# Effect of Temperature and Preconditioning on Photoperiodic Response of Pharbitis nil [1, 2]

**Atsushi Takimoto** [3] **and Karl C. Hamner**
**Department of Botany and Plant Biochemistry, University of California, Los Angeles**

In both short-day and long-day plants, endogenous rhythms apparently are involved in photoperiodic response. One line of the evidence is a rhythmic flowering response to cycle length (1, 3, 4, 6, 9), and another is the rhythmic effectiveness of light breaks given at different points in very long dark periods (2, 4, 6, 8, 10).

*Pharbitis nil*, strain Violet, is an extremely sensitive short-day plant. A single dark period of 16 hours is sufficient to induce a maximum flowering response, and further increases in the dark period do not change the response. A brief exposure to light may inhibit flowering when it is given 6 to 12 hours after the beginning of a long dark period, but has no effect at any other points (11). Thus, it seems that there has been no evidence to indicate that endogenous rhythms were involved in the photoperiodic response of *Pharbitis nil*. Most of the experiments with *Pharbitis nil*, however, have been done at optimal temperatures, i.e., at 25 to 30°. At these temperatures, all plants develop terminal flower buds with a single dark period of 16 hours. Therefore, even if further increases in the length of the dark period caused fluctuations in the photoperiodic stimulus, the number of flower buds formed at optimum temperatures might not reflect these fluctuations since the measure of the photoperiodic response (number of flower buds) reaches an upper limit (formation of a terminal flower bud). It was thought worthwhile to investigate the photoperiodic response of *Pharbitis nil* at suboptimal temperatures. At lower temperatures, it was expected that a single long dark period would not be enough to induce the maximum flowering response, and a more detailed photoperiodic response might be recognized.

The first half of the present experiments was designed to investigate the effect of temperature on photoperiodic response of *Pharbitis nil* exposed to very long dark periods. The last half of the experiments was carried out at suboptimal temperatures, and designed to determine whether or not endogenous rhythms are involved in the photoperiodic response. Endogenous rhythms are known to fade away when plants are exposed to continuous illumination or continuous darkness for long times, but should be reset by light-off or light-on signals (2, 4, 7). Therefore, special consideration was given to preconditioning which may initiate endogenous rhythms.

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[3] Permanent address: Laboratory of Applied Botany, Faculty of Agriculture, Kyoto University, Kyoto, Japan.

## Materials and Methods

Seedlings of *Pharbitis nil*, strain Violet, were used for all experiments. To obtain uniform germination, the seeds were treated with concentrated sulfuric acid for 25 to 30 minutes, washed thoroughly in running water overnight, and spread on moistened sand in Petri dishes. They were then kept at room temperature for about 24 hours; at this time the radicle had emerged. The sprouted seeds were selected for uniformity, and planted in 10 × 10 × 6 cm plastic pots filled with soil. The soil used in the present experiments consists of 30 % vermiculite, 7.5 % peat moss, 7.5 % sand, and 55 % compost soil. Fertilizer (200 g super phosphate, [contains 20 % available phosphoric acid, 51 % anhydrous calcium sulphate to provide 12 % sulfur] 30 g potassium sulphate and 200 g fish meal were mixed with 16 gallons of soil) was also mixed with the soil.

Nine seeds were planted in each pot, and the pots were placed in a temperature-controlled room. The temperature was maintained at 30 to 32° during the first 24 to 30 hours, at the end of which all seedlings had appeared above ground. After this, temperature was maintained at 20 ± 1°. The plants were exposed to continuous illumination of about 400 ft-c from fluorescent lamps. Four days after planting, the plants were subjected to experimental treatments. After the experimental treatments, the plants were placed under continuous illumination of about 400 ft-c from fluorescent lamps at 20° for at least 24 hours, and subsequently transferred to benches in the greenhouse. In the greenhouse the plants were exposed to 18-hour photoperiods by supplementing daylight with incandescent light of about 50 ft-c at night. Temperature in the greenhouse varied with season and ranged from 15 to 35°. About 2 weeks after the treatment, plants were harvested and dissected to determine the number of flower buds initiated. All of the dark treatments were given in small cabinets placed in temperature-controlled rooms. Tempera-

ture in the dark rooms fluctuated 2°. However, in the cabinets it varied only 0.2°.

