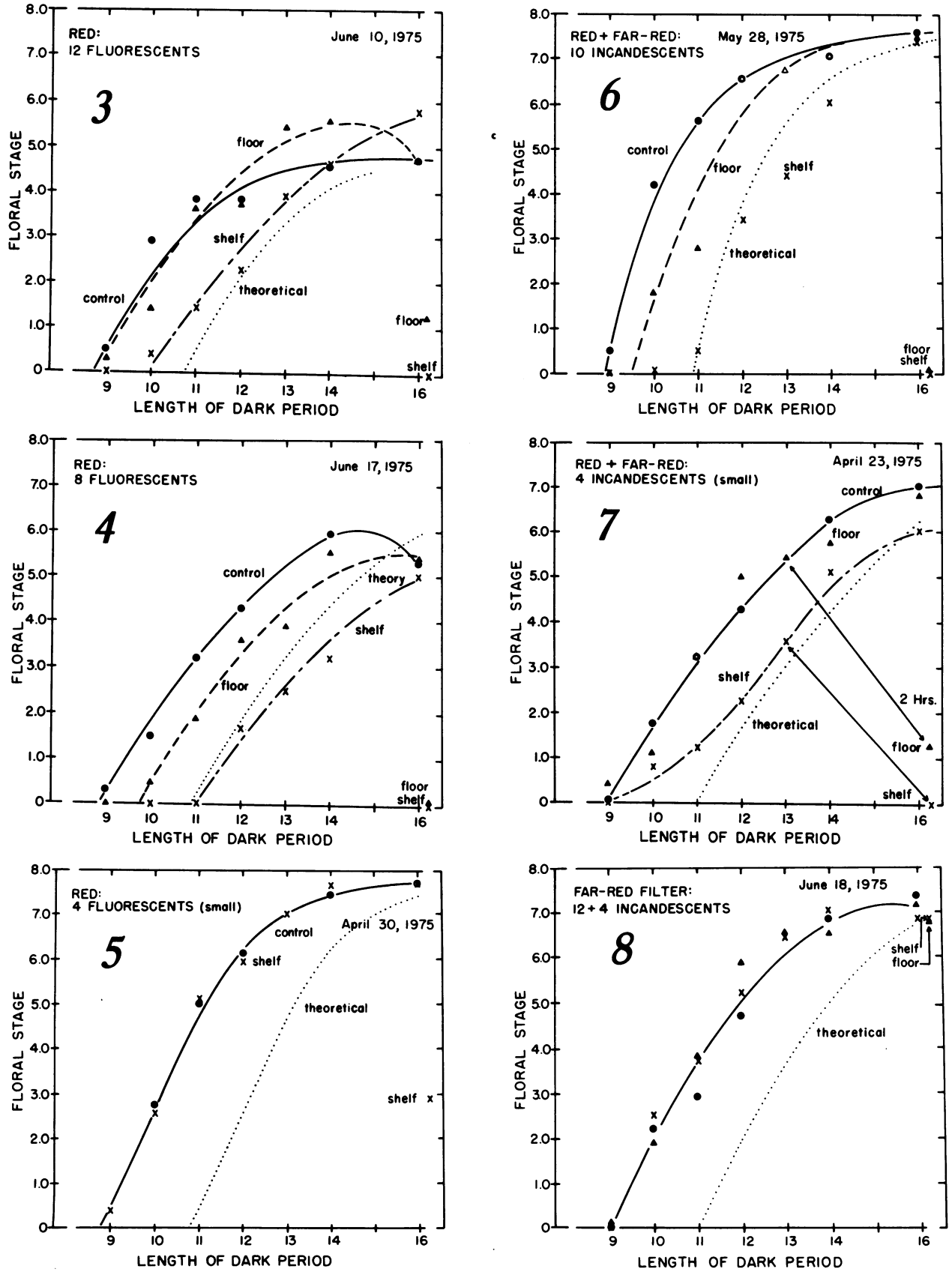

FIG. 3. Results of an experiment in which plants were exposed to two levels of red light (shelf and floor) for either the first 2 h of the inductive dark period or for the middle 2 h of a 16-h dark period, compared with controls not exposed to light but receiving dark periods of comparable lengths. Light levels are given in Table I.

