The major absorption change attributed to the photooxidation of cytochrome, a negative peak at 420 mμ, was the predominant change in *Porphyridium*. A smaller positive peak at 450 mμ, associated with cytochrome, was also observed. A positive change at 395 mμ, attributed to cytochrome, decayed with a significantly shorter half-life than that at 420 mμ. A tentative explanation was advanced.

Several lines of evidence were presented for the similarity of the 520 mμ absorption change observed in *Porphyridium* to the 515 mμ change in *Chlorella*.

Literature Cited

1. ALLEN, F. L. 1955. Observations on photosynthesis and related systems. I. Influence of anaerobiosis on photosynthetic rates during continuous irradiation. Arch. Biochem. Biophys. 55: 38–53.
2. DUYSENS, L. N. M. 1954. Reversible changes in the absorption spectrum of *Chlorella* upon irradiation. Science 120: 353–54.
3. DUYSENS, L. N. M. 1955. Role of cytochrome and pyridine nucleotide in algal photosynthesis. Science 121: 210–11.
4. INSELBERG, E. AND J. L. ROSENBERG. 1963. Interference of emission changes with fast absorption changes in the flash spectroscopy of algae. In: Photosynthetic Mechanisms of Green Plants, p 717–25, Publ. 1145, Natl. Acad. Sci.—Natl. Res. Council, Washington.
5. KOK, B. 1957. Light induced absorption changes in photosynthetic organisms. Acta Botan. Neerl. 6: 316–36.
6. MÜLLER, A., B. RUMBERG, AND H. T. WITT. 1963. On the mechanism of photosynthesis. Proc. Roy. Soc. B 157: 313–32.
7. WITT, H. T. 1955. Kurzzeitige Absorptionsänderungen beim Primärprozess der Photosynthese. Naturwissenschaften 42: 72–73.
8. WITT, H. T., R. MORAW, AND A. MÜLLER. 1959. Blitzlichtphotometrie. Z. Phys. Chem. (NF), 20, ¾: 193–205.

Conditions Determining Effects of Far-Red and Red Irradiations on Flowering Response of Pharbitis nil [1]

H. Fredericq [2]

U. S. Department of Agriculture, Agricultural Research Service, Beltsville, Maryland

Flowering of *Pharbitis nil* Chois. (*Ipomoea nil* L. Roth.) seedlings occurs in short photoperiods but is inhibited by far red at the end of the photoperiod (7, 10). Dependence of this response on conditions during the photoperiod was appreciated but had not been examined. The purpose here was to investigate the conditions during the photoperiod that influence flowering and to examine the nature of phytochrome action both immediately after the photoperiod and at various later times in the dark period.

Materials and Methods

Seed of *P. nil* for these experiments was from a supply used by Nakayama et al. (7). The seeds were macerated for about 40 minutes in concentrated sulfuric acid, then rinsed overnight in water, sown in wet perlite the next morning, and placed in an incubator at 27.5° in darkness. Forty-eight hours later the seedlings were transplanted to Knop's solution containing microelements and ferric citrate.

For the 1962 experiments they were precultivated under continuous light for 2½ days in the greenhouse. The natural light was supplemented at night with incandescent-filament light, following the procedure of Nakayama et al. (7). For the 1963 experiments, to which the tables of this paper refer, the precultivation was done in a growth room with daily cycles of 10 hours of fluorescent light (1,000 ft-c) and 14 hours of incandescent light (80 ft-c). With these modifications seedling growth and chlorophyll formation were improved. The precultivation under continuous light was separated from the first inductive cycle by a short night of 7 hours. After 3 inductive cycles the seedlings were returned to the greenhouse, where they received continuous light until the flowering response was measured.

The average number of flower buds per plant and the number of plants bearing terminal buds, which are formed only under optimal conditions of induction, were recorded. There were 9, 12, or 18 plants per treatment. Control plants grown under continuous light after germination in the dark all remained strictly vegetative. Temperatures during the inductive cycles were 25° during the photoperiods and 22° during the nights.

The far-red radiation was obtained by filtering

[1] Received Feb. 14, 1964.

[2] Permanent address: Laboratory of Plant Physiology, Ledeganckstraat, 39, Ghent, Belgium.

the light of four 150-w incandescent flood lamps through 5 cm of water and through 2 layers each of red and dark-blue cellophane. The irradiance was 20 $\mu w/cm^2/m\mu$ at 740 $m\mu$ (tables I–V) and 40 μw (tables VI–VII). Four layers of blue cellophane instead of two were used for the latter. The higher energies used in the experiments of tables VI and VII were obtained by decreasing the distance between the plants and the radiation source. The red radiation was about 60 $\mu w/cm^2/m\mu$ at 660 $m\mu$. It was obtained by filtering light from standard cool-white fluorescent lamps through 2 layers of red cellophane.