Artificial light was used exclusively from the start of the germination to at least 24 hours after the end of the experimental treatments. Lighting used in all of the present experiments was obtained from cool white fluorescent lamps. Light intensity at the leaf surfaces was about 400 ft-c, and the temperature during the light period was 20° in all of the experiments. In some experiments, red light was used. The red light was obtained from Gro-lux fluorescent lamps filtered with 2 layers of red cellophane, and in all of the experiments presented here, the intensity was about 3300 ergs/cm²/sec at the leaf surfaces. The plants were exposed to red light in the dark room in which the cabinets for dark treatments were placed. Therefore, the temperature during the red light illumination was the same as that during the dark period.

In all experiments, 18 to 27 plants were used for each treatment, and the average number of flower buds per plant was used as an indicator of the photoperiodic responses.

## Experimentation and Results

*Photoperiodic Response of Pharbitis nil at Various Temperatures.* All of the plants used in Experiments 1, 2, 3 and 4 were exposed to continuous illumination at 20° prior to the experimental dark treatments.

Experiment 1: Plants were subjected to a single dark period of various lengths at various temperatures. Results are summarized in figure 1. The lower the temperature, the longer the dark periods required to initiate flower buds, and flowering responses increased almost linearly with increasing duration of the dark period. At 18°, even a dark period of 48 hours was not enough to induce maximum flowering response. None of the plants subjected to a single long dark period at 15° initiated flower buds.

Experiment 2: Since a slight difference in temperature caused significant differences in flowering responses, particularly at the temperatures of 17 to 20°, in the following experiments the temperatures were controlled very carefully. Plants were subjected to a 24-hour dark period at 20 and 25°, and were exposed to red light for 5 minutes at different times in the dark period. Flowering responses are shown in figure 2. At both temperatures, red light given 8 hours after the beginning of the dark period was most effective in inhibiting flowering, and the inhibition curve was more sharp at 25° than at 20°.

Experiment 3: Plants were subjected to a 48-hour dark period at various temperatures, and were exposed to red light for 5 minutes at different times. From the results shown in figure 3, it is clear that at any temperature red light given 8 hours after the beginning of the dark period is most inhibitory, and the inhibitory effect is increased by lowering the temperature. Five minutes of red light given in the last half of the 48-hour dark period had no effect at temperatures of 20° or higher, but had some inhibitory effect at 18 and 18.5°. At higher temperatures, all