FIG. 4. Results of an experiment similar to that of Figure 3 but with lower light levels.

FIG. 5. Results of an experiment similar to that of Figure 3 but with still lower light levels.

FIG. 6. Results of an experiment similar to that of Figure 3 but with a mixture of R plus FR.

FIG. 7. Results of an experiment similar to that of Figure 6 but with lower light levels.

FIG. 8. Results of an experiment similar to that of Figure 3 but with FR light only. Note relatively high light levels given in Table I.

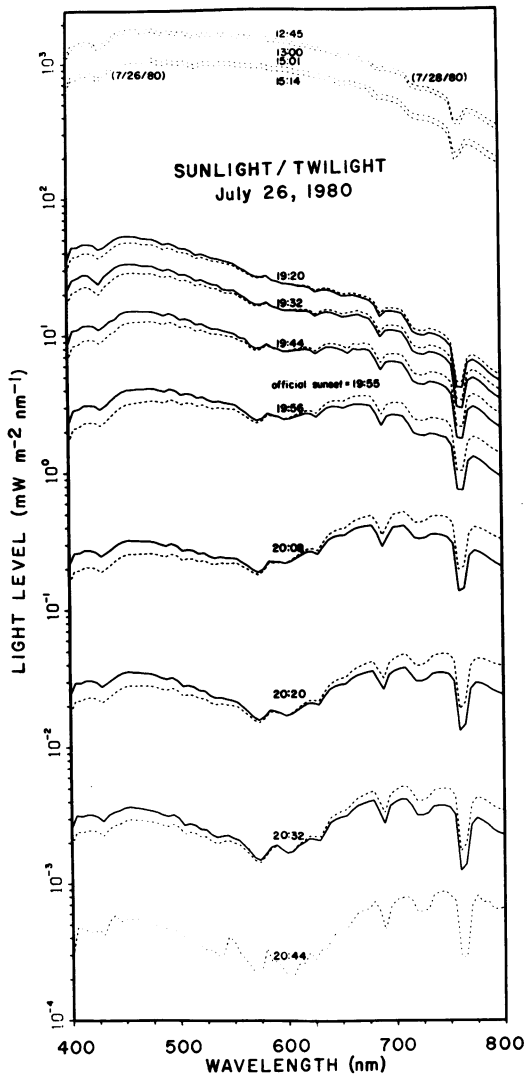

FIG. 9. Spectral distribution of light energies during twilight on July 26, 1980. For comparison, four full sunlight curves have also been included (two measured on July 28, 1980). Measured data (- - -); data corrected for the time during the scan (——). Curves appear relatively flat because of the logarithmic scale required to show seven orders of magnitude (10 million-fold). Times shown are MST; they are given for the middle time of each scan, representing times for the corrected spectral curves. The dips in the curves around 688 and 762 nm are absorption bands of O_2 in the atmosphere, and the band centering at 723 nm is caused by H_2O. See Table II for description of conditions during measurements and for integrated energy values. (Figure computer drawn.)

