# Studies on Red Light Interruption in Relation to Timing Mechanisms Involved in the Photoperiodic Response of Pharbitis nil[1, 2]

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In a previous paper (2) it has been suggested that at least 3 kinds of timing mechanisms are involved 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. This component is very sensitive to temperature. The second component is an endogenous circadian rhythm which starts at the beginning of the light period, and for which the temperature sensitivity has not been determined. 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 sensitivity about 8 hours after the onset of darkness. The first and the second components have been detected by giving different lengths of dark periods, and the third component by giving a red light interruption during 24- or 48-hour dark periods. In the previous paper (2) an effect of red light interruptions given during the last half of a 48-hour dark period was noted but was not discussed. The present experiments were designed to obtain more detailed information on these timing mechanisms with emphasis upon the effects of red light interruptions given at different times during a long dark period.

## Materials and Methods

Seedlings of *Pharbitis nil*, strain Violet, were used for all of the experiments. Experimental methods and procedures were quite similar to those described in a previous paper (2). After germination plants were exposed at 20° to continuous illumination of about 400 ft-c from cool white fluorescent lamps. Four days after germination the plants were subjected to experimental treatments, and then placed under continuous illumination of about 400 ft-c from the fluorescent lamps for at least 24 hours at 20°. After that the plants were transferred to benches in the greenhouse where they were exposed to 18-hour photoperiods by extending daylight with incandescent light of about 50 ft-c. About 2 weeks after the treatment plants were harvested and dissected to determine the number of flower buds initiated. Temperature during the experimental dark period was controlled carefully so that, with the particular dark period used, the flowering response of the controls would be at the most sensitive level. Red light was obtained from Gro-lux fluorescent lamps filtered with 2 layers of red cellophane, and in all experiments presented here the intensity was about 3300 ergs/cm$^2$ per second at the leaf surfaces. The quality of the irradiation is shown in figure 1 of the following paper (3). In all experiments 18 to 27 plants were used for each treatment, and the average number of flower buds per plant was used as in indicator of the photoperiodic response.

## Experimental Results and Discussion

Plants were subjected to a single dark period of various lengths, during which red light interruptions were given at different times. The flowering responses are shown in figures 1 to 4. In all of these curves maximum inhibition of flowering is seen when red light was given about 8 hours after the beginning of the dark period, thus confirming the results discussed in a previous paper (2). All of these curves are somewhat undulating subsequent to this initial dip. In every case the curve shows a second dip 30 to 40 hours after the onset of darkness; in other words, about 24 hours after the maximum depression at the 8-hour point. There is some evidence, therefore, that a circadian rhythm is exhibited in this response. This rhythm is suggested by all of the curves, including those in which the plants received continuous light prior to the main dark period. In the previous studies (2) an endogenous circadian rhythm seemed to be associated with the light-on signal of the pretreatments when the plants were exposed to noninductive photoperiodic treatments prior to the main dark period. The present experiments, however, suggest that the onset of darkness may induce another endogenous circadian rhythm which results in high sensitivity to red light 8 hours after the onset of darkness, and a second weaker pulsation of sensitivity to red light occurring about 24 hours later. This rhythm will hereafter be called the light-off rhythm to distinguish it from the rhythm which

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was previously described, and which may be called the light-on rhythm.

As was noted before (2), if plants exposed to continuous illumination prior to the main dark period are compared with plants which received a pretreatment of 8 hours of darkness and 8 hours of light prior to the main dark period, the flowering responses to the red light interruptions given during the first 16 hours of the dark period are influenced by the pretreatment. The initial dip in the curve for the latter plants is more delayed and extends further into the dark period. This effect was considered to be based on the interaction between the first pulsation of the light-off rhythm and the pulsations of the light-on rhythm (2). It appears from the present experiments that this interaction continues through the second pulsation of the light-off rhythm since pretreatment of the plants with 8 hours of dark and 8 hours of light prior to the main dark period causes similar effects on the second dip in the curve. When the dark period was 48 hours (fig 4), the second dip in the curve was not influenced by pretreatment. In this case, however, an interaction between the red light interruption and the following light period might have a predominant effect since the red light given during the last 20 hours of the dark period slightly inhibited flowering irrespective of the length of the dark period.