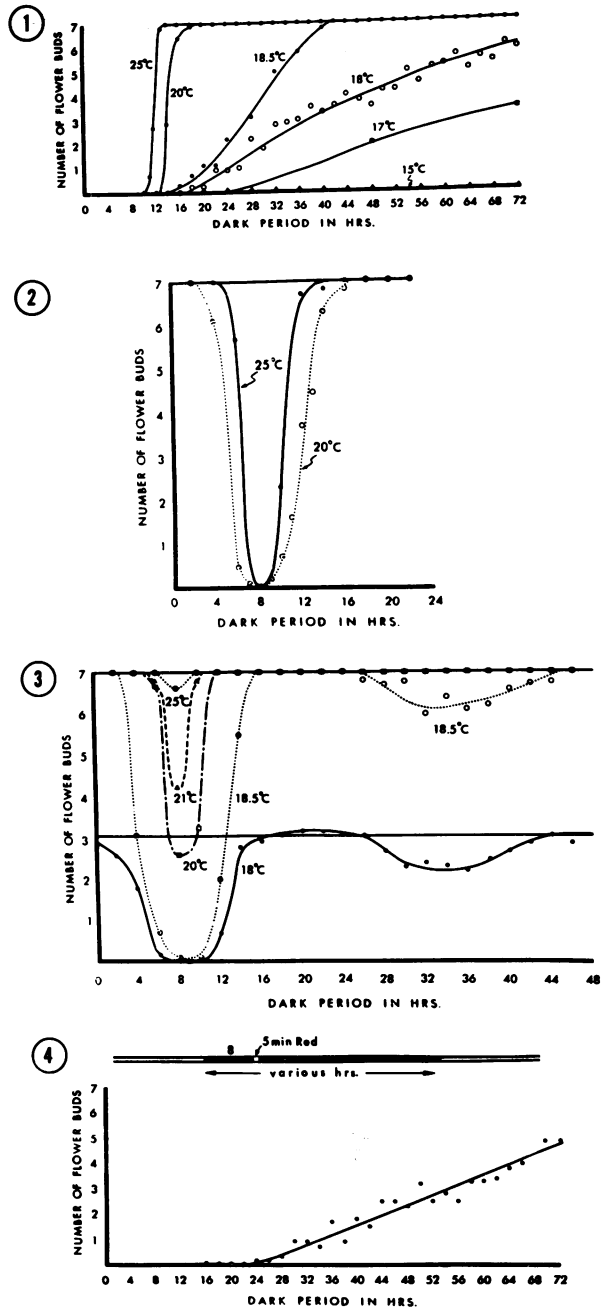


FIG. 1. Flowering response of *Pharbitis nil* exposed to a single dark period of various durations at different temperatures. The plants were exposed to continuous illumination before the dark treatments.

FIG. 2 and 3. Flowering responses of *Pharbitis nil* at 2 different temperatures when exposed to 5 minutes of red light at different times in a 24- or 48-hour dark period. The plants were exposed to continuous illumination before the dark treatment.

FIG. 4. Flowering response of *Pharbitis nil* exposed to single dark periods of various durations which were interrupted with 5 minutes of red light 8 hours after the beginning of each dark period. The plants were exposed to continuous illumination before the dark treatment, and dark-period temperature was 20°.

of the control plants which were not exposed to red light initiated terminal flower buds. Flower inhibition caused by the red light which was given in the last half of the 48-hour dark period was apparently masked at higher temperatures because the response was saturated.

Experiment 4: Five minutes of red light given 8 hours after the beginning of the dark period inhibited flower initiation completely at 20 and 25° when the dark period was 24 hours (Experiment 2), but not completely at those temperatures when the dark period was 48 hours (Experiment 3). The inhibitory effect of red light given 8 hours after the beginning of the dark period varies with the length of the dark period. In the present experiment the relation between the flower inhibitory effect of red light given 8 hours after the beginning of the dark period and the length of the dark period were investigated. Plants were subjected to a dark period of various lengths at 20°. All of the plants were exposed to red light for 5 minutes 8 hours after the beginning of the dark periods. As shown in figure 4, flowering responses increased almost linearly with increasing duration of dark periods beyond 24 hours.

*Effect of Preconditioning on Photoperiodic Response of Pharbitis nil.* The flowering response of *Pharbitis nil* increased nearly linearly over a certain range with increasing duration of the dark period at any temperatures when the plants were kept under continuous illumination before the dark period (fig. 1). There was no indication to show the participation of endogenous rhythms in photoperiodic response of *Pharbitis nil.* Endogenous rhythms, however, may fade away under continuous illumination and may be reset by light-on or light-off signals. In the following experiments, various kinds of light-on and -off signals were given prior to a main dark period.

Experiment 5: One group of the plants (A) was kept under continuous illumination before the dark period of various lengths. Another group (B) was subjected to an 8-hour dark period followed by an 8-hour light period preceding the main dark period which was of various lengths. Temperature during the dark period was 18°. Flowering responses are shown in figure 5. Flowering responses of group A increased linearly with increasing duration of the dark period. However, those of group B increased stepwise. In group B, very sharp increases in the flowering response were observed when the dark periods were between 40 and 46 hours and also for dark periods between 62 and 68 hours in duration.

Experiment 6: Another experiment similar to Experiment 5 was carried out. In this experiment, one group of the plants (A) was exposed to an 8-hour dark period, a 12-hour light period and then to the main dark period of various lengths. Another group (B) was exposed to an 8-hour dark period, an 8-hour light period, and then to the main dark period of various lengths. The results shown in figure 6 are very similar to those in Experiment 5. The flowering responses of group A increased sharply with increasing duration of the dark period from 36 to 40 and from 60 to 64 hours, but those of group B did so with increasing duration of the dark period from 40 to 42 and from 62 to 68 hours.