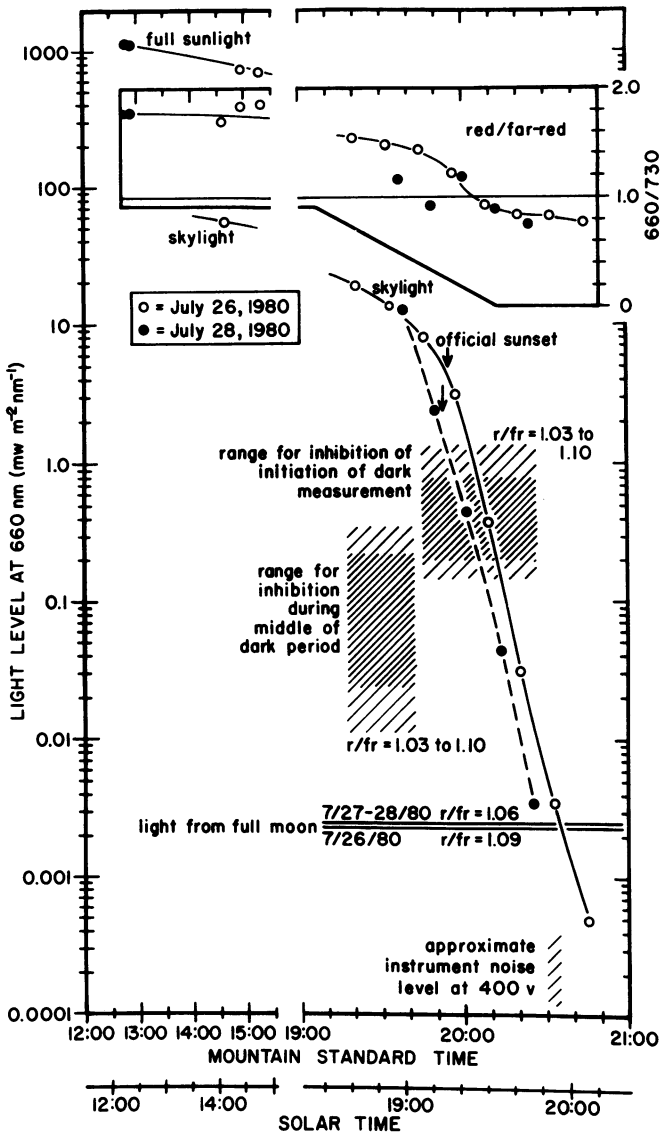

FIG. 10. Light levels at 660 nm as a function of time on July 26 and 28, 1980 at Logan, Utah, including light from a nearly full moon. The cross-hatched areas represent ranges of light levels that inhibit initiation of dark timing or inhibit flowering during the middle of the dark period; they are based on the data of Table I and Figures 6 and 7. The inserted graph shows ratios of light levels at 660 nm to levels at 730 nm for the sunlight and twilight measurements; other R:FR ratios are given as numerals. Note the relatively brief times required for twilight levels to drop through the range of delay of dark measurement. See Table II for description of conditions during measurements and for integrated energy values.

detected by experiments such as those of Figure 8? Or is it necessary to use extremely high levels for short times, rather than the long exposures of Figure 8? More work needs to be done, but direct and indirect measurements suggest that Pfr can drop to low levels in less than 30 to 60 min (*e.g.*, 7, 15, 30).

Exposure to temperatures of 10 C during the first 2 h of a critical dark period experiment delayed initiation of dark measurement (lengthened critical night) by about 55 min, but exposure to 10 C between the 5th and 7th h (again with dark periods of various lengths) had no effect on timing (21). The conclusion is that initiation of dark timing is indeed a temperature-sensitive metabolic process, but dark measurement itself is temperature insensitive—a common feature of circadian-type clocks (*e.g.*, 10 and 30). The temperature-sensitive process that initiates dark measurement could well be conversion and/or destruction of Pfr.

Second. Could the initiation of timing be a response to some critical ratio of $Pfr:P_{total}$? This is apparently true for stem elongation and has been suggested for photoperiodic timing (11, 16, 27). If so, the critical night should be strongly influenced by the light quality just before darkness (*i.e.*, establishment of various phytochrome ratios by light), and there should be some balance of R and FR that would be detected as darkness at any brightness level, at least during the first part of the dark period before sensitivity to light increases as reported in this paper. The "null" experiments in which mixtures of R and FR are sought that have no effect (*e.g.*, 8) provide some support for this idea. Furthermore, light quality just before dark strongly influences flowering in *Pharbitis nil* (R promoting and FR inhibiting; 14, 17) and in Wintex barley (FR promoting; 6). Actually, Figure 8 shows that nearly pure FR