Experimental Results

Far Red at the Beginning of Dark Periods of Various Lengths. Results of a typical experiment (table I) show flowering of control plants to be completely induced with formation of a typical terminal bud on photoperiods of 2 to 10 hours. Four minutes of far red at the beginning of the dark period completely suppressed flowering induced by a 2-hour photoperiod but was less effective for longer photoperiods. The inhibition was reversed by red. Higher far-red irradiances applied after 8-hour photoperiods were also ineffective. Flowering was promoted by far red at the end of 12-hour photoperiods and this effect was reversed with red as had been observed under similar conditions by Takimoto and Naito (11).

The far-red irradiations enhanced elongation of hypocotyls, reduced the angle between the cotyledonary petioles, and inhibited anthocyanin formation in hypocotyls and petioles. These effects were reduced as the photoperiod increased but were always of the same type in contrast to flowering, which was qualitatively opposite, changing from suppression to induction.

Light Intensity during the Photoperiod. The inhibition of flowering by far red at the end of a 4-hour photoperiod was enhanced as the intensity of the light during the photoperiod was decreased (table II). This effect was also observed with 8-hour photoperiods but only when the illuminance was lowered to 100 ft-c, which was adequate for full induction of controls. The effect was reversed with red.

Far Red at Various Times after the Beginning of the Dark Period. Results in tables III and IV show the same general trend. Far red is gradually less inhibitory after increasing intervals of darkness or low intensity of fluorescent light (3 ft-c illuminance)

Table I. *Effect of 4 Minutes of Far Red at End of Various Daily Photoperiods on Flowering of Pharbitis nil*

The illumination during the photoperiod was 2,000 ft-c from cool-white fluorescent lamps.

Daily photoperiod (hr)	Mean flower buds per plant (number)		Plants with terminal flower bud (%)	
	Control	FR	Control	FR
0.5	1.6	0.0	0	0
1	4.7	0.0	45	0
2	5.6	0.2	100	0
3	5.4	2.1	100	0
4	6.1	4.2	100	18
6	6.5	6.4	100	96
8	6.5	6.8	100	91
10	6.1	6.5	100	97
11	4.2	4.6	0	0
12	0.3	2.6	0	0
13	0.0	0.0	0	0

Table II. *Effect of 4 Minutes of Far Red at End of Various Daily Photoperiods on Flowering of Pharbitis nil*

The illumination during the photoperiod was from cool-white fluorescent lamps.

Length and illuminance of photoperiod	Mean flower buds per plant (number)		Plants with terminal flower bud (%)	
	Control	FR	Control	FR
4 hours:				
1,700 ft-c	6.3	5.8	100	67
900 ft-c	6.2	3.3	100	0
400 ft-c	6.3	0.0	100	0
8 hours:				
1,700 ft-c	6.6	6.7	100	100
900 ft-c	6.3	6.3	100	100
400 ft-c	6.3	6.3	100	100
12 hours:				
1,700 ft-c	1.2	3.3	0	0
900 ft-c	1.1	4.0	0	0
400 ft-c	1.3	3.4	0	0

Table III. *Effect of 4 Minutes of Far Red at Various Times after Daily 2-Hour Photoperiods on Flowering of Pharbitis nil*

The illumination during the photoperiod was 1,600 or 800 ft-c from cool-white fluorescent lamps.

Treatment after and illuminance during 2-hour photoperiods	Mean flower buds per plant (number)		Plants with terminal flower bud (%)	
	Control	FR	Control	FR
FR immediately:				
1,600 ft-c	5.8	0.0	100	0
800 ft-c	5.7	0.0	100	0
FR after 3 hours of darkness:				
1,600 ft-c	5.8	1.2	100	0
800 ft-c	5.7	0.3	100	0
FR after 6 hours of darkness:				
1,600 ft-c	5.8	5.7	100	89
800 ft-c	5.7	3.0	100	22
FR after 8 hours of darkness:				
1,600 ft-c	5.8	5.9	100	100
800 ft-c	5.7	5.2	100	67
FR after 11 hours of darkness (= middle of 22-hour night):				
1,600 ft-c	5.8	6.2	100	100
800 ft-c	5.7	6.1	100	100

Table IV. *Effect of 4 Minutes of Far Red at Various Times after Daily 2-Hour Photoperiods on Flowering of Pharbitis nil*

The illumination during the photoperiod was 1,400,900, or 400 ft-c from cool-white fluorescent lamps.