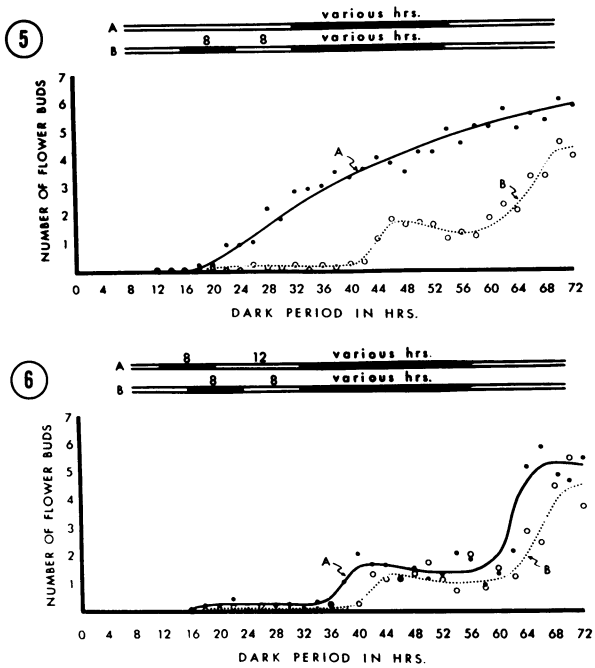


FIG. 5 and 6. Flowering response of *Pharbitis nil* at 18°, exposed to a single dark period of various durations preceded by different light conditions. Light conditions preceding the main dark period are shown diagrammatically.

In both groups, sharp increases in the flowering responses were observed 48 to 52 and 70 to 76 hours after the beginning of the preceding light period. It is considered that the 8 hours of dark and 8 to 12 hours of light given before the main dark period initiated some endogenous rhythm which affects the photoperiodic response of *Pharbitis nil.*

Experiment 7: Three groups of the plants were subjected to an 8-hour dark period followed by 8-, 10- or 12-hour light periods. Thereafter, all groups were placed in darkness for 48 hours and exposed to 5 minutes of red light at different times in the first 18 hours of the dark period. The dark temperature was 19°. Another group, which served as control, was kept under continuous illumination before the 48-hour dark period and exposed to 5 minutes of red light at different times in the first 18 hours of the dark period. Flower inhibitory effects of the red light are shown in figure 7.

Another experiment similar to that mentioned above was carried out, but in this experiment, 2, 4 and 6 hours of light were given before the 48-hour dark period instead of 8, 10 and 12 hours of light, and dark-period temperature was 18.5° (fig. 8).

Figures 7 and 8 show that the time of effectiveness of the red light interruption was influenced by pre-

conditions. The times at which red light was inhibitory were somewhat delayed and extended with decreasing duration of the light period preceding the main dark period. However, when only 2 hours of light was given before the dark period, 5 minutes of red light given in the first 18 hours of the 48-hour dark period did not inhibit flowering at any point, but was, instead, stimulatory to flowering.