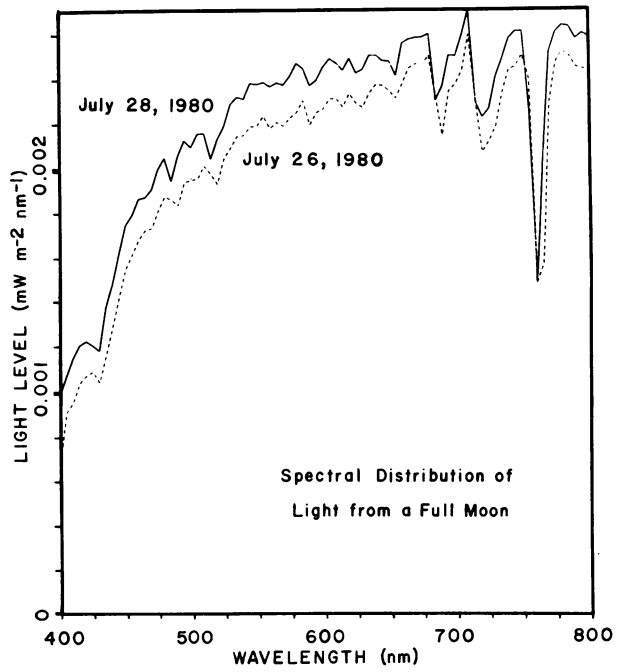

FIG. 11. Spectral distribution of light from the nearly full moon at Logan, Utah, on July 26, 27 to 28, 1980 (moon was full on July 27). The moon was approximately 30° above the southern horizon during the measurements, made with a fiber optics probe. Note that energies are shown on a linear rather than a logarithmic scale. The curves represent averages for three and five scans, as shown in Table II, where integrated energy values are also given. Note the H_2O absorption band at 723 nm and the O_2 bands at 688 and 762 nm. The moon appears somewhat yellow because its reflectance is low in the blue part of the spectrum. (Figure computer drawn.)

is perceived as darkness.

Third. Could the detection of darkness be a slowing in the rate of cycling between Pfr and Pr? This interesting idea has been suggested by Jose and Vince-Prue (12). It is not easy to reconcile with the results presented in this paper, however. When FR is present, levels of R must be higher to achieve a comparable delay in the initiation of dark measurement (Table I), but this should produce a faster rate of cycling than the lower levels of pure R that have equal effectiveness and presumably would push most of the pigment into the Pfr form. These results best agree with a level of Pfr model rather than a cycling model. Furthermore, cycling would probably be rather temperature insensitive, while initiation of dark timing is delayed at low temperatures (see above discussion and ref. 21).

Fourth. Could ratios or cycling rates in some way influence rates of synthesis and/or destruction? The key to understanding might lie somewhere in this area, but more must be learned before the idea can be properly developed.

The above possibilities are based on phytochrome action at low irradiance levels, but the initiation of dark measurement also has characteristics of the so-called HIR. It is (1) dependent on irradiance (requiring several times higher light levels than inhibition in the middle of the dark period), (2) dependent on the R:FR ratio, and (3) not readily reversible. The observations of this paper roughly agree with the model of Schäfer (26), which suggests that a pool of Pfr associated with some subcellular component is required for maintenance of the HIR. Conditions that either increase or stabilize this pool should lead to an increased response. As the pool decreases, dark measurement might be initiated. Inhibition of the initiation of dark measurement by low temperatures (see above discussion) would be understood as slowing the dissipation of the pool at reduced temperatures. Clearly, such an HIR model is closely related to the simple idea of Pfr dropping to some critical level, but if the HIR is involved in dark initiation while only Pfr accounts for inhibition of flowering (7th to 9th h), then the difference in light sensitivity might be explained. Deitzer *et al.* (6) suggest that the HIR is implicated in the photoperiodism responses of Wintex barley.

Results presented here suggest several experiments. For example, various R:FR ratios should be studied in relation to light levels at the beginning of the dark period. It might also be valuable to examine both the time of maximum sensitivity to a light interruption of the inductive dark period as well as critical night as indications of dark timing (15, 20, 30).

When During Twilight does the Plant Change from Its Day to Its Night Mode of Photoperiodic Response? Note in Figure 10 that, on the clear day (July 26, 1980), the R:FR ratio in natural twilight changed somewhat (a relative increase in FR) as official sunset was approached, but note that clouds on July 28, 1980 (Table II) increased the proportion of FR even before sunset (see also 11, 30). Thus, the R:FR ratio may not be dependable at sunset from day to day. (It is desirable to consider the entire spectrum rather than just 660 to 730 nm, but these levels are probably representative.) In any case, ratios for the experiments used to show the range of light levels effective in delaying dark measurement were similar to ratios during twilight.