Treatment after and illuminance during 2-hour photoperiods	Mean flower buds per plant (number)		Plants with terminal flower bud (%)	
	Control	FR	Control	FR
FR immediately:				
1,400 ft-c	5.8	0.0	100	0
900 ft-c	6.2	0.0	100	0
400 ft-c	5.5	0.0	82	0
FR after 6 hours of darkness:				
1,400 ft-c	5.8	2.8	100	11
900 ft-c	6.2	0.7	100	0
400 ft-c	5.5	0.0	82	0
FR after 6 hours of 3 ft-c fluorescent light:				
1,400 ft-c	5.8	3.9	100	30
900 ft-c	5.8	1.1	100	0
400 ft-c	5.3	0.2	100	0

used to maintain phytochrome in the P_{fr} form. This decrease depends strongly on the light intensity during the photoperiod, being most pronounced at the higher intensities.

Other Conditions Influencing Inhibitory Action of Far Red. Effects of far red at the beginning of dark periods were more pronounced following photoperiods in sunlight than in fluorescent light of similar illuminances (table V). Irradiation in a growth room with carbon arcs and supplementary incandescent-filament lamps gave results similar to sunlight.

When far red followed a single inductive photoperiod of 8 hours, flowering was inhibited, but when it followed each of 2 or more daily 8-hour photoperiods, it was without apparent effect. Two or more successive inductive photoperiods caused normal flower-bud development, but a single one led to suboptimal production. All the above-mentioned experiments were done with 5-day-old seedlings precultivated under continuous light for 2½ days. Flowering in them was not inhibited by far red either at the end of 8-hour photoperiods of high-intensity fluorescent light or in the middle of the 16-hour night.

Eight-day-old seedlings precultivated for 5½ instead of 2½ days, however, were more sensitive to far red; 4 minutes inhibited rather strongly at the end of 8-hour periods of high intensity and also in the middle of the night.

Reversibility in the Middle of the Night. Substantial repromotion of flowering occurred when a 30-second far-red irradiance immediately followed an inhibitory 30-second red one in the middle of 16-hour nights (table VI). Repromotion failed when longer irradiations were used and when 3 minutes of darkness was inserted between ½-minute irradiations. Both 5- and 8-day-old seedlings were equally well reversed. Inhibition by far red at the beginning of darkness was evident only in the 8-day seedlings.

Reversal of response to 30-second irradiances of red and far red in the middle of 22-hour nights did not occur but was successfully repeated in the middle

Table V. *Effect of 4 Minutes of Far Red at End of Daily Photoperiods of Different Lengths and Kinds of Light on Flowering of Pharbitis nil*

The fluorescent illumination was 1,700 ft-c from cool-white fluorescent lamps. The plants in the greenhouse were shaded to reduce the natural illumination to about 1,700 ft-c.

Length of photoperiod and kind of light	Mean flower buds per plant (number)		Plants with terminal flower bud (%)	
	Control	FR	Control	FR
2 hours:				
Fluorescent	6.3	0.0	100	0
Natural	6.0	0.0	92	0
3 hours:				
Fluorescent	6.0	2.0	100	0
Natural	6.1	0.8	100	0
4 hours:				
Fluorescent	6.2	6.4	100	67
Natural	5.9	3.3	100	0
6 hours:				
Fluorescent	6.0	7.1	100	100
Natural	6.3	4.5	100	25
8 hours:				
Fluorescent	...	...	...	...
Natural	6.5	5.1	100	44

Table VI. *Effect of Alternate Red and Far-Red Irradiations in Middle of Daily 16-Hour Dark Periods on Flowering of Pharbitis nil*

The illumination during the photoperiod was 1,700 ft-c from cool-white fluorescent lamps.

Duration and kind of irradiation in middle of night	Mean flower buds per plant of indicated age (number)		Plants of indicated age with terminal flower buds (%)	
	5 days	8 days	5 days	8 days
Control	6.6	7.6	100	92
30 sec of R	0.7	0.3	0	0
30 sec of R + 30 sec of FR	3.7	3.8	0	0
1 min of R	0.0	0.0	0	0
1 min of R + 1 min of FR	1.4	2.6	0	0
2 min of R	0.0	0.0	0	0
2 min of R + 2 min of FR	0.0	0.8	0	0
30 sec of R + 3 min of darkness + 30 sec of FR	0.4	...	0	...
4 min of FR at end of photoperiod	6.7	3.3	100	0

Table VII. *Effect of Alternate Red and Far-Red Irradiations on Flowering of Pharbitis nil*

The illumination during the photoperiods was 2,000 ft-c from cool-white fluorescent lamps. Photoperiodic cycles were 8 hours light, 16 hours dark and 2 hours light, 22 hours dark.