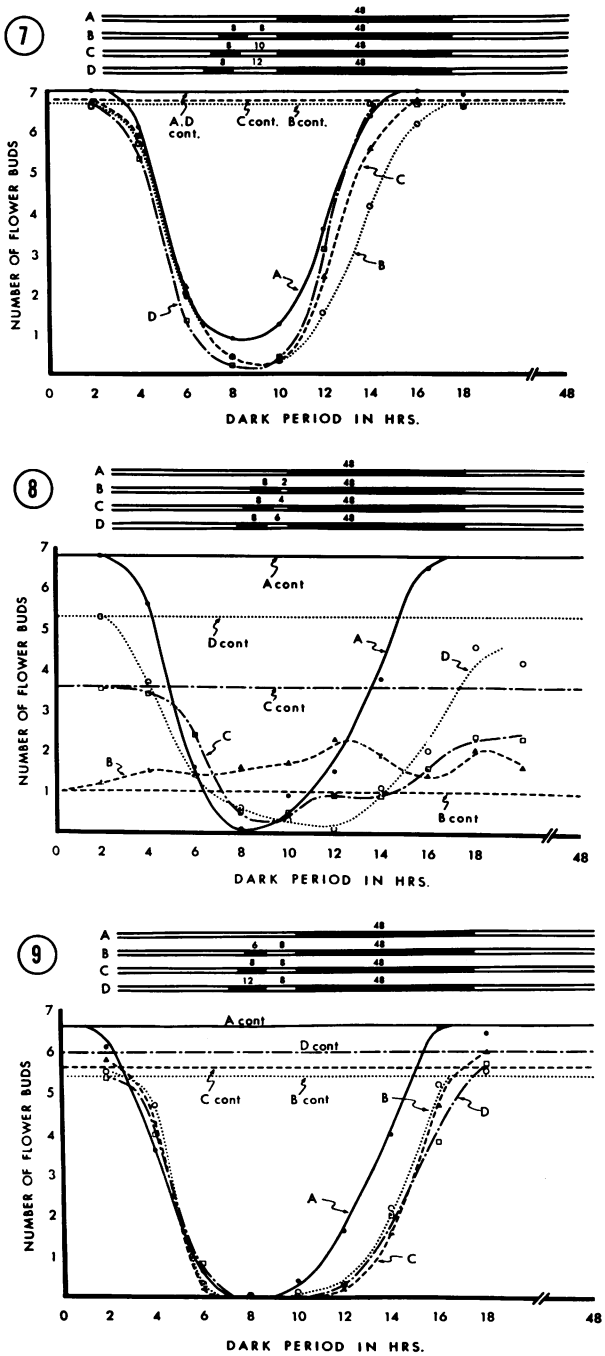


FIG. 7, 8 and 9. Flowering responses to red light interruptions given at different times in the first half of a 48-hour dark period which was preceded by various light conditions. The light conditions preceding the 48-hour dark period are shown diagrammatically. Dark-period temperatures were 19° in fig. 7, and 18.5° in fig. 8 and 9.

Experiment 8: In the experiments mentioned above, an 8-hour dark period was separated from the main dark period by various lengths of light periods. In the present experiment, plants were subjected to various lengths of dark period followed by 8 hours of light preceding the main dark period, and the time of effectiveness of red light interruptions was investigated. Three groups of the plants were exposed to 6-, 8- or 12-hour dark periods followed by 8 hours of light preceding a 48-hour dark period. Five minutes of red light was given at different times in the first 20 hours of the 48-hour dark period. A control group was kept under continuous illumination before the 48-hour dark period in which 5 minutes of red light was given in the same way in other experimental groups. Results are shown in figure 9. All of the experimental groups showed similar responses to a 5-minute red interruption and all deviated from the controls in the same way.

## Discussion

The photoperiodic response of *Pharbitis nil* is very sensitive to temperature. The lower the temperature, the longer the dark periods required to initiate flower buds. Flowering responses increased nearly linearly with increasing duration of the dark period if the plants were kept under continuous illumination before the dark period. However, if 8 hours of darkness followed by 8 to 12 hours of light were given before the main dark period, flowering responses increased stepwise with increasing duration of the dark period. This indicates that an endogenous rhythm is participating in photoperiodic response of *Pharbitis nil.* Sharp increases in flowering response were observed 48 to 54 and 70 to 76 hours after the beginning of the light period irrespective of the length of the light period. It is considered that some endogenous rhythm was initiated by light-on or light-off signals given before the main dark period.