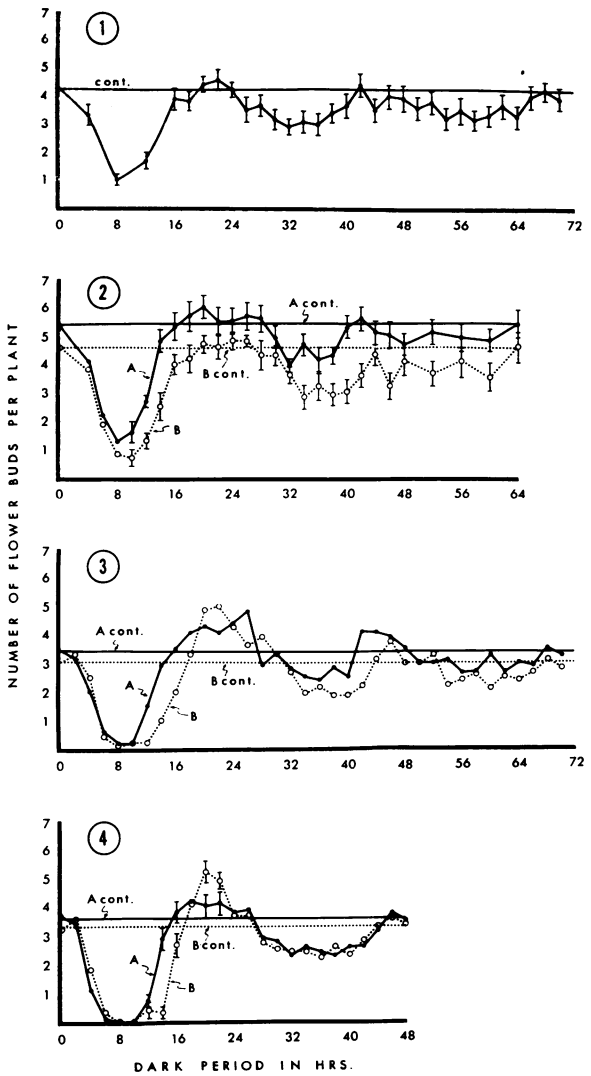


FIG. 1-4: Flowering responses to red light interruptions given at different times in a long dark period. The vertical lines indicate the standard error. FIG. 1. Plants kept under continuous light were subjected to a 72-hour dark period at 17.5°. Five minutes of red light was given at different times. FIG. 2. Plants kept under continuous light (A) and those subjected to an 8-hour dark and an 8-hour light period (B) were exposed to a 64-hour dark period at 18°. Five minutes of red light was given at different times. FIG. 3. Plant kept under continuous light (A), and those subjected to an 8-hour dark and an 8-hour light period (B) were given a 72-hour dark period at 17°. Thirty minutes of red light was applied at different times during the 72-hour dark period. FIG. 4. Similar to figure 3, but the main dark period was 48 hours and the dark temperature was 18°.

Five minutes of red light given at different times in a long dark period did not promote flowering in any of these experiments. However, as has been reported in soybean (1), 5 minutes of red light might not be enough to produce stimulatory effects even though the endogenous rhythms were going through stimulatory phases of their cycles. On the other hand, 30 minutes of red light slightly promoted flowering when it was given 16 to 24 hours after the beginning of the dark period. This stimulatory effect was observed in *Pharbitis* even when the plants were kept under continuous light before the dark period (fig 3, 4). Since there is no light-on rhythm when the plants are pretreated with continuous light, this result suggests that the light-off rhythm which is initiated by the onset of darkness has both stimulatory and inhibitory phases.