Duration and kind of irradiation in middle of night	Mean flower buds per plant of indicated age (number)		Plants of indicated age with terminal flower buds (%)	
	5 days	8 days	5 days	8 days
After 8-hour photoperiods:				
Control	6.8	7.6	100	88
30 sec of R	1.0	0.5	0	0
30 sec of R + 30 sec of FR	6.3	3.5	67	0
After 2-hour photoperiods:				
Control	5.9	6.7	100	89
30 sec of R	1.3	0.0	0	0
30 sec of R + 30 sec of FR	1.6	0.0	0	0
4 min of FR at end of 8-hour photoperiod	...	3.9	...	0
4 min of FR at end of 2-hour photoperiod	0.9	0.0	0	0

of 16-hour ones (table VII). Control plants were fully induced by 2- and 8-hour photoperiods.

Discussion

Far red applied immediately after photoperiods of different lengths is inhibitory after very short ones (2–4 hours), has no obvious effect after intermediate ones (8–10 hours), and is promotive after longer ones (11–12 hours). These effects result from conversion of P_{fr} to P_r. The inhibitory effects of far red after very short photoperiods indicate that the P_{fr} has not completed its flower-promoting function at the close of photoperiod and must continue to act during the first part of the dark period. This explanation is supported by the fact that the far-red inhibition is fully reversed by red at the beginning of the dark period, which reestablishes P_{fr}. It is also considerably less effective if a few hours of darkness intervene before far red is applied. Moreover, attempts to induce flowering in seedlings grown in darkness on sucrose failed (12) or were only partially successful (9). These observations are in agreement with results obtained in a somewhat different way for flowering of *Chenopodium amaranticolor* (6). A similar action of far red on tuberization of *Begonia evansiana* has also been described (5).

The requirement for the presence of P_{fr} phytochrome during the first part of each inductive cycle has been demonstrated for *Chenopodium rubrum* (3). However, several of our results indicate that the need for P_{fr} in the beginning of each dark period depends on light conditions occurring during the preceding photoperiods. Thus, the loss of inhibition when far red is applied 6 hours instead of immediately after the end of the 2-hour photoperiod is less rapid if the intensity is lower during the 2-hour period (tables III–IV). It is possible that the dark reversion of P_{fr} to P_r, which takes several hours (2), may be dependent upon the amount of substrates synthesized. The degree of inhibition by far red at the end of 4-hour photoperiods is also strongly dependent upon the light intensity during these periods (table II). Finally, far red given immediately after an 8-hour period of red light had no effect if the intensity of the red light was high but inhibited if the intensity was moderate. These last 2 points indicate that some other factor related to the light intensity and independent from time is also very important; possibly the amount of substrate(s) with which phytochrome reacts could be involved here.

The lack of reversibility of flowering by red and far red in the middle of the dark period, reported earlier for *Pharbitis* (7) and *Lemna* (8), appears to be due to very rapid action of P_{fr} instead of some peculiar qualitative difference between *Pharbitis* and other short-day species such as *Xanthium*. Reversibility was still present in *Xanthium* (4) after 15 minutes, but in *Pharbitis* it disappeared in more than 1 but less than 4 minutes (table VI).

The inhibitory effects of far red at the beginning of the dark period were also observed in *Kalanchoe blossfeldiana* and here, as in *Pharbitis,* the inhibition was less after 8 hours than after shorter photoperiods. This inhibition was also reversible by red as it was in *Pharbitis*. Conditions that led to strong far-red inhibition at the end of the photoperiod were ones that led to failure of reversibility in the middle of the night in both species. Recent experiments of Borthwick and Downs (1) show that *Xanthium* also reacts in this peculiar *Pharbitis* way if the photoperiod is shortened or the light intensity lowered.

Summary

Far red at the end of 2- or 4-hour photoperiods inhibits flowering. It is also inhibitory after 8-hour ones if the intensity of illumination during the photoperiod is low. This inhibition by far red at the beginning of darkness results from conversion of P_{fr} to P_r before the P_{fr} has completed a necessary flower-promoting function. The inhibition by far red at the beginning of the dark period is completely and repeatedly reversible.

Far-red reversibility of flowering in the middle of the dark period is successful if the red and far-red irradiances are no more than 30 seconds each and are not separated by darkness. Failure of reversal occurs when longer irradiances or a 3-minute dark period between two 30-second irradiances are used. Very rapid action of P_{fr} probably accounts for the previous failures of others to obtain reversal.