A hypothetical oscillation curve of the rhythm which is probably initiated by a light-on signal is shown in figure 10, curve a. This curve shows a sensitivity to light which stimulates or inhibits the flowering response of *Pharbitis nil.* Curve b in figure 10 shows the flowering response of *Pharbitis nil* which was subjected to various lengths of dark period preceded by continuous illumination (dark temperature, 18°). In this case the endogenous rhythm had faded away because no light-on signal was given before the main dark period. When the light-on signal is given before the main dark period, the expected flowering responses are shown by curve c in figure 10, A and B, and represents an integration of curves a and b. Curve c in A and B shows the theoretical flowering responses of the plants exposed to 8 hours of darkness followed by 8 and 12 hours of light preceding the main dark period of various lengths,

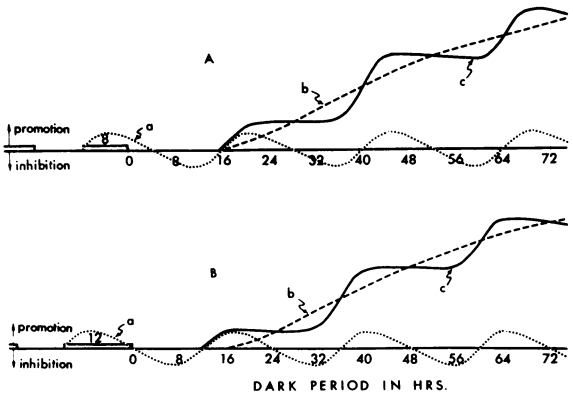


FIG. 10. Theoretical curves for flowering responses of *Pharbitis nil* exposed to a single dark period of various lengths. For details see text.

respectively. These curves are very similar to those experimental curves shown in figures 5 and 6.

The time of effectiveness of a red light interruption is also influenced by preconditions. However, if the dark period was preceded by a light period of 4 or more hours, red light given 8 hours after the beginning of the dark period strikingly inhibited flowering irrespective of the preconditioning and the dark-period temperature. This means that some timing mechanism which is temperature compensated starts at the beginning of the dark period and becomes very sensitive to light after 8 hours.

However, as has been described above, the light-on signal is considered to initiate some endogenous rhythm which gives a rhythmic response to the length of dark period. Five minutes of red light given in the dark period, also gives a light-on signal. Assuming that 5 minutes of red light may also initiate the same kind of rhythm, it seemed conceivable that the 5 minutes of red light given 8 hours after the beginning of the dark period would result in an inhibitory phase at the end of the 48-hour dark period. Thus, even the flower inhibitory effect of red light given in the first half of the 48-hour dark period might partly depend on an interaction with the following light period. If this were true, rhythmic responses to the length of dark period should be expected in Experiment 4, in which the plants were exposed to various lengths of dark periods which were in turn interrupted with 5 minutes of red light 8 hours after the beginning of each dark period. This was not the case in Experiment 4. That is, the 5 minutes of red light is not enough to initiate an endogenous rhythm. Therefore, if the plants were exposed to continuous illumination before the dark period it is considered that the time of effectiveness of a red light interruption given in the first half of the 48-hour dark period is controlled mainly by the timing mechanisms which start at the beginning of the dark period (i.e. the light-off signal). Nevertheless, the time of effectiveness of red light interruptions was influenced by the duration of the light period which was given before the main dark period. This is considered to depend on the effect of the endogenous rhythm which was initiated by the light-on signal given before the main dark period. In Experiment 8, 3 groups of the plants were exposed to 6-, 8- or 12-hour dark periods followed by 8 hours of light preceding a main dark period. All of these groups showed similar responses to a red light interruption. This gives support to the hypothesis that an endogenous rhythm is initiated by the beginning of the light period (light-on signal).

Theoretical curves of flowering responses to red light interruptions given in the first half of a 48-hour dark period are shown in figure 11 A–D. Curve b in each graph of figure 11 A–D shows a timing component which presumably starts at the beginning of the dark period and becomes very sensitive to red light after 8 hours. Figure 8 showed that the flower inhibitory effect of red light given 8 hours