One might expect, as was found with the light-on rhythm, that this light-off rhythm would be reflected in a stepwise increase in flowering with increasing lengths of dark period, but such is not the case. As reported previously (2), plants subjected to various lengths of dark show stepwise increases in the flowering response (evidence of a circadian rhythm) only when the plants are pretreated with a light-dark regime and not when pretreated with continuous light. The only evidence for the participation of an endogenous rhythm associated with the light-off signal was obtained when the long dark period was interrupted at various times with red light. A possible explanation is as follows:

In the previous paper (2) one component of the time measurement of the dark period showed an hourglass type of response. This component was very sensitive to temperature, and a red light interruption during an inhibitory phase of the dark period seemed to slow down this particular reaction. For

example, at 20° a dark period of about 16 hours is enough to obtain a maximum flowering response. However, if after 2 hours of darkness the plant is illuminated with red light, the flowering response increases slowly with increasing duration of the subsequent dark period, and a dark period of 72 hours is not enough to induce the maximum flowering response (fig 4 in Literature 2). In other words, a red light interruption given in the inhibitory phase of a dark period seems to slow down the hourglass component of the timing mechanism to produce a result similar to that obtained by lowered temperature (2). Therefore, in order for a red light interruption to produce its effect, the interruption must be followed by a minimum dark period. When the dark period is terminated with continuous light the hourglass component of the timing mechanism apparently stops at the end of the dark period. In this case the effect of the light-off rhythm may not be reflected in the flowering response since the amount of flowering is determined solely by the length of the dark period. The light-on rhythm, on the other hand, is reflected both in a light-break effect and in the flowering response to increasing lengths of dark period.

The oscillations of the light-off rhythm appear to be damped rapidly after 1 cycle, as is shown in figures 1 to 4 (solid line). However, this damping may be more apparent than real since the curves show only the flowering response to red light interruptions which is controlled by the light-off rhythm. We could assume, for example, that red light given at either the 8-hour point or at the 32-hour point slows down the hourglass component to the same extent. However, red light given at the 32-hour point produces much less inhibition of flowering since the plant is exposed to 32 hours of darkness prior to the red light interruptions, and presumably up to the point of the interruption the hourglass component of the reaction proceeded in a normal fashion.

In considering the results of this paper together with those of the previous paper (2) one may conclude not only that 2 endogenous circadian rhythms, one induced by the light-on signal and the other by the light-off signal, are involved in the photoperiodic responses of this plant; but also, that these 2 rhythms are not identical in their physiological reaction. The light-on rhythm is apparent not only through a rhythmic sensitivity to red light during the dark period, but also is shown by a rhythmic sensitivity to length of dark period resulting in a stepwise increase in flowering with variable dark period lengths. On the other hand, the light-off rhythm is apparent only through a rhythmic sensitivity to red light. These differences in the physiological responses to the 2 rhythms may be of considerable importance in comparing the photoperiodic responses of various plants. Perhaps some plants use only one or the other of the 2 rhythms as the primary mechanism in timing the photoperiodic response.

Finally mention should be made of the possibility that the so-called biological clock may also involve 2 endogenous rhythms, one induced by the light-on signal and the other by the light-off signal. It is well known, for example, that the biological clock adjusts to the light-dark regime of the environment. The time sense of organisms moved from east to west or vice versa adjusts to a new local time within a few days. The question has arisen as to just how the biological clock adjusts to compensate for changing day length. If the clock were reset by the dawn of each day or by the dusk of each day, then it is difficult to see how it would be properly set in the short days of the winter as compared to the long days of the summer. On the other hand, if the biological clock is set both by dawn and dusk, a satisfactory explanation for the accuracy of the clock both during the winter and the summer would be provided.

## Summary

Seedlings of *Pharbitis nil*, strain Violet, were subjected to a long dark period and exposed to 5 or 30 minutes of red light at different times. When the dark period was preceded by continuous illumination, red light given about 8 hours after the onset of darkness produced maximum inhibition, and a second interval of inhibition was observed when the red light was given between 30 and 40 hours after the onset of darkness, irrespective of the length of the dark period. Thirty minutes of red light given 16 to 24 hours after the beginning of the dark period slightly promoted flowering. These phenomena suggest that one of the timing mechanisms which is initiated at the beginning of the dark period is an endogenous circadian rhythm.

When the plants were subjected to an 8-hour dark period followed by an 8-hour light period before the main dark period, the time of effectiveness of red light interruption was somewhat extended and delayed at both the 8- and 32-hour points. This effect is considered to depend on another endogenous circadian rhythm which is initiated at the beginning of the light period. It is supposed that at least 2 kinds of endogenous circadian rhythms are participating in the photoperiodic reaction of *Pharbitis*.

## Literature Cited

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