Acknowledgments

I wish to thank Dr. Borthwick and Dr. Hendricks, and the other physiologists of Beltsville for their valuable suggestions and constant interest in my experiments. I thank the United States Educational Foundation in Belgium for the award of a Fulbright Travel Grant.

Literature Cited

1. Borthwick, H. A. and R. J. Downs. 1964. The roles of active phytochrome in the control of flowering of *Xanthium pensylvanicum*. Botan. Gaz.
2. Butler, W. L., H. C. Lane, and H. W. Siegelman. 1963. Nonphotochemical transformations of phytochrome in vivo. Plant Physiol. 38: 514–19.
3. Cumming, B. G. 1963. Evidence of a requirement for phytochrome-P_{fr} in the floral initiation of *Chenopodium rubrum*. Can. J. Botany. 41: 901–26.
4. Downs, R. J. 1956. Photoreversibility of flower initiation. Plant Physiol. 31: 279–84.
5. Esashi, Y. 1963. Studies on the formation and sprouting of aerial tubers in *Begonia evansiana* Andr. VIII. Dual effect of near infrared. Plant Cell Physiol. 4: 135–43.
6. Könitz, W. 1958. Blühhemmung bei Kurztagpflanzen durch Hellrot-und Dunkelrotlicht in der Photo- und Skotophilen Phase. Planta 51: 1–29.
7. Nakayama, S., H. A. Borthwick, and S. B. Hendricks. 1960. Failure of photoreversible control of flowering in *Pharbitis nil*. Botan. Gaz. 121: 237–43.
8. Purves, W. K. 1961. Dark reactions in the flowering of *Lemna perpusilla* 6746. Planta 56: 684–90.
9. Takimoto, A. 1960. Effect of sucrose on flower initiation of *Pharbitis nil* in aseptic culture. Plant Cell Physiol. 1: 241–46.
10. Takimoto, A. and K. Ikeda. 1960. Studies on the light controlling flower initiation of *Pharbitis nil*. VII. Light-break. Botan. Mag. (Tokyo) 73: 341–47.
11. Takimoto, A. and Y. Naito. 1962. Studies on the light controlling flower initiation of *Pharbitis nil*. IX. Further studies on the effect of far-red preceding the inductive dark period. Botan. Mag. (Tokyo) 75: 205–11.
12. Tashima, Y. and S. Imamura. 1953. Flower initiation in total darkness in *Pharbitis nil*, a short-day plant. Proc. Japan Acad. 29: 581–85.

Interaction of Growth Substances in Growth and Organ Initiation in the Embryos of Capsella [1, 2]

V. Raghavan

Department of Botany, University of Malaya, Kuala Lumpur, Malaysia

The morphological changes that accompany progressive differentiation in plant embryos have been characterized for a number of species, but the nature of the factors that regulate these changes is largely unknown. In an earlier work on embryos of *Capsella* it was shown that normal growth and development in culture of small globular embryos ($<80\ \mu$ long) were regulated by the presence in the medium of a balanced mixture of IAA, kinetin and adenine sulfate (6). For the normal differentiation of heart-shaped and older embryos ($>80\ \mu$ long) a requirement for exogenous growth substances was not observed; however, when grown in media supplemented with suitable concentrations of IAA, gibberellic acid (GA), kinetin or adenine sulfate, these embryos showed increased growth and characteristic morphogenetic responses like elongation of the primary root, hypocotyl and cotyledons, expansion of the embryonic leaves and callus growth (7). In further work it was found that the pattern of growth and organ initiation in embryos grown in media supplemented with combinations of IAA and GA, IAA and kinetin, and GA and kinetin was modified to some extent; the results of these studies are presented in this paper. Attention is given to the influences of the different combinations of growth substances on the morphogenesis of the embryos, and on the tolerance, stimulation or inhibition in growth of the embryonic organs.

Materials and Methods

Embryos of *Capsella bursa-pastoris* Medic. were obtained from plants grown in the greenhouse in a mixture of equal parts of sand, perlite and leaf mold under a supplementary incandescent illumination of ca. 200 ft-c light given during the daytime. The composition of the culture medium and methods of culture were similar to those described previously

[1] Received Dec. 17, 1963.

[2] Work performed at the Biological Laboratories, Harvard University, Cambridge 38, Mass., with a grant from the National Science Foundation (G-18684) to Professor John G. Torrey. Appreciation is expressed to Professor Torrey for his interest in this work.