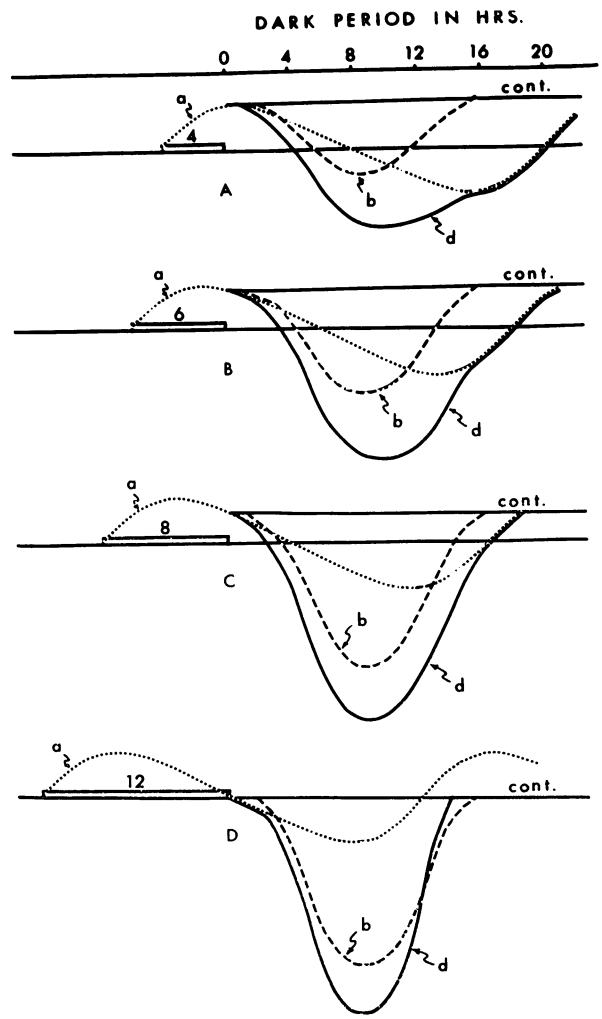


FIG. 11. Theoretical curves for flowering responses of *Pharbitis nil* exposed to 5 minutes of red light at different times in a 48-hour dark period. For details see text.

after the beginning of the dark period decreased with a decreased duration of the light period preceding the main dark period. When a light period of only 2 hours was given preceding the main dark period, the red light given 8 hours after the beginning of the dark period did not inhibit flowering, but was, instead, stimulatory to flowering. It is assumed, therefore, that to initiate this component of the timing mechanism, a light period of 4 hours or more is required before the dark period and that the amplitude is related to the length of the previous light period. In figure 11, the amplitude of curve b was increased with increasing duration of the light periods given prior to the dark period. Curve b in figure 11 D is assumed to be the same as with continuous light (cf. fig. 3). A light period of 2 hours may not be enough to initiate this component of the timing mechanism, and details on this problem will be discussed in another paper (unpublished). Curve a in figure 11 A–D shows an endogenous rhythm which starts at the beginning of the light period. This is the same curve as that shown in figure 10. The flowering response of control plants which were not exposed to red light during the dark period is influenced by the phase of the rhythm at the end of the dark period (cf. Experiment 5, 6). The dark period is 48 hours and the cycle length of the rhythm is 24 hours. Therefore, the phase of the rhythm at the end of the dark period is the same as that at the beginning of the dark period. In figure 11 A–D the flowering levels of controls are shown by straight lines (cont.).

The flower inhibitory effect of red light given during the dark period depends on both of the timing mechanisms mentioned above. Assuming that the effects are additive, the flowering responses which are shown by curve d in figure 11 A–D are expected. The theoretical curves d in figure 11 A–D are very similar to those experimental curves shown in figures 7 and 8.

Data presented here suggest that there are at least 3 kinds of timing mechanisms in the photoperiodic response of *Pharbitis nil*. The first timing component is similar to an hourglass in that a linear increase in the flowering response results with increasing duration of the dark period. Furthermore this component is very sensitive to temperature (cf. fig. 1 and curve b in fig. 10). The second component is an endogenous circadian rhythm which starts at the beginning of the light period (cf. curve a in fig. 10, and 11). The third component, which is temperature-insensitive starts at the beginning of the dark period and has a very light-sensitive phase with the maximum 8 hours after the onset of darkness (cf. fig. 2, 3 and fig. 11, curve b).