Depending on how the lines are drawn between twilight points, and considering either the wide or the narrow range of levels effective in inhibiting dark measurement, the transition from day to night mode is surprisingly rapid: only about 5.5 to 11.5 min. This is a fascinating result. The human eye can reliably estimate light levels over the entire seven orders of magnitude from full sunlight to dark night (Fig. 10), but the photoperiodism response of cocklebur plant seems to change from day to night as light changes only about one order of magnitude—in only minutes instead of 2 or 3 h.

Nearly 20 years ago, Takimoto and Ikeda (29) covered plants at various times during both evening and morning twilight, comparing their flowering with plants left uncovered. Thus, they could observe the level of twilight that was perceived by the plant as darkness. They found considerable variation among five short-day species: *Oryza sativa* was relatively insensitive to light both in the morning and in the evening; *Glycine max, Perilla frutescens,* and *Pharbitis nil* were relatively insensitive at dusk but more sensitive at dawn; and *Xanthium saccaratum* (probably a hybrid of imported *X. strumarium* with local varieties) was highly sensitive in the evening and less so in the morning. Light levels were measured as ft-c, and no measurements were made of R:FR ratios. In an earlier paper (28), they also studied effects of clouds during twilight, concluding that clouds might well influence photoperiodic time measurement, but perhaps less so for plants that are most sensitive to light during dusk and/or dawn (the lowest light levels being somewhat less influenced by clouds). There seems to be room for much work of this type, especially if modern light-measuring equipment were used.

Is the Photoperiodic Response of Cocklebur Plants Influenced by Moonlight? As it turns out, the quality of moonlight is not unlike the quality of light just after sunset: lower in blue and enriched in the red end of the spectrum (Fig. 11). Ratios of R:FR were also similar for moonlight (see also ref. 11 and references therein). Figure 10 suggests that maximum levels of moonlight are not high enough to influence flowering in the middle of the dark period, even though sensitivity to light increases by about another order of magnitude at that time, compared to dusk. This must be a tentative conclusion, however, since the experiments were not designed to detect the lowest levels effective during the middle of the dark period (although it would not be difficult to do so).

Because of misgivings about comparing cosine-receptor mea-

surements with fiber optics probe measurements, light from a nearly full moon was again measured on Sept. 25 to 26, 1980, using the cosine receptor and photomultiplier tube set at 600 v. The moon reached an elevation of about 50° at its zenith (1:09 MST), and sky conditions were clear. Light level at 660 nm, averaged for five scans, was 0.00229 mw m^{-2} nm^{-1} on September 25 to 26, compared with 0.00237 and 0.00255 mw m^{-2} nm^{-1} on July 26 and 27 to 28, respectively. Spectra obtained with the cosine receptor (not shown) were higher in the blue and lower in the red parts of the spectrum than spectra obtained with the fiber optics probe, however. This might be because the cosine receptor responded to light from much of the sky, whereas the fiber optics probe responded only to moonlight. Different atmospheric conditions and moon elevations on the two dates might also help account for the differences. More studies are needed.

Actually, values for moonlight in Figures 10 and 11 are close to maximum levels for the summer full moon. Aiming the probe directly at the moon is equivalent to a leaf surface being at right angles to the moon's rays, but of course many leaves would not be so positioned, and many would be shaded by other leaves. Because the moon travels close to the path of the ecliptic, the full moon is often relatively low in the night sky in summer (sun is high at noon in summer) and high at midnight in winter. The full moon near its zenith was only 30° above the southern horizon when the measurements of Figure 11 were made. These considerations provide little support for the suggestion (3, 4) that sleep movements position leaves so they are nearly parallel to rays from the full moon overhead at midnight so leaves are least sensitive to these rays. In summer at northern and southern latitudes, the moon is not overhead, and its intensity is apparently not high enough to influence cocklebur photoperiodism, anyway. Furthermore, cocklebur plants reach maximum sensitivity to light 6 or 7 h after dusk—close to morning in summer; the full moon would be descending in the southwest sky.

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