In Experiment 3, 5 minutes of red light given during the last half of the 48-hour dark period inhibited flowering to some extent even when the plants were exposed to continuous illumination before the dark period. This inhibitory effect might be due to an interaction with the following light period, but the details on this phenomena will also be reserved for discussion in another paper (unpublished).

## Summary

Photoperiodic response of *Pharbitis nil* is very sensitive to dark temperature, but the time of effectiveness of a red light interruption remains constant at any temperature.

If *Pharbitis* plants were exposed to continuous illumination before a single dark period of various durations, flowering responses increased almost linearly with increasing duration of the dark period. However, if they were subjected to an 8-hour dark period followed by an 8- or 12-hour light period preceding the main dark period, flowering responses increased stepwise with increasing duration of the main dark period, indicating that an endogenous rhythm is participating in the photoperiodic response of *Pharbitis nil*. This rhythm is considered to be initiated at the beginning of the light period.

The time of effectiveness of a red light interruption was also influenced by preconditioning. The times at which red light was inhibitory were somewhat delayed and extended with decreasing duration of the light period given before the main dark period. However, the red light which was given 8 hours after the beginning of the dark period inhibited flowering irrespective of the length of the light period preceding the dark period, if the light period was 4 hours or more.

Data presented here suggest that there are at least 3 kinds of timing mechanisms in the photoperiodic response of *Pharbitis nil*. The first timing component is similar to an hourglass in that a linear increase in the flowering response results with increasing duration of the dark period. Furthermore this component is very sensitive to temperature. The second component is an endogenous circadian rhythm which starts at the beginning of the light period. The third component, which is temperature-insensitive, starts at the beginning of the dark period and has a very light-sensitive phase with the maximum 8 hours after the onset of darkness. To start the last component of the timing mechanism, a light period of 4 hours or more may be necessary before the dark period.

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# Alkaloids and Plant Metabolism VII. The Kinetin-Produced Elevation in Tyramine Methylpherase Levels [1, 2]

**Carol E. Steinhart, Jay D. Mann,[3] and S. Harvey Mudd**

**Laboratory of Cellular Pharmacology, National Institute of Mental Health, U.S. Department of Health, Education and Welfare, Bethesda 14, Maryland**

Hordenine and *N*-methyltyramine are synthesized in roots of germinating barley by successive *N*-methylation of tyramine (10, 12). The greatest concentrations of these alkaloids are found on the fourth or fifth day of germination. We have shown that the highest activity of tyramine methylpherase (*S*-adenosylmethionine: tyramine methyltransferase), the enzyme which catalyzes the formation of *N*-methyltyramine, also occurs on the fourth or fifth day of seedling germination. A similar time course of enzyme increase and subsequent decrease was found in plants grown from isolated embryos (13).

When kinetin is supplied to barley embryos, the tyramine methylpherase activity after the fifth day is elevated in comparison to that in untreated controls (13). We now find that there is some specificity in this effect for both the hormone and the enzyme involved. The increased enzyme activity results from stimulation of enzyme synthesis, rather than retardation of inactivation.

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[2] This is the 32nd paper in a series on enzymatic mechanisms in transmethylation.

[3] Present address: Department of Horticultural Sciences, University of California, Riverside, California.

## Methods

Seeds of *Hordeum vulgare* L. var. *Betzes* were used throughout these experiments. In our early work we used only seeds from the 1960 crop. Recently we have also employed seeds from the 1957, 1961, and 1962 harvests.

Methods for the culture of barley embryos and the assay of tyramine methylpherase have been described (13). Supplements to the basal culture medium were sterilized by passage through Millipore filters. Whenever possible, they were added to the sterile medium before it solidified. When treatment occurred after embryos were growing in the medium, 0.5 ml of solution per flask was added from a syringe equipped with a sterile Swinny adapter and Millipore filter. Seeds were soaked for 2 to 2.5 hours before the embryos were excised and planted. The term "day 2" means 48 hours after planting. Scutella, which do not contain tyramine methylpherase activity, were customarily included with the roots for convenience in harvesting, but the shoots were discarded. Chromatographically pure kinetin was purchased from Mann Research Laboratories.

Roots were extracted at 4° with 1 of 3 different extracting fluids, according to the use to